



WORLD AQUACULTURE

DARWIN AUSTRALIA

MAY 29 TO JUNE 1, 2023

"Supporting Strength in Aquaculture"

The Annual International Conference & Exposition of
World Aquaculture Society
and
Asian Pacific Aquaculture 2023,
Annual meeting of Asian Pacific Chapter, WAS

DARWIN CONVENTION CENTRE
Darwin, Northern Territory, Australia

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WELCOME

Dear colleagues and friends,

Welcome to Darwin, Northern Territory, Australia, and to World Aquaculture 2023. On behalf of the WA23 Steering Committee and the WAS Board, I warmly welcome you to the event at a special venue, the Darwin Convention Centre on the Darwin Waterfront, and in a perfect tropical dry season.

This is Larrakia Country, and we acknowledge the Larrakia people as traditional custodians of the land on which we meet for WA23. We pay our respects to Larrakia Elders past and present, and to their emerging leaders.

Situated in the far north of Australia, and so close to Southeast Asia, Darwin is a culturally diverse city with a range of activities to discover, especially in the outdoors. In between WA23 conference sessions and exploring the trade show, the organising team hope that you will find time to explore local restaurants, experience the beauty of the local natural environment and wildlife, wander the night markets, and immerse yourself in art and culture in Darwin. And take the opportunity to enjoy premium quality seafood.

World Aquaculture 2023 is the first time in 9 years that Australia has hosted this international aquaculture event. Aquaculture production in Australia has increased from 80,000 tonnes valued at AU$1 billion to 131,600 tonnes and AU$1.73 billion in that time. Industries in the tropical north, especially shrimp ('prawns' for the Aussies), barramundi and pearl oysters are contributing to that growth. In the south of Australia, salmonids, oysters, tuna and abalone are the largest sectors. WA23 provides a forum to share technical and business experiences through this growth period with international colleagues, and to learn from knowledge generated around the world, to **support strength in aquaculture** globally.

World Aquaculture events are a vital contributor to communication among producers, suppliers, researchers, government and all stakeholders in the aquaculture landscape. The success of these meetings is only possible with support of sponsors and all exhibitors, and we sincerely thank them for their involvement in WA23.

We also thank the conference organisers and members of the Steering, Program, Student Activities, and Regional Organising Committees that have been working hard to plan, coordinate and bring the technical program, trade show and associated meetings together.

Thank you to all participants for joining us to make this a memorable event.

Enjoy WA23 in Darwin.

Assoc Prof Jennifer Blair

Chair of WA2023

President, World Aquaculture Society

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ABSTRACTS

WOMEN IN THE AUSTRALIAN AQUACULTURE INDUSTRY – CHALLENGES AND OPPORTUNITIES

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Until recently, Australia had little data on women's contributions to the seafood industry and the roles women play. Furthermore, there was little industry-wide understanding of women's experiences working in the industry, if and where there are inequalities and gendered differences in experiences, and where barriers lie to women fully participating and progressing in the industry.

In 2021-22, Women in Seafood Australasia (WISA) and the Fisheries Research and Development Corporation funded research to comprehensively understand women's participation and engagement in the seafood industry, an industry that currently needs to attract new entrants and retain them.

The research questions were:
1. What is the contribution of women in the Australian seafood industry?
2. Who are the women in the Australian seafood industry?
3. What needs to change for women to have a career and succeed in the Australian Seafood Industry?

This national project used a combination of data sources, including Australian Bureau of Statistics Census data, in depth key informant interviews, and an online survey, to reveal answers to these questions first asked by WISA twenty years ago.

This presentation will present the findings of this research within the context of the Australian aquaculture industry, highlighting the challenges and opportunities for women in aquaculture.

The presentation will form part of a think-tank session chaired by WISA, where we look deeper into the barriers women face to participate and reach their full potential in aquaculture, and towards solutions – what can individuals, small and large businesses and organisations do to attract and retain women in aquaculture? We will discuss the deeper tensions for businesses and organisations to make change, opportunities for increasing the attractiveness of the aquaculture industry to women and other under-represented groups entering and thriving in the industry, and actionable solutions for the aquaculture industry in different environments in both the short and long term.

NEW ANTHROPOGENIC CHALLENGES THREATENING THE INDIAN OCEAN AREA OF AFRICA: MITIGATION AND ADAPTATION ANTHROPOGENIC CHALLENGES THREATENING THE INDIAN OCEAN AREA OF AFRICA: MITIGATION AND ADAPTATION

Abubaker

Background:
There are many ongoing anthropogenic activities threatening the sustainable use of marine biodiversity, improve food safety & security as well as providing protections and preservations of the Indian ocean environment and its diverse resources.

Some of these challenges are as highlighted below:

1. Dynamite fishing which is also called blast fishing is a major issue affecting the Indian Ocean in Africa. The coasts of the Eastern part of Africa is blessed with extensive network of coral reefs whose biodiversity and beauty supports major artisanal fishing and tourism industries in the East-African countries like Tanzania, Kenya, Mozambique, Mauritius, Comoros islands, Madagascar and South Africa among others. Unfortunately, due to the fact that explosives are cheap and easily accessible to fishers/fishermen in some of these East and South Eastern-African countries the fisher men in the region use them for fishing purposes. The explosives are used for blasting to kill fish inside the Indian Ocean territories in order to catch more fish at once or in one outing. A blast can lead to a catch of up to 400 kg of fish and a profit of US$1,800 in market sales, a lucrative short-term profit despite the long-term destruction left behind.

These Dynamites/Bombs or Explosives are usually sourced from mining, demolition, and road construction enterprises for these blast fishing sometimes they are even made at home locally such as the Bottle bombs made with kerosene and fertilizer and diesel.

Fig.1: A photo of an unexploded bottle Bomb:

Fig.2: Showing a photo of Dynamite fishing in the Indian Ocean:

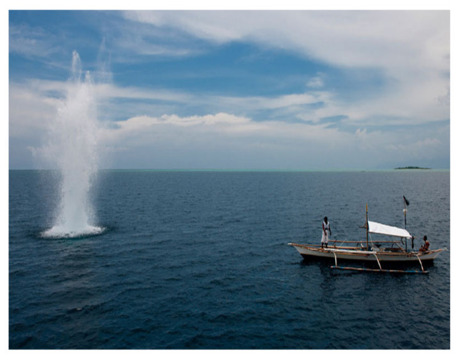

Fig. 3: A marine traffic coordinator using AIS and radar:

(Continued on next page)

A single blast in blast fishing can kill hundreds of fish in seconds, destroys large numbers of fish and other marine animals and their nearby habitats and yet indirectly also kills and damage many of the coral reefs around which supports rich array of marine animals of the Indian ocean

2. Many of the Indian Ocean coastal cities/towns in the eastern part of Africa bordering the Indian ocean such as Mogadishu, Port Louis, Kisi-mayo, Mombasa, Dares Salam, Victoria, Pemba, Zanzibar, Toamasina, Beira and to some extent even Maputo, Durban, Port Elizabeth in South Africa keeps generating local wastes that are mostly ending up in the Indian oceans as their final destinations.

3. Piracy is another addition to the issues affecting livelihood and economic activities in the Indian Ocean. Piracy in the Indian Ocean off the coast of Somalia has been a threat to international shipping since the second phase of the Somali Civil War in the early 21st century to an extent that it impeded the delivery of shipments and increased shipping expenses, costing an estimated $6.6 to $6.9 billion a year in global trade in 2011 according to Oceans Beyond Piracy (OBP).

INCREASING THE SUSTAINABILITY OF A POND FARM: A COMPREHENSIVE APPROACH

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Presented are methods for improving sustainability in extensive finfish pond farming and the results of their implementation on a specific farm. The results included an increase in outlet water quality, a reduction in water consumption, improved disease control, a drop in FCR, and zero use of wild-caught fish in fish feed.

First, cultivation of chlorella algae on-site and its introduction to the ponds increased DO and suppressed blue-green algae. This improved the fishes' health, increased natural productivity, and reduced organic contamination in the discharge water. Second, a technique was developed to bypass the need for transferring fingerling from rearing ponds to grow-out ponds, reducing water discharge and intake and the fishes' susceptibility to disease. The stocking density was then evaluated by catching a portion of the fish and using a Neutral Red dye. Finally, probiotics were added to the fishes' diet and a potassium iodide and lactic acid mixture to the transport water as additional disease control measures.

Zero use of wild-caught fish was achieved by producing fish protein concentrate from the wastes of on-site fish processing and using it alongside yeast as major protein sources for self-produced extruded feed (pellet dissolution was minimised by using reflex feeders). This diet was complemented by the ponds' natural productivity, which was boosted by bottom cultivation and vika-oatmeal mixture sowing prior to the pond being filled, resulting in pond bottom aeration. The plants' remains shifted the water's C:N ratio in favour of biofloc development and provided a substrate for periphyton.

Biography:

Graduated from the Moscow Institute of Physics and Technology in 2008. Started working at an almost abandoned 240-hectare carp pond farm in 2012. Recultivated the ponds that were overgrown with weeds and bushes and made the farm the largest and most successful freshwater fish producer in the region.

BEHAVIOURAL CHANGES AND HISTOPATHOLOGICAL EFFECT OF GLYPHOSATE AQUATIC HERBICIDE (FORCE UP) ON TISSUES OF SUB ADULT AFRICAN CATFISH *(Clarias gariepinus)*

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This experiment determined the toxicity and behavioural changes of glyphosate aquatic herbicide (Force up) on African catfish *Clarias gariepinus* sub adult. A total of 120 live and apparently healthy *Clarias gariepinus* sub adult measuring 18.3-30cm standard length and average weight of 123g was randomly distributed into twelve (53.5cm X 33cm X 34cm) glass tanks of 60litres capacity each were filled with 20litres aerated bore-holes water, Six treatment in duplicate was set up for the experiment. Ten (10) *Clarias gariepinus* sub adult was distributed randomly in triplicate per treatment for experiment involving the sub adult *Clarias gariepinus*. The toxicant was introduced at different concentrations (0mg/l, 40mg/l, 70mg/l, 100mg/l, 130mg/l, 160mg/l) in triplicate per treatment. Fish mortality and behavioral changes was monitored and recorded for the first 24hours, 48 hours, 72 hours and for the 96hours. The inability of the fish to respond to external stimuli was used as an index of death. Dead fish were removed immediately with a scoop net to avoid contamination due to rotting. Behavioral changes exhibited by the fish include erratic swimming, air gulping, loss of reflex, molting, discoloration, barbel deformation and excessive mucus secretion in fish exposed to higher concentration of glyphosate aquatic herbicides. Histopathology of the organs after 96 hours; In the liver, there was nuclear vacuolization (NV) with irregular shaped nuclei, moderately damaged tissue and hepatocyte (H) regeneration which indicates that recuperation is still possible while in the skin; there was cellular abnormalities, shrinkages, hypertrophy of tissue, absence of dermal layer and necrosis. The 96h LC50 of glyphosate aquatic herbicide (Force up) to Sub Adult African Catfish *Clarias gariepinus* is 123.784mg/l with the maximum safe concentration ranged between 1.24mg/l to 12.38mg/l, the safe level of a compound is derived by multiplying the 96h LC50 with an application factor of 0.01-0.1. Such application factor are applied to acute toxicity test data to estimate the concentration that is safe for chronic exposure. The results of the study revealed that Glyphosate aquatic herbicide (Force up) is toxic to fish organs and causes histopathological changes in different organs such as skin and liver; therefore, indiscriminate use by farmers should be discouraged particularly in aquatic bodies.

ATTRACTABIILITY AND PALATABILITY OF FORMULATED DIETS INCORPORATED WITH CHICKEN FEATHER AND ALGAL MEALS FOR JUVENILE GILTHEAD SEABREAM *Sparus aurata*

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Feed ingredients of aquatic animal origin such as fishmeal, krill meal, shrimp meal and squid meal are rich in chemical compounds such as free amino acids (FAA), nucleotides, amines and nucleosides that are readily recognizable by the chemosensory systems of the fish in the process of locating and ingesting food. Rendered animal and algae proteins are a potential source of digestible protein, amino acids (AAs), vitamins and minerals. This study was conducted to evaluate the attractability and palatability of chicken feather and algal meals to replace fish meal, in the formulated diets for juvenile of seabream, *Sparus aurata*. Treated chicken feather meal with 20, 35 and 50% replacement of fish meal (20, 35, 50 CHF), algal meal (*Ulva fasciata*) with 20 and 35% replacement of fish meal (20, 35 AM) and control diet (CNL) were tested for attractability and palatability to juvenile gilthead seabream, *Sparus aurata*.

Algal meal accorded considerable feeding effector properties to seabream feeds at high levels of inclusion (20 and 35% AM), but is probably only slightly superior to fishmeal in terms of attractability and palatability. Chicken feather meal was not an effective feeding effector to seabream at 20, 35 and 50% inclusion level. However, 50% CHF was more palatable than CNL, 20 and 35% CHF. Overall, 20 AM is the best diet in terms of both attractability and palatability. Among the various biochemical parameters analyzed in the present study, levels of algae replacement in the ingredients closely related with the effectiveness of the ingredients as attractants and palatability enhancers for seabream.

Table 1. Proximate composition of treated feather meal, algal meal and fish meal used in the experiment. Values are means and standard deviation of three replicates. Means in a column with different superscripts are significantly different ($p<0.05$).

Sample Name	Crude Protein (%)	Crude Lipid (%)	Ash (%)	Fibre (%)	Dry Matter (%)
Fish meal (sardine)	$55^{b} \pm 0.36$	$9.00^{a} \pm 0.02$	$15.70^{b} \pm 0.03$	$2.20^{b} \pm 0.62$	$90.40^{a} \pm 0.08$
Treated Feather meal	$97.99^{a} \pm 0.02$	$1.01^{b} \pm 0.07$	$4.95^{c} \pm 0.04$	$1.06^{c} \pm 0.53$	$84.01^{b} \pm 0.05$
Algal meal (*Ulva fasciata*)	$13.92^{c} \pm 0.05$	$0.08^{c} \pm 0.09$	$24.22^{a} \pm 0.02$	$5.45^{a} \pm 0.33$	$90.22^{a} \pm 0.02$

Table 2. Attractability and palatability of feeds formulated to contain treated chicken feather meal and algal meal as attractants and palatability enhancers in the experiment.

Feed	Attractability (%)	Palatability(%)
	Mean	Mean
CNL	58.57^{a}	15.27^{c}
20CHF	27.14^{b}	16.45^{bc}
35CHF	24.29^{b}	15.46^{c}
50CHF	17.14^{b}	21.41^{a}
20AM	70.00^{a}	18.70^{ab}
35AM	70.00^{a}	17.59^{b}

PROSPECTS OF DEEP-SEA CORALS REARING: A SUCCESS STORY AT SEALABS

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Seawater Laboratories for Aquatic Biosystem Simulations (SeaLABS) of the King Abdullah University of Science and Technology (KAUST), located on the central Red Sea coast of Saudi Arabia, provides outdoor and indoor wet labs for culture experimentation under controlled or simulated environments. SeaLABS is supported by a fully automated Life Support System (LSS) that supplies different water types, aeration, power and internet connectivity in all wet labs. Here, we report the success of SeaLABS in long-term rearing of deep-sea corals (DSCs) from the Red Sea. During a KAUST scientific expedition in the Red Sea in 2013, DSC species *Dasmosmilia valida, Dendrophyllia* sp., *Eguchipsammia fistula*, and *Rhizotrochus typus* were collected using a remotely operated vehicle (ROV), with four-function manipulator arm. After a prior search by high-resolution acoustic seabed mapping for potential DSC habitats, several ROV dives yielded live specimens that were collected using a specially designed lander with holding compartments that were closed and opened by the ROV manipulator arm. After slowly buoying up the lander and retrieved onboard the research vessel, the DSC samples were immediately transferred into acrylic tanks with seawater chilled to 21±0.5^{0}C, resembling the temperature range at their natural habitats. In the SeaLABS, the DSCs were maintained in completely dark setting through and fed with commercial frozen mysis shrimps, with proper thawing, almost twice a week. In their first four years in captivity, the DSCs were maintained in four 125-L low-density polyethylene tanks assembled in a closed-loop recirculating system, then transferred into open glass tanks with 21^{0}C chilled filtered seawater supplied by the LSS in flow-through mode at 60 L min^{-1}. At present, the DSCs are surviving in good conditions and providing readily available live samples for various biomolecular and ecological studies. This SeaLABS experience demonstrates the feasibility of a long-term DSC culture under simulated wet lab settings.

ACIAR REVIEW OF THE BARRIERS TO THE EXPANSION OF TILAPIA AQUACULTURE FOR DEVELOPMENT IN PNG, SOLOMON ISLANDS AND TIMOR LESTE.

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The Australian Centre for International Agricultural Research (ACIAR) has commissioned a desk-to review to identify barriers to the expansion of tilapia aquaculture, with a focus on feeds and fingerlings in PNG, Solomon Islands and Timor-Leste. This review examines literature, connects with previous and current ACIAR projects, and ensures country leaders from PNG, Solomon Islands (SI) and Timor-Leste (TL) are fully involved. The Pacific Island Aquaculture Session will assist this review by consulting with experts from the region.

Despite being identified as having considerable potential for the Pacific, and significant investment by Pacific Island countries and partners, growth of tilapia aquaculture in PNG, SI and TL has been modest with reported total production of less than 2kt/yr in 2019[#]. Tilapia production globally contributes widely to nutrition and livelihoods, due to its well-established culture methods, low input costs and consumer acceptance. These characteristics have led to tilapia becoming the second-most farmed fish in the world (6.2 Mt in 2019)[#], leading ACIAR to ask the question; what are the barriers to development in PNG, SI and TL and what more can be done to help?

Challenges and potential options for further investment will be discussed.

Challenges:

1. ***Scale issues***. Pacific Island countries have much smaller populations than neighbouring countries in Southeast Asia meaning much smaller domestic markets.
2. ***Remote terrain***. Tilapia farming in Pacific Islands often occurs in remote areas with poor infrastructure, difficulty accessing basic supplies, including feeds and fingerlings, and essential chemicals. Some areas even lack a functional economy.
3. ***Access to information & technology.*** Problems with scale & remoteness exacerbate problems with communication, training, & availability of qualified people.

Some initial options that might help:

1. Recognising that individual solutions are needed for ***different culture systems***.
2. ***Subsidised importation of commercial*** **feeds** to rapidly evaluate production potential, for different culture systems.
3. Evaluating the potential of ***black soldier fly*** (BSF) larvae and other vermiculture species as feed sources, especially for more extensive culture systems.
4. Expansion of ***satellite hatcheries*** & creation of a ***Pacific Island network for hatcheries***.
5. Helping import difficulties with 17 methyl-testosterone, vitamin & mineral premixes, etc.
6. ***Increased focus on capacity building, training, and information exchange***.
7. Addressing scarcity and expense of feed ingredients.
8. Support for local enterprises to establish small-scale fish feed manufacturing.

[#] FAO, 2021. FAO Yearbook. Fishery and Aquaculture Statistics 2019. Rome.

PACIFIC ISLAND AQUACULTURE PANEL SESSION

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The Pacific Island Aquaculture Session covers the status of aquaculture in the Pacific, the challenges, and the opportunities. Invited and unsolicited contributions will summarise key learnings from success stories, examine current trends and directions and identify challenges and priorities. Particular attention will be paid to tilapia aquaculture and the challenges with supply of feeds and fingerlings. However, the session is also open for presentations on the role of other aquaculture systems – in inland and coastal regions – for contributing to future food supplies and social and economic development within the region. All aspects of aquaculture production and the value chain, from production systems, feed and seed supply, private enterprise development, investment, market systems and social, gender, economic and environmental dimensions may be discussed.

The Session will conclude with a one-hour panel session which will solicit expert knowledge from the invited panel and the audience. The panel members will include: Geoff Allan, Leo Nankervis, Michael Phillips, Jacob Wani, Jes Sammut, Joshua Noiney, Tim Pickering, Chinthaka Hewavitharane, Jharendu Pant, Zechariah Harohau. A series of open-ended questions will be posed, including:

1. What are the reasons aquaculture development has not progressed more rapidly in the Pacific island Nations (PINs) generally and PNG, SI and TL specifically?
2. What are the priorities to help overcome these challenges?
3. Is there a role for assisted purchase of commercial feeds to help establish the potential of the farming systems?
4. How can the small-scale production of aquaculture feeds be supported?
5. Can the success of satellite hatcheries in PNG be replicated elsewhere?
6. How can the challenges of importing small amounts of chemicals (e.g. 17α-methyltestosterone (17 MT) for single-sex fingerlings and vitamin/mineral premix for feeds) be overcome?
7. How can we rapidly build capacity, enhance training and encourage information exchange? Is this a priority in all PINs?

INLAND SALINE AQUACULTURE IN AUSTRALIA: PAST PROGRESS CHALLENGES AND OPPORTUUNITIES

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The Fisheries Research and Development Corporation (FRDC) and other Australian governments invested substantially in research, demonstration and coordination to identify, evaluate and facilitate commercialisation for inland saline aquaculture in Australia in the early 2000s. Other investment in inland saline aquaculture occurred in several other countries, e.g. USA, Israel and India.

This investment has led to significant industry development in some areas (e.g. India) but commercial progress in Australia has been slow. There is renewed interest in inland saline aquaculture and new investment has been proposed.

Why did commercial inland saline aquaculture work in some areas but not others? Are there common features that might help predict which ventures are more likely to succeed? Despite numerous comprehensive reports and publications from previous work, many of the previous outputs have not been digitised and are difficult to access for a new generation of scientists, policy makers and farmers who are interested in exploring opportunities for inland saline aquaculture. Are we at risk of repeating the mistakes of the past?

The FRDC and NSW DPI seek to address this problem by making sure previous information is readily available, to review why commercial opportunities failed to eventuate, and to examine if recent developments present new opportunities for inland saline aquaculture and, if so, how to best realise those opportunities.

INLAND SALINE AQUACULTURE IN AUSTRALIA: PANEL DISCUSSION

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The Fisheries Research and Development Corporation (FRDC) and NSW Department of Primary Industries (NSW DPI) are supporting a project on inland saline aquaculture (ISA). The project aims to collate existing documents and publications over the last twenty years, to examine commercial developments and impacts from previous research, to identify new opportunities, and to recommend ways these opportunities might be further explored and captured.

The Inland Saline Aquaculture (ISA) Session includes invited and unsolicited contributions that will summarise developments, key learnings from success stories, current trends and directions and identify challenges and priorities.

Keynote speakers for the Session will include Professor Kevin Fitzsimmons (Arizona University) and Dr Tincy Varghese (CIFE, India). Australian expert speakers include Dr Stewart Fielder NSW DPI), Dr Brett Ingram (VFA), Dr Gavin Partridge (Harvest Road, WA) and xxx from SA. Particular attention will be paid to why commercial ISA didn't develop in Australia, as expected in the early 2000s. Attempts at commercial ventures failed to thrive and, if we are to avoid this in the future, we need to know why.

The Session will conclude with a one-hour panel session which will solicit expert knowledge from the invited panel and the audience. The panel members will include keynote and invited Australian experts. A series of open-ended questions will be posed, including:

1. What characterises successful ISA?
2. Are there some fundamentals that are important for commercial potential?
3. What did previous R&D miss?
4. What has prevented ISA ventures from thriving?
5. Is there still potential for ISA in Australia?
6. If so, where and for what species?
7. What can be done to limit future failure of ISA?

MONITORING FOR RESTORATIVE AQUACULTURE: MEASURING ENVIRONMENTAL BENEFITS ACROSS SPECIES, ECOSYSTEMS AND GEOGRAPHIES

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When it utilizes the right practices and species and occurs in the right places, aquaculture can actively support the recovery of degraded ecosystems while also meeting increasing demands for food and livelihood. One approach, to simultaneously meeting environmental and social-economic needs, is restorative aquaculture, which we identify as commercial or subsistence aquaculture that provides direct ecological benefits to the environment with the potential to generate net positive environmental outcomes (Figure 1). To ensure restorative aquaculture can effectively and consistently meet these multiple and often conflicting objectives, however, a clearer understanding of the ways in which practices and systems provide environmental benefits is needed, along with widespread and ongoing monitoring.

We detail a cross-sectoral, -ecosystem and -geographic Monitoring, Evaluation and Learning Framework for Restorative Aquaculture, which we developed to support evidence-based decisions about sustainable aquaculture, regenerative food strategies, and restoration activities linked to aquaculture. This framework draws on case studies of seaweed, shellfish, marine finfish and inland aquaculture to describe an ‘industry-ready’ M&E approach, inclusive of environmental and social targets, objectives, Key Performance Indicators, and a suite of suggested, low-cost monitoring methods for ecosystem services associated with water treatment and quality, provision of habitat, and climate change and resilience.

A consistent and transparent approach to monitoring will enable industry, government, community, and investors to identify the positive environmental impacts of aquaculture, and measure and monitor their ecological, social-economic values. It will also foster development of supporting mechanisms, such as effective policy, consumer awareness, and offsets.

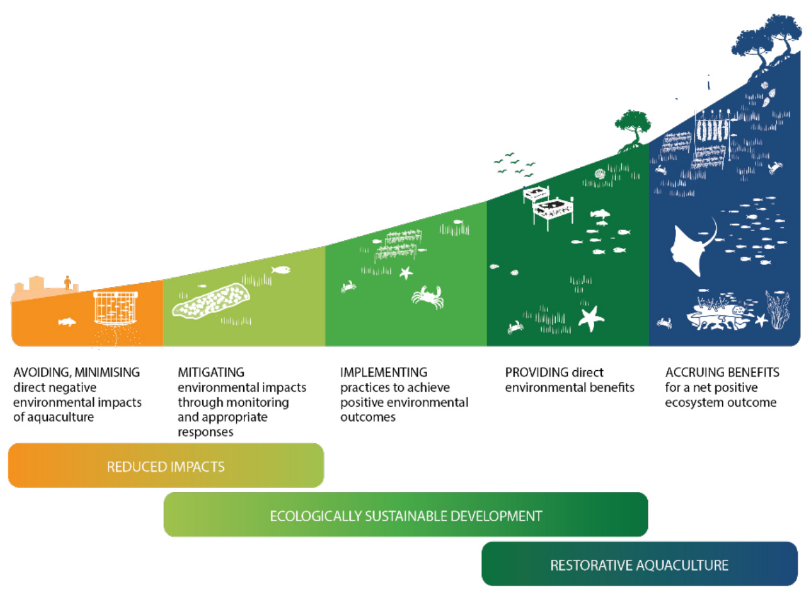

Figure 1. The restorative aquaculture pathway; the reduction of negative risks and effects through risk mitigation and ecologically sustainable development, to deliver, and over time accrue, environmental benefits in local ecosystems

AN OUTBREAK OF LYMPHOCYSTIS DISEASE VIRUS INFECTION IN CULTURED JUVENILE JOHN'S SNAPPER (*Lutjanus johnii*) IN SELANGOR, MALAYSIA

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Snappers (Lutjanidae) are a popular fish species for mariculture due to the high market demand and ease of culture in captivity. In Malaysia, the production of snappers was approximately 14,000 tonnes in 2020, with an estimated wholesale value of USD 88 million. However, intensification of aquaculture lead to various diseases in cultured marine fishes. This study reports an outbreak of lymphocystis disease virus infection in cultured juvenile John's snapper (*Lutjanus johnii*) in Selangor, Malaysia. In January 2021, approximately 19,000 juvenile John's snapper exhibited signs of lethargy and isolating themselves from the schooling group, developed lymphocysts all over the body and fins, and died. The outbreak started at day 3 following the introduction of fish into the hatchery, and the severity of the disease was observed between day 7 and 14, resulting in 53% mortality. No bacteria was isolated, while a low prevalence of protozoan parasite was observed at 5% of fish samples. Histopathology examination revealed focal nodular dermal fibroblast hypertrophy with karyomegaly and hyaline capsule formation. Basophilic intracytoplasmic inclusions were observed in the diseased fish. PCR and sequence analyses revealed that all affected fish were positive to lymphocystis disease virus (LCDV), which was absent from the clinically healthy fish. Phylogenetic analysis revealed that the isolates from this outbreak were from the cluster of LCDV genotype 2. This paper reports the first documentation of LCDV infection in cultured John's snapper in Malaysia. Farmers and authorities should aware of the impacts LCDV infection, as it can cause high mortality and economic losses in cultured fish.

EFFECT OF DIFFERENT CARBON SOURCES ON THE CULTURE OF A ROTIFER *Brachionus plicatilis* IN BIOFLOC SYSTEM

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The rotifer *Brachionus plicatilis* is a universal live feed in marine fishes and crustacean larval hatcheries as their first live diet. The mass production of this rotifer in tropical hatcheries is unpredictable due to sudden crush and limited facilities in order to produce high quality microalgae. Recently developed biofloc based system is considered a potentially viable technique that proliferates natural probiotic bacteria and various microorganisms along with rotifer, *Brachionus* but the monoculture of rotifer has not initiated in a biofloc system. A 4-days rotifer *B. plicatilis* batch culture was conducted in biofloc system using molasses, rice bran, maize starch, palm kernel expeller versus a control (without carbon source). This study was maintained the carbon:nitrogen ratio at 10.

Daily water quality parameters and rotifer population density were evaluated. Fifteen 125 L polyethylene tanks with water volume of 100 L were used for this experiment. Each tank was stocked with 5×10^6 rotifer (50 rotifer ml^{-1}). Each treatment was randomly assigned in triplicate. TAN concentration and pH values were significantly lower in the four biofloc systems than control ($p < 0.05$) (Table 1). Significantly higher biofloc volume was obtained in the molasses and rice bran adding biofloc systems than those adding the maize starch or palm kernel expeller ($p < 0.05$) (Table 1). Significantly higher *B. plicatilis* population density and their specific growth rate were in the molasses and rice bran adding bioflocs treatments ($p < 0.05$) and these were followed by palm kernel expeller, maize starch and control, respectively (Table 2). The present study indicated that molasses and rice bran carbon source enhanced the production of *B. plicatilis* in the biofloc system.

TABLE 1. Water quality parameters in the control and the various carbon sources using biofloc treatments during four-days culture of rotifer *Brachionus plicatilis*

Variables	Control	Carbon sources			
		MO	RB	MS	PKE
Temperature (ºC)	27.07^a	28.08^a	28.18^a	28.11^a	28.11^a
pH	7.37^a	7.06^b	7.09^b	7.11^b	7.08^b
DO (mg L^{-1})	7.55^a	6.70^{ab}	6.91^{ab}	6.76^{ab}	6.66^b
TAN (mg L^{-1})	3.33^a	0.43^b	0.58^b	0.79^b	0.52^b
NO_2-N (mg L^{-1})	0.18^a	0.33^a	0.35^a	0.37^a	0.33^a
NO_3-N (mg L^{-1})	1.25^b	8.75^a	5.83^{ab}	5.83^{ab}	5.41^{ab}
SS (ml L^{-1})	0.58^b	7.16^a	7.25^a	1.83^b	2.41^{ab}

Superscript similar letter in same row did not differ ($p > 0.05$) significantly and different letters differed ($p < 0.05$) significantly.

TABLE 2. The rotifer *Brachionus plicatilis* density, specific growth rate (SGR) and length in the control and various carbon source using biofloc treatments after 4 days of culture

Variables	Control	Carbon sources			
		MO	RB	MS	PKE
Density (ind. ml^{-1})	95.0^c	1058.67^a	886.34^a	156.0^c	584.0^b
SGR (r)	0.16^d	0.76^a	0.72^a	0.28^c	0.61^b
Length (μm)	171.97^a	169.42^{ab}	165.98^b	172.07^a	166.37^b

[NB: SGR: specific growth rate. Superscript similar letter in same row did not differ ($p > 0.05$) significantly and different letters differed ($p < 0.05$) significantly]

FISH MEAL AND ITS ALTERNATIVES: A REVIEW ARTICLE

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In any aquaculture practices, feed is one of the most important input cost to run the fish farming unit. In a good aquaculture system, the difference between income and the gross profit is indicated by production costs.This implies that every aquaculture venture's financial success is determined by the income and production costs of the products sold.According to the United Nations' Food and Agriculture Organization, prices for key ingredients in fish feed, such as fish meal, have risen by as much as 50% in recent years. According to a recent FAO research, fish feed accounts for more than half of fish farmers' production expenses, and the average price of the materials widely used in fish feed has risen by 20 to 92 percent. The aquaculture industry is not immune to this worldwide phenomena, and the main issue is how it will affect the industry, according to the FAO, which also stated that commodity prices are "very unlikely" to decline in the foreseeable future.Depending on the species fed, fish feed prices range from a few hundred dollars per tonne to more than $1,000 per tonne. The major elements of any Aquaculture rely on readily accessible resources such as mustard oil cake, soybean, maize, fishmeal, fish oil, rice, and wheat, however prices of these commodities, notably fish meal, have skyrocketed since 2005.If we want to expand fish farming on a large scale and make it profitable, then it is high time to look into alternatives to fish meal. As we know returns depend on input costs and one such input cost is correct feed and feeding.

DHA-RICH PROTIST MICROORGANISM *Aurantiochytrium* sp.: IN VITRO ANTI-INFLAMMATORY POTENTIAL

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Aurantiochytrium sp., a marine heterotrophic eukaryotic protist that inhabits tropical waters of the Indian and Pacific Oceans and temperate and cold waters of Australia, Arctica, and Antarctica, has attracted interest in the aquaculture industry due to its nutritional profile and ability to enrich fish and shrimp feeds with long-chain polyunsaturated fatty acids (arachidonic acid – ARA and large amounts of docosahexaenoic acid – DHA. *Aurantiochytrium* sp. also produces astaxanthin and β-carotene. Dietary intake of *Aurantiochytrium* sp. as a feed additive may improve zootechnical performance and pathogen resistance by enhancing the immune system of fish and shrimp.

To better understand the composition and potential of *Aurantiochytrium* sp., test samples were analysed by CSIRO at the Queensland Bioscience Precinct Analytical Laboratory using established best practice methods. Analyses were based on methods prescribed by the Association of Official Agricultural Chemists (AOAC, 2016). Results are shown in Table 1 and show enrichment of *Aurantiochytrium* sp. with lipids, compared to a benchmark ingredient – squid meal.

To explore possible anti-inflammatory activity of *Aurantiochytrium* flours, an in vitro assay was performed using the mouse macrophage-like cell line, RAW264.7. Bacterial lipopolysaccharide (LPS) was used to induce production of nitric oxide (NO), a signalling molecule that plays an important role in the development of inflammation. Quercetin, a well-known plant-derived anti-inflammatory molecule, was used as the positive control, and squid meal was also assayed for comparison. Decreases in LPS-stimulated NO production following treatment with quercetin, squid meal, or *Aurantiochytrium* flours indicated anti-inflammatory potential. To ensure anti-inflammatory activity was not related to cytotoxicity, cell viability was measured using the MTT assay and ranged from 80 to 100%. The samples explored in the anti-inflammatory assay are described in Table 2.

Table 1 – Chemical analysis of tested ingredients

	TC 20[a]*	**ALL-G-RICH[b]**	**Squid Meal[c]**
Dry Matter (%)		97.54	89.57
Ash (%)		3.06	4.51
Total Lipid (%)		68.87	8.06
Crude Protein (%)		10.87	74.51
Carbohydrate (%)		14.73	12.91

*Data analyses in progress - will be presented at the event. [a]Aurantiochytrium sp. from Australian National Algae Culture Collection, strain number CS-997, GenBank accession number JN675250. [b]Aurantiochytrium sp. ALL-G-RICH produced and supplied by Alltech Inc. (Nicholasville, Kentucky, USA) and imported by Alltech do Brasil Agroindustrial Ltda (Araucaria, Paraná, Brazil). [c]Squid meal – freeze-dried whole squid from Australian local market and meal made at BIRC – CSIRO.

Table 2 – Ingredients used in the mouse cell assay

Ingredient tested	**Treatments**
Aurantiochytrium TC 20	AU
Aurantiochytrium ALL-G-RICH	ALL
Squid meal	SM
Quercetin (Internal Control)	Quercetin

PROTIST MICROORGANISM *Aurantiochytrium* sp. AS AN ADDITIVE TO *P. vannamei* IN SUBOPTIMAL TEMPERATURE: PRELIMINARY RESULTS

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The Pacific white shrimp is a tropical and ectothermic species, thermoregulating inefficiently and at a high energy cost. The ideal temperature for its cultivation ranges from 27-30 ºC and autumn and winter temperatures or subtropical regions can affect its performance. Thus, the use of biotechnological additives that include long-chain fatty acids in shrimp nutrition can be an alternative, aiming at greater permeability of the cell membrane and better performance of the animals.

Aurantiochytrium sp. is defined as a marine protist, heterotrophic and has occurrence ranges from the tropical waters to the temperate. It can produce bioactive compounds and accumulate large amounts of polyunsaturated fatty acid DHA (docosahexaenoic acid). The inclusion of *Aurantiochytrium* flour in the diet may be the key to better maintenance of shrimps in low temperatures by increasing their immune system and the use of nutrients. Test ingredient was analyzed by CSIRO staff using established best practice methods at the Queensland Bioscience Precinct Analytical Laboratory for chemical composition and fatty acids and the results are shown in Table 1. The analyzes were based on the Association of Official Agricultural Chemists (AOAC, 2016). Five treatments with three replications each were carried out with the inclusion of *Aurantiochytrium* flour doses (0, 1, 2, 3 and 4%) in practical diets for *P. vannamei*, cultivated in clear water at a temperature of 22 ºC. The experiment lasted 63 days and the experimental units had 400 L of useful volume. The stocking density was 100 shrimp per m^3. The initial weight of the animals was 3.8±0.02 g. The water quality parameters were maintained within the rates defined for the experiment, Temperature: 22.1±0.2 ºC, Dissolved Oxygen 7.0±0.1 mg/L, Ammonia 1.1±0.4 mg/L and Nitrite 0.2±0.2 mg/L. The results of zootechnical performance are shown in Table 2. The average survival was 98.0±2.0%. The results showed no statistical differences. The experiment resulted in excellent survival, considerable weight gain and satisfactory productivity when considering that the entire cultivation took place at a suboptimal temperature. Therefore, the use of *Aurantiochytrium* flour was equivalent to the control diet, without harming the performance of the shrimp and with satisfactory results for the temperature used. Different inclusion doses can be tested.

Table 1 – Proximal analysis of *Aurantiochytrium* sp. meal used in this trial

***Aurantiochytrium* sp. ALL-G-RICH[a]**			
Chemical composition		**Fatty acids composition**	
Dry matter	975.35	16:00	62.98
Crude protein	108.68	18:4n-3	0.26
Lipid	688.73	20:5n-3	0.12
Ash	306.38	22:6n-3	26.62
Gross energy	31.90	LC-PUFA	27.13

[a]Aurantiochytrium sp. ALL-G-RICH produced and supplied by Alltech Inc. (Nicholasville, Kentucky, USA) and imported by Alltech do Brasil Agroindustrial Ltda (Araucaria, Paraná, Brazil).

Table 2 – Zootechnical performance results

	Final weight (g)	**Weekly weight gain (g)**	**Productivity (kg/m³)**
Control	13.1±0.67	1.04±0.07	1.35±0,10
1%	12.6±0.78	0.98±0.08	1.24±0,04
2%	12.7±0.78	0.99±0.09	1.25±0,08
3%	13.9±0.07	1.10±0.03	1.34±0,02
4%	12.9±0.41	1,01±0.04	1.25±0,02
p value	0.2411	0.2364	0.1364

TREATMENT OF SLUDGE FROM INTENSIVE *Penaeus monodon* PONDS BY PROBIOTICS

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Aquaculture effluents with high concentration of nitrogen and phosphorus compounds can cause negative impacts on the environment and aquatic wildlife. This trial evaluated the possibility to treat sludge collected from intensive ponds for black tiger prawn *Penaeus monodon*. The experimental sludge contained 2,150 ± 282 mg/L COD, 9.4 ± 1.0 mg/L ammonia–nitrogen, 0.5 ± 0.1 mg/L nitrite–nitrogen (NO^{2-}–N), 7.1 ± 2.8 mg/L nitrate–nitrogen (NO^{3-}–N), 2.83 mg/L PO_4^{3-}–P (phosphate–phosphorus). Anaerobic treatment was applied using a commercial probiotics (Wawiz Greezon, Green Guard Biotechnology Co.) for 4 weeks at 0.1 ppt weekly. Sampling was conducted every seven days to monitor ammonia-N, nitrite, nitrate, phosphorus, COD and microbial structure.

Results showed that the probiotics treatment removed completely (100%) ammonia and 89.6% phosphorus from the sludge when the concentration of these two parameters remained high in the control. Nitrite and nitrate slightly increased in the probiotics treatment in the first week but gradually declined towards the end of the trial. The variable regions V1-V3 of the 16S r RNA gene was amplified by PCR and was sent to AGRF for paired-end sequencing 300 bp paired end chemistry on the Illumina MiSeq platform (Illumina). Within a week, probiotics treatment significantly drove microbial community to divert from the control group and the initial bacterial composition. These changes did not occur to most of the dominant taxa of the bacteria as the top three phyla (Chlorobi, Proteobacteria and Bacteroidetes) were relatively persisted. These results indicate that even with the low abundance in the community, probiotic bacteria still showed their high effectiveness in removing nitrogen and phosphorus waste from prawn farming wastewater. These results suggest that using anaerobic probiotics can be used effectively for sludge treatment in intensive shrimp farming.

RISK FACTORS FOR SUMMER MORTALITY IN AUSTRALIAN ABALONE: A CASE-CONTROL STUDY

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In Australian aquaculture, abalone mortality during summer has been termed summer mortality. We conducted a case-control study to identify risk factors associated with summer mortality in farmed Australian abalone. We reviewed scientific literature, ten years of abalone laboratory submissions and collaborated with Australian abalone farmers to develop a case definition for summer mortality. Farm mortality data (2015-2021) were used to identify tanks which fit the case definition (cases) and tanks which did not (controls). We constructed a model to predict summer mortality risk using logistic regression and forward variable selection. Modelling considered 61 potential risk factors (examples included water quality, husbandry, climate, biology) with the final selected model including six main effects and five two-way interactions.

Except for maximum water temperature in the week prior to the case/control date, all other parameters selected in the final model were involved in one or more interactions. The model included interactions between age and each of previous year summer mortality, size at weaning and feed rate. There were also interactive effects of previous year summer mortality and size at weaning, and of feed rate and post-grading mortality. Risk of summer mortality was two times more likely for every 2 °C increase in maximum weekly water temperature. Post-grading mortality was associated with an increased risk of summer mortality, but relative risk decreased at higher feed rates. Risk of summer mortality in younger abalone increased with higher feed rates, while increasing feed rates for 30-month abalone did not. Abalone in tanks which experienced summer mortality the previous year were at a higher risk, regardless of age. Risk of summer mortality increased with abalone age for abalone that did not experience summer mortality the year prior.

We also investigated summer mortality events in 2021 and 2022 (January to April) for primary pathogens and infectious agents. Farmers submitted 30 abalone from case tanks and 10 abalone from control tanks to veterinary diagnostic laboratories for gross and histopathological examination and microbiological culture. *Vibrio* spp. were detected in both case and control abalone, with a higher prevalence in case animals. Bacterial presence did not always translate to clinical disease. In some animals from case tanks, *V. harveyi* and *Vibrio* spp. caused disease as there were gross and histopathological findings consistent with vibriosis. However, mixed bacterial growth, and not one uniform pathogen, was detected in both case and control abalone. There was no evidence that *V. harveyi* and *Vibrio* spp. were consistently the primary cause of mortality. The bacterial pathogens detected were secondary (opportunistic) to primary stressor(s).

HELP, I NEED SOMEBODY! HELP, NOT JUST ANYBODY! GETTING THE BEST OF TALENT INTO AQUACULTURE

Morris Pizzuto*, Leo Nankervis*, James Garde*, Rachel Mara*, Brando Westley*, Sally Roberts*, Peter Cheesman*, Geoff Barbaro
with acknowledgement for the support of the Cooperative Research Centre for Northern Australia

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The world is experiencing tight labour markets, and aquaculture is struggling to obtain the farming, technical, management and scientific staff needed to meet growing industry demand. During 2021 – 2022, research funded by the Cooperative Research Centre for Northern Australia examined the support to address workforce needs that had been identified in a previous study (Cobcroft et al, 2020).

The research found that while relevant training at all levels was generally available, there remains a very significant gap in the number of learners being trained and the needs of industry. The study also highlighted the importance of on-farm, vocational experiences, including internships and work-integrated-learning programs.

So what does aquaculture as an industry need to get the workforce that's needed? This session will present a diversity of research, experiences, programs and an active panel discussion, with plenty of opportunities for audience questions. We'll explore the options to attract and retain farming, technical, management and scientific staff, and try to identify what may be the best approaches in a variety of situations.

The session will introduce some of the development of new training resources taking an immersive approach, programs encouraging local involvement and increased diversity and other pathways that are taking place, and whether these approaches are available for aquaculture employers. We'll discuss the ways learners can approach potential employers and how to find the opportunities they seek.

We encourage you to bring your questions, viewpoints and ideas for a respectful discussion aimed at benefitting the whole industry across all disciplines.

USING E-LEARNING TO IMPROVE FISH WELFARE

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Fish welfare was until recently an alien term to many fish professionals. This status quo is now changing. In this presentation, we will explore how FAI Academy e-learning courses in five different languages are bringing to the farmer the hidden scientific information on fish welfare assessment.

In the last seven years over 13, 000 research papers were published and many codes of good practice updated for some fish species. Increasing public awareness led several organizations to create fish welfare campaigns forcing some retailers to commit to better fish welfare in their supply chains. But to implement fish welfare, it is crucial that the information on good practices reach fish farmers. The information contained in the 13,000 publications - all written in English - is essential but is not a tool to implement fish welfare at the farm level in non-English speaking countries. To unlock this information, FAI Academy is creating e-learning courses available for free in Portuguese, Spanish, Thai, Chinese, and English. E-Learning, electronic learning, or computer-based learning is a form of distance learning: it is flexible, convenient, and accessible.

In this presentation, we will share FAI's journey to creating a framework on best practices for building e-learning courses targeting the aquaculture sector. We will explore the methodologies available to train not the traditional student, but to train the fish farmers and other fish professionals – all those who can improve fish welfare.

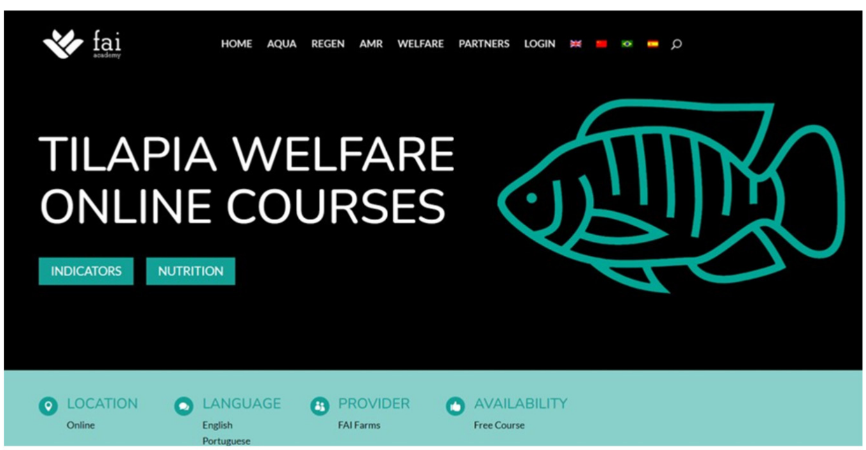

Figure 1 Screenshot of FAI Academy website showing the tilapia welfare courses webpage https://fai.academy/aquaculture/welfare-in-tilapia-production-guideline-series/

COMMUNICATING APPLIED RESEARCH: A *SHINY*-BASED WEB APP FOR SALMON FARMERS

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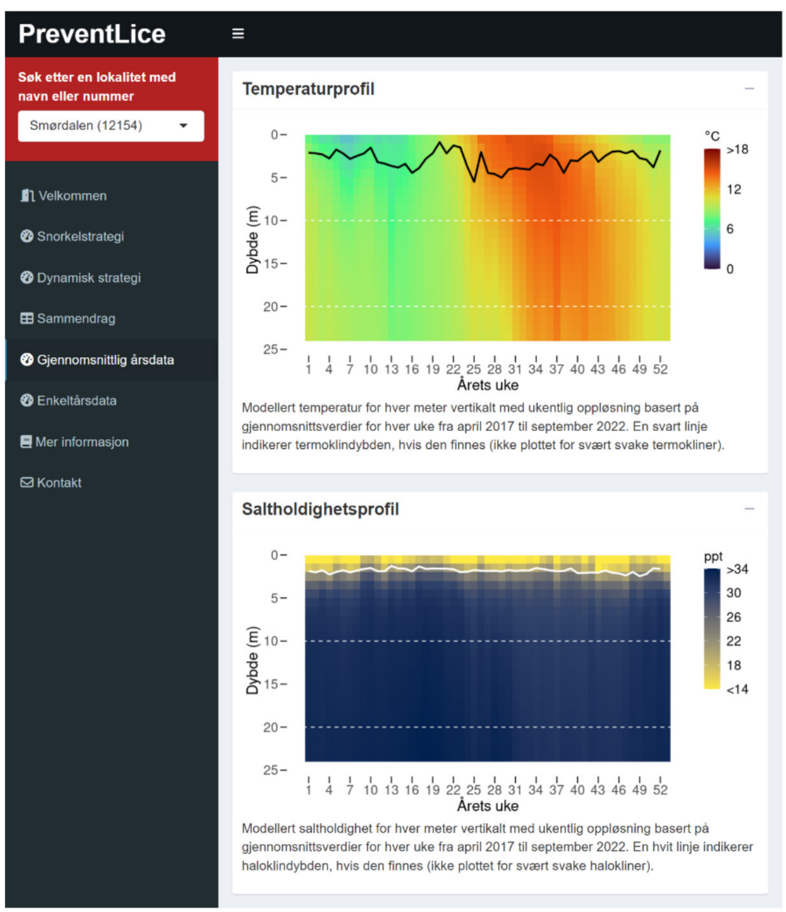
Applied aquaculture research sometimes fails to have impact, especially when findings are communicated via scientific publications that are inaccessible to those outside the academic field. Researchers can boost real-world impact by collaborating with industry partners, holding workshops, and publishing plain-language news articles. Another approach is to develop interactive apps, ranging from simple educational tools to complete planning tools that perform complex analyses on user inputs. This type of app development has become far more achievable with the *shiny* package for *R*. We developed a *shiny* app that provides site-specific parasite-prevention advice for over 1000 Norwegian salmon farms. Sea lice, especially salmon lice (*Lepeophtheirus salmonis)* are the perhaps the greatest challenge facing Atlantic salmon aquaculture globally. Effective treatments have side effects, so farmers are increasingly looking to prevent infestations by installing barriers than span the highest-risk depth bands, preventing the planktonic larvae from entering sea-cages. However, the intensity and depth of infestation pressure varies through time and between sites, making it difficult for farmers to implement these strategies optimally. We investigated how salinity, temperature, density, currents, and waves influence the efficacy of lice barriers, and by combining this knowledge with local predictions from a national-scale hydrodynamic model, generated bespoke initial recommendations for every active salmon farm in Norway. This information, including the hydrodynamic data underlying the recommendations, is made available to farmers via a free web app and an open-access data archive.

Figure 1. Screengrab from the freely accessible PreventLice web app, available (in Norwegian) at: https://t.ly/9V7f



NORTHERN TERRITORY GOVERNMENT BARRAMUNDI STOCKING PROGRAM

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Recreational fishing is an important aspect of life in the Northern Territory (NT), injecting over $50 million into the economy per annum and contributing to a significant part of the NT's cultural identity. To enhance recreational fishing in the greater Darwin region, the Northern Territory Government's Darwin Aquaculture Centre (DAC) has been stocking barramundi (*Lates calcarifer*) – an iconic NT angling species – since 2004 into waterways where natural recruitment of barramundi is limited or restricted. This benefits local communities by providing angling opportunities close to urban zones and in safe recreational areas. This includes urban and rural lakes and lagoons, as well as Manton Dam, the most significant freshwater recreation area in the greater Darwin region. Since this programs inception, the DAC has stocked over 1 million barramundi, with stocking densities calculated based the fish's size and the area of the waterway being stocked. Historically, most barramundi stocked have been between 30-100 mm in length. Since 2019, the DAC has begun stocking 'advanced' size barramundi over 400 mm in length which offer immediate fishing opportunity and high recapture rates in some locations. More recently, the DAC has begun tagging larger barramundi so survival and growth of stocked fish can be quantified through angler recaptures. Future research potential includes expanding tagging efforts in stocked fish across different sizes as locations, as well as investigating survival, growth and diet habits of stocked barramundi to inform best grow out environments for the program.

HARMFUL ALGAL BLOOMS AND AQUACULTURE: FISH, SHELLFISH AND HUMAN HEALTH

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Several environmental constraints have been hampering the development of aquaculture, in both marine and freshwater environments. One of these constraints are Harmful Algal Blooms (HAB) which are caused by over 400 species of microscopic, pelagic and benthic microalgae. These HAB pose a threat to coastal ecology, biodiversity, human health, and economic activities. There is a global consensus that HAB have been increasing in incidence, severity and geographical distribution linked to human activities and climate change, and that these HAB are problematic for fisheries and aquaculture as well as human health. Indeed, HAB cause illnesses to mass mortalities in several aquatic animals, including shellfish and fish in aquaculture farms. They are as well producers of highly potent toxins that can affect humans via the ingestion of shellfish and fish that have accumulated their toxins through direct ingestion or via bioaccumulation and bioamplification throughout food webs, or via exposure to dissolved toxins in drinking waters and/or bloom water aerosols. In this presentation, we present our analyses of 30 years data of HAB occurrence and their effects on cultured shellfish and fish, as well as associated human sicknesses, based on available global data.

OYSTER FARMING IN THE TROPICAL NORTH

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Oyster aquaculture is one of the most sustainable and environmentally friendly agribusinesses in the world. Blacklip rock oysters (*Saccostrea echinata*) are a native species of tropical oyster found across the north Australian coast. Research is underway into the farming potential of Blacklip rock oyster in northern Australia. Trial grow out sites are located near the remote communities of Warrawi on South Goulburn Island and Alyangula on Groote Eylandt. The Northern Territory Government's Darwin Aquaculture Centre in collaboration with the Yagbani Aboriginal Corporation and Groote Aqua Aboriginal Corporation have been working on a development project supported by the Cooperative Research Centre for Developing Northern Australia. Working together, this project has assessed farming methods and infrastructure suitable to our unique tropical growing conditions and there effects on oyster growth and marketability. This poster will highlight the research and development behind the tropical rock oyster programs success; which has grown from a community vision and is currently transitioning into small-scale commercial production. We will also summarise future research and development needs to support the community with their ultimate aim of supplying markets with a premium, native oyster, which has been sustainably grown by Aboriginal Territorians in the pristine waters of northern Australia.

EFFECTIVE DISEASE CONTROL FOR *Infectious spleen and kidney necrosis virus* (ISKNV): REDUCING THE RISK OF TRANSBOUNDARY SPREAD

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Infectious spleen and kidney necrosis virus (ISKNV) is a globally important pathogen that infects more than 165 species of freshwater and marine fish. Infections may be subclinical or cause severe disease epidemics in wild fish, aquaculture, and ornamental fish typically with 20-60% and up to 100% mortality. The species ISKNV includes three closely related but distinct genotypes: red seabream iridovirus (RSIV), ISKNV-genotype, and turbot reddish body iridovirus (TRBIV). The recognised host and geographical range of each genotype has recently expanded reflecting an emerging disease situation.

The World Organization for Animal Health (WOAH) recognized the importance of ISKNV by listing red sea bream iridoviral disease (RSIVD) as notifiable under a definition including infection with RSIV and ISKNV-like viruses (WOAH, 2019). Presently, infection with TRBIV is not included in this definition, although this is being reviewed by the Aquatic Animal Standards Commission. TRBIV infection was originally considered to be restricted to flatfishes until recent reports of disease in food and ornamental fish, such as barramundi (*Lates calcarifer*) and rock bream (*Opleghathus fasiatus*). Currently, ISKNV, RSIV and TRBIV are reported to be endemic in SE Asia and cause recurrent moderate to severe epidemics in aquaculture with sporadic emergence of disease in additional locations.

This paper will review current literature and make recommendations to address barriers to minimizing the spread and impacts of ISKNV. Firstly, a uniform and simplified nomenclature that reflects functional differences in viruses is recommended. ISKNV disease control and revision of WOAH standards are currently hampered by confusing nomenclature. A plethora of virus names are in use due to the wide host range and terminology derived from the host fish and the virus family (*Iridoviridae*), without indicating the important genotype designation. At genotype level, confusion derives from the use of ISKNV as the name of the species and one of the genotypes. Further confusion arises when referring to the genotypes collectively by the genus name (*Megalocytivirus*) as this includes other important virus species. Improved and efficient methods for characterisation and classification of viruses are required because ISKNV is challenging to grow in cell culture. To support vaccination programs, improved genetic characterisation is required because of the close relationship of different genotypes and their association with similar pathology in an overlapping host range without showing evidence of cross protection. Finally, there is the need for further validation of fit-for-purpose diagnostic tests to facilitate international trade. These require defined purposes reflecting appropriate virus nomenclature and the different needs of disease diagnosis and high-throughput surveillance including detection of subclinical infection. Together, these recommendations will support regional surveillance and reporting to promote improved biosecurity related to ISKNV.

CALCULATING AUSTRALIA'S SEAFOOD'S CARBON FINPRINT

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Funded by the FRDC, this project is the first overview of energy use and carbon emissions in the Australian aquaculture sectors, benchmarked against wild catch fisheries. Previously, some work had been done on energy consumption and efficiency improvements, but the carbon footprint, or 'fin'print, of Australian produced seafood had never been calculated. Measuring the carbon footprint of the aquaculture sectors was a complicated task requiring an account of all the emissions generated directly and indirectly by the sectors. This includes fuel burnt directly to power vessels, to purchased electricity, refrigeration emissions and the emissions from products bought from external suppliers such as aquaculture feed. When aquaculture operators understand how their businesses create emissions, they can make appropriate changes to reduce both emissions and energy costs.

The study assessed the energy use and carbon emissions of Australia's largest aquaculture & fisheries industries, constituting about 82% of Australia's domestic seafood production by gross value of production (GVP). Whilst comparisons of relative footprints within aquaculture are useful, it is potentially more useful to compare footprints more broadly across a range of seafood products. Seafood consumers are increasingly wanting to know the stories behind the products they're buying, including efforts by fishers and farmers to reduce their carbon footprint. Carbon information for produced seafood is vital for providing a competitive advantage when it comes to consumer preferences for low-emission proteins. It also highlights how seafood production may need to adapt to remain competitive and sustainable.

Results from the study are shown in Table 1, with GHG emissions calculations for aquaculture averaging 9.7kg CO2-e /kg of seafood produced, and wildcatch 4.4kg CO2-e /kg of seafood produced. As an output of the project, a 'toolbox' of resources is available including a new self-assessment tool helping smaller operators convert their costs into a carbon number, track their operations, and compare themselves within, or to other, sector(s).

Table 1: Australian aquaculture & fisheries sector emissions by sector and scope (2019-20)

Sector Production					**Scope 1**	**Scope 2**	**Scope 3**
	GVP $	**Vol t**	**Emissions (tot)**	**CO_2-e/kg**	**% of tot**	**% of tot**	**% of tot**
Aquaculture	**1,390**	**92,872**	**901,614**	**9.7**	**19%**	**19%**	**63%**
%	**54%**	**39%**	**59%**				
Salmonids	$890	66,015	561,128	8.5	21%	13%	66%
Tuna	$137	8,345	149,459	17.9	10%	12%	78%
Oysters	$137	8,345	42,248	5.1	55%	26%	35%
Barra	$91	3,427	46,265	13.5	13%	45%	41%
Prawns	$135	6,740	102,515	15.2	9%	45%	46%
Fishing	**1,201**	**143,554**	**625,337**	**4.4**	**49%**	**15%**	**36%**
%	**46%**	**61%**	**41%**				
Sardines	$30	42,277	68,066	1.6	32%	19%	50%
Other Finfish	$372	62,280	280,260	4.5	49%	18%	33%
Prawns WC	$233	17,640	117,749	6.7	66%	12%	22%
Rock Lobsters	$521	8,002	89,851	11.2	37%	7%	56%
Scallops	$18	6,615	41,741	6.3	64%	13%	24%
Shark rays	$27	6,740	27,671	4.1	40%	15%	45%
TOTALS	**2,591**	**236,426**	**1,526,951**	**6.5**	**31%**	**17%**	**52%**

TECHNOLOGY-BUSINESS-MANAGEMENT OF SMALL-SCALE RECIRECULATING AQUACULTURE SYSTEM (RAS) FOR SUSTIANBLE URBAN FARMING IN SUB-SAHARAN AFRICA: A REVIEW OF CHALLENGES AND OPPORTUNITIES

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The population of cities in developing countries, especially Sub-Saharan Africa, is rapidly increasing. As cities grow, so does the demand for fish protein, means of livelihood and threat of climate change. While flow-through aquaculture can provide fresh, healthy and nutritious fish protein, it is plagued by extensive land requirement as well as effluent discharge. Alternatively, micro and small-scale Recirculating Aquaculture System (RAS) can improve food and nutritional security (FNS), livelihoods as well as reduce environmental degradation in urban areas. The question however remains - what are the key techno-economics issues, surrounding small-scale RAS in urban farming? This study reviews the RAS prototype of the Sustainable Aquaponics for Nutritional and Food Security in Urban Sub-Saharan Africa (SANFU) II project. The analysis is based on a simple mass balance, stock density, and cash-flow analysis. The results suggest that a concentration of solids out from the filtration of 0.015 kg/l in high fish stock density with limited space i.e. approx. 138 African Catfish - Clarias Garipinus in 600-liter tank can be achieved. An average monthly fixed and variable costs as well as revenues of ₦16,700 (US$29) and ₦32,535 (US$56), respecteively, is attainable under certain conditions. Government policies on urban farming should promote RAS for FNS, environmental justice, and food system resilience.

ADDITION OF *Yucca schidigera* TO REDUCE AMMONIA-NITROGEN EXCRETION IN SHRIMP postlarvae (*Penaeus Vannamei*) DURING PACKING

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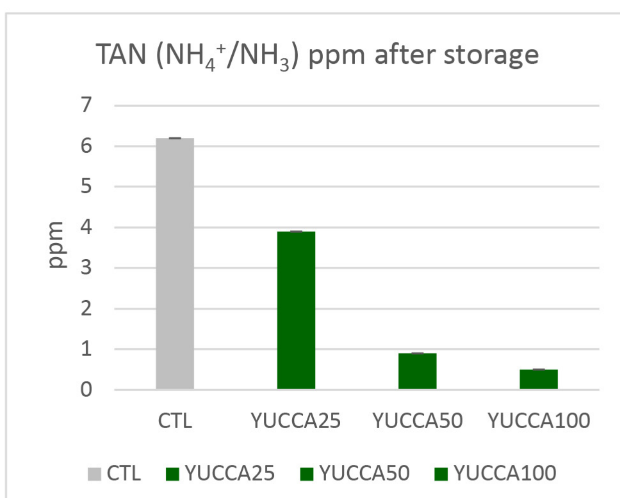
Post larvae shrimp are usually transported in plastic bags over long distances. During transport, the stress applied to the shrimp causes a metabolic change resulting in a decrease of water quality and an increase of ammonia, which can influence the physical condition of the post larvae shrimp and lead to increased mortality. These diminished conditions are often mistakenly considered by farmers as a weakness of the alevins.

The aim of this study was to evaluate the effect of addition of *Yucca schidegera* (Norponin® L, Nor-Feed, France) on the ammonia-nitrogen excretion of shrimps post larvae (*Penaeus vannamei*) during packing.

This study was performed in an experimental station (Indonesia). Shrimps post larvae were randomly divided into 3 groups contained 3,480 post larvae in bag of 5 L: one control groups (CTL) with 2.5 g of active carbon, (YUCCA25) with 25 ppm of *Yucca,* (YUCCA50) with 50 ppm of *Yucca* and (YUCCA100) with 100 ppm of *Yucca*. Each group are performed in triplicate with a storage of 12 hours similar to the packing process. Measurement of TAN (NH_4^+/NH_3) concentration occurred at the end of the storage. Mortality was assessed at the end of storage for each group.

Results evidenced that YUCCA groups have a beneficial effect in reducing significantly ammonia-nitrogen excretion after storage with a difference of -37% for YUCCA25, -85% for YUCCA50 and -92% for YUCCA100 compared to the CTL group. Mortality after storage was not significantly different for each group (CTL: 2.46 ± 0.69%, YUCCA25: 3.41 ± 0.93%, YUCCA50: 3.25 ± 0.64% and YUCCA100 3.92 ± 0.91%).

Overall, the present study demonstrates that an addition from 25 ppm of *Yucca schidigera* can reduce ammonia nitrogen excretion and potentially maintain conditions of alevins during storage. However, other studies are necessary to assess zootechnic parameters of shrimps post larvae after storage with *Yucca schidigera.*



DECAPOD CRUSTACEAN WELFARE – CONSIDERATIONS FOR AN EMERGING FIELD

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Welfare of decapod crustaceans is an area of growing interest in the context of animal ethics, production, and research. The prevailing literature suggests decapod crustaceans are sentient and demonstrate a "pain-like" experience, with research spread across a diverse range of crabs, lobsters, and freshwater shrimp. These studies consistently highlight the lack of knowledge on pain, nociception, sentience and stress in decapod crustaceans, and the paucity of novel methods that quantify stress or assess welfare of decapod crustaceans. These knowledge gaps make it difficult to provide science-based recommendations regarding the welfare of crustaceans, however, there is growing sentiment to treat decapod crustaceans with care, provide optimal handling and minimise their stress and suffering. The increased scrutiny and concern for welfare in crustaceans provides an opportunity to develop objective measures of welfare to validate, improve practices in a range of settings ensuring recommendations rising from the research are objective and based in science.

This presentation will discuss the crustacean behaviour and welfare, highlight knowledge gaps and future research opportunities to improve welfare of farmed crustaceans.

REPLACE OF FISHMEAL BY FIELD CRICKET (*Gryllus bimaculatus*) MEAL (FCM) IN THE DIETS FOR SEX-REVERSAL AND NURSING OF NILE TILAPIA (*Oreochromis niloticus*)

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Fishmeal is used as a sole ingredient in the standard feed for the production of monosex tilapia fry. Attempts to reduce fishmeal use have been tried. In this study, field cricket (*Gryllus bimaculatus*) meal (FCM) was tested to replace fishmeal (FM) at the rates of 0, 20, 40, 60, 80 and 100% in terms of sex-reversal efficiency, survival, growth and feed utilization. Six diets with similar levels of crude protein (55.7±0.2%) were formulated and fed to Nile tilapia fry (8.0±0.1 mg) stocking in 18 aquaria (100 L) at the density of 300 each. They were fed five times a day for 21 days as per the standard protocol. They were raised additional 63 days. Results showed that replacement of fish meal by FCM did not affect the percentage of males which remained high (range 97.2-98.6%) regardless of the replacement rate. During sex-reversal, when the replacement of fishmeal increased from 20% to 80%, growth, survival and feed utilization indicators continue to improve. However, 100% replacement of fishmeal by FCM reduced the growth and survival (Fig. 1). Broken line analysis showed that 80% replacement of fishmeal resulted in highest survival during sex reversal and 85% replacement during the first nursing and no effects on second phase nursing. Highest biomass gains and feed conversion efficiencies were also obtained at 80% replacement for all the phases.

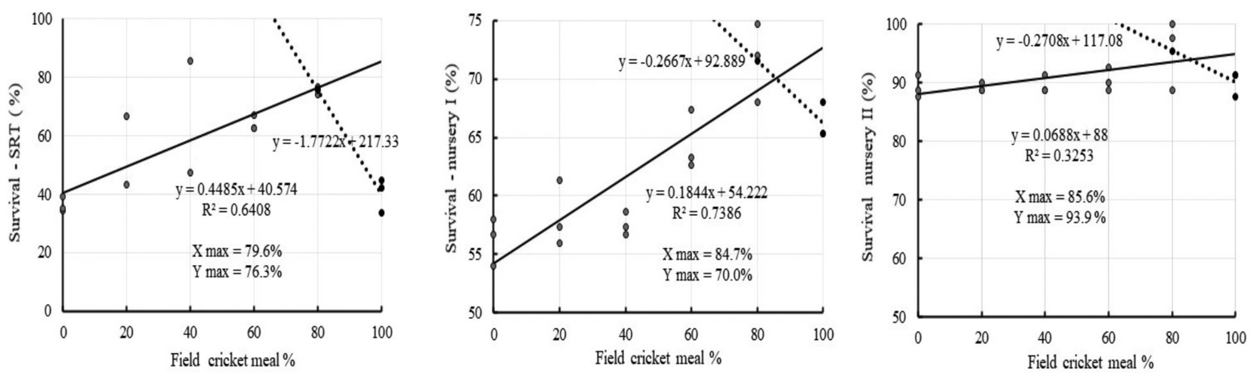

Fig. 1 Survival (%) of Nile tilapia fry during sex-reversal of 21 days (left) and nursery phase I (middle) and II (right)

DISSEMINATION OF TILAPIA HATCHERY TECHNOLOGY WORLDWIDE

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Tilapia culture has a special history in Thailand. Nile tilapia (*Oreochromis niloticus*) was granted as "gift" by the former prince of Japan to the former king of Thailand in 1965. Perceiving the potential value of phenomenal self-recruitment as seen in the pond in *Chitralada* palace where they were maintained, the King gave 10,000 fingerlings to the Department of Fisheries (DoF) and asked to distribute to the farmers. The DoF distributed the fish to 15 inland fisheries research stations to distribute to the public. Tilapia was the focus of AIT's research since early 1980s. AIT maintained the stock in hapas-in-pond, conducted series of research trials over a decade, developed practical techniques of fry production and grow-out. Low-cost grow-out technique in green water coupled with nursing of fry in hapas at early stage was transferred through Aqua Outreach program in Asia. Due to the promotional efforts of various organizations, Nile tilapia gained increasing interest among farmers. It became the most widely cultured fish in Thailand overtaking catfish in total production since 90s. However, wide adoption of tilapia farming was still hindered by unavailability of large quantity of quality fry which was realized by researchers at AIT and attempts were made to find a practical solution to this. A success of producing consistently high-quality mono-sex fry on a mass scale using hormonal sex-reversal helped improve consistency of marketable size and improved profitability and encouraged intensification of tilapia farming. The mono-sex production method consists of techniques of maintaining large number of broodfish in hapas, collection and artificial incubation of their eggs (Fig. 1), and a practical method of sex-reversal. This combination of techniques developed at AIT was successfully transferred to the private sector in addition to the public sector with contractual arrangements. As a result, now there are over 100 hatcheries of its kind in Thailand alone; some of them supply up to 10-20 million monosex fry per month. This technology has now spread around the globe starting from Fiji in the east Bangladesh, Brazil, Myanmar, Nepal, PR China, the Philippines, Vietnam, and others. This paper describes the approaches and strategies applied by AIT, and some success stories/impacts which should be useful for many others.

Fig. 1 Large commercial tilapia hatchery in Thailand

THE COMMERCIALISATION JOURNEY OF TROPICAL ROCK LOBSTER PRODUCTION FROM HATCHERY TO GROW-OUT

Jennifer Blair*, Scott Parkinson, John Breen, Sandra Infante-Villamil, Tony Barton, Steven Gill, Quinn P. Fitzgibbon, Chris G. Carter, Andrew J. Trotter, Basseer M. Codabaccus, Greg G. Smith

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Successful production of spiny lobsters in hatcheries required decades of aquaculture research. Hatchery technology for the tropical rock lobster *Panulirus ornatus* developed by the Institute for Marine and Antarctic Studies, University of Tasmania has been commercialised by Ornatas. The vision to establish a new tropical rock lobster aquaculture industry in northern Australia, founded on hatchery-produced juveniles, has been driving investment by Ornatas in commercial scale systems, operations, ongoing research, and building the capabilities of our talented team.

Together with university and business partners Ornatas is investigating technical and biological challenges at the nursery and grow-out stages. These challenges include developing a commercial feed and minimising cannibalism in juveniles. Nutrition and nursery systems form core components of science in the Australian Research Council, 'Industrial Transformation Research Hub – Sustainable Onshore Lobster Aquaculture'. The Cooperative Research Centre for Developing Northern Australia (CRCNA) and Fisheries Research and Development Corporation (FRDC) are supporting research to provide an improved understanding of grow-out production systems, biosecurity requirements, feeding strategies and growth to produce market-ready, premium lobster product, through the project 'Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia'.

The tropical rock lobster hatchery in North Queensland has been fully operational since the end of 2021 and is consistently producing 1000s of puerulus and first stage juveniles. Ornatas' newly commissioned nursery has the capacity to produce 100,000 lobsters p.a. for the grow-out operation. Company activity in 2023-2024 will be focussed on the expansion of our pilot grow-out facilities to support a commercial farming scale of 50 tonnes p.a. initially and then scaling up proven systems. To meet production targets, the company projects our current staff of 30 will increase to 120 people will by 2032. For a new aquaculture industry, the composition of the team requires different technical and scientific backgrounds at a variety of qualification levels, with lobster-specific training provided 'in-house'. The growth of this new aquaculture industry is dependent upon strong partnerships, infrastructure investment, sustainable practices, and equipping our people to innovate.

Fig. 1. Hatchery-produced juveniles *Panulirus ornatus*.

BIOSECURITY BEYOND COMPLIANCE TO DEVELOP A RESILIENT TROPICAL ROCK LOBSTER AQUACULTURE INDUSTRY IN AUSTRALIA

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Diseases are one of the challenges limiting the sustainable development of the aquaculture industry. The occurrence, introduction and spread of pathogens of aquatic animals has increased over time due to the intensification of aquaculture, trade of aquatic animals and their derived products, resistance to antimicrobials and climate change. In addition, as new aquaculture species are explored and the industry expands geographically, diseases can surprise aquaculturists over time and affect productivity of stock and profitability of operations. The intent of a proactive approach to prevent diseases is captured in the 13th century Latin saying "it is better and more useful to meet a problem in time than to seek a remedy after the damage is done". Meeting the problem in time for pioneering tropical rock lobster aquaculturists involves tackling the challenges posed by exotic, endemic and emerging diseases in a responsible and efficient manner to ensure the sustainability of the industry. The first step towards prevention and preparedness in commercial-scale tropical rock lobster aquaculture in Australia, was to understand the risk and pathways of potential pathogen entry and disease outbreak. Equipped with an understanding of risk, the next step was development of an enterprise-level Hatchery Biosecurity Plan and implementation of protocols to reduce risk. Coupled with the Biosecurity Plan, a Health Surveillance and Management Plan was designed to deliver an early detection and rapid response mechanism for disease outbreaks and for new and emerging diseases. It is critical to train our people to understand pathogen risks, implement management measures, and be alert to signs and symptoms of disease in lobsters – all documented in the Biosecurity Plan. Staff involvement in system operations and husbandry are essential to succeed in our aim of disease prevention, early detection and control. A challenge for lobster aquaculture is the gap in knowledge of endemic lobster pathogens, detection tools, and the expense of surveillance. Strong collaboration with universities, government agencies, animal health laboratories, veterinarians and consultants to carry out R&D activities, apply diagnostic tools, and advise on management approaches is another critical component of the biosecurity system for the new tropical rock lobster aquaculture industry in Australia.

There are several benefits to a well-designed enterprise-level biosecurity plan. A good plan documents the processes to meet government regulatory requirements, opens opportunities for national and international markets, and contributes to the sustainable development of a productive and profitable lobster aquaculture industry in Australia.

A DEEP LOOK INTO CULTURED *Asparagopsis taxiformis* MICROBIOME

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The red seaweed genus *Asparagopsis* is renowned for producing antimicrobial compounds that can be used in a range of applications (reducing methane emissions from cattle, cosmetic skin care and boosting fish immune system) and that also benefits the seaweed itself by controlling its microbiome. However, it is unclear how any specific bacteria on the seaweed surface interact with the growth and health of *Asparagopsis* in culture. This study demonstrates that domesticated seaweed has very different microbiomes from wild samples. We subsequently explore the effects of manipulating the microbiome of the domesticated seaweed using different antibiotics, quantifying the change in the bacterial community and densities using fluorescence microscopy, and exploring any correlations to the growth rates of the seaweed. At the same time, we explore the impact of antibiotic treatments on the production of halogenated compounds, the key metabolites responsible for methane reduction and antimicrobial activity. These compounds include bromoform ($CHBr_3$) as the most prevalent and another 5-10 major compounds. We then isolated various bacterial strains which can be reinoculated onto *A. taxiformis*. These results are discussed as the potential for use of isolated epiphytic bacteria as probiotics for seaweed culture.

EXAMINATION OF CORN FERMENTED PROTEINS TO REPLACE FISHMEAL IN JUVENILE RAINBOW TROUT DIETS

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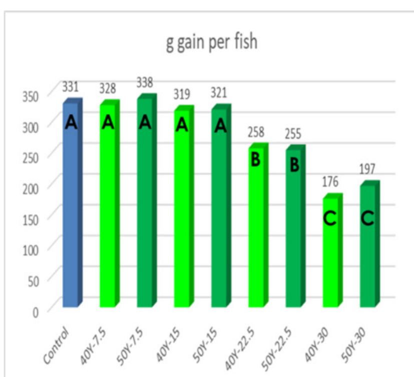
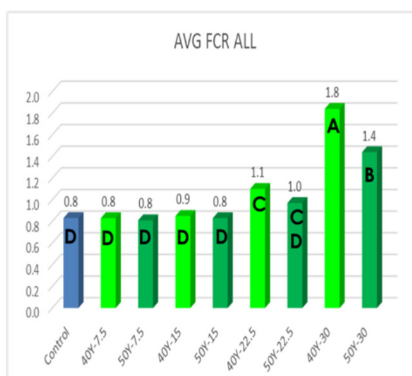
The ability of high value plant protein concentrates to replace fish meal in diets for rainbow trout depends on their available nutrient composition, cost, and consistency. The aim of this study was to assess the effects of two novel corn fermented protein products (ANDVantage™ 40Y and ANDVantage™ 50Y, The Andersons, Inc.) on growth performance of juvenile rainbow trout.

A 2 x 5 factorial design was applied with test products included at 0, 7.5, 15, 22.5 and 30% diet dry weight replacing dietary fish meal and poultry meal on a digestible protein (DP) basis. All diets were formulated to 42% DP and 18% crude lipid, supplemented with Lys, Met and Thr to targets of 3.8, 1.3 and 2.1%, respectively, and manufactured by cooking extrusion. Diets were randomly assigned to triplicate tanks of rainbow trout (*Oncorhynchus mykiss*, Troutlodge Inc., Sumner, WA USA) with a mean initial weight of 38 ± 0.7 g (mean ± SD). Fish were cultured in poly tanks (320 L) at n = 20 fish per tank in a recirculating system with a flow rate of 4-6 L min^{-1}, temperature at 15 °C, and a 13:11 light:dark cycle, and fed twice daily to apparent satiation six days per week for 12 weeks.

Including ANDVantage products at levels above 22.5% decreased g gain fish ($P<0.0001$). A significant interaction was observed for feed conversion ratio (FCR; $P<0.0001$) wherein fish fed ANDVantage™ 40Y had significantly higher FCR than fish fed ANDVantage™ 50Y when fed levels above 22.5%.

Optimized inclusion levels, determined by regression analysis for combined data or for each ingredient when interactive effects occurred, indicate that inclusion levels for ANDVantage 40Y and ANDVantage 50Y in rainbow trout diets range from 13.2 to 19.8% depending on the performance variable assessed.





Maximum inclusion capacity of ANDVantage products in rainbow trout diets. Limits identified for various performance parameters using regression analysis.

Inclusion capacity[1]	Product	Parameter[2]	P value	R^2	Equation
13.55	Combined	Growth (g gain)	< 0.0001	0.95	$Y = 329.4 + 2.716X - 0.2524X^2$
14.29	40Y	FI (% BW d^{-1})	< 0.0001	0.94	$Y = 1.935 - 0.04284X + 0.002616X^2$
19.75	50Y	FI (% BW d^{-1})	0.0002	0.87	$Y = 1.907 - 0.02141X + 0.001163X^2$
13.23	40Y	FE	< 0.0001	0.99	$Y = 1.251 + 0.01467X - 0.00123X^2$
15.13	50Y	FE	< 0.0001	0.97	$Y = 1.253 + 0.01451X - 0.001019X^2$

[1] % diet dry weight.
[2] Feed intake (FI), feed efficiency (FE)

AQUAVIP - UNRAVELING THE ROLE OF VIPERIN IN THE TROUT ANTIVIRAL RESPONSE

Felipe Schoninger*, Rita Azeredo, Sergio Fernández-Boo, Teresa Martins, Carolina Tafalla, Lourenço Ramos-Pinto and Marina Machado

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The high economic impact of viral outbreaks in aquaculture supports the need for the development of prophylactic and therapeutic solutions to assist animal health management. In fact virus are responsible for 8 of the 10 diseases with great social and economic and/or public health repercussion or even present a potential risk for the aquaculture industry. However, some effort has been devoted towards the study of viral response in fish, the underpinning antiviral mechanisms activated and the application of vaccines. Yet in order to do so, a better understand of fish basic antiviral mechanisms is necessary. The AQUAVIP project gathers a team mostly composed of researchers with important knowledge on the modulation of fish immune responses and disease resistance, experts in genetics and researchers with an important know-how on the field of antiviral mechanisms of fish. All aligned, the AQUAVIP team intends to create the tools to the creation of innovative and sustainable strategies to improve fish antiviral response and resistance to viral pathogens.

Antiviral response is mostly sustained by non-specific mechanisms with interferons (INF) comprising the central effector system for antiviral response in all animals, including fish. A recent attention has been given to a particular IFN effector, viperin (VIP) that despite being identified for more than 20 years, its action mechanisms have been only recently described. VIP antiviral activity is proposed to be due its interaction with viral RNA. By catalysing the conversion of CTP to ddhCTP, VIP inhibits viral replication since the modified nucleotide, when fused into the nascent chain of the viral RNA, acts as a chain terminator impairing its polymerization. This antiviral mechanism is widely recognized in mammals, however, multiple other roles of VIP, besides its role as antiviral ribonuclease are described. The VIP multi-action mechanisms recently described for mammals point to its importance for antiviral response. Therefore, VIP characterization and antiviral role in fish is necessary. For that, the AQUAVIP project uses a multidisciplinary approach, with up to date methodologies, to characterize VIP antiviral activity and to take a look on its role during Rainbow trout (*Oncorhynchus mykiss*) innate immune response to virus.

Ultimately, the AQUAVIP project will contribute to the creation of new tools to improve fish immune response and resistance to virus, allowing to better recognize the innate immune mechanisms activated in response to virus with focus on the modulation of the antiviral pathways with close relationship with VIP.

Acknowledgements: This project was financed by national funds through the FCT – Foundation for Science and the Technology, IP, EXPL/CVT-CVT/0458/2021.

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EFFECT OF PHYTOCEE SUPPLEMENTATION ON POST LARVAE SHRIMP *Litopenaeus vannamei* GROWTH PWEFORMANCE, HEPATOPANCREAS AND INTESTINAL HEALTH

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This study aimed to investigate whether Phytocee (Natural Remedies Company), a natural plant extract, can improve the growth performance and gut health of post-larvae shrimp *Litopenaeus vannamei* under oxygen depletion stress, compared to vitamin C-phosphate. Oxygen depletion is a common stress in shrimp aquaculture, and it can negatively affect shrimp health and growth including mortality. Therefore, finding effective ways to mitigate the negative effects of stress on shrimp is crucial for sustainable and profitable shrimp farming. Phytocee is a natural plant extract that contains polyphenols, withanolides and triterpenoids , which are known to have antioxidant and immune-boosting properties. These properties may make Phytocee to be an effective alternative to vitamin C in supporting shrimp health and productivity, especially under stressful conditions, while promoting sustainable and environmentally friendly aquaculture practices. The feeding trial was conducted in CRD with 3 treatments and 4 replicates as shown in Table 1.

Vitamin C-phosphate and Phytocee were top up on commercial feed and fed to shrimp for 21 days, then challenge the dissolved oxygen stress tolerance test. Healthy white shrimp PL 15 stage were stocked in 300L tank at density of 3 shrimp/L. All shrimps were fed 5 times a day. After the 21-day feeding, a low dissolved oxygen (DO) stress test was achieved by stopping aeration for 4 hours trial. DO of normal and depleted oxygen conditions was about 5 ppm and 1 ppm, respectively. Survival shrimps were collected to analyze hepatopancreas health, lipid deposition and EHP infection. The trial results were shown in Figure 1 and 2 which displayed that no significant differences (P> 0.05) were observed in final body weight and feed conversion ratio as well as survival rate among all treatments. Results under low DO condition found that there were not significant differences (P> 0.05) in survival rates. Supplementation of Phytocee (T3) improved survival by 3-5% of numerical value when compared to control (T1) and vitamin C-phosphate (T2). After oxygen depletion, hepatopancreas health of shrimp fed T3 and T1 have more constriction than T2. The lipid deposition in shrimp fed T3 have more improvement than T1 and T2 by 5% and 6%, respectively. In addition, evaluation of EHP showed the efficacy of Phytocee to reduce EHP infection by 5% lower than control T1 and 4% lower than T2. The intestinal health was in the same range. In conclusion, Phytocee 2g/Kg diet has the potential to use as a natural alternative for vitamin C-phosphate to support shrimp health and productivity under stressful conditions.

TABLE 1. Diet description of shrimp feed formula

Treatment	Diet Description
T1	Commercial diet
T2	Commercial diet + Vitamin C phosphate 2 g/kg diet
T3	Commercial diet + Phytocee 2 g/kg diet

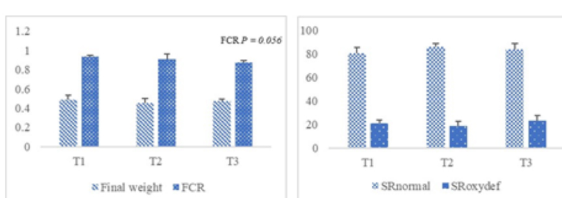

Figure1 Growth performance and survival rate under normal and oxygen depletion conditions

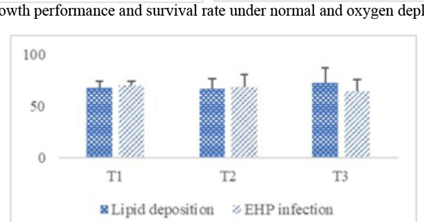

Figure 2 Lipid deposition and EHP infection

EVALUATION OF COMMERCIALLY AVAILABLE POLYELECTROLYTE FLOCCULANTS IN HARVESTING *Nannochloropsis oceanica* (CS-702)

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The ability to harvest microalgae at a commercial scale continues to be a major economic problem for the algal biomass industry. Due to their small cell size and relatively low concentration, large volumes of water are required to be removed to produce a commercially viable product, making the process both energy, and cost prohibitive. Among current commercially available harvesting methods flocculation has been identified as an efficient and cost-effective technique to concentrate algae biomass. For a flocculant to be suitable for commercial use it must be inexpensive, efficient, effective at low concentrations, and non-toxic for applications in food and feed. Additionally, the flocculant is expected to maintain the biochemical integrity of the harvested biomass.

Twenty commercially available polyelectrolyte flocculants were evaluated for their flocculation efficiency of high-density indoor cultures of *Nannochloropsis oceanica* (CS-702). Flocculation efficiency varied considerably ranging from 1 to 99% (OD750). Flocculants POLY SEPAR CFL 20 and CFL 40 showed the highest flocculation efficiency in the medium to high concentration range (50-100mg L^{-1}) ranging from 91-99%.

POLY SEPAR CFL40 was chosen for subsequent scaled-up harvest trials of *N. oceanica* cultivated in 2300L outdoor raceways due to its high flocculation efficiency and low toxicity. Pilot-scale flocculation of 2300L of raceway cultures was trialled using CFL40 at concentrations of 80 and 120mg L^{-1} with a reluctant flocculation efficiency of 83 and 85%, respectively.

The biochemical composition of *N. oceanica* flocculated using CFL40 at concentrations of 80 and 120mg L^{-1} prior to centrifugation versus biomass harvested via centrifugation was evaluated. Among the various biochemical parameters monitored the addition of flocculant increased the ash content and decreased the lipid and energy content. However, flocculated algal biomass did not register any variation in protein and carbohydrates when compared to algal biomass harvested using a centrifuge.

KELP MARICULTURE'S POTENTIAL TO REDUCE NEGATIVE EFFECTS OF FINFISH AQUACULTURE ON WATER QUALITY

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Finfish aquaculture often poses harm to the marine ecosystem through the associated output of nitrogen waste primarily in the form of ammonium (1 p.3). Managing this nitrogen output must therefore be an important consideration of aquaculture farmers and inform the way they set up and manage their farms. Growing kelp alongside finfish farms represents a potential solution to nitrogen pollution as well as providing other co-benefits and the Southern Ocean Carbon Company is participating in practical research through the Blue Economy Co-operative Research Council in part to realise this potential. The deployment of *Macrocystis pyrifera* (Giant Kelp) at sites adjacent to finfish aquaculture farms may provide a solution due to the nitrogen harvesting of giant kelp (1 p. 1). Modelling has shown that growing kelp next to farms can drastically improve the water quality of the system and provide economic co-benefits to practitioners (1, p.1).

Not only do these systems have the potential to benefit finfish aquaculture farms through improving water quality through processing their nitrogen waste, but in processing this waste there is an associated benefit of significantly improved seaweed growth as a result of the increased levels of nitrogen (Figure 2).

This suggests that close proximity to nitrogen sources is the ideal place to install giant kelp aquaculture in order to maximize production. This giant kelp biomass provides further value through a number of co-benefits. These include applications as a soil additive for terrestrial farming, as food, as an emerging alternative to conventional plastics, and also as a carbon sequestering agent to address climate change.

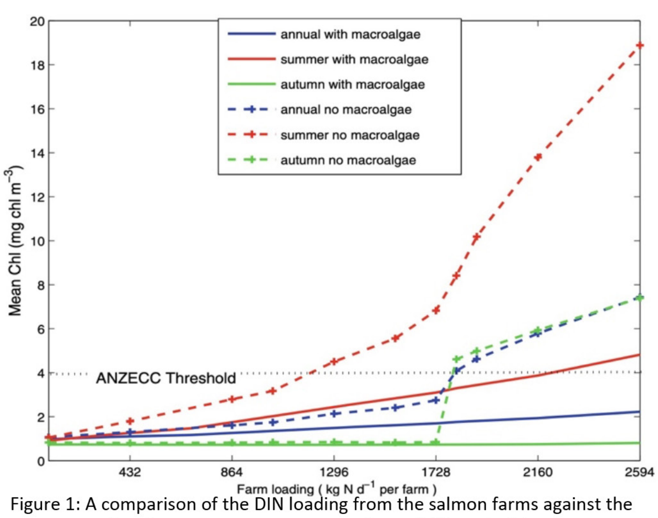

Figure 1: A comparison of the DIN loading from the salmon farms against the mean annual and mean summer concentrations of chlorophyll for the estuary. Key features are the strong nonlinear response of Chl to farm loadings above 1730 kg N day−1 per farm, and the strongly mitigating effect of IMTA macroalgae. Source: Hadley et. al 2015

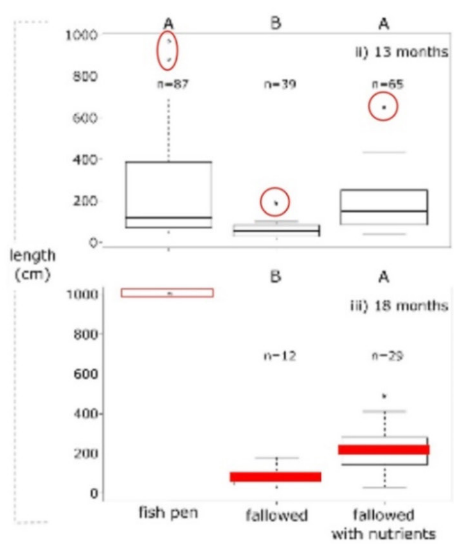

Figure 2: (Unpublished data via Cayne Layton)

UNCOVERING THE MECHANISMS OF TRAINED IMMUNITY IN *Panulirus ornatus*: INSIGHTS FROM IMMUNE GENE EXPRESSION AND ANTIMICROBIAL ACTIVITY

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Disease is a major threat to the sustainable growth and profitability of lobster aquaculture, with periodic disease outbreaks leading to significant mortalities in commercial species. Due to the absence of an antibody-mediated adaptive system in invertebrates, true vaccination against commercially important diseases is not possible. However, trained immunity (TI) may offer a potential disease management strategy for the emerging commercial species, *Panulirus ornatus*. Trained immunity is a process by which the innate immune system of an organism can develop a memory-like response to certain pathogens or stimuli, resulting in an enhanced response upon secondary exposure. While there is evidence to suggest that trained immunity exists for *P. ornatus*, the mechanisms by which it works are not yet fully understood. Current thinking is that the process involves epigenetic reprogramming of innate immune cells, leading to changes in their gene expression profiles and functional responses. However, the specific molecular pathways and mechanisms involved in this process are poorly understood.

We present initial results that highlight specific changes in immune gene expression and the production of the antimicrobial peptide defensin in *P. ornatus* from initial and secondary exposure to a formalin-killed bacterial pathogen. Haemolymph samples were collected from an experiment conducted over a 10-week period. Groups received either a single exposure to the bacterin or an initial followed by a secondary exposure at differing time points. Circulating haemocytes were separated from plasma and immune gene expression profiles assessed while the antimicrobial activity of the remaining plasma was assessed as a measure of an immune response likely to improve disease resistance.

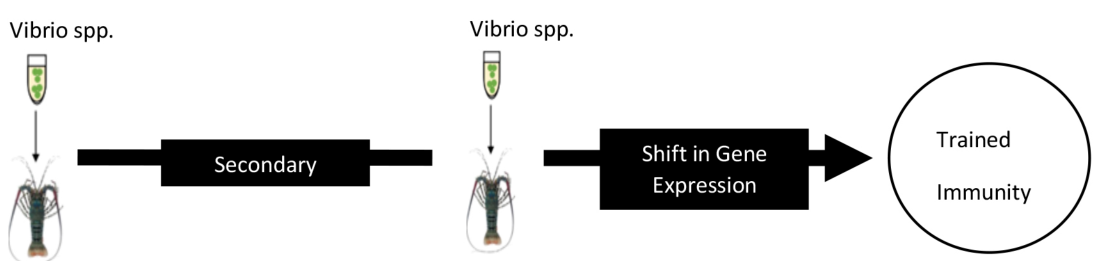

Figure 1. Timeline of an individual receiving an A) initial dose and B) secondary dose of inactivated bacteria and the posited shift in gene expression that facilitates the establishment of trained Immunity .

MODELLING HIGH SURVIVAL OUTCOMES IN VANNAMEI PRAWN FARMING USING DATA FROM CENTRAL PHILIPPINES

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The contribution of aquaculture to global aquatic food production is expected to increase 5-fold by 2030. The massive growth in this sector has created a need to efficiently manage resources to maximize productivity. However, issues such as climate change and emerging diseases contributes to the challenges in aquatic food farming. For instance, these factors greatly affect the survival of aquatic culture. This can impact yield outcome creating shortage in food supply and an economic loss for aquaculture farmers. Therefore, it is essential to explore and leverage innovative technologies.

We analysed industry data consisting of 3 harvest cycles from 9 *P. vannamei* shrimp ponds in the Philippines. The dataset includes daily physio-chemical properties, feed and supplement data, and water management input data. The survival rate, indicating the health outcome of the collective shrimp culture at the end of each harvest cycle, was taken as the target variable for the study. The survival rate threshold was set to 100%, and a high survival outcome was treated as a classification problem. We labelled 870 data points resulting to below 100% survival as class 1, while the remaining 174 data points resulting to 100% and above as class 0. We trained 3 models based on various configurations of the variables: using all variables, using only physio-chemical properties, and using only feed variables. We used the XGBoost algorithm using Python to train these models. XGBoost selects variables based on feature importance and removes redundant variables sharing a high correlation with another. A smote function was implemented to balance the data by generating synthetic data points. Parameters were tuned to reduce model overfitting. Cross-validation was applied to test the model. We compared the model performance of using each variable configuration based on accuracy, sensitivity, and specificity.

Results show that using all variables increases model performance with a prediction accuracy of up to 85.17%. Using only physio-chemical variables reduces model accuracy to 67.94% while using only feed variables brings this down further to 63.16%. This result indicates the importance of implementing thorough and accurate data collection practices in farm settings. This study also indicates the potential for predicting survival and yield in a farm culture.

Table 1. Model performance

	All variables*	Physio-chemical variables	Feed variables
Accuracy	85.17%	67.94%	63.16%
Sensitivity	85.57%	31.43%	94.29%
Specificity	85.06%	75.29%	56.90%

*variables with high correlation were filtered out

EFFECTS OF USING NOVAQPRO® MICROBIAL BIOMASS IN COMMERCIAL DIETS FED TO WHITELEGGED SHRIMP *Penaeus vannamei*

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Introduction: This paper discusses the results of two tank-based studies carried out at the Andaman Aquatic Research Centre (AARC) in Thailand testing the effects of Novacq™ microbial biomass on growth, survival and FCR of *P. vannamei* fed commercial diets containing varying levels of this ingredient in its original form: Novacq™, and an improved form produced by Ridley called NovaqPro®.

Methods: Trial 1 was conducted in a 76 * 200 litre tank system stocking shrimp juveniles of 0.7g initial weight, which were fed 19 high quality iso-nitrogenous and iso-calorific diets per trial with 4 reps per treatment for 6 weeks to apparent satiation. Trial 1 used varying levels (2.5-15%) of 3 different types of dried Novacq™ powder (original CSIRO Novacq™, Thai and Australian produced NovaqPro®), replacing wheat flour in otherwise similar diets. Trial 2 used the same system, stocking 0.6g juveniles, using 4 replicates of 7 treatments in 28 tanks, fed for 6 weeks. A high quality Ridley diet (43% protein) was used with and without 5, 7.5, or 10% of 2 different autoclaved Novacqs™: 1 original Novacq™ from CSIRO and 1 NovaqPro® type of Novacq™ developed and produced by Ridley in Australia. Results were analysed by two-way ANOVAs with Novacq™ treatment and inclusion rate as factors. Significant differences against the control diet were tested by one-way ANOVAs and post-hoc comparisons using Dunnett's two-sided multiple-comparison test.

Results: Trial 1 showed that shrimp accepted all feeds with survival above 91% across dietary treatments. Production performance metrics were statistically affected by Novacq™ type and inclusion levels with all microbial biomass treatments significantly outperforming the control. Thai and Australian-produced NovaqPro® were similar and outperformed the original Novacq™. Generally, there was a direct correlation between NovaqPro® inclusion level and growth, which was increased by up to 58% over the non-microbial biomass control diet at a 12.5% inclusion level, whilst original Novacq™ increased growth rate by up to 35% over the control. Trial 2 again showed an average 94% survival and good growth. Results showed that there was a generally direct relationship between Novacq™ inclusion level and shrimp growth rates for the two types of Novacq™. However, this was most pronounced for the Ridley NovaqPro®, which, even when autoclaved, increased growth rate by 24% over the control at 5%, rising to 33% at 7.5-10% inclusion, which was 31-45% better than that achieved by the autoclaved original Novacq™.

Conclusions: Use of dried NovaqPro® microbial biomass powder has been shown to increase growth (with little effect on survival or FCR) in direct relation to dietary inclusion rates by up to 58% at up to 12.5% inclusion in high quality Australian diets under laboratory conditions. When this product was autoclaved to sterilize it, relative performance decreased, but was still highest for the modified and improved Ridley NovaqPro® version, which promoted growth at significantly greater rates than that of the original Novacq™. The new NovaqPro® microbial biomass has thus been shown to be a significant improvement over the original Novacq™ microbial biomass product.

PERFORMANCE THRESHOLDS FOR SEXUAL MATURATION ONSET IN ATLANTIC SALMON *Salmo salar*: A CANDIDATE FOR SELECTION ON REPRODUCTION?

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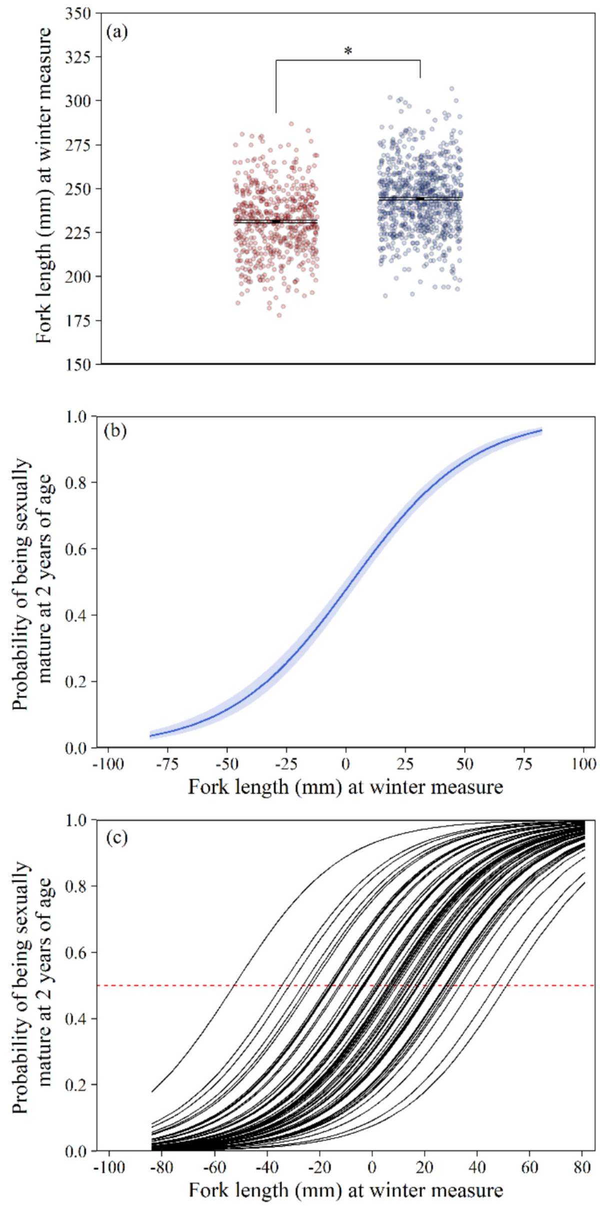
The onset of sexual maturation in salmonids is conditional on performance surpassing a threshold. Although the basis on which this performance threshold is founded is still not clear, the primary signal(s) appears to be associated with size and condition. The magnitude of the performance threshold, which can vary across populations, influences the incidence of sexual maturation within a population. Applying selection pressure to the performance threshold could be an effective approach to improve control of sexual maturation in cultured populations. However, the prospect of intra-population variation in the performance threshold for sexual maturation onset remains largely unexplored.

We investigated the relationship between size, condition and sexual maturation outcome in Atlantic salmon (*Salmo salar*) from the SALTAS selective breeding program. Males that were sexually mature at 2 years of age (maiden spawn) had, on average, greater size and condition than their immature counterparts in the previous winter. For every 10 mm increase in winter fork length or 0.1 K increase in winter condition, the odds of being sexually mature at 2 years of age increased by 1.48 times or 1.24 times, respectively. Family explained 35.66 % of the variation in sexual maturation outcome amongst 2 year-old males that was not attributable to the average effects of fork length and condition. Our findings suggest that the performance level required for sexual maturation onset varies between families from the SALTAS population. This could support the application of selection to advance or delay sexual maturation onset inthe studied population.



(a): Fork length in winter (~12 months post hatch) in sexually immature (●) and mature (●) 2 year-old male Atlantic salmon.
(b): Fitted logistic regression of the probability of being sexually mature at 2 years of age based on fork length in winter.
(c): Estimated probabilistic maturation reaction norm for families from the SALTAS population.
For panels (b) & (c), fork length data has been zero-centred such that zero is equal to the population mean.

IMPACT OF BACTERIAL COINFECTIONS IN CHANNEL CATFISH *Ictalurus punctatus* CULTURE

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Catfish farming is the largest sector of U.S. aquaculture and is of major economic importance for Southern U.S. agriculture. Maintaining and improving catfish health is a primary concern for producers, and bacterial pathogens can cause large-scale losses in production ponds. *Edwardsiella ictaluri*, *Aeromonas hydrophila*, and columnaris-causing bacteria (*Flavobacterium* spp.) are predominant bacterial pathogens causing mortality within production ponds. In a pond environment, catfish are simultaneously exposed to multiple aquatic pathogens, and issues with water quality and stress can influence pathogen and host dynamics. Bacterial coinfections may increase the severity of the constituent pathogens and elevate mortality, thus creating economic losses. A recent project assessed and characterized the effects of bacterial coinfections on juvenile channel catfish. Three *in vivo* pathogen challenge trials compared exposure to single and coinfective bacterial doses. Mixed combinations of *F. covae* (ALG-530-00), virulent *Aeromonas hydrophila* (vAh; ML09-119), and *E. ictaluri* (S97-773) were incorporated into the experimental design.

With respect to experimental coinfection with vAh and *F. covae*, at 96 h post-challenge, the single virulent *A. hydrophila* infection (immersed in 2.3 x 10^7 CFU mL^{-1}) resulted in final cumulative percent mortality (CPM) of 28.3 ± 9.5 %. The full-dose *F. covae* group (immersed in 5.2 x 10^6 CFU mL^{-1}) was 23.3 ± 12.9 %. A coinfection full-dose combination (98.3 ± 1.4 %) and a half-dose administration (76.7 ± 17.1 %) greatly increased mortality ($P < 0.001$).

Concerning experimental coinfection with vAh and *E. ictaluri*, at ten days post-challenge, the full-dose, single vAh infection (immersed in 1.9 x 10^7 CFU mL^{-1}) resulted in a final CPM of 25.0 ± 2.9 %. The CPM for the full-dose *E. ictaluri* group (immersed in 4.0 x 10^5 CFU mL^{-1}) was 11.7 ± 4.4 %. When both pathogens were administered together, the full-dose combination (41.7 ± 7.3%) and half-dose combination (40.0 ± 10.4%) demonstrated pronounced mortality.

Experimental findings indicate changes in both mortality levels and trends from exposure to multiple bacterial pathogens. Reducing disease outbreaks in catfish farming is critical to enhancing production yields and quality products. As more information is available on polymicrobial infections, new methods of treatment or prevention may be possible, such as more informed decisions on targeted antibiotic applications or vaccination strategies.

GENOME EDITING FOR GENETIC IMPROVEMENT IN FISH AND SHELLFISH AQUACULTURE

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Increasing ability to harness the power of genomics is forcing a rethinking of aquaculture genetic improvement strategies. Successful breeding programs will always be built on the careful selection of the next generation of broodstock, detailed record keeping, and accurate collection of phenotypic data. Genomics allows this base of phenotypic selection to be enhanced, and ultimately accelerated to increase genetic gain per generation. This is currently done in both advanced finfish and shellfish programs at the most sophisticated level through the use of Genomic Selection. However, another exciting technology is on the horizon that will fundamentally change how we deliver genetic improvement. This technology is Genome Editing.

Genome Editing is a technology that can thought of as “precision breeding”. It will be an important tool in the future toolbox for genetic improvement in aquaculture. The current state of the art in Genome Editing in aquaculture is impressive and on the cusp of significant commercial application. The basic concept is that enzymatic tools (such as CRISPR technologies) can be used to create variants in specific DNA sequences that create a desired phenotype (such as sterility, monosex, rapid growth, or disease resistance). The technique does not involve adding new DNA, so is not transgenic and does not create a GMO. It simply involves understanding the genomics and underlying genetic variant that is needed for a trait to be expressed, and harnessing natural processes to create that variant rather than sorting through many thousands of broodstock and many generations to achieve the same effect.

The power of genomic research is that we are beginning to understand the exact genes involved in performance traits, and how variation in those genes leads to improved performance. Harnessing the power of Genome Editing allows us to transfer this knowledge to application in commercial breeding programs for heritable, quantum advances in genetic improvement. Importantly, sterility will be a requirement in most applications of GE in aquaculture as a method of biocontainment to prevent escape to the environment, or the inadvertent application of genetically improved animals.

This presentation will provide background on how genome editing works, an update on regulation, and how this tool will be used to improve aquaculture fish and shellfish genetics in the very near future.

INVESTIGATING SALMON BEHAVIOUR AND INTERACTIONS WITH THE ENVIRONMENT TO AUTOMATE FEEDING AND IMPROVE WELFARE IN AQUACULTURE FARMS

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Aquaculture is expanding globally, valued at £159 billion in 2022, with Atlantic salmon dominating finfish production at 3.5 million tonnes annually. As the industry grows, more sophisticated technology is needed to monitor farms and ensure their sustainability. Using behaviour as a non-invasive form of monitoring, in combination with artificial intelligence and machine learning, can allow for higher control over farm management. For instance, the development of algorithms to analyse fish behaviour related to feeding may be used to fully automate the feeding process and reduce environmental and economical waste. The goal of this study is to identify changes to Atlantic salmon (*Salmo salar*) behaviour related to responses to environmental conditions (*e.g.,* currents, oxygen, temperature), operational procedures, therapeutic mechanical or medicinal treatments or health status of the fish (AGD, PGD, sea lice burden). Therefore, it is essential to understand how the fish are distributed within a cage, and where best to place cameras to gather reliable behavioural data. For this study, 5 cameras were deployed at a Scottish Atlantic salmon farm consisting of 10 cages, each 100 m in circumference and ~15 meters in depth. The cameras were deployed in one cage in the following orientation: 3 down the centre (4 m, 9 m, 14 m), 2 at 9 m on the inner and outer areas of the cage, respectively. An algorithm was created by Observe Technologies to process video footage from these cameras and transform it into behavioural data useful for farmers (*e.g.,* activity, speed, schooling). First analysis shows that with respect to activity, the camera in the centre of the cage at 9 m is the most accurate for tracking feed times, indicating that this is a valuable placement of the camera to gather data on feed behaviour. Additionally, there is significantly higher activity on the side of the cage closer to the inner farm compared to the opposite, more exposed side, and more activity at the bottom of the cage compared to the surface. This suggests that the fish are congregating in these two locations, possibly to avoid stronger currents and for fear of predation at the surface, respectively. Further work is underway to investigate how environmental or health conditions affect this positioning to ultimately automate feeding and reduce feed waste.

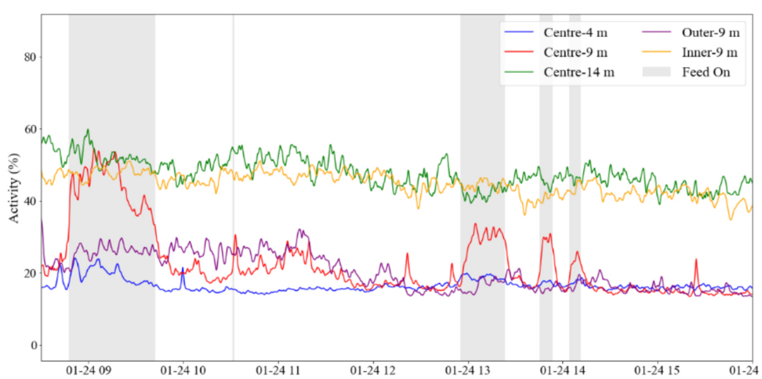

Figure 1 Fish activity (%) observed by 5 cameras (coloured lines) on Jan. 24, 2023. Feed times are indicated by the grey bars.

POSITIVE IMPACTS OF NUTRITIONAL SUPPLEMENTATION WITH DIFFERENT ANTIOXIDANT SOURCES IN MARINE FISH GAMETOGENESIS AND SPERM TRAITS

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The use of antioxidant supplements in broodstock diets proved to be beneficial for the reproductive performance of some marine fish. Protection against oxidative stress is important during spermatogenesis, because of the high levels of reactive oxygen species (ROS) inherent to this extremely active replicative process. Spermatogonia and spermatocytes have a natural mechanism of protection against ROS associated to enzymes and cytoplasmic molecules that act as scavengers. However, this protection diminishes in spermatozoa, in which the cytoplasmic content is reduced. As a biological response to oxidative stress and to protect spermatozoa, seminal plasma can be a powerful source of antioxidants, where diet composition has a pivotal role. In fact, not only seminal or plasma membrane composition may be modulated through the incorporation of certain compounds in the feeds, such as trace elements or vitamins, but also other sperm traits can be affected. Studies have been conducted to search for novel feed formulations with incorporation of micro and macroalgae or directly by the increase of compounds such as zinc, selenium and vitamin C and E, known by their antioxidant properties. Having algae a diversity of bioactive compounds with considerable antioxidant capacity, they are clearly good candidates. However, it is not always clear the effect of different antioxidant sources and most of the research conducted had positive and negative effects depending on the fish species.

In the present study gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) and Senegalese sole (*Solea senegalensis*) breeder diets were supplemented with macroalgae (*Plocamium* or *Sargassum*), as natural antioxidants sources, or directly by administrating zinc, selenium and vitamins C and E into the feeds. Experiments were conducted during gametogenesis, spermiation and post-spermiation in some species to identify putative effects on gonadal antioxidant system and sperm traits. Sperm samples were collected and several quality tests were determined: spermatozoa motility, viability, ROS, DNA fragmentation and lipid peroxidation. Expression of apoptotic, anti-apoptotic, antioxidant genes and enzymes as well as total antioxidant capacity (TAS) were determined in reproductive tissues and cells.

Results demonstrated a clear effect of antioxidants in sperm traits, very dependent on the species and antioxidant used. In Senegalese sole the supplementation of 5% *Plocamium cartilagineum* increased spermatozoa motility and viability when compared with control or with *Sargassum vulgare,* although in terms of cells protection against ROS, *Sargassum* supplementation proved to be more efficient, probably due to a high phenolic content present in this algae. In seabream and seabass, there was higher total antioxidant activity during gametogenesis than spermiation, with an increase of TAS in fish fed on vitamin supplementation. In seminal plasma, higher levels of vitamins and selenium were detected when compared with control. Sperm GSR and GPx activity was positively affected by treatments. In seabream, *bax* expression (pro-apoptosis) was downregulated in Se and Zn treatments, where *bclx* (anti-apoptosis) was upregulated. Funding: Projects SPERMANTIOX and GERMROS (PTDC/CVT-CVT/4109/2020 and EXPL/CVT/0305/2021, FCT), BREEDFLAT (PT-INNOVATION-0080-EEA grants), and CCMAR and LA Strategic Projects (UIDB/04326/2020, LA/P/0101/2020).

MARINE SPATIAL PLANNING TOOL FOR DIGITAL ENVIRONMENTAL IMPACT ASSESSMENT: CASE STUDY APPLICATION FOR THE SCOTTISH SALMON AQUACULTURE INDUSTRY

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Marine spatial planning (MSP), incorporating multiple factors for consideration in the development of offshore aquaculture, is commonly used in site selection as well as a key tool for digital environmental impact assessments (D-EIA). By accounting for cumulative impacts from multiple farms and associated linkages, MSP tools are critical to ensure the impacts of a new developments are not underestimated within the context of existing developments. We demonstrate the application of MSP as a D-EIA tool for site selection of salmon farms in the west coast of Scotland with a focus on sea lice infestation risk and management. With plans to double production by 2030, the aquaculture industry in Scotland is developing innovative solutions to optimise site selection for reduced environmental impact.

The MSP tool combines multiple layers in a GIS workbook to include sensitive habitats, alternative industry uses, base line monitoring, impact maps for sea lice, treatment toxicants and farm waste. By integrating spatial mapping, background data and model output from both existing and proposed farms into the D-EIA process, commercial operators as well as regulators can perform multi-criteria assessments as part of the site selection process (Figure 1). For example, sea lice infestation risk from regionally linked farms as well as the environmental impacts of bath treatments. The MSP tool supports a holistic, system scale assessment of environmental impacts of individual farm operations while incorporating cumulative impacts associated with multiple farms within a distinct region. This allows regulators to understand the broader impact of multiple farms, while enabling industry to visualise the site selection process using a virtual environment with quantitative measures of risk.

Region	Aquaculture factors				Environmental / socio-economic factors			
	Sea lice infestation risk	Risk of connectivity between sites	Water temperature	Current speeds	Commercial fishing usage	Sewage inputs	Significant habitats	Climate change risk (forecasting)
Zone / site 1	High	High	Not suitable	Not suitable	High	Low	High	Low
Zone / site 2 (for further exploration)	Moderate	Moderate	Marginal	Suitable	Low	Low	High	Low
Zone / site 3	High	High	Not suitable	Suitable	High	Moderate	High	Low
Zone / site 4	High	High	Not suitable	Not suitable	High	Moderate	High	Moderate

Figure 1 – Example multi-criteria assessment for site selection based on environmental impact assessment

THE BLUE ECONOMY COOPERATIVE RESEARCH CENTRE PATH TO SUSTAINABLE INTEGRATED SYSTEMS FOR OFFSHORE AQUACULTURE 3: BUILDING BLOCKS FOR MULTISPECIES SYSTEMS

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Australia and New Zealand have two of the world's largest Exclusive Economic Zones, they offer extensive offshore access with unrealised potential for aquaculture (Gentry et al., 2017) and renewable energy. To be able to tap into this potential, the Blue Economy Cooperative Research Centre (BECRC) was established and will operate until the end of this decade. The BECRC brings together over 40 partners from 10 countries. The overarching aim of the BECRC is to support research that develops new seafood and renewable energy systems that move production offshore safely, economically and sustainably. Within the BECRC, there are five interconnected Research Programs that have been developed in collaboration with industry and other partners (BECRC, 2023). The '*Seafood and Marine Products*' program supports the development of offshore aquaculture systems that provide viable and sustainable growth opportunities. This presentation will overview existing R&D and address the need for R&D building blocks and pathways to achieving sustainable offshore aquaculture based around "multispecies systems" - multiple aquaculture species grown in the same geographical area.

The initial step will be to scope out and prioritise established and emerging aquaculture species suitable for novel offshore and potential production systems and in relation to prevalent abiotic factors in the different geographic regions available to the BECRC. By taking a collaborative approach, an initial scoping project will hold a series of workshops to rank and prioritise identified species and systems. This process will also identify gaps in knowledge for R&D as well as understand practical limitations to deployment and achieving success. The scope will span from temperate to tropical aquaculture, and consider a wide range of taxa including microorganisms, seaweeds, shellfish, and finfish. It will also determine the most appropriate aquaculture systems, whether monoculture, multispecies or integrated marine trophic aquaculture (IMTA). The aim is to create recommendations for decision-making and prioritisation of future BECRC R&D projects.

BECRC: Blue Economy Cooperative Research Centre. 2023. *Seafood and Marine Products* [Online]. https://blueeconomycrc.com.au/research/seafood-marine-products/. Available: https://blueeconomycrc.com.au/ [Accessed 01.01.2023 2023].
GENTRY, R. R., FROEHLICH, H. E., GRIMM, D., KAREIVA, P., PARKE, M., RUST, M., GAINES, S. D. & HALPERN, B. S. 2017. Mapping the global potential for marine aquaculture. *Nature Ecology & Evolution,* 1**,** 1317-1324

CRYOBANK GENETIC DIVERSITY ASSESSMENT AND STORAGE OPTIMIZATION USING GENOMIC IMPUTATION AND HAPLOTYPE ANALYSIS

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Cryopreservation of sperm cells (cryobank) is a strategy used by some aquaculture and livestock breeding programs to conserve and, if necessary, recover the genetic diversity of a population. The effectiveness of this strategy depends primarily on the methodology of cryopreservation, the viability of mass artificial fertilization with cryopreserved milt/semen, and the selection and culling decisions of the individuals to have their sperm stored, given constraints imposed by storage capacity and related costs. Ideally, the population represented in the cryobank is as genetically diverse as possible and, in some cases, present relatively superior genetic merit. The latter for cryobanks that also have the aim of serving as a backup population of elite broodstock. Different methods to assess and optimize the genetic diversity of a population can be used to assist in determining the storage and management policy of cryobanks. If genotypic information is available, genetic diversity can be optimized at the haplotype level, maximizing the count of unique haplotypes of a given subset of individuals. Modern imputation methods can also have a role in estimating the diversity held within ungenotyped individuals. The objectives of this study were to assess the genetic diversity of the Saltas (Salmon Enterprises of Tasmania Pty Ltd) cryobank and develop a strategy to optimize decision making on which samples to be stored. The Saltas cryobank was primarily developed to preserve the genetic diversity of the Saltas Atlantic salmon selective breeding program (SBP) but subsequently has also been strategically designed to serve as a backup source of nucleus sires. Currently, it stores milt from over 300 sires, from twelve different year classes (2007-2018), in approximately 2,000 Squarepacks® (Cryogenetics Inc). Its storage policy has been based mainly on information from pedigree and estimated breeding values. As 50K SNP genotype information is available for a large proportion of sires in the cryobank and for all broodstock candidates, the genetic diversity in the present study was assessed by computing the number of unique haplotypes from genomic regions (~1,500 windows) comprised by 30 consecutive SNP markers. The results indicated that the Saltas cryobank is currently storing approximately half of the haplotypes segregating in the SBP population. Cryobank samples and broodstock candidates were ranked according to their contribution for the genetic diversity of the SBP population, allowing the development of an optimized approach for future storage policy. The haplotype analyses of available broodstock candidates revealed no loss of genetic diversity in the most recent generations and, consequently, no current need to use cryobank material to recover genetic diversity. This result can primarily be explained by the size of the population (around 200 families produced annually) and by the optimum contribution and mate allocation strategies implemented in the SBP, which maximize genetic gain under constrained levels of coancestry and inbreeding. Opportunities to combine haplotype diversity and optimum contribution theory, for cryobank storage optimization purpose, were envisioned and will be further explored.

GENETIC VARIATION IN RESIDUAL VARIANCE REVEALS OPPORTUNITY TO SELECT OYSTERS FOR UNIFORMITY OF PRODUCTION

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Uniformity can significantly impact productivity and profitability of commercial oyster production. For instance, grading management activities (maintaining oysters of similar size to be cultivated together) correspond to one of the main variable costs for oyster growers. Different strategies can be adopted to increase uniformity. If genetic variation for uniformity of a given trait exists, it could be efficiently improved by selective breeding. In the present study, we investigated the existence of genetic variation on uniformity of different production traits in oysters, by modelling the genetic heterogeneity of residual variance, also referred as micro-environmental sensitivity. To avoid misleading inference due to eventual problems related with data structure or statistical artefacts from certain analysis, genetic heterogeneity of residual variance was assessed for two different oyster species, three different traits, and using two statistical methods. The analysed traits were harvest weight, shell length and width index (width to length ratio). The phenotypic measurements came from the ASI Pacific Oyster and the Sydney Rock Oyster Australian breeding populations. The analyses for each trait and dataset used information of approximately 50,000 oysters from about 1,000 families. The statistical methods adopted were the two-step REML and the double hierarchical generalized linear model, both applied under a sire-dam model. The genetic parameters used to assess the feasibility of selection for uniformity were: the genetic coefficient of variation of residual variance (GCV_E), also referred as ‘evolvability’, which provides the potential response to selection for residual variance; the heritability of residual variance, used to determine the accuracy of prediction of breeding values for the residual variance; and the genetic correlation between mean and variability. The results showed compelling evidence of genetic control of variability for the different traits, species/datasets and statistical methods evaluated. Evolvability estimates (0.109-0.499) indicated great opportunity to select oysters for uniformity of production, in agreement with estimates from other aquaculture and livestock species. The GCV_E estimate of 0.499, for example, indicates that for every change of one standard deviation of the additive genetic variance (of variability), the residual variance is expected to be reduced by 49.9%. The heritability estimates of residual variances were low (0.001-0.025) highlighting the necessity of large progeny size to accurately predict the breeding values for variability or, alternatively, the necessity to implement genomic selection to increase accuracy of prediction. The genetic correlation estimates between the mean and the residual variance were positive and, in general, moderate to high (0.133-0.729) indicating difficulty to simultaneously increase the mean and reduce variability, although some families with this pattern were observed. In summary, the results supported the existence of genetic control of variability for different production traits in oysters, revealing the potential to improve uniformity of oyster production through selective breeding.

THE USE OF CERTIFICATION SCHEMES TO ENSURE GOOD WELFARE PRACTISES IN THE AQUACULTURE INDUSTRY

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The aquaculture industry represents a critical food protein source to alleviate the pressure on global fish stocks and meet the increasing demand for seafood products. Its impacts are considered and addressed through legislation and voluntary certification programmes in many degrees. But the degree of coverage varies from country to country, and there needs to be a globally accepted approach to many of the challenges aquaculture poses. This was the case of fish welfare that had been overlooked until it became a buzzword for public perception and product acceptance.

The vision of the Aquaculture Stewardship Council (ASC) is a world where aquaculture plays a major role in supplying food with a minimum negative impact on the environment. Fish welfare was identified as a topic to expand. Supporting factors for inclusion were:

- Planet: Welfare is a key factor of sustainable production.
- Fish: Fish are sentient beings, and current aquaculture practices may impair welfare needs and thus need to be addressed.
- Science: There is sufficient applicable scientific research available on fish welfare for ASC to support expanding its current health and welfare-related indicators for different species.
- People: Consumer acceptance of aquaculture products will be increasingly influenced by the extent to which the industry is perceived to be dealing with fish welfare.

During this speech, ASC will present its ongoing work on developing a set of welfare indicators applicable across a range of species and which strive to move the aquaculture sector one step further in terms of producing seafood in a welfare-friendly manner which adds an extra value to the ASC label.

COMPARING THE GUT MICROBIOME OF WILD YELLOWTAIL KINGFISH *Seriola lalandi* TO FARMED INDIVIDUALS WITH UNDERLYING DISEASE CONDITIONS AND EXPLORING THE USE OF MODULATION TO MANIPULATE THE MICROBIOME TO IMPROVE HEALTH OUTCOMES

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The indigenous microbiota (or microbiome) of fin-fish is of topical interest in fish health and nutrition research, as parallel studies in human and animal models have highlighted the importance of a balanced microbiota in maintaining host health. The microbiota is composed of an ecological community of commensal, symbiotic and pathogenic microorganisms and includes bacteria (which are the dominant microbes), archaea, viruses, protozoa and fungi. The fin-fish gut microbiota contributes to digestion, nutrient acquisition and the immune response, which can in turn affect growth, reproduction, development and vulnerability of the host to disease. Knowledge of the gut microbiome has been shown to be of relevance for the identification of both favourable and dysbiotic (or pathogenic) phenotypes and offers the prospect for downstream manipulation for optimising host health and nutrition and consequentially the productivity of farmed species.

In this study, the active components of the gut bacterial microbiome of wild Yellowtail Kingfish (YTK) were characterised and compared to the microbiome of the surrounding water and the gut of farmed YTK exhibiting signs of an underlying disease condition. The ability to manipulate the gut microbiome of farmed poor-performing YTK was then explored in a trial undertaken at the SARDI pool-farm facility (West Beach, South Australia, Australia) using antibiotics and faecal microbiota transplantation (FMT), with inoculum collected and prepared from ‘healthy’ farmed YTK donors (n=102 individuals).

Differences were observed in the bacterial assemblages of the surrounding water environment and the gut of wild and farmed YTK, highlighting that YTK can select, regulate and maintain their own environmentally-independent communities. Differences were also observed between the gut microbiome of wild and farmed YTK that were exhibiting signs of an underlying disease condition. This was characterised by reduced microbial species richness, diversity and evenness, and the occurrence of one or more dominant bacterial taxa, highlighting a shift towards a dysbiotic state with potential loss of functionality.

Following antibiotic treatment and FMT, using inoculum prepared from ‘healthy’ on-farm YTK donors and administered via gavage, an increase in bacterial taxonomic diversity and species evenness was observed two days post inoculum in some poor-performing YTK recipients, attributed to an increase in bacterial phyla and decrease in the abundance of potential opportunistic pathogens. Differences were also observed at 8 days post inoculum, followed by a reversion to the ‘normal’ state at 15 days post inoculum. These results suggest that the gut microbiome of poor-performing YTK can be modulated to potentially improve health outcomes, but requires further investigation and optimisation of the approach to allow for more longer-term favourable health outcomes.

PLANT EXTRACTS: A PROMISING SOLUTION TO FIGHT EMS

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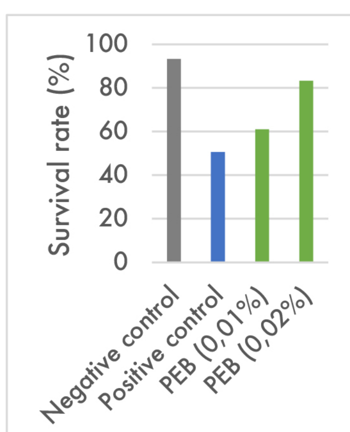
Marine shrimp aquaculture has become a worldwide industry with farms mostly located in Asia and Latin America, ranging from Mexico to Peru, and China to Australia. Despite different environments, ecosystems, farming systems and technology, expansion of shrimp farming came with its diseases, which also are today widespread around the globe. Among pathogens involved, bacteria from the *Vibrio* genus are very common in seawater and different strains from different species are known for causing pathologies like Early Mortality Disease (EMS), currently known as Acute Hepatopancreatic Necrosis Disease (AHPND). As shrimp are fragile animals with no acquired immunity system, alternatives to antibiotic treatments are sought for by the industry, to stimulate natural defences. Among sustainable candidates, plant extracts have shown promising results to limit *Vibrio* infection in aquaculture. In this context, several experiments were conducted in to evaluate the potential of a plant extract blend (PEB) against *Vibrio* bacteria.

Firstly, *in vitro* assays were done using a liquid formulation based on the plant extract blend to evaluate the inhibition effect against several *Vibrio* strains from different locations worldwide. Secondly, an *in vivo* trial was performed by infecting shrimp with *Vibrio parahaemolyticus*. The challenge dose of 1.5×10^5 CFU/mL was selected as the official challenge dose for the main trial after doing a calibration test and calculating with probit analysis. Then, 500 Specific pathogen free (SPF) whiteleg shrimp (*Litopeneaus vannamei*) with an average initial weight of ~1 g/shrimp were divided into 20 tanks. The set-up was 4 different treatments and 5 replicates for each treatment. The 4 treatments included negative control (no infection), positive control (infection without any product), and two PEB treatments (infection, with PEB in powder included as a preventive product at 0,1% and 0,2% in extruded feeds). Trial duration was 27 days (2 days of acclimation + 14 days of pre-challenge + 1 day of main challenge + 10 days of post-challenge). During the trial, survival rate as well as immunological parameters were measured.

The use of the PEB led to a global inhibition of *Vibrio* bacteria growth *in vitro*. At the end of the *in vivo* trial, improvement of survival rate after infection with *Vibrio parahaemolyticus* was observed (Fig.1). Other results including immunological parameters like phenoloxidase activity suggest that the PEB was able to stimulate shrimp defences and therefore limit disease consequences after *V. parahaemolyticus* infection, underlining PEB as a promising solution to vibriosis in shrimp farming.



Fg.1 Survival rate on day 10 post-challenge

PLANT EXTRACTS AS AN ALTERNATIVE TO FIGHT EARLY MORTALITY SYNDROME/ ACUTE HEPATOPANCREATIC NECROSIS DISEASE (EMS/AHPND)

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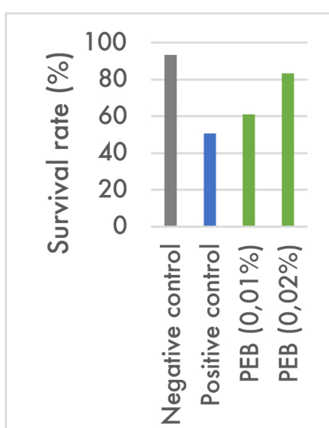

Fg.1 Survival rate on day 10 post-challenge

APPLICATION OF BILE ACID ON GROWTH PERFORMANCE AND IMMUNITY OF WHITE SHRIMP, *Penaeus vannamei*

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White shrimp (*Penaeus vannamei*) is the most economically significant marine shrimp species in Thailand in terms of production. Numerous feed additives are available to promote growth and immune response in aquatic animals, and one such additive is bile acid. Bile acid is a type of organic compound that is produced by the liver and secreted into the intestine. However, bile acid can play a role as feed additive to make many benefits. The valuable role of bile acid is improving growth rates and feed utilization by increasing the digestibility of dietary fats and proteins. Bile acids are secreted into the intestine to aid in the digestion and absorption of fats, and it can also promote the activity of certain digestive enzymes, such as lipase. This can support the shrimp to break down and absorb nutrients more efficiently, leading to improved growth rates and feed conversion ratios. In addition, bile acids enhance the activity of certain immune cells, like hemocytes and lymphocytes, and increase the production of immune molecules, lysozyme and phenol oxidase. The objective of this present study is to study the effects of bile acid supplementation in diets on shrimp growth performance and immune response.

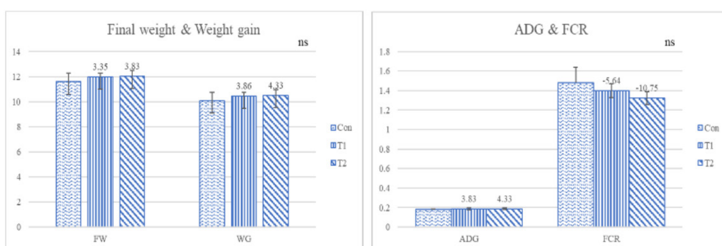

Figure 1. Growth performance of white shrimp fed diets with different levels of bile acid.

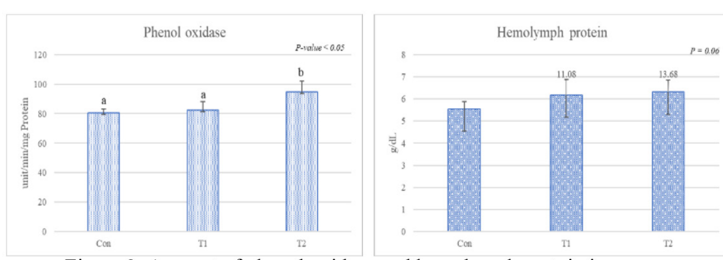

Figure 2. Amount of phenol oxidase and hemolymph protein in hemolymph shrimp fed diet with different levels of bile acid.

META-ANALYSIS OF STUDIES ON PROTECTION PROVIDED BY DIFFERENT PROPHYLACTIC AGENTS, THEIR ROUTES OF ADMINISTRATION AND INCUBATION TIMES AGAINST NODAVIRUS INFECTION IN *Macrobrachium rosenbergii*

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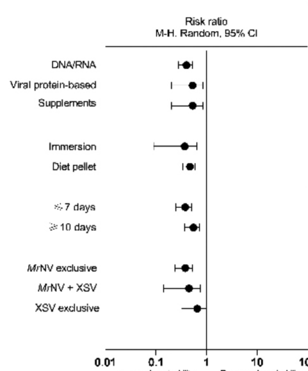
White tail disease caused by Macrobrachium rosenbergii nodavirus (MrNV) and extra small virus (XSV) is an infection that primarily affects giant freshwater prawns, Macrobrachium rosenbergii, which in turn causes significant economic losses in aquaculture farms worldwide. In response, different prophylactic agents have been studied to improve resistance to this infection. This meta-analysis aims to assess the effectiveness of different prophylactic agents in promoting shrimp survival. Embase, Google Scholar, PubMed, and Scopus search engines were used to search for relevant articles from 2000 to 2022. Articles were screened to only select articles studying crustaceans being given prophylaxis before the disease challenge. Treatments were analyzed on overall survival, and we performed subgroup analysis of treatment type, administration routes, treatment incubation time pre-infection, and whether the prophylactic agents were derived from MrNV, XSV, or a mixture of both. Between the 3 major prophylaxis types, DNA/RNA prophylaxis, viral protein-based prophylaxis, and supplements, all conferred protection against MrNV infection, though no differences between groups were observed. A shorter incubation period (≤ 7 days) conferred better protection, and prophylactic agents from MrNV origin were found to be more effective. A high degree of heterogeneity was detected between studies, necessitating future large-scale studies addressing possible bias.



	n	RPS (%)	Z	I^2	Subgroup Diffferences
DNA/RNA	19	60	P < 0.00001	74	P < 0.16
Viral protein-based	12	47	P < 0.0001	94	
Supplements	14	50	P < 0.0001	96	
Immersion	5	63	P < 0.00001	96	P < 0.25
Diet pellet	40	53	P < 0.00001	88	
≤7 days	20	62	P < 0.00001	87	P < 0.02
≥10 days	25	45	P < 0.00001	86	
*Mr*NV exclusive	17	62	P < 0.00001	82	P < 0.004
*Mr*NV + XSV	9	55	P < 0.00001	91	
XSV exclusive	5	35	P < 0.0008	83	

Table 1. Subgroup analysis comparing the effects of different prophylactic agents against nodavirus infection in Macrobrachium rosenbergii. Each individual plot represents the pooled results of all the weighted averages of each study involved in the subgroup. The horizontal bar represents the 95% confidence interval. The total number of studies involved in each subgroup is represented by n, the relative percentage survival by RPS, the total overall effect by Z (P value for Z-test) and heterogeneity by I^2. Significant differences contributed by specific variable factors in each subgroup analysis were also analyzed.

EVALUATION OF ACTUAL PROTEIN LEVEL IN MEALWORM MEAL AND ITS POTENTIALITY TO REPLACE DIETARY FISH MEAL FOR GIANT GROUPER *Epinephelus lanceolatus*

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Chitin, a polymer of N-acetylglucosamine, is rich in insect. While insect protein is used as feed ingredient, the nitrogen content in chitin may lead to the overestimation of its actual protein content that analyzed by Kjekdah method. Our present study aimed to evaluate the actual protein level in mealworm meal and its potentiality to replace dietary fish meal for giant grouper (*Epinephelus lanceolatus*). The crude protein and chitin content in the mealworm meal were analyzed to be 74.63% and 13.21%, respectively. After correction with nitrogen content in chitin, the actual protein of mealworm meal was 69.29%. Based on the results, dietary fish meal protein was replaced by mealworm meal at 0%, 10%, 20%, and 30%. Total of four experimental diets were each fed to triplicate groups of juvenile grouper (initial weight: 20.65 ± 0.08 g) in a recirculation system for 8 weeks. Weight gain, protein retention, protein efficiency ratio, feed efficiency, survival, plasma triglyceride and cholesterol concentrations were not significantly ($p>0.05$) affected by the dietary treatments. The fish in-fish out ratio decreased from 1.82 to 1.37 while the replacement level increased from 0% to 30%. The oleic acid (C18:1n9) and monounsaturated fatty acids (ΣMUFA) significantly increased in muscle with the increasing dietary levels of mealworm. Results indicated that the mealworm meal can replace dietary fish meal protein up to 30% for giant grouper without any negative effect on growth performance. The use of mealworm meal to replace fish meal can reduce the consumption of marine resource and achieve the goal of sustainable aquaculture.

Weight gain (WG), feed efficiency (FE) and fish in-fish out ratio (FIFOs) of grouper fed different diets for 8 weeks.[1]

	Fishmeal protein replaced by mealworm meal level (%)				
	0	10	20	30	*p* value
WG (%)	599.29±12.98	596.03±13.98	582.60±22.11	552.84±7.49	0.365
FE	1.20±0.02	1.19±0.00	1.19±0.01	1.17±0.01	0.228
FIFOs (%)	1.82±0.03^{d}	1.67±0.00^{c}	1.51±0.02^{b}	1.37±0.02^{a}	$p<0.001$

Values with different superscripts in the same row are significantly different ($p<0.05$).

[1]All values are presented as mean values with standard deviation (n=3).

TEMPERATURE MODULATION ON INTERFERONS REGULATED IMMUNE SYSTEM IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

Tzong-Yueh Chen*, Zhi Yan Chong, Yuan-Ning Wang, Hong Ye Ong

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This study aimed to investigate the temperature effects on interferons (IFNs) signaling pathway and IFNs-regulated immune system in orange-spotted grouper. As climate changes resulted in temperature variation in recent years, it leads to widespread outbreaks of diseases frequently in aquaculture, which eventually cause heavy impacts on the global aquaculture industries. Fish are ectothermic animals, and hence their immune system is deeply modulated by ambient temperature changes. Therefore, understanding the molecular mechanism of how temperature changes influence the immune system in fish will contribute to developing the strategy for disease prevention and control. However, relevant research about this issue is relatively less, so this study aims to investigate the effects of temperature variation on IFNs-regulated mechanisms in orange-spotted grouper (*Epinephelus coioides*). In this work, with the treatment of different temperatures, the comparative analysis of type I IFNs, type II IFNs, and other related immune molecules involved in antiviral responses (*Mx1*, *ISG15*, *Viperin* and *IFNR1*) was conducted, which provided the fundamental information to explore environment temperature factors regulating antiviral molecular mechanism. The comparative analysis of the IFNs system in orange-spotted grouper along with the immune stimulation was performed at high and low temperatures respectively. The results indicated IFN-regulated genes were suppressed at the late time point upon high-temperature treatment. The cloning of *Mx1*, *Mx2*, and *Mx3* promoters was done, meanwhile, we proved that temperature modulation could influence the *Mx* promoters' activity and their transcript expression level. Thereby, our study provided evidence of how temperature modulation affects the IFN-regulated system for future antiviral response studies, which would be possible to develop a new strategy to overcome the impact of climate change on aquaculture.

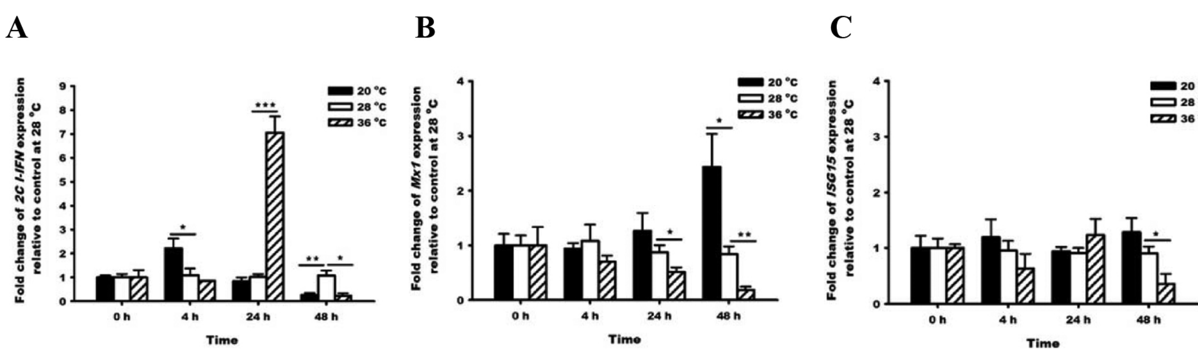

Figure 1. Expression of IFNs-regulated genes upon temperature modulation.

Expression of (A) *type I IFN* (*2C I-IFN*), (B) *Mx1* and (C) *ISG15* were measured by qPCR and normalized with *β-actin*. The expression at 28 °C at each time point was set as 1 for comparisons. Results represented the mean ± standard deviation (SD) (n=6). Values were compared using the t-test (*p < 0.05, **p < 0.01, and ***p < 0.001).

AQUACULTURE MIGHT INCREASE THE AQUATIC GREENHOUSE GASES CONCENTRATIONS, CASE STUDY OF *Litopenaeus vannamei*

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After the Industrial Revolution, the atmospheric greenhouse gases concentrations have increased as well as the human population. With growing population, demands for aquatic animal protein increases and this has greatly promoted the rapid expansion of global aquaculture industry. Previous studies have pointed out that the aquatic ecosystem is an important source of greenhouse gases. However, scarce researches have focused on the greenhouse gases emissions from the aquaculture. In addition, greenhouse gases emissions vary between different types of aquaculture ponds, species and pond management practices. In view of these, we investigated greenhouse gases concentrations of the monoculture *Litopenaeus vannamei* in semi-indoor and indoor concrete aquaculture ponds. During the farming period to harvest, aquatic environments have been monitored and greenhouse gases concentrations have been analyzed.

The results of pCO_2, CH_4 and N_2O concentrations during the whole farming period are listed in table 1.

Seawaters which had slight variations of greenhouse gases concentrations were pumped respectively from the aquaculture farm's adjacent sea area to the semi-indoor and indoor ponds as inflow water in the table 1. With increasing farming days, ascending trends of greenhouse gases concentrations in both the pond and outflow waters were observed in the semi-indoor ponds as well as indoor ones. In conclusion, monoculture *Litopenaeus vannamei* in both semi-indoor and indoor concrete ponds act as sources of greenhouse gases to the atmosphere.

Table 1

Greenhouse gases concentrations of the monoculture *Litopenaeus vannamei* in semi-indoor and indoor concrete ponds during the farming period.

		Semi-indoor			Indoor		
		pCO_2 (μatm)	CH_4 (nM)	N_2O (nM)	pCO_2 (μatm)	CH_4 (nM)	N_2O (nM)
Inflow water		1118.2±117.0	4.5±0.3	13.1±3.8	532.4±76.9	5.6±1.1	11.5±0.6
30 day	Pond water	536.7±13.3	3.8±0.4	17.3±3.9	606.9	3.5±0.2	10.7±0.1
	Outflow water	ND	ND	ND	654.7	4.3±0.1	10.8±0.1
120 day	Pond water	4945.9±454.4	3.6±0.5	52.1±9.0	3684.2	18.2±0.7	94.1±0.6
	Outflow water	3976.6±992.1	552.34±34.9	154.4±37.6	3477.7	1295.5±11.7	377.5±2.1

ND indicates no data.

Results were summarized as mean ± standard error.

THE PREBIOTIC, CACAO POD HUSK PECTIN AND PROBIOTIC, *Lactobacillus plantarum*, COMBINED AS SYMBIOTIC TO IMPROVE GROWTH PERFORMANCE AND IMMUNOCOMPETENCE OF *Litopenaeus vannamei*

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The cacao pod husk (CPH) produced as an agricultural waste by-product during the cacao bean processing. To reach the sustainable development goals on waste recycling, CPH was applied to extract CPH pectin for developing the potential for diverse application in aquaculture, minimizing CPH impact to the environment and bringing benefits to the agriculture and aquaculture industries. There are five treatments in this study including (1) basal diet (BD), (2) CPH pectin (5 g/kg diet, CPH), (3) *Lactobacillus plantarum* (10^{10} cfu/kg diet, LP10), and two synbiotic combinations of (4) CPH pectin at 5 g/kg diet + LP at 10^{7} cfu/kg diet (CPH+LP7) (5) CPH pectin at 5 g/kg diet + LP at 10^{10} cfu/kg diet (CPH+LP10). After the 56-day feeding trial, significantly elevated percent weight gain, percent length gains and feeding efficiency in *Litopenaeus vannamei* were only observed in CPH+LP7, and the remainder of the treatments remained consistently similar to the BD. The total haemocyte count, granular cells, phenoloxidase activity, and respiratory bursts of *L. vannamei* fed with CPH+LP7 and CPH+LP10 are significantly increases at 7–28 days, accompanied by significant promotion of phagocytic activity and clearance efficiency in response to *Vibrio alginolyticus* challenge during 56 days of feeding trial. Furthermore, at the end of the 56 days of feeding trial, shrimp receiving CPH pectin and/or LP treatments showed a significantly higher survival ratio against *V. alginolyticus* infection and hypothermal stress. It was therefore concluded that the synbiotic combination of CPH pectin and LP exhibited complementary and synergistic effects on growth performance and immunocompetence in *L. vannamei* within 56 days of feeding trial.

APPLICATION OF CHITOSAN-BASED 4-HYDROXYBENZOIC ACID HYDROGEL TO REMOVE AND DETECT AMMONIUM FOR WASTEWATER TREATMENT IN RECIRCULATING AQUACULTURE SYSTEM (RAS)

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For the adsorption development of a dissolved ammonium removal method in RAS, chitosan-based hydrogel (Cht-HG) modified with 0.3 mM of p-hydroxybenzoic acid (Cht-0.3PHBA-HG) was devised. An additional function of ammonium detection under Ultraviolet (UV) displaying fluorescence depends on ammonium concentration. Between control hydrogel, Cht-HG and Cht-0.3PHBA-HG were compared in the ammonium adsorption test, and the maximum adsorption capacities (q_m) of Cht-HG and Cht-0.3PHBA-HG were 11.6 mg g^{-1} and 19.9 mg g^{-1}. Cht-0.3PHBA-HG exhibited a relatively elevated adsorption capacity (i.e., 1.71-fold higher adsorption efficiency) due to the morphological and chemical modifications attributed to p-hydroxybenzoic acid (PHBA). The influence of the adsorbed ammonium into Cht-0.3PHBA-HG was investigated under UV light and exhibited a steady fluorescence quenching at 396.5 nm with the addition of increasingly higher ammonium concentrations. Therefore, Cht-0.3PHBA-HG could be used as an ammonium sensor. Furthermore, the correlation coefficient (R^2) was 0.951 for ammonium determination ranging from 0.1 to 5.0 mM, and the limit of detection (LOD) was 0.1 mM. For the practical application, Cht-HG and Cht-0.3PHBA-HG were assessed with an actual effluent including 2.24 mg L^{-1} ammonium concentration of a fish farm and the ammonium adsorption ratio were 33.0% and 60.1%, respectively. These results explain the modified hydrogel has the improved practical adsorption efficiency and sensing ability for ammonium.

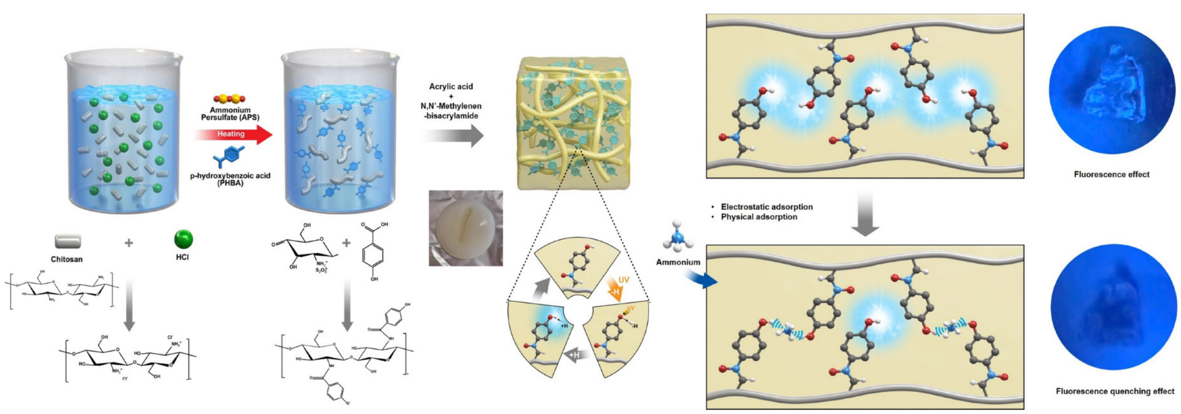

Fig. 1. Schematic diagram of chitosan-based 4-hydroxybenzoic acid hydrogel synthesis and fluorescence quenching effect depends on adsorbed ammonium concentration.

PROBIOTIC (*Lactobacillus rhamnosus*) PRETREATMENT SIGNIFICANTLY REDUCED MUSCLE CONCENTRATIONS OF FLORFENICOL AND WITHDRAWAL TIME IN ASIAN SEABASS (*Lates calcarifer*)

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Dietary supplement of probiotics as feed additive is becoming a common practice in aquaculture. While the majority of studies mainly focus on the beneficial effects of probiotics in aquaculture, few studies concern the “adverse effect” of probiotic supplement that may reduce the efficacy of the drug treatment. The study of the effect of probiotics on the pharmacokinetics of antibiotics may open a new and more complete overview of the advantageous use of probiotics in aquaculture.

Florfenicol (FF) was orally administered for 5 days at 10 mg/kg/day to 4 Asian seabass pre-treated with saline or *Lactobacillus rhamnosus* (2.5×10^7 CFU/fish/day) for 7 days at 25°C. The muscle/skin (in natural proportion) concentrations of FF, FF amine (FFA) and FF+FFA were found consistently about 1.5-2.5 folds lower in the probiotic-treated fish. (Table 1), suggesting that the probiotic treated fish had lower tissue levels of FF and metabolites. Whether or not the drug reduction was a result of reduced gut absorption of increased liver metabolism warrants further elucidation.

The tissue depletion curve of FF+FFA was used to build an estimation of the withdrawal time (WDT) following EMEA standard (with a 95% confidence, the time when the upper one-sided 95% tolerance limit is below the MRL). Under Taiwan governmental regulated dosages of FF at 10 mg/kg for 5 days and MRL of 1 ppm, the WDT in the probiotic-treated fish is 4 days, shortened by 2 days in comparison to the 6 days in the control group.

The 7-day probiotic supplements might beneficially shorten the WDT after FF treatment but might also undesirably reduce therapeutic efficacy by reducing drug concentrations in Asian seabass.

TABLE 1. Muscle depletion of FF (µg/mL) with or without probiotic pre-treatment. FF was given P.O. at 10 mg/kg/day for 5 days at 25°C.

Time *	Control group 10 mg/kg/day at 25°C		
	FF	FFA	FF+FFA
Day 1	2.89±1.75	0.29±0.11	3.05±1.69
Day 3	0.37±0.22	0.18±0.07	0.56±0.28
Day 5	0.12±0.04	0.10±0.06	0.22±0.07
Time *	Probiotic group 10 mg/kg/day at 25°C		
	FF	FFA	FF+FFA
Day 1	1.55±0.80	0.10±0.07	1.65±0.86
Day 3	0.29±0.13	0.08±0.03	0.37±0.16
Day 5	0.07±0.01	0.04±0.03	0.11±0.04

* Time indicates time after the last dose

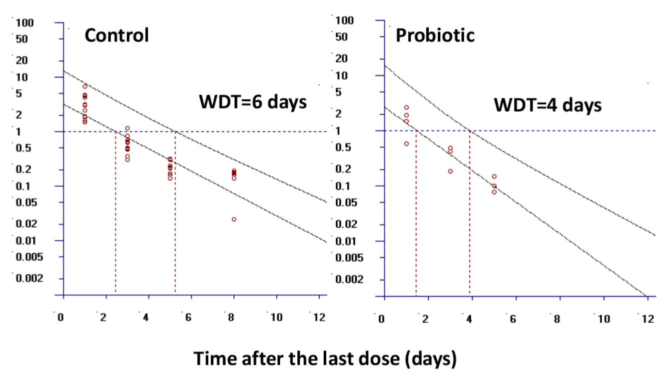

FIGURE 1. Comparison of FF withdrawal times (WDT) with or without probiotic pre-treatment. Tissue residue depletion of FF+FFA in the muscle/skin of Asian seabass following oral dosage (10 mg/kg/day once daily for 5 days (n=4).

EFFECTS OF GRADED LEVELS OF STANDARDIZED NATURAL CITRUS EXTRACTS IN FEED ON GROWTH AND SURVIVAL OF NILE TILAPIA *{Oreochromis niloticus)* BEFORE AND AFTER A CHALLENGE WITH *Aeromonas hydrophila*

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Citrus extracts are increasingly used in aquaculture production, due to the benefits they provide to animals. Such a process is driven by the market demand for natural products. However, depending on their origin, Citrus extract can vary a lot in terms of composition and concentration of active compounds. The present study was undertaken to study the effect of graded inclusion levels of a standardized natural citrus extract (SNCE) in the feed of Nile tilapia. In parallel, the SNCE has been characterized in order to identify its active compounds.

This study was performed at the University of Vale do Säo Francisco experimental station in Brazil. 380 juvenile Nile tilapias (mean initial weight: 12.8 ± 0.17 g) were randomly divided into 6 groups with 3 replicates. Each treatment was fed with a standard diet supplemented with SNCE at different doses described in Table 1 for 60 days.

Final weight was evaluated at the end of the experiment. statistical analysis was performed using ANOVA. Then, 30 fish per treatment were randomly selected and inoculated with *Aeromonas hydrophila* (0.2ml of a $1*10^{7}$ CFU/ml solution) and mortality was assessed 8 days post challenge. In parallel, 5 batches of SNCE have been analyzed using HPLC-UV-DAD-DEDL and HPLC-UV-MSMS analyzers to characterize the SNCE.

	T0	T1	T2	T3	T4	T5
SNCE inclusion rate (ppm)	0	200	400	800	1600	3200

Table 1:SNCE inclusion rate used in feed for each evaluated treatment.

Results show a positive effect of the increasing inclusion of NSAB on growth (Fig. 1).

Survival after the challenge with *A. hydrophila* was also positively influenced by the increased inclusion rate of the citrus extract (Fig. 2).

Regarding SNCE characterization, analysis performed on 5 batches didn't show any difference between the 5 DEDL profiles of the SNCE. Pectic oligosaccharides, eriocitrin, and hesperidin were identified as SNCE's major components.

Our results demonstrate the positive effects of the graded inclusion of a standardized natural citrus extract on zootechnical performances on juvenile Nile tilapias grown in unchallenging conditions. They also underline its interest when fish are challenged with a pathogenic bacteria. These results may be explained by the modulation of Nile tilapias' intestinal microbiota. In fact, some compounds of SNCE such as hesperidin and POS are well known for their beneficial effect on intestinal microbiota. More studies will be necessary to confirm these hypotheses.

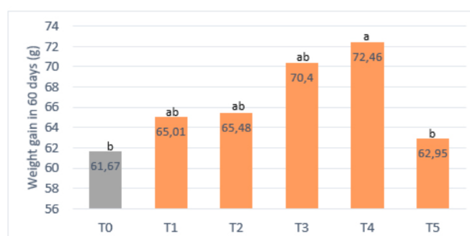

Figure 2: Average weight gain of Nile tilapia after 60 days of treatment

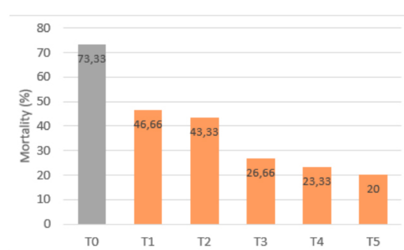

Figure 1: Effect of SNCE on Nile Tilapia after 8 days of bacterial challenge with A. hydrophila.

DIETARY SUPPLEMENTATION OF A MIXTURE OF SAPONIN-RICH PLANTS TO REDUCE AMMONIA-NITROGEN EXCRETION IN SHRIMP *Penaeus Vannamei*

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The intensification in aquaculture production leads to increasing attention on the management of ammonia from its waste. Natural solutions such as *Yucca schidigera* extracts have shown beneficial effects on shrimps [1]. However, *Y. schidigera* is collected from the wild and its slow development make it an endangered natural resource that tends to be an expensive solution [2]. Alternative solutions, economically and environmentally improved, appear to be necessary to manage ammoniac. The aim of this study was to evaluate the effect of a dietary supplementation of a mixture of saponin-rich plants consisting of four saponin plants (Norponin Opti®, Nor-Feed) on the ammonia-nitrogen excretion of shrimps (*Penaeus vannamei*).

This study was performed in an experimental station (Halieutica, France). 138 juvenile shrimps were randomly divided into 2 groups with 3 replicates each: control groups (CTL), fed with a standard diet and supplemented groups (OPTI) fed with the standard diet supplemented with 500 g/T of the commercial blend of saponin-rich plants during 30 days. Measurement of TAN (NH_4^+/NH_3) concentration in each group occurred once a day, 5 days a week using a colorimetric method assisted by a spectrophotometer. Individual performance measurements were made at the beginning and at the end of the experiment.

Results evidenced that OPTI group has a beneficial effect in reducing ammonia-nitrogen excretion during the whole experiment with an average difference of -13% and significantly ($p<0,05$) at week 3 of the experimentation with a difference of -20%. Weight gain during the experiment was not different significantly (CTL: 0.73 ± 0.15 g vs OPTI: 0.69 ± 0.15 g). Overall, the present study demonstrates that a supplementation with 500 ppm of the saponin-rich feed additive as positively impacting ammonia-nitrogen management.

References:
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[2] Arce-Montoya M, Hernández-González JA, Rodríguez-Álvarez M, et al. Plant Cell Tiss Organ Cult 2007; 88: 35–40.

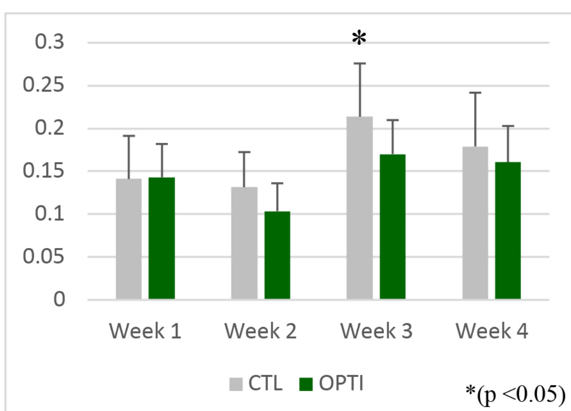

Figure 1: TAN (NH_4^+/NH_3) levels during the trial

AQUAPONICS AS A CROSS CURRICULAR TEACHING TOOL

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Aquaculture has, perhaps more than any other agriculture or academic content area, the potential for interdisciplinary and collaborative instruction. The multifaceted nature of aquaculture provides numerous opportunities and classroom activities to engage students in any number of subject matter areas. Students are confronted with complex problems that allow them to experiment and build their problem solving and cooperative learning skills. These problems help translate the academic principles; they are required to learn, into real-world applications. The hands-on nature of many of these activities helps hold student interest and provide ongoing motivation. Concepts learned can be applied in many other fields and can help better prepare students for higher education. Table 1 indicates other academic areas that relate directly or indirectly to aquaculture.

The connections to biology and chemistry are readily apparent but connections to other disciplines require more effort. Our role as Extension educators and content specialists is to help teachers make and explore these connections. This presentation describes methods to maximize the cross curricular capacity and effectiveness of an aquaculture teaching platform.

Biology	Chemistry	Physics
Math	Economics	Plumbing
Mechanical Systems	Construction	Sales
Marketing	Hydraulics	Language Arts
Business Planning	Finance	Home Economics
Food Sanitation & Safety	Nutrition	Physiology
Morphology	Fish Health	Fish Reproduction
Genetics	Art	History
Sociology	Carpentry	Masonry
Hydroponics	Computer Technology	Public Relations

DEVELOPMENT OF THE VIET UC *Penaeus monodon* BREEDING PROGRAM

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The Viet Uc Seafood Corporation commenced a breeding program for the black tiger prawn, *Penaeus monodon*, in Ca Mau Province, Vietnam in 2017. The intention of this program was to develop quality high-health domesticated and genetically-improved seed for the local *Penaeus monodon* farming industry from a Vietnamese-based program using Vietnamese genetics and supply the commercial needs of the local *Penaeus monodon* farming industries. CSIRO has played an advisory and supporting role in the program to date, initially by providing guidance on health management and husbandry of the domesticated lines, and more recently by advising on genetic management of the program.

The program commenced by evaluating the genetic diversity and similarity of two available wild stocks of Vietnamese *Penaeus monodon* using an existing genotyping tool, which ended in selection of candidates from Ca Mau coastal waters. Founders were introduced in three batches over a 12-month period and were screened repeatedly for a range of pathogens considered important for the Vietnamese *Penaeus monodon* farming industry and establishment of the program more generally. Rigorous pathogen screening continued, coupled with rigorous biosecurity measures, over the course of rearing the domesticated generations since. The program now operates three cycles per year, with between 50 and 100 families produced each generation, and with genetic links made between cycles to enable the development of a singular breeding population. The program has progressed well since establishment, with program families reaching their fifth generation by mid-2023. The program is well poised having progressed from the domestication to the genetic improvement phase, and with the focus now switched from establishing a high health domesticated genetic resource to enhancing performance gains to enable development of commercially competitive lines.

Here we discuss the development of the Viet Uc *Penaeus monodon* program and focus on the rationale behind the program's establishment; the general approaches taken to progress the program; and considering the outlook of the program to develop quality stocks suited to the Vietnamese farming environment and to support the sustainability of the Vietnamese industry.

LEARNINGS AND OPPORTUNITIES IN THE DEVELOPMENT OF FIT-FOR-PURPOSE DATA SYSTEMS FOR AQUACULTURE SELECTIVE BREEDING PROGRAMS

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Over more than a decade CSIRO have partnered with aquaculture industry in building breeding programs aimed at realising improved gains from selectively bred animals. The need to build computer systems to support selective breeding has been a key element of success for these partnerships. The approach has been to build computer systems to enable efficient data management and to empower the industry technical managers of the breeding programs to make good and timely breeding decisions. Data is core to selective breeding, the data system enables critical timelines to be met, provides risk mitigation strategies, it frees staff from routine and mundane data management tasks, and in effect defines standard operating protocols.

The processes and learnings from the design and development of databases for varying species has driven continuous improvement and necessitated new design features to stay apace with increasing sophistication. This presentation highlights several key observations pertinent to the development of data systems for finfish, prawn and oyster selective breeding programs and highlights differences and commonalities across each. Advanced breeding techniques, such as genomic selection, have required the implementation of processes for the quality control and storage of SNP data, and the practical challenges this presents are discussed. We also consider the benefits of incorporating breeding-specific functionality such as optimum contribution selection, mate allocation, inbreeding and gains trends and pedigree reporting into the data systems.

THE EBB AND FLOW OF CRITICAL WATER QUALITY PARAMETERS WITHIN A COMMERCIAL ABALONE/ULVA IMTA SYSTEM: EFFECTS OF INCREASED RECIRCULATION

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Several commercially operated land-based abalone farms in South Africa employ integrated aquaculture (IMTA) technology to grow the local abalone, *Haliotis midae*, together with the seaweed *Ulva lacinulata*. The studied farm routinely operates at 50% water recirculation, where ammonia removal by *Ulva* enables continuous recirculation. *Ulva* is also harvested and used as supplementary feed. The farm is divided into platforms, each containing four independent modular clusters, which consist of a single paddle raceway containing ca.1 ton Ulva (volume 300,000l) linked with ca. 10, 000 -15,000 kg of abalone within 42 tanks (volume of each tank 8,000l).

In this study a series of experiments examined system water quality parameters at standard farm operation (50% recirculation), increased recirculation (75%), and the effects of short-term 100% recirculation. The latter designed to test the potential to prevent Harmful Algal Bloom (HABs) intake during external environmental events. At 50% recirculation, TAN removal across the *Ulva* biofilters ranged from 65-85% and pH ranged from 7.8 (daytime) to 7.4 (night). Data suggested a strong positive linear relationship between TAN removal and TAN load to the biofilter, with %TAN removal higher during the day. No significant differences in temperature, pH, TAN or FAN were observed between the 50% and 75% recirculation clusters. At 100% recirculation, temperature was consistently 1°C higher, and pH was around 0.5 unit higher. TAN and FAN increased rapidly at 100% recirculation, with TAN values ranging from 0.3-0.8 mg l^{-1} compared with values less than 0.1mg l^{-1} at 50%. Oxygen levels were not considered problematic at any stage in the experiments and increase at 100% recirculation. It is clear from the data that this system could feasibly run for extended periods at 75% recirculation, with relatively little effect on water quality compared with 50% recirculation.

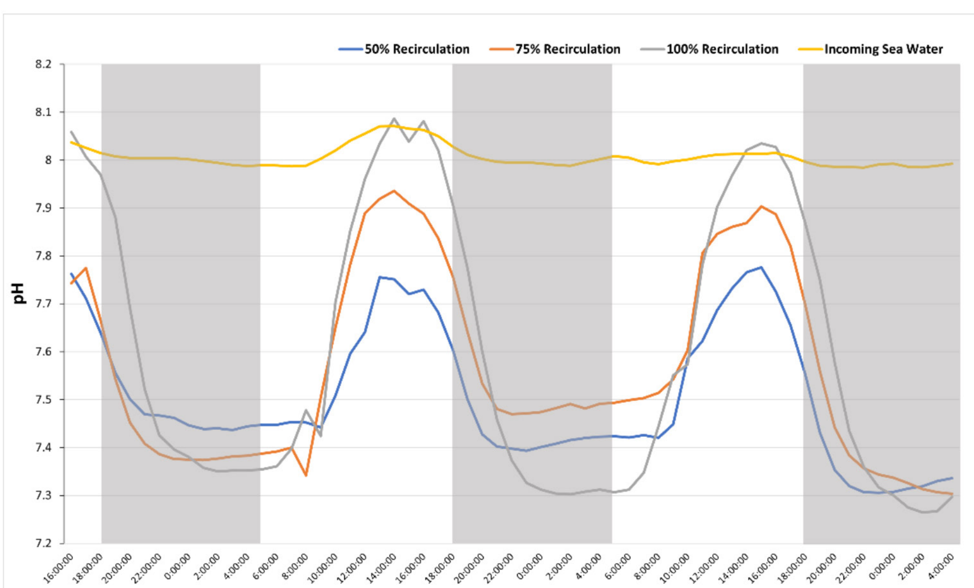

Figure 1: pH fluctuations due to varying recirculation rates within a commercial IMTA system integrating Abalone and the seaweed Ulva

EFFICACY OF NOVACQPRO™ BACTERIAL BIOMASS AS A BIOACIVE COMPONENT OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* FEEDS

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Good shrimp growth is a primary production consideration for good economic returns. Hence, there is considerable interest in developing diets that promote growth and survival. A number of bioactive feed components are supplemented in shrimp feed to improve feed intake and growth. NovacqPro™ is a high ash low protein marine microbial biomass proven to improve *Litopenaeus vannamei* growth. The present study evaluated the effects of supplementing NovacqPro™ on growth, feed intake, and digestibility of juvenile *L.vannamei*. Six isonitrogenous (36% crude protein) and isolipidic (6% crude lipid) diets were prepared to include graded levels (0-20% of the diet) of Novacq™. Diets were used in two growth trials, where 15 juvenile *L.vannamei* (0.27±0.01g and 0.58±0.02g, respectively) were size sorted and stocked in an indoors recirculating aquaculture system. Each diet was randomly assigned to five replicate tanks. In the second trial, two additional treatments consisting of providing the basal and the 10% Novacq diets in excess (+15% of the standard ration), were added. Additionally, feed intake was assessed by offering shrimp one gram of "as is" feed, then siphoning leftovers after 30 minutes to calculate feed consumption. Feed intake was also assessed via passive acoustic monitoring of shrimp feeding behavior. Afterwards, digestibility of NovacqPro™ was evaluated by offering shrimp diets containing 5% and 10% of the product with 1% titanium oxide as inert marker. Results of the present study suggest that NovacqPro™ can be supplemented at up to 20% of shrimp diets without significant effects on growth, survival, and FCR (*p>0.05*) (Table 1). However, when offered in excess, diets containing 10% NovacqPro™ resulted in better growth, and FCR. Acoustics data support the growth results. NovacqPro™ as bioactive feed component promotes shrimp growth, and helps imrpove production and increase profit.

Table 1. Growth performance of *L. vannamei* (average initial individual weight 0.58 ± 0.02g) offered diets containing increasing levels of Novacq for 42 days (n=5).

Diet	Final biomass (g)	Final average individual weight (g)	Weight gain (%)	FCR	Survival (%)
Basal	86.03^{b}	7.26	1206.20	1.63	80.00
2.5% Novacq	100.80^{ab}	8.03	1290.14	1.45	84.00
5% Novacq	111.59^{a}	8.21	1292.55	1.42	90.67
7.5% Novacq	109.60^{a}	7.88	1286.67	1.49	93.33
10% Novacq	107.45^{a}	8.57	1362.37	1.34	84.00
20% Novacq	86.64^{b}	7.75	1235.98	1.54	76.00
PSE*	5.21	0.34	60.66	0.07	5.47
p value	0.003	0.1742	0.5696	0.0990	0.2613

*Pooled standard error.

PASSIVE ACOUSTIC MONITORING AS A TOOL TO ASSESS FEED RESPONSE AND GROWTH OF SHRIMP IN PONDS AND RESEARCH SYSTEMS

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It is well understood that shrimp are opportunistic grazers that eat a wide variety of organisms. Yet diets with low or no inclusion of fish meal are often regarded as of lower quality because the farmer does not smell fish and the color is not dark. In most production situations, the farmer must rely on qualitative judgement of feed intake based on visual observations of such factors as gut fill or feed disappearance from feed trays. Similarly, in the laboratory we have relied on traditional methods of observation. These are highly subjective measures which are not always easily interpreted. With the development of passive acoustic monitoring (PAM) we are finding a quantitative measure to help us better understand the response of shrimp to various diet types as well as ingredient matrixes. We are currently working to identify facts that influence the acoustic signal as well as confirming the efficacy of PAM in the laboratory. We will update you on our findings for various pellet sizes, hardness and use of attractants. Such technologies we feel will be able to provide more unbiassed data to address issues of consumption and use of attractants to improve feed formulations.

At the farm level, with the advent and adoption of automated feeding systems we are re-learning feed management and finding traditional feed management is highly restrictive. As we move further towards technological solutions to feed management the use of passive acoustic technologies have become more prevalent. Over the past 7 years we have moved our research system from traditional feed management relying on 120-day production cycle to automatic feeder systems allowing for a 90-day production cycle while producing higher biomasses and larger shrimp. This presentation will summarize or work using acoustic as a laboratory tool to better understand the factors effecting feed intake as well as our pond production work transitioning to passive acoustic feeding and evaluation of the technology in the field.

THE EFFECTS OF DIFFERENT SOYBEAN PROTEIN SOURCES ON GROWTH PERFORMANCE, FEED UTILIZATION EFFICIENCY, INTESTINE HISTOLOGY, AND GENE EXPRESSION OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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The goal of this study was to evaluate the impacts of one animal-based diet and four soybean meal sources at different replacement which were the products of solvent-extracted soybean meal with low oligosaccharide (SBM-LO) content, soy protein concentrate (SPC), fermented (FerSBM), and expeller-pressed (EPSBM) process on the growth performance, feed utilization efficiency, intestine histology, and gene expression. The trial was conducted in a green water recirculation system with the stocking density at 30 inds/ tank, at the initial weight of 0.42 ± 0.01 g. Shrimps were fed in eight weeks using four replicate tanks, with eight experimental diets including SBM-LO, SPC, FerSBM at 50% and 100% protein replacement, expeller-pressed soybean meal from at 100% protein replacement, animal diet with 50% fishmeal and 50% meal as protein source, and one basal diet as reference. Trial results revealed significant differences amongst treatments, especially between animal-based and plant-based diets for all growth parameters (*p<0.05*) except for survival rate (*p>0.05*). With respect to feed utilization efficiency, we observed interesting trends with significant higher in phosphorus retention for the BrightDay diets. Concerning histomorphology and gene expression, no statistical changes were observed (*p>0.05*). Based on these findings, more research on different processed soybean sources needs to be done to optimize the diets matrix, diversify the ingredients source for better animal growth performance and optimize profits for practical applications.

Growth Parameters	Basal[a]	SBM-LO 50%[b]	SBM-LO 100%[b]	SPC 50%[b]	SPC 100%[a]	FerSBM 50%[b]	FerSBM 100%[b]	EPSBM 100%[b]	Animal[b]	*P-value*
Survival Rate (%)	92.22	95.83	99.17	95.83	93.33	100.00	95.83	99.17	95.83	0.065
Final Weight (g)	20.88[a]	20.95[a]	20.26[abc]	20.53[ab]	18.36[de]	19.43[bcd]	19.24[cd]	18.06[e]	19.11[cde]	<0.001
Weekly Gain (g/ week)	2.56[a]	2.57[a]	2.48[abc]	2.51[ab]	2.24[de]	2.38[bcd]	2.35[cd]	2.20[e]	2.34[cde]	<0.001
Feed Conversion Ratio	1.10[bcd]	1.05[d]	1.05[d]	1.07[cd]	1.24[a]	1.09[cd]	1.15[abc]	1.18[ab]	1.16[abc]	<0.001
Apparent Net Protein Retention (%)	56.80[a]	56.30[a]	54.18[ab]	55.30[ab]	45.81[c]	53.54[ab]	52.56[ab]	51.90[ab]	50.27[bc]	<0.001

DEVELOPMENT OF A SEX-SPECIFIC MOLECULAR MARKER IN NSW MULLOWAY *Argyrosomus japonicus*

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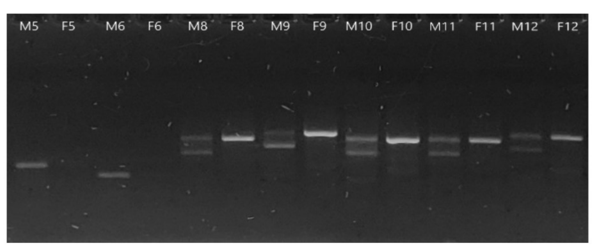
The mulloway (*Argyrosomus japonicus*) is an iconic Australian gamefish species, known as the 'silver ghost', prized for being one of the largest fish that you can land from the beach. They grow to 75kg and almost 2m in length. In NSW, mulloway stocks have collapsed. As a result, this species is the focus of restocking efforts. Mulloways are sexually monomorphic externally; lacking distinguishing features present in other fish species. Sex can typically only be determined by lethal sampling or gonadal biopsy via gonopore cannulation. Gonadal biopsy is only possible on fish in the advanced stages of gametogenesis shortly before or during the reproductive season and can injure the fish. Sexual maturation takes 2–3 years in captivity, so non-lethal sexual identification can only be performed after this period. In this study we report the development of molecular markers for the purpose of determining sex in mulloway non-invasively. This marker is based on a 95bp indel in the dmrt1 gene only present in males. This marker was validated on known sex mulloway caught for restocking programs in NSW.

Fish sampled were NSWDPI broodfish of known sex, currently used for restocking. DNA was extracted from finclips of 6 females and 6 males and sent for Illumina sequencing. The resulting dataset had an average read depth of 40x for each fish. Each sequence was aligned to a published mulloway genome reference (ASM1571009v1). Both the aligned male and female sequences were next searched for known sex-associated genes; these gene sequences were inspected for differences between sexes. The most prominent differences were used to create oligonucleotide primers. These primers were tested on known sex individuals and the products of the most robust pair were sequenced (Figure 1).

After sequencing, areas of heterogeneity identified in the products were used to develop a second round of primers. This second round of primers were tested in all possible combinations and the most effective 'sex marker' pair were determined.

These markers were validated on 31 mature, known sex, mulloway. In all instances (100%), sex markers matched observed sex. These markers are based on the promotor region of the dmrt1 gene and target a 95bp deletion in the male sequence. This deletion is most likely located on the y sex chromosome as males are heterozygous.

These markers enable sex to be determined for sub-adult or non-spawning mulloway. They have applications for managing the sex ratio of mulloway broodstock and can be utilised to answer biological questions such as natural sex ratio, habitat usage and dispersal of wild mulloway. Additionally, the techniques demonstrated here present a pipeline for the development of an effective tool for developing sex markers for other sexually monomorphic species.



(Figure 1: 1.5% agarose gel showing differing DNA PCR products patterns between females and males.)

ANIMAL WELFARE: THE LINK BETWEEN HARVEST STRESS, QUALITY AND VALUE CHAIN PERFORMANCE

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In the aquaculture industry, understanding any potentially real or perceived animal welfare risks is becoming increasingly important. Within animal welfare definitions, the freedom from stress or suffering when slaughtered will most likely be applied to fish in order to secure animal welfare as part of any current or future animal protection laws. In farm environments, the impacts of stress have been well documented in terms of growth, reproductive physiology, and the immunocompetence of fish in farm environments.

Despite being very important factors in a successful aquaculture operation, the aforementioned are production focussed, and it is important to remember that the final product of any aquaculture industry is not only a fish but a food item with appearance, flavour, texture and storage characteristics that compete with other items of food. Even though the production of a farmed fish can take up to 20,000 hours, the flesh and product quality attributes of the fish can be significantly impacted upon by high levels of harvest stress in the last hour.

With both the levels and types of stress varying during the harvest of farmed fish, we examined the effects of different types of harvest stress. During this presentation, we will be presenting the effects of stress on the quality attributes of farmed Australian Southern bluefin tuna in Japan and farmed barramundi in Singapore.

ANALYSING MICROPLASTICS IN WATERS AND SEDIMENTS AROUND FARMS IN URBANIZED AREAS

Huajuan Mo, Su Ting Ng, Nur Shoofiyah, Cheong Yan Ong, Nur Sara, Shumin Yu, Boon Seng Kong, Niranjala Perera, Raymond Ong, Wei Chern Foong, Umid Joshi, Guillaume Drillet*

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The capabilities to monitor for the presence of microplastics in aquaculture in view of evaluating sources and protecting the industry from potential negative impacts reside in the implementation of methods which can be implemented at large scale. Comparability of results across studies is a necessity but is proven is difficult because different methods are used globally, and the focus of published studies often differ.

We used a combination of FTIR-ATR and microFTIR to evaluate the presence of microplastic potentially impacting aquaculture productions. To ensure comparability of our results with other published studies we made use of the freeware siMPle which is implemented in large number of organizations worldwide and allows to speed up the time spent for image analysis. We validated the development of our methods and include an evaluation of the recovery rates of microplastics through the process of density separation using NaCl and polytungstate and oxidation using H2O2 in view of preparing samples for microFTIR analysis.

FISH FEEDING OPTIMIZATION IN AQUAPONICS USING A REINFORCEMENT LEARNING APPROACH INFORMED BY MODEL PREDICTIVE CONTROL

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Fish feeding optimization contributes to the balance of controlling fish growth and ensuring water quality in aquaponic system. The fish feeding control aims to minimize the gap between the target and actual individual fish weight at harvest time while minimizing feeding amount. The aim of our research is to develop a new fish feeding control strategy in aquaponic system based on reinforcement learning (RL) technology informed by model predictive control (MPC). We employ the fundamental RL based feeding control method using Q learning to solve feeding optimization problem. A fish feeding control method based on RL informed by MPC is proposed. As shown in figure 1, it integrates the feeding control decision of MPC into RL. In this process, MPC is firstly applied on the known aquaponic system model to obtain feeding strategy. Then, the obtained feeding control trajectory is considered as the background information of aquaponic system to determine initial Q table. The proposed methods are validated by simulated aquaponic system model. Different feeding control methods are compared to show performances with respect to final individual fish weight and feed conversion ratio (FCR). The simulation results show that the proposed feeding control method based on RL informed by MPC can make effective feeding decisions in terms of optimised fish growth, water quality of fish and plant tanks. The introduction of background knowledge improves the performances of fundamental RL based feeding control method, which would decrease 7.19% in FCR and improve 41.89% in final individual fish weight. Our work offers a simple way to solve feeding optimization method based on RL. The proposed approach can be applied to the feeding decision optimization in aquaponic site based on the specific production data collected.

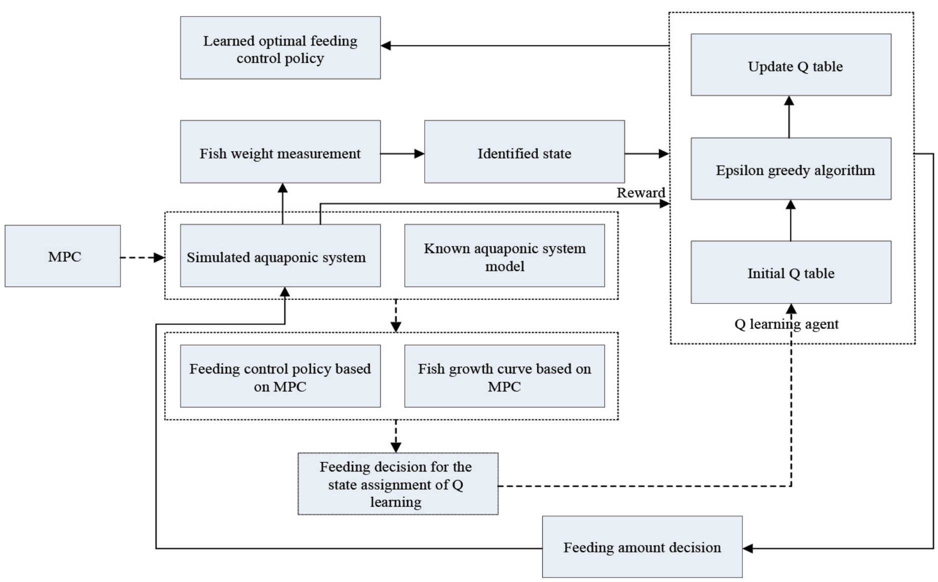

Fig. 1 The framework of fish feeding control method based on RL informed by MPC in aquaponic system

THE 2023 TASMANIAN SALMON PLAN: PROCESS AND REGULATORY IMPEMENTATION PRIORITIES

Ian Dutton*, Lynn Albert, John Adams, Eric Brain and Laura Fatovich

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The Tasmanian salmon industry is Tasmania's largest primary industry and makes a considerable contribution to the seafood sector in Tasmania and nationally. The industry operates under a diverse array of laws, regulations and strategies and involves businesses which operate from local to global scales. Tasmania's primary industries, including salmon, are managed under Tasmania's Resource Management and Planning System, which is based on the principles of sustainable development.

In September 2021, the Tasmanian Government embarked on a process to develop a new long-term salmon plan, building on the strong foundation and demonstrable improvements in practices and regulation achieved through the 2017 Industry Growth Plan for the Salmon Industry. That process involved extensive public and industry consultation that was undertaken in conjunction with the Blue Economy Cooperative Research Centre. More than 1200 submissions were received on the Discussion paper and Draft Plan, reflecting significant public interest in the future of aquaculture in Tasmania.

The 2023 Tasmanian Salmon Industry Plan (the Plan) was launched on May 1, 2023. The Plan is an enduring strategic framework to support an innovative and sustainable salmon industry in Tasmania, which meets expectations of the community. It guides the Government's long-term priorities for the salmon industry – being responsive to the changing needs of salmon farmers, supporting businesses and the wider community.

The Plan has four inter-related priority outcomes: Priority outcome 1: Sustainable industry Priority outcome 2: Healthy ecosystems Priority outcome 3: Prosperous communities Priority outcome 4: Contemporary governance There are 21 strategic pathways, aligned to the priority outcomes, that represent activity areas for delivery in the near, medium and longer term.

This presentation outlines the process under which the plan was developed and describes key implementation priorities that are relevant to other aquaculture regulators.

THE 2023 TASMANIAN SALMON PLAN: PROCESS AND REGULATORY IMPEMENTATION PRIORITIES

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ADVANCES IN LAND-BASED CORAL AQUACULTURE FOR REEF RESTORATION AND THE AQUARIUM TRADE

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Coral aquaculture is a rapidly growing sector for restoration initiatives and the marine aquarium trade. Until recently the global aquarium trade has relied heavily on wild collection and export of live coral from nations surrounded by tropical coral reefs. However, the fishery has come under increased scrutiny as concerns rise for coral reefs under current and projected climate scenarios, frequent bleaching events, and severe weather events. Unreliable collection data has also contributed to rising uncertainty over the suitability and sustainability of current catch quotas for coral across Australian tropical reefs.

As government backed initiatives to better manage, restore and rehabilitate tropical coral reefs gain momentum, there are increasing opportunities for coral fishery operators to adapt and collaborate on large-scale coral aquaculture programs.

Monsoon Aquatics (MA) has been scaling up land-based coral aquaculture via asexual propagation ('fragging'), and recently through coral spawning, to meet the demand of the aquarium trade, but also to increase capacity for future large-scale reef rehabilitation initiatives. MA has successfully implemented a fragging program that produces > 2000 corals frags weekly of 60 different species (Fig. 1). Since the start of the coral spawning program in 2022, MA has successfully spawned 6 species (Table 1), including a world-first in captive spawning and settlement of the 'scoly', *Homophyllia australis* (Fig. 2), from gravid wild-caught stock. This has given MA key insight into spawning activity, larval rearing, settlement and growout of these coral species leading to a scale-up of current land-based coral spawning operations. MA has identified key challenges to address for future work:

Broodstock conditioning and spawning induction:
- Feeds, feeding and nutrition.
- Conditions to trigger gametogenesis and gamete release.
- Predicting spawning times

Maximising settlement success:
- Settlement substrates
- Optimal physico-chemical conditions
- Triggering and controlling settlement

Increasing survival and growth of spat
- Pest and predator control
- Feeds, Feeding and nutrition.

Table 1: List of coral species spawned at Monsoon Aquatics

Common Name	Species	Spawning mode	Spawning	Settlement	Grown to saleable size
Acan	*Micromussa lordhowensis*	Broadcast	2021,2022	✓	TBD
Acro	*Acropora tenuis*	Broadcast	2022	✓	TBD
Scoly	*Homophyllia australis*	Broadcast	2021,2022	✓	✓
Torch	*Euphyllia glabrescens*	Brooder	2020-2023	✓	✓
Trachy	*Trachyphyllia geoffroyi*	Broadcast	2021	×	×
Hammer	*E. parancora*	Brooder	2020-2022	✓	✓

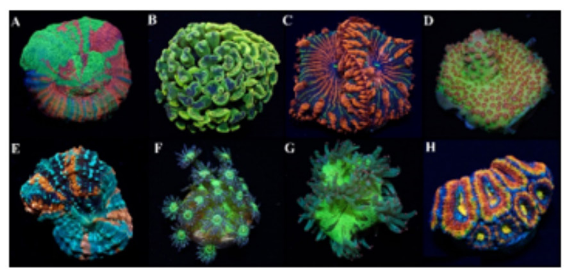

Figure 1: A selection of coral frags routinely produced at Monsoon Aquatics. **(A):** 'Scoly' – *H. australis*, **(B)**: 'Hammer' – *E. glabrescens*, **(C)**: 'Blasto' - *Blastomuussa vivida*, **(D)**: 'Acro' – *A. microclados* **(E)**: 'Deshi'- *Acanthophyllia deshayesiana*, **(F)**: 'Goni'- *Goniopora* sp. **(G)**: 'Elegance coral' – *Catalaphyllia jardinei* **(H)** 'Acan' - *M. lordhowensis*

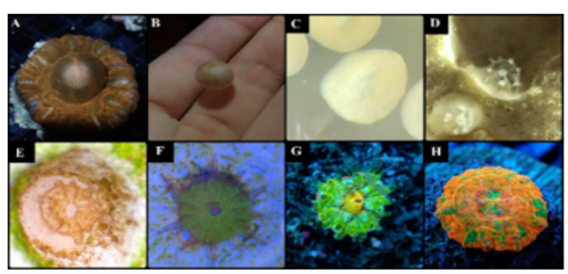

Figure 2: Progression from spawning to 1 year of growth in the Scoly *Homophyllia australis* **(A)**: Adult Scoly preparing to release a gamete bundle, **(B)**: Gamete bundle prior to dissociating, **(C)**: 'prawn chip' stage of egg development, **(D)** newly settled spat, **(E)** spat with newly acquired zooxanthellae, **(F)** developing pigment, **(G)** developing adult form, **(H)** 1yr old spat showing adult form and colouration.

INTENSIVE CULTURE OF REDCLAW CRAYFISH *Cherax quadricarinatus* USING VERTICAL FARMING TECHNOLOGIES

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Freshwater crayfish are in increasing global demand. Global markets are large (US$10 billion) however demand consistently exceeds supply. The Australian freshwater redclaw crayfish (*Cherax quadricarinatus*), endemic to northern parts of Queensland and Northern Territory, are considered an outstanding candidate for major industry development due to its rapid growth, ease of culture, and tolerance to a range of environmental factors. Despite the great potential and market demand widely recognised for redclaw crayfish, the development of large-scale production of redclaw has been limited by a lack of established and proven intensive production technologies. Traditional pond-based methods result in unpredictable yields primarily due to adverse environmental conditions, inbreeding, predation, cannibalism, large size variations and escapees. To date the Australian industry average is 3-4 tonne per hectare, falling well short of market demand.

Vertical farming technologies provide efficient use of space, increased yield/hectare, environmental control, year-round production, and can be established anywhere. It was proposed that redclaw could be well suited to vertical farming overcoming the issues associated with traditional production methods. Commercial crab fattening boxes from Thailand were used in this 4 year trial. A total of 1000 individual boxes were arranged in systems comprising of 6 boxes horizontally and 8 or 9 boxes high, each box holding a single crayfish (unless breeding). All systems were RAS with zero water exchange and a total footprint less than 300m^2. Breeding and production of eggs was successful year round with 2nd and 3rd generation females producing significantly more eggs per spawning. All breeding crayfish were microchipped enabling full traceability and the establishment of a selective breeding program. There were no mortalities due to cannibalism in the individual culture and growth rates showed significantly less variation. The outcomes of this four-year trial prove that redclaw crayfish are well suited to vertical culture for breeding and growout with a potential yield of 16 tonne/hectare/yr. Vertical farming technologies will enable the redclaw industry to become one of the most sustainable and market competitive aquaculture industries globally.

Table 1: Example figures of production metrics for vertical redclaw farming.

Operations summary				
Floor space required (m^2)	770	1550	2000	4000
Number of crayfish	50,000	100,000	200,000	400,000
Cycle p.a.	2	2	2	2
Annual production in Kg (75g adult, 80% survival rate)	12,000	24,000	48,000	96,000
Price per Kg of adult redclaw (AUD domestic sales only)	$25	$25	$25	$25
Sales p.a. (AUD)	$300,000	$600,000	$1,200,000	$2,400,000

INNOVATIONS, OPPORTUNITIES AND CHALLENGES WITH THE INTENSIFICATION OF Penaeid SHRIMP CULTURE

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The intensification of shrimp culture in many regions of the global shrimp farming industry has been predominately driven by consecutive incidences of global disease outbreaks, which have caused enormous economic loss, and steered a growing segment of the shrimp farming industry towards production systems with higher biosecurity and operating control to mitigate disease risks. Superintensive systems provide a means to achieve a high level of control over the inputs and outputs of shrimp culture and thus can minimise the entry and spread of diseases. However, successful super-intensive shrimp production is reliant on an advanced understanding of many important biological and economic parameters in the farming system, coupled with effective monitoring, to maintain optimal production.

Compared to traditional extensive or semi-intensive systems, super-intensive systems require higher inputs of feed, energy, labour, and supplements. These systems are highly sensitive to the interactions between these different inputs and require that the biological and economical parameters of farming are carefully balanced to ensure success. More than 12 different superintensive farming systems have now developed to accommodate local conditions and farming preferences, ranging from clearwater, RAS, semi-biofloc and biofloc systems.

In this presentation we discuss the innovations, opportunities and challenges relating to intensification of Penaeid culture, working from a recent review article covering this topic involving the presenting authors. Fundamental principles that enable super-intensive systems to operate effectively are presented, with a focus on achieving a balance in the system. We review the role of water quality management to ensure the health and stability of the system itself and the growth and immunocompetence of the shrimp. The importance of advancing nutritional knowledge and tools to support consistent and efficient production of shrimp in these high-cost super-intensive systems is discussed. The paper also outlines the role of breeding for developing lines suited for these challenging production systems. Keys issues of animal welfare and social license pertinent to super-intensive shrimp farming are also discussed. Understanding synergies between the keys areas of production systems, nutrition, breeding, and welfare are crucial for super-intensive farming as all areas coalesce to influence the health of shrimp and commercial farming success.

RECENT ADVANCES ON AQUAPONICS RESEARCH AT CSIRO: CHALLENGES AND FUTURE PERSPECTIVES

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Aquaponics production is emerging as an alternative for aquaculture diversification, and as 'bio-converter' tool to enhance the circularity in Agri-Aqua food systems. Aquaponics allows growers to save water, maximise production from their farm footprint and make productive use of nutrient-rich effluents. As a result of these benefits, commercial aquaponics farms are growing in a rate of 17%/year worldwide. However, high construction and running costs (e.g. labor, aquafeeds, electricity) are one of the main constraints for further development. The proper nutrient ratio and feed loads will dictate the size of the 'aquaculture component', heavily impacting the construction costs. The business profitability is also linked to establishing a market for the produce and species selection, as well as system optimization.

In 2021 CSIRO started applied research in Aquaponics with the aim of generating knowledge to help the industry grow and scale. The key focuses so far have been (i) creation of a R&D foundation and new capabilities, and (ii) establish connection, understanding and dialague with the industry. To date, three experimental trials have been conducted with barramundi (*Lates calcarifer)* and Jade perch (*Scortum barcoo*) juveniles, exploring the nursery phase (~10 to 30g as initial weights, over ~8 weeks). We evaluated the fish performance in aquaponics (AQP) versus conventional recirculating aquaculture systems (RAS); and the plant performance (butterhead lettuce *Lactuca sativa*, as an initial biological model) in aquaponics versus conventional hydroponics (HYD). We also explored different nutrient ratios (e.g. 1 to 3g grams of fish feed/ /plant/day) and key mineral supplementation, and their impact on the incidence of visual symptoms of plant nutrient deficiencies. The system design in RAS consisted of a fish tank, clarifier and biological filter. In AQP, similar design was applied plus a floating bed hydroponic units. In HYD, floating bed and reservoir (same fish tank) were utilised. The results demonstrated (fish and plant) performance differences according to systems, feeding ratios and mineral supplementation. We also conducted an industry survey aiming to understand the barriers of commercial aquaponics in Australia. In addition, during the past 18 months, several industry engagements were carried-out aiming to better understand the field constraints and potential needs for R&D.

Looking towards the future, a pilot-scale Aquaponics facility is being built at CSIRO and will enable research at scale, allowing us to produce and collect "real-world" data to feed into economic-sustainabilily models & Decision Support Tools.

A META-ANALYSIS ON THE GROWTH PROMOTING EFFECT OF FEEDING NOVAQPRO™ TO *P. vannamei* AND *P. monodon*

Ricardo G Esquerra[1], Matt Briggs, Camila Marinelli Martins, Cedric Simon and Artur Rombenso

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Introduction: Novacq™ is a microbial biomass ingredient with a unique blend of bioactive molecules that is produced via a novel fermentation technology developed by Commonwealth Science and Industrial Research Organisation, Australia (CSIRO) and licensed to Ridley. Prawns and shrimp fed with Novacq^{TM} grow on average faster and are healthier. Significant improvements in the production methodologies for Novacq™ have led to the development of NovaqPro™, a version of Novacq™ with greater biopotency. To estimate the biopotency of NovaqPro™ when fed to *Penaeus vannamei* and *P. monodon*, a meta-analysis was carried out using data from 5 and 2 trials, respectively. Trials consisted of 2 to 23 treatments of 4 replicates each and treatments differed amongst each other by having 10% of NovaqPro™ coming from different production batches, and appropriate negative controls without NovaqPro™. The basal diets were the same within each trial. All *P. vannamei* trials were conducted at Andaman Aquatic Research Centre (AARC), Thailand, and *P. monodon* trials were carried out by CSIRO in its Bribie Island site in Queensland, Australia. The experimental layout was similar across trials which used juvenile animals with about 0.5g initial weight, and were fed experimental feeds for 6 weeks keeping the animals under standard site practices. When describing the results of the trials, NovaqPro™ biopotency is measured as the percentage difference in weight gain between each replicate and its relevant control group. This dataset yielded 236 and 156 independent datapoints for *P. vannamei* and *P. monodon*, respectively.

Statistical methods: The inverse variance method was used to combine results of biopotency and estimate pooled effects of all treatments in both *P. vannamei* and *P. monodon* populations. **Heterogeneity test:** I-square test was used to verify the heterogeneity between treatments. As heterogeneity was statistically significant, random effects model was used. **Bias test:** Egger test was used to verify that the results did not have direction or bias. **Plotting results:** Forest, Funnel and Baujat Plots allowed for the visualization of all treatments included in the study, their corresponding standard error, the heterogeneity and variability of each treatment, and its contribution to the final result verifying the absence of bias.

Results: The biopotency findings for these trials can be interpreted as the capacity of NocaqPro™ to improve, on average, weight gain for *P. vannamei* or *P. monodon* fed at 10% in a commercial-type feed, under the standard experimental protocol described herein. Results are summarized in the Tables below.

Conclusions: Feeding NovaqPro™ to *P. vannamei* and *P. monodon* at 10% in a commercial-type feed in trials significantly improved weight gain, on average, by 51.64 and 39.31%, respectively. The large number of data point comparisons (236 and 156 for *P. vannamei* and *P. monodon*, respectively) provides a high degree of robustness to the current analysis.

Penaeus vannamei			**Biopotency**
Meta-analysis	Pooled effect		51.64%
	Confidence interval 95%	Lower	48.63%
		Upper	54.65%
Test of heterogeneity			I^2 = 96.8%; p<0.001
Test of bias			P=0.949

Penaeus monodon			**Biopotency**
Meta-analysis	Pooled effect		39.31%
	Confidence interval 95%	Lower	32.98%
		Upper	45.64%
Test of heterogeneity			I^2 = 82%; p<0.001
Test of bias			p=0.1846

No bias was detected among treatments in either animal species.

IN VITRO CULTURE OF SENEGALESE SOLE SPERMATOGONIA: CAN GROWTH FACTORS BE USED FOR SPERMATOGONIA PROLIFERATION?

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Spermatogonia are increasingly used in biotechnological innovation related to transplantation in endangered or commercially cultured fish species and to cryopreserve genetic material. In some species, spermatogonia enrichment and *in vitro* cultivation are crucial steps to obtain enough of these germ cells. Senegalese sole (*Solea senegalensis*) is a flatfish species farmed in the South of Europe with a high market price, but that presents a reproductive disorder which does not allow to close the live cycle in captivity, making this species propitious to carry out spermatogonial biotechnological applications. While a protocol using strainers has been recently implemented to enrich Senegalese sole spermatogonia, the parameters required for the *in vitro* culture of spermatogonia are still unknown for this species. In this context, the aim of this study was to determine the growth factors needed for the *in vitro* cultivation of Senegalese sole spermatogonia.

For this purpose, testes from juvenile fish (~1 year old; 35-50 g) were pooled (3 pools; 12 testes per pool) and processed for spermatogonia (SPG) enrichment. Briefly, testes were cut into 1 mm^3 fragments, dissociated using a mechanical and enzymatic treatment, and passed through a 100 µm-strainer to remove fragments. Cells present in the flowthrough were passed sequentially through two 5 µm-strainers to enrich cell suspension in SPG (> 5 µm). Cells were seeded in a 6-well gelatine-coated plate at 2x05 cells/well density in triplicates for each experimental group. Experimental groups were: control (without growth factors), GF (a cocktail of recombinant murine growth factors: GDNF, IGF-1, and bFGF) and S (Senegalese sole serum). Cells were maintained at 18 ºC and the number of spermatogonia was determined at 24- and 48-hours post-seeding (hps) from 6 images taken using an inverted microscope equipped with phase contrast.

While the number of SPG was significantly higher in GF (40 ± 24 cells; $P = 0.03$) than in Control (14 ± 7 cells) at 24-hps, the number of SPG in S (27 ± 13 cells) was not different from Control or GF. No differences were observed among the experimental groups at 48-hps. Interestingly, the number of SPG increased in all groups from 24 to 48-hps (Control: 97 ± 45; GF: 103 ± 45; S: 92 ± 39). This suggests that the presence of growth factors in the media is important until 24-hps but not at 48-hps. However, the use of growth factors, including serum, boosted spermatogonia proliferation faster than the control group after 24 hours of SPG culture. The initial seeding density seems to be very important for SPG proliferation. More studies are needed to validate these conditions and test other growth factors to finally describe a protocol for spermatogonia cultivation in Senegalese sole.

This study was funded by Portuguese national funds (FCT) through projects GERMROS (EXPL/CVT-CVT/0305/2021), UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020, and by European funds through project BREEDFLAT (PT-INNOVATION-0080-EEA grants). Contract 2020.04181.CEECIND was awarded to E. Fatsini. European Union's Horizon 2020 research and innovation programme under grant agreement No. 871108 (AQUAEXCEL3.0).

ASSESSING THE BIOSECURITY RISK OF IMPORTED UNCOOKED, WHOLE AND HEAD-ON EVISCERATED, BARRAMUNDI AND NON-SALMONID FINFISH IN RELATION TO EXOTIC VIRUSES

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Australian barramundi (*Lates calcarifer*) farming industry presently enjoys freedom from numerous internationally significant diseases, including all of the Megalocytivirus genus of iridoviruses that are known to be causing severe impacts on finfish species globally. Australia currently allows the importation of uncooked, farmed and wild caught, non-salmonid finfish products for human consumption from several countries, as whole, and head-on eviscerated products. The importation of barramundi and other finfish products for human consumption had not been fully assessed as a risk pathway for disease incursion prior to this project. It was hypothesized that importation of uncooked, whole and head-on eviscerated non-salmonid finfish products may contain significant exotic viruses of concern, such as red sea bream iridovirus (RSIV), infectious spleen and kidney necrosis virus (ISKNV), scale drop disease virus (SDDV), Singapore grouper iridovirus (SGIV), or turbot reddish body iridovirus (TRBIV). These viruses are considered exotic to Australia and could present a significant biosecurity and disease threat, due to their highly contagious and pathogenic nature, if infection were to occur within an aquaculture or naïve wild fishery setting. It was also hypothesized that imported whole and head-on eviscerated uncooked non-salmonid finfish products may contain a substantial volume of high-risk processing wastes, which could make their way into waterways, therefore facilitating an incursion pathway for exotic diseases.

119 imported, uncooked, non-salmonid finfish products were sampled from seafood markets, seafood retailers and supermarkets across Australia, between June and October 2020. Tissues sub-sampled for diagnostic testing included gill, liver, and haematopoietic organs (spleen and kidney) on whole fish, and remnant tissue from above organs, from head-on eviscerated fish products. Samples were tested for SDDV, ISKNV (including the genotypes ISKNV, RSIV and TRBIV) and SGIV using qPCR. Confirmatory re-testing was performed on any samples that tested positive or inconclusive using the original nucleic acid extract and an additional nucleic acid sample prepared from the tissue homogenate by Australian Centre for Disease Preparedness (ACDP) Fish Diseases Laboratory. Sampled finfish products were assessed in relation to their compliance to import conditions. Results will be presented along with recommendations that were provided to help bolster Australia's aquaculture industry from exotic disease risks.

The project was funded by the Australian Barramundi Farming Association (ABFA) and Fisheries Research and Development Corporation (FRDC).

POSITIVE PRAWN PRODUCTION THROUGH INVESTIGATION OF EMERGING CAUSES OF DISEASE IN A COMMERCIAL AUSTRALIAN BLACK TIGER PRAWN (*Penaus monodon*) FARM

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In late 2018, a commercial Australian black tiger prawn (*Penaeus monodon*) farm suffered unprecedented mortality events within the hatchery and subsequently earthen pond grow-out production. Complete loss of over half of its hatchery tank populations and grow-out pond populations occurred, with total cumulative mortality in excess of 200 million animals. Investigations performed during the mortality events failed to identify a definitive infectious cause. A project was developed from the need to better understand the range of potential trigger(s) and determining factors that led to the mortality events in both the hatchery and grow-out stages of production. Next Generation Sequencing (NGS) were employed to investigate potential emerging pathogens and corresponding qPCR were developed for the three selected bacterial toxin genes; YaFO, RtX, and zon occludens (also referred to as ZOT). Further testing of archived hatchery post larval samples was conducted to determine the potential significance of the identified toxin genes. A prospective trial was conducted using three commercial grow-out ponds to allow for multidisciplinary diagnostic testing of samples that were purposively collected every 2-3 days to be performed (including microscopy, necropsy, histopathology, and molecular biology), should mass mortality re-occur in these ponds. Passive water sampling devices were also deployed in one of the prospective trial ponds to explore the possible involvement of agri-chemicals pollutants in the mortality events. Finally, an epidemiological analysis of hatchery and grow-out farm (stocking and mortality records) as well as test data was performed to identify potential risk factors associated with mortality events encountered during the 2018/19 commercial production. A better understanding the possible cause(s) of the mass mortality event is likely to be of value to all farms across the Australian prawn farming industry, which may encounter similar events in the future.

The presence or apparent quantity of the YaFO, RtX, and zon occludens toxin genes was not found to be significantly associated with the hatchery or grow-out stage mortalities. No other infectious organism (i.e. virus, bacteria, fungi or parasite) could be identified as a likely cause of the mortality events either in the hatchery or grow-out stage of production. None of the three prospective trial ponds experienced the mass mortalities equivalent to the previous season on the farm. Retrospective epidemiological analysis identified a range of risk factors, associated with the mortality events in the hatchery and grow-out, which helped the farm implement system changes for the following season. Further project findings will be presented.

The project was funded by the Australian Prawn Farming Association (APFA) and Fisheries Research and Development Corporation (FRDC).

COMPREHENSIVE ANALYSIS OF CIRCULAR RNA EXPRESSION AND THEIR REGULATORY NETWORK IN NILE TILAPIA MUSCLE

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Circular RNAs (circRNAs) are functional (mostly) non-coding transcripts produced by head-to-tail splicing in multicellular organisms. They participate in various biological processes by acting as miRNA sponges, RNA-binding protein regulators and protein translation templates. In recent years, There is mounting evidence that circRNAs modulate muscle growth in farmed animals but the regulatory mechanisms of circRNAs in fish are still poorly understood. Thus, this study aimed to investigate the expression of circRNAs in Nile tilapia and explore their regulatory mechanism through a multi-omics approach. We compared the circRNA, mRNA and miRNA transcriptomes in fast muscle collected from fast- and slow-growing Nile tilapia (*Oreochromis niloticus*) from the third generation of our in-house domestication program.

A total of 1947 mRNAs, 9 microRNAs (miRNAs), and 4 circRNAs were differentially expressed between fast- and slow-growing fish. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses showed that the differentially expressed mRNAs are related to muscle fibre development, striated muscle cell development, and spliceosome. Further, we focused on a novel circRNA (circMef2c) produced from *myocyte-specific enhancer factor 2C-like*, and verified its expression and back splice junction using reverse transcription quantitative PCR and Sanger sequencing, respectively. As circRNAs participate in biological regulation through competing endogenous RNA (ceRNA), we identified the miRNA targets of circMef2c. *In silico* prediction showed that circMef2c could bind to 3 miRNAs, and these miRNAs could target 65 mRNAs. Among the genes in this ceRNA network, circMef2c-oni-miR-34-*igfbp2*, circMef2c-oni-miR-130b-5p-*myod1*, and circMef2c-oni-miR-202-*fgf14* are likely to play a key role in muscle growth. Our results provide a comprehensive overview of a novel ceRNA regulatory network in Nile tilapia muscle. In addition to deepening our knowledge of muscle growth in teleosts, they pave the way for exploring novel growth biomarkers in farmed fish.

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Reference:

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INLAND SALINE AQUACULTURE IN NSW: 10 YEARS OF RESEARCH & DEVELOPMENT

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Investigation into opportunities for the use of inland saline groundwater for aquaculture in NSW started in the late 1990's. A comprehensive inventory of NSW inland saline groundwater resources was completed, including ephemeral and permanent lakes, saline water from coal seam gas mining and sub-surface drainage schemes where saline groundwater was pumped to protect the root zone of crops. Research to identify suitable sites, species and production systems was followed by commercialisation plans and identification of investment opportunities.

NSW DPI and industry partner, Murray Irrigation Limited (MIL), constructed an aquaculture R&D facility on the bank of a 2000 ha evaporation basin within the Wakool Tullakool Sub-surface Drainage Scheme. The WTSSDS included 54 pumps initially producing ~35 ML/d of saline water keeping the groundwater 1m below the surface to protect the root zone.

Initially, the chemistry of the saline groundwater, which is different to coastal seawater, was measured and cost-effective methods of ameliorating deficiencies were developed allowing evaluation of species including marine fish (Australian snapper and mulloway), freshwater fish (rainbow trout and silver perch), marine prawns (black tiger and Japanese tiger) and Sydney rock oysters. Mulloway and rainbow trout were selected for long-term "commercial-scale" grow-out studies in ponds to provide data over annual production cycles and to highlight potential problems associated with grow-out in a semi-arid, inland environment. Market-size fish were sold into the local community to gauge market acceptance.

The culture of rainbow trout in raceways was identified as having the best opportunity for industry development. Commercialisation of this activity was started and consortium including an agribusiness R&D group (Lonsec), commercial trout farmers (Aquatic Solutions Australia), saline groundwater managers MIL and the Australian Seafood CRC. A business plan was developed and funding approved for a commercial demonstration farm at WTSSDS.

Advanced modelling showed that provided 20 ML/d of saline groundwater could be guaranteed, the farm could produce 200t trout/year and would be a showcase for inland saline trout farming, anticipating the technology would be transferred and adopted at other inland saline sites. Unfortunately, an ongoing drought through the mid-late 2000's meant that irrigation of crops in the inland areas of NSW largely ceased. The saline groundwater table receded and precluded the need to pump groundwater into the WTSSDS. The volume of saline groundwater at the WTSSDS decreased from an average of ~ 35 ML/d in "normal" years to 4-5 ML/d in "drought" years. Water security could not be guaranteed by MIL and the project did not proceed.

A large collection of data, including inland saline groundwater resources, suitable species for inland saline aquaculture and economic analyses for development of commercial aquaculture using inland saline groundwater was produced by NSW DPI and is available for new enterprises looking to invest in inland saline aquaculture.

SUSTAINABLE TROPICAL LOBSTER AQUACULTURE – OVERVIEW OF THE RESEARCH FOCUS

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Closed cycle spiny lobster propagation has long been considered a unattainable holy grail of aquaculture principally due to difficulties in culturing lobsters through their long and complex larval development cycle. However, research at the University of Tasmania (UTAS) has developed reliable commercial processes for the larval culture of spiny lobster (*Panulirus ornatus*). This breakthrough in hatchery production has paved the way for the development sustainable commercial lobster aquaculture in Australia. The research team at UTAS has now partnered with Ornatas Pty Ltd to develop tools for sustainable juvenile lobster culture and to establish the world's first onshore spiny lobster aquaculture facility.

Spiny lobsters are cultured in other regions of the world using wild seedstock and on-growing in seacages utilizing wild caught feeds (trash fish). This is not viable in Australia due to the unavailability of wild harvested seedstock and strict environmental regulations. Instead, sustainable juvenile culture of lobsters is best achieved in onshore systems where culture parameters can be better controlled, and environmental impacts limited. To resolve the challenges of onshore culture, UTAS and Ornatas have joined forces in a multi-partnered Australian Research Council project, the Industrial Transformation Research Hub for Sustainable Onshore Lobster Aquaculture. Key research foci to facilitate immediate commercialisation of *P. ornatus* includes: the development of a sustainable manufactured feed and systems to reduce the impacts of cannibalism. Other areas of research focus include: the development of systems for improved seedstock quality and transport, and a understanding of the social, environmental, and economic impacts of a developing industry.

For a sustainable lobster industry to flourish, a manufactured feed is needed. The feed must have a suitable nutritional profile, be water stable, palatable, have a low FCR, use economical, sustainable ingredients and be applicable to commercial production. From an experimental perspective many of these criteria have been achieved with a focus now on the commercialisation phase. The second key focus of research is the establishment of systems to prevent cannibalism, especially in juveniles. Cannibalism generally occurs on moulting animals, they are readily attacked and consumed by conspecifics. Intensive video work and behavioural studies are being conducted to gain insight into ways that cannibalism can be mitigated in a commercial setting. The outcome of this work will facilitate the development of new and novel culture techniques for this and other antagonistic crustacean species.

THERMAL PHYSIOLOGY OF TROPICAL ROCK LOBSTER (*Panulirus ornatus*); DEFINING PHYSOLOGICAL CONSTRAINTS TO HIGH TEMPERATURE

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This study investigated the effect of chronic temperature acclimation (24, 26, 28, 30, and 32 °C) on growth, aerobic metabolism, feeding, and gut evacuation rate of early juvenile spiny lobster, *Panulirus ornatus* (initial weight = 0.48 ± 0. 03 g). Groups of 7 juvenile lobsters were communally cultured (n=4) for 45 days post-acclimation and solely fed on a formulated feed. Lobster specific growth rate (SGR), apparent feed intake (AFI), and specific feed consumption (SFC) displayed unimodal response to temperature peaking at 28.0, 27.9, and 26.2 °C, respectively. Similarly, survival had a significant quadratic relationship with temperature; however, it displayed an inverse relationship to SGR, AFI, and SFC where the lowest survival was found at 29.1 °C. Continuous exponential increases in lobster standard, routine and maximum metabolic rates, and aerobic scope were observed up to a maximum non-lethal temperature of 32 °C. Gastrointestinal evacuation rate (GIER) (>96%) increased minimally between 24 to 30 °C from 2.5 h to 2.9 h and substantially rose to 5.1 h at 32 °C. While the feed conversion ratio improved with increasing temperature, the temperature optimum for metabolic feeding efficiency closely matched specific growth rate and apparent feed intake. These findings show that optimum temperature for growth performance of juvenile *P. ornatus* does not align with maximum aerobic scope, rather growth was limited by prolonged GIER (up to 5.1 h) at high temperatures (32 °C) which constrained food consumption. These findings contribute to the growing body of literature debating the universality of aerobic scope to define physiological thermal boundaries of aquatic ectotherms suggesting thermal performance of lobsters under sub-lethal temperatures to be regulated by feed intake and driven by digestive processes such as GIER. Future research should focus on lobster feed formulation and its influence on digestive processes such as GIER, foregut evacuation, and digestibility which may influence the thermal tolerance of spiny lobsters in culture.

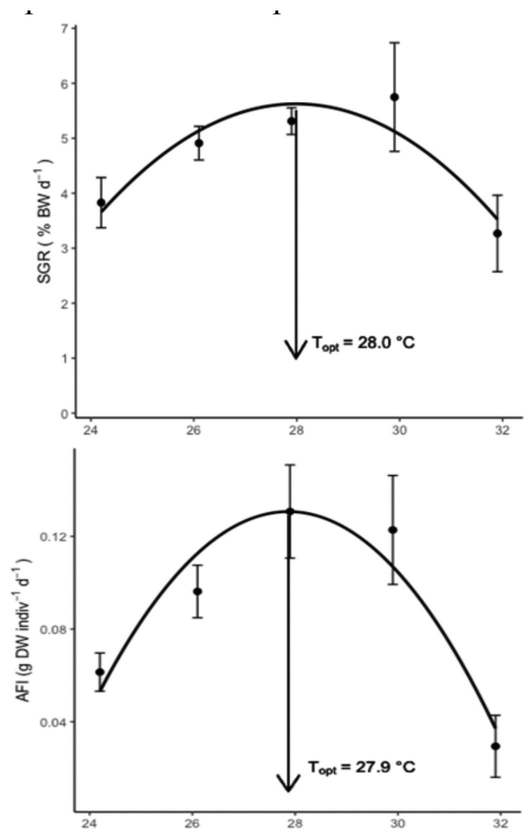

Figure 1. Temperature dependant specific growth rate (SGR) and apparent feed intake of *P. Ornatus* juveniles.

INLAND SALINE AQUACULTURE – USA AND ISRAEL PERSPECTIVES

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Inland saline culture in Arizona and Israel share many characteristics. Most of the interest and production has occurred in the driest and hottest portions of each country. Saline aquifers occur in these regions from either the slow accumulation of water leaching salts from the rare rain events or large-scale irrigation, or from geothermal activity dissolving underground salt deposits. The differences in ionic balances derived from these processes have a critical impact on water and feed management for inland saline aquaculture. In Arizona, most of the inland saline aquaculture efforts have been with shrimp and barramundi (*Lates calcarifer*) culture. However, farming of tilapia in saline water has been the most successful commercially. In 2005 three shrimp commercial-scale farms were operating in southwestern Arizona. PL's were imported from Florida or Latin America and reared in three separate locations. One farm was able to use groundwater without any feed adjustments or water treatment. Another farm used a special formula mineral premix to deal with an unbalanced ionic ratio in the water. The third farm used potash additions as a water treatment method to balance ionic concentrations. In all cases, the goal was to balance ions to more closely resemble dilute seawater. By 2023, one farm ceased operation, one was converted first to a successful tilapia farm for 15 years and in the last couple of years was converted to a very successful barramundi farm. The third farm has struggled with a mix of partners, marketing plans and intermittent production and in 2023 was not operating. The barramundi farm, and a cluster of smaller tilapia farms, appears to be the future path for inland culture in Arizona. In Alabama, one shrimp farm has been operating for many years with inland saline waters. Similarly in Texas, one shrimp farm has been in fairly continuous production.

In Israel, inland saline aquaculture has followed a similar pattern of expansion, some contraction, and some diversification. Use of saline aquifers for extensive culture of euryhaline species including tilapia, carp, flathead mullet (*Mugil cephalus*) and to a lesser extent; European seabass, red drum barramundi, white leg shrimp (*Litopenaeus vannamei*) and gilthead sea bream (*Sparus aurata*) have operated for many years. Most of these operations use extensive methods such as earthen or cement ponds with paddle wheels for aeration but some have been using intensive methods (RAS) with variable degrees of success. The largest RAS facility in Israel can produce around 1000 tons of barramundi per year. Total production of fish for human consumption in Israel is at about 20,000 tons per year which represents about 10% of the fish consumption in the country. Focus on species of lesser economic value (such as carp and tilapia) has caused a steady deterioration in the success of these operations which are currently on their brink of closure. Small-scale production of euryhaline and marine ornamental fishes has expanded in recent years using intensive recirculating aquaculture systems. A few RAS hatcheries produce fingerlings for local production and export. Current research is focused on intensification and development of more cost-effective diets, acclimation of high value marine species to saline water, aquaculture wastewater treatment, trials to utilize in-pond raceways and split pond designs, larger scale in-door RAS, and high salinity broodstock and hatchery for ocean spawners are planned or underway.

TILAPIA PRODUCTION AND MARKETS – A GLOBAL PERSPECTIVE

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It was a banner year for tilapia production and consumption in 2022. The minor exception seems to have been China, where the continued Covid restrictions for most of the year hurt production from farms, sales in stores and consumption in restaurants. Otherwise, the global covid recovery has seen bounce backs in production and consumption in most countries. The industry has noted a significant increase in feed prices globally, mostly due to commodity shortages in grains and oilseeds related to Russia's invasion of Ukraine, along with the overall global inflation rate.

However, global inflation has a silver lining for the tilapia market. As one of the lower cost protein sources in many countries, tilapia demand increased considerably. As the pandemic abated, the major importing countries reported increasing grocery sales of fresh and frozen fillets to be prepared for home consumption. High fuel costs also led to considerable price increases for most wild caught seafood, leaving even more space for tilapia (and some of the other farmed white fish) to gain market share. The unraveling of supply chain bottlenecks for refrigerated containers and port facilities was especially helpful for tilapia as so much of the product is transported internationally.

Most tilapia consumption still occurs in the domestic markets of producing countries. Often, tilapia farmers and their neighbors are the single biggest consumers. This floor of demand held up as producers were able to increase production to supply increases in international demand.

Another bright spot has been the increase in positive press for tilapia. As consumers search for lower cost protein and especially healthy seafood, many writers are suggesting tilapia as a high quality and safe product. The false claims filling the internet from years past seem to have finally been replaced by truthful and accurate information.

Brazil and Bangladesh have probably been the brightest spots for increased tilapia production (and consumption) in 2022. Correspondents from Brazil have reported 2022 production exceeded 550,000 mt. and Bangladesh reported 370,000 mt. In both countries their domestic demand and export markets increased. Vietnam also expects to exceed 300,000 mt. in production, with most staying in domestic markets. Egypt and Indonesia also expect to see moderate increases in production and demand. Besides the slowdown in China, Ecuador was reported to have reduced tilapia production, switching some production from tilapia back to more lucrative shrimp farming. While US consumption has increased in 2022, domestic production has been static. One of the largest US farms shifted production to barramundi, with the production barely replaced with increases from other farms.

Overall, the best estimates suggest that global production increased in 2022 to 6.9 million mt. 2023 is looking positive and should see increasing US demand along with international production and demand. Prices will increase due to overall inflationary pressure, but much less than wild-caught seafoods.

F3: ACCELERATING THE RACE TO REPLACE THE FISH IN FISH FEED

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Fishmeal (FM) and fish oil (FO) have historically been the ingredients of choice to provide nutritional advantages in aquaculture feeds. While these ingredients represent efficient nutritional packages, the uncertain future of forage fish stocks means that feed formulators need more options in their pantry to adapt to supply chain disruptions and ensure global food security. To address the growing concern about the availability of FMFO in the future, Future of Fish Feed (F3) was formed as a collaborative effort between NGOs, researchers, and private partnerships to accelerate the commercialization of innovative, substitute aquaculture feed ingredients to replace wild-caught forage fishes.

F3 focuses on three areas:

- F3 Challenge, a series of industry contests to develop and sell fish-free feeds and fish oil replacements within the aquaculture sector.
- F3 meetings and webinars to facilitate networking and collaboration between ingredient suppliers, feed companies, farmers, and investors.
- Feed Innovation Network (FIN) to support the innovation and widespread adoption of fish-free feed ingredients by providing experimental protocols, open formulas, and connections to testing facilities and ingredient providers.

Through these major areas of focus, the F3 team has brought together the aquafeed industry to address the challenges facing the sector and provide opportunities for alternative ingredient providers and fish farmers to connect. Industry feedback on the technical difficulty of developing fish oil substitutes and replacing FMFO in carnivorous fish diets led to the F3 Fish Oil and Carnivore Challenges. F3 meetings brought together emerging alternative ingredient suppliers, investors, and some of the world's largest feed manufacturers to spark new ideas to facilitate FMFO replacement among members of the aquaculture industry. The Feed Innovation Network was launched in response to requests for a repository of alternative ingredient information. F3 feed research trials continue to address knowledge gaps in fish-free ingredient research. Since marine fishes and shrimps claim a disproportionately high value in the seafood market and include some of the biggest users of FMFO, these species are priorities for F3 research.

F3 continues to evolve as the needs of the aquafeed sector change and new opportunities for alternative ingredients arise. New ingredients are coming online every day, but the race to replace fishmeal and fish oil will only advance through collaboration across the seafood industry. The responsiveness of industry will be a key factor in its ability to feed the growing population and protect the world's oceans.

DEVELOPING FLEXIBLE DECISION SUPPORT TOOLS FOR AQUACULTURE BREEDING PROGRAMS

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Strong applied breeding programs rely on carefully balancing the management of genetic diversity with maximising genetic gain. Multiple approaches exist to optimise different decision-making needs within a breeding program, which are often either compelling in theory, but challenging to apply, or are not designed with the specific needs of aquaculture breeding and species-specific reproductive biology in mind.

Based on real-world experience delivering industry-scale aquaculture breeding programs, we highlight the importance of developing efficient and flexible decision support tools to select and direct individual-level crossing of genetically superior broodstock, and to electronically capture data generated during spawning activities. This presentation demonstrates the value of using fast and efficient Optimum Contribution Selection and Simulated Annealing approaches in aquaculture breeding programs. We give particular emphasis on the needs of various aquaculture species and provide examples of the specific biological and practical challenges encountered in *Salmo salar, Penaeus vannamei, Penaeus monodon* and *Pangasianodon hypophthalmus* breeding programs during the development of the CSIRO Dating Service software. We also highlight the types of user-controlled parameters that provide practitioners the ability to adapt to the dynamic needs of aquaculture breeding programs when preparing broodstock candidate lists and mate plans. Finally, we demonstrate how accurate and efficient data capture can be used to dynamically adjust subsequent mating plans, to ensure the overall genetic gain and diversity levels remain balanced to achieve a program's strategic goals.

ARTIFICIAL MOONLIGHT INDUCES SPAWNING IN THE HONEYCOMB GROUPER *Epinephelus merra*, A LUNAR-SYNCHRONIZED SPAWNER

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Lunar-synchronized spawning is a rhythmic event observed in several marine animals, including corals and fish inhabiting temperate and tropical water. In the case of Epinephelinae, fish migrate from the habitual site before the species-specific moon phase and form aggregation at the spawning site. However, it remains unknown how they perceive cues from the moon for initiating gonadal development and subsequent spawning. We hypothesized that fish use moonlight to synchronize reproductive events.

Using the honeycomb grouper *Epinephelus merra*, a tropical Serranidae, spawns around the time of the full moon, we demonstrate the successful induction of spawning in this species by controlling light condition at night. Following three months of acclimatization under long days at 28°C, mature fish were reared under an artificial full moon or new moon conditions at night.

Released eggs during the experiment were collected and quantified. In the result of that many eggs laid by the fish in the full moon group, but not by the new moon group, at 3 weeks after moonlight treatment (Table 1). Moreover, fish in the full moon group had a high gonadosomatic index and abundant yolk-laden oocytes in the ovary (Fig. 1). Therefore, our study demonstrated that the moonlight triggers gonadal development and subsequent spawning in lunar-synchronized grouper with a full-moon preference.

TABLE 1. The presence of spawning through the experiment

Experimental phases	Rearing condition	Weeks after artificial moonlight			
		1	2	3	4
Acclimatization	New moon	N.D.	-	-	+
First month	Full moon	++	+	+++	++
	New moon	-	+	+	+
Second month	Full moon	-	-	+++	-
	New moon	-	-	+	-

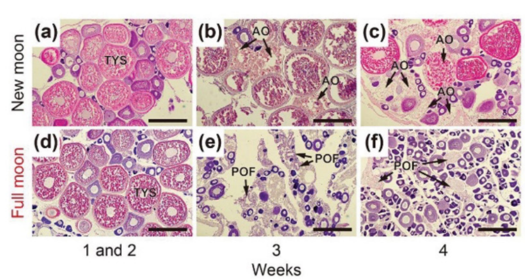

FIGURE 1. The effect of artificial moonlight on the oocyte stage composition

POTENTIAL USE OF SMALL-SCALE INSECT FARMING FOR AQUACULTURE FEEDING WITH SUSTAINABILITY ASPECTS

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Of key importance in the search of alternative sources of proteins seem to be new systems of agricultural production, orientated towards mass production of protein with the use of such organisms as insects, which are able to use nutrients from agricultural by-products. Bioconversion of agricultural waste with the use of insects may supplement the future demand for protein, but such residues are still an untapped food resource. Due to the lack of technical and legal support, the current mass insect production systems are poorly developed, hence insect protein is marketed mainly as an ingredient in animal feeds. Among the insect products with an especially high potential to be used for nutritional purposes in terms of the economic efficiency of producing high value food and feed products, it is worth mentioning the black solder fly larvae (*Hermetia illucens*).

The edible insect industry is strategic to ensure a constant supply of feed components for aquaculture. However, due to the fact that insects are a protein component in fish nutrition, fluctuations in demand and supply, correlated with this industry, may be susceptible and thus may directly affect the price of this substrate. Insect protein reached its record price level, i.e. USD 2,300, in 2015. These fluctuations relate to components from *H. illucens*. It is also important that the insect production market is constantly growing, and economists forecast an increase to about 1.200 million tons in 2025. Despite this, in aquaculture it is necessary to ensure constant access to this feed component, shorten supply chains and use by-products, which would result in meeting the goals of sustainable development.

The purpose of these R&D studies is to present the possibility of using small-scale *H. illucens* cultures for use in fish farms. The research assumes the implementation of sustainable up-cycling of agricultural residues: modular cascading waste conversion system into *H. illucens* larvae as a feed component. It is predicted that the main use of this type of insect raw materials in food systems will be the reduction of amounts and the recycling of waste biomass from food production and consumption, followed by its auxiliary application, as a protein source for animals. The technological system for the production of *H. illucens* dry larvae was divided into 4 stages. Stage 1 was the setting up of the larval culture in boxes/trays and the preparation of the substrate, Stage 2 was larvae feeding, Stage 3 was the collection and hygienisation of the larvae and Stage 4 was the drying up and storing the product in the freezer. The planned concept of the technological process was designed to make use of various side streams from the agricultural and food industry. The presented assumptions may be important for the eco-friendliness of fish farming and may be economically justified due to the additional stream of feed components available directly in the aquaculture farm.

Acknowledgement. Project financially supported by a research project entitled "Sustainable up-cycling of agricultural residues: modular cascading waste conversion system"; research grant agreement No. FACCE SURPLUS/III/UpWaste/02/2020, research is supported by the National (Polish) Centre for Research and Development (NCBiR) (Project FACCE SURPLUS/III/UpWaste/02/2020).

IMPROVED PERFORMANCE OF TROPICAL MARINE FISH *Epinephelus fuscoguttatus* THROUGH THE APPLICATION OF A HEALTH PROMOTING ADDITIVE INTENDED FOR THE PREVENTION OF ECTOPARASITE INFESTATIONS

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Ectoparasites are one of the most important bottlenecks for sustainable aquaculture production of tropical marine finfish species. External parasitic infestations are commonly associated with secondary bacterial infections, and this multifactorial pathogen combination constrains survival, growth performance and profitability. Health promoting additives are commonly used in commercial feeds as preventive strategies to reduce the severity of ectoparasite infestations.

This research investigated the effect of an essential-oil based health additive (Apex®Prefend, Adisseo) on growth performance and on prevalence and infestation intensity of ectoparasites in brown marble grouper (*Epinephelus fuscoguttatus*). The trial was conducted in an earthen pond farm located the North of Vietnam (Hanoi province). The additive was supplemented in feed at 0% (control), 0.3% (medium-dose) and 0.7% (high-dose) during the first 3 months of grow-out. The experiment was designed with three replicates per treatment group. Each replicate was in a net pen within the same earthen pond. Four ectoparasite species, including *Trichodina* sp., *Caligus* sp., *Zeylanicobdella* sp., and *Dactylogyrus* sp., were naturally detected in fish.

Results showed a relatively high survival for all treatment groups, ranging between 91.6-92.9%. However, the additive effects were detected in growth performance. The medium and high inclusions of Apex®Prefend improved daily weight wain (DWG) by 10% and by 14%, respectively. Likewise, the feed conversion ratio (FCR) was reduced by 7% and 10% by the medium and high inclusions, respectively. The prevalence and infection intensity were significantly reduced by 80% and 70% respectively in the medium-dose additive group, and by 70% and 60% in the high-dose additive group.

This study confirms under real field conditions that the essential oil-based functional additive Apex ®Prefend is an effective strategy to support grouper fish in dealing with ectoparasite infestations and therefore, to improve growth performance and health.

PREVENTIVE STRATEGIES BASED ON HEALTH PROMOTING ADDITIVES TO COPE WITH MULTIPLE INFECTIONS IN SHRIMP FARMING

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Shrimp production is currently limited by emerging infectious diseases outbreaks. Most of these outbreaks are not caused by a single pathogen, but by a combination of pathogens, with a significant contribution to pathogenicity from environmental factors and high stress levels on farm animals. Shrimp humoral immune response is key to responding to these infectious pathogens and promoting faster recovery. Antimicrobial peptides (AMPs), such as penaidin and crustin, are extremely important in this immunocompetence, efficient infection response, disease recovery and maintenance of performance. This work presents laboratory and field studies evaluating the feed application of a phytobiotic-based additive (Sanacore GM) under conditions of co-infection and multiple infections.

The first part of the study aimed to evaluate the impact of the additive (Sanacore® GM, Adisseo) supplementation towards immunocompetence optimization. The relative mRNA expression of AMPs penaidin and crustin in hepatopancreatic tissue was measured after a period of supplementation of 28 days with treated feeds. Test animals were challenged with WSDV (2.7 x 10^9 WSDV copies/shrimp) and *Vibrio parahaemolytycus* (4.6 x 10^8 CFU/ml) applied in feed with a 48hours-time difference between pathogens. Results show a three- and seven-fold statistically significant increase in crustin and penaidin levels in hepatopancreas, respectively, in the additive group. These results are aligned with a numerical improvement in survival of 40% in the additive group at 24 hours after challenge.

The second part of the study aimed to evaluate the effect of different application strategies of the health promoting additive (Sanacore® GM) in a shrimp farm with a history of multiple outbreak infections, including WSDV, EHP, IMNV and *Vibrio* spp. Two strategies were evaluated: (1) corrective strategy, and (2) combined preventive + corrective strategy. The two strategies were compared to reference historical data. Results show improvements in survival of 58% and 169% by the corrective and the combined strategy, respectively. Feed conversion ratio (FCR) was statistically significant reduced by 5% and 32% by the corrective and combined strategy, respectively. The corrective strategy also increased statistically biomass by 10%, while the combined strategy achieved a 64% improvement. These two trials clearly demonstrate the efficacy of the phytobiotic-based additive (Sanacore® GM) as a strategy to minimize the impact of co-infections and multiple infections and accelerate recovery in shrimp farming.

OVERVIEW OF CURRENT STATUS, CHALLENGES AND FUTURE TRENDS ON SEAWEED FARMING IN THE PACIFIC REGION

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The Pacific Island Countries and Territories (PICTs) have actively collected wild seaweed and cultivated various species of seaweeds over the past decades. Even though seaweed farming production has declined over the last 5 years in most of the Pacific producing countries and territories, these commodities continue to play an important role at the economic and rural development levels in various countries and territories within the region.

The production of seaweeds is largely based on the cultivation of species of the genus *Kapapphycus,* intended for export to obtain hydrocolloids (e.g. agar, alginates, carrageenan). These species are not native to the Pacific region and were introduced in different waves of introduction of exotic genetic material from the 1950s and 1960s. Countries like the Solomon Islands have maintained a relatively constant production of these species, destined for export. In addition, other countries, mainly located in Polynesia, have focused their production on local species of the genus *Caulerpa* (among others), destined for domestic and export markets – mainly Asian markets, but in this case for direct human consumption. Furthermore, in several Polynesian countries, such as Tonga, the production of "mozuku" seaweed (*Cladosiphon* spp.) either for domestic consumption and for exports is gaining a lot of attention from national governments, investors, farmers and exporters.

The production of seaweeds in PICTs presents numerous limitations for its sustainable maintenance and responsible expansion, among others: optimization of production systems, management of disease and epiphyte risks, improvement of harvesting, storage, pre-processing, and processing strategies, as well as facilitation of negotiations for export. On the other hand, challenges associated with climate change are notably affecting the consistent production and high quality of seaweed crops in the Pacific, including rising temperatures, increased eutrophication of coastal waters, drastic changes in salinity and pH – mostly associated with extreme weather events, increased prevalence and incidence of infectious diseases (e.g. ice-ice disease syndrome) and pests (e.g. epiphytes), as well as existing conflicts with other users of the same coastal resources. It can be said that main challenges to seaweed production in PICTs are to maintain a constant and adequate production volume – considering its geographical isolation and disadvantages with other producing regions, such as Asia – and to ensure optimal and constant quality throughout the production system and storage.

The cultivation of seaweeds currently plays and may play in the future, an important positive role on Pacific communities, facilitating food security, improving their quality of life, enhancing livelihoods, and diversifying income generation activities, but coherent and rapid action is required in order to minimize production risks and find feasible solutions to the main constraints faced in seaweed production and marketing.

CAN TRAINING BE A VEHICLE TO DRIVE CULTURE CHANGE IN AN INDUSTRY?

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After the Australian Seafood Industry conference (Seafood Directions) in 2022, Seafood and Maritime Training (SMT), Tasmanian Seafood Industry Council (TSIC) and Women in Seafood Australasia (WISA) teamed up to look for an opportunity to raise awareness of the benefits of a diverse workforce and shifting towards gender equality in the Seafood Industry and community.

During this meeting an opportunity was recognised for SMT's current training programs to play a major role in shifting the balance. This was highlighted based on:

- SMT is the Tasmanian Seafood Industry's preferred provider and trains over 1000 students in a variety of commercial fishing, aquaculture, and maritime courses each year.
- Of these students 95% are employed in the Tasmanian Seafood and Maritime industry and many of them are undertaking the training as they have just been employed for the first time.
- The student cohort are also majority male aged between 16 and 30 which we believe are a target group to promote change in the way they think about the benefits of diversity
- With more than 8000 people being directly or indirectly employed by the Tasmanian Seafood Industry, within a small State both in size and population, SMT's student enrolment represents a significant proportion of the industry and community and provides an opportunity to drive change.

To capture this opportunity the team set about developing a strategy on how best to bring the diversity conversation into SMT training which included:

- The development of a vision and purpose for the project that promoted and guided the team on what they wanted to achieve.
- Scheduling facilitated workshops with SMT Training, Administration and Management staff plus key industry stake holders to tease out the gender inclusion and diversity conversation that could be woven into the training.
- Looking for partners to help capture the outcomes of this work as part of an ongoing study of training driven change and a diversity of thought score card for the industry.

At the time of submitting this abstract the teams project vison is - For the Tasmanian Seafood Industry to become and be recognised as a champion of gender inclusion and diversity. With the purpose - To change an industry culture by embedding the benefits of gender equality and diversity of thought, into the training that industry participants are already engaging in.

The workshop is planned for May 2023 and we are looking forward to implementing the diversity conversation into SMTs training courses and gauging the initial feedback from students and employers.

The presentation at World Aquaculture will showcase a case study of how this gender equality and diversity conversation has progressed, outline the process SMT have gone through to start this program of work to achieve our vision for the Tasmanian Seafood Industry, and discuss the challenges and opportunities.

MARITIME SPATIAL PLANNING: HOW CAN AQUACULTURE AND FISHERIES COEXIST?

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Small scale fisheries (SSF) have a strong representation in Portugal, encompassing more than 80% of the fishing fleet. Closely related to SSF, shellfish harvesting is also an ancestral activity that involves more than 3000 fishers. Both activities are very important at social, cultural, economic and environmental levels. Currently, SSF and harvesting face several challenges, especially the competition for using the maritime space with other economic activities, mainly aquaculture. However, and despite the importance of these activities, the deficiency of basic data on SSF and harvesting as well as lack of high-resolution geo-spatial and temporal data which may compromise the protection of their main fishing grounds, preventing them from being occupied by other activities. New information and communication technologies (ICTs) and the advances in mobile phone technology, associated with GPS and the transmitting capacity to the coast, improved data collection, storage, and communication, revolutionizing data acquisition in fisheries. In this context, the present work presents the implementation and use of the mobile application "PescApanha" (Fig. 1) developed with the coordination of IPMA to fill information and data gaps on Portuguese SSF and harvesting activities, information of upmost importance to design a comprehensive Maritime Spatial Planning where all activities should and must coexist. This app was developed to be a lower cost system and to encourage the voluntary participation of professional fishers and harvesters in order to obtain relevant data on their activities. Therefore, this app aims to collect high-resolution geo-spatial data and information related to the fishing gears used and weight of the three main species caught. Overall, the app-generated data allow the estimation and mapping of the spatial-temporal distribution fishing effort per metier. With this information it is possible to identify the main fishing grounds, to promote the long-term sustainable management of SSF and harvesting activities and resources, and to support an integrated and effective management of the maritime space.

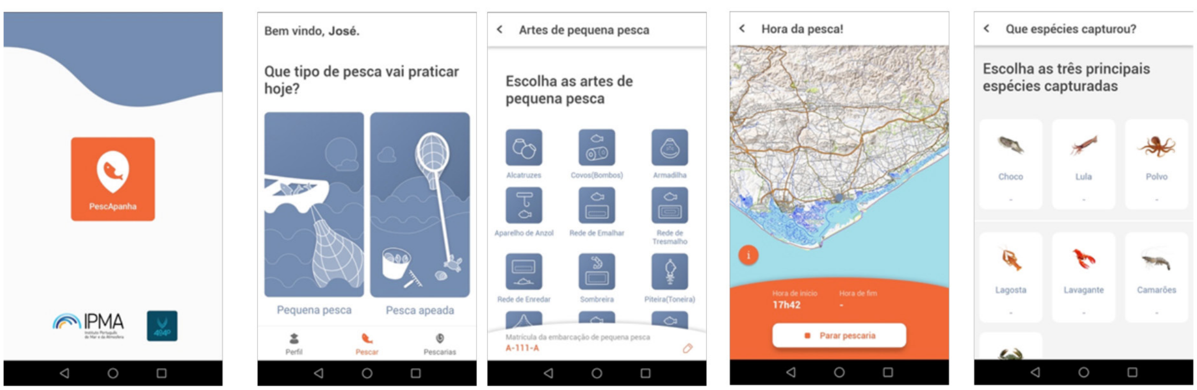

Figure 1. Screenshots of the app PESCAPANHA.

THE PATHOLOGY OF *Lates calcarifer* HERPESVIRAL DISEASE – DISSEMINATED INTRAVASCULAR COAGULATION EXPLAINS MORTALITY SPIKES

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Lates calcarifer herpesvirus (LCHV) is an emerging serious disease in aquaculture. Sudden drops in feed rates and mortality spikes exceeding 40-50% often accompany LCHV infections in juvenile *L. calcarifer,* soon after transfer into sea cages. Affected fish have patchy white skin and fins, corneal opacity, and frequently hang on surface water column like 'ghost' or 'zombie' fish. Fish have pale gills, fluid-filled intestines with yellowish casts, lipid depleted liver, enlarged spleen and kidney, and reddened brain.

Epithelial hyperplasia, apoptosis, marginated nuclear chromatin, amphophilic intranuclear inclusion bodies, and the occasional multinucleated cells are observed in gills, skin, intestines, liver and kidney. These are often accompanied by monocytic-lymphocytic infiltration and extensive necrosis in gills, skin and intestines. Martius scarlet blue stains indicate presence of fibrin in brain, intestines, kidney and liver, or disseminated intravascular coagulation (DIC). DIC has been reported in human herpesviral infections. Multifocal lifting of intestinal epithelium with proteinaceous exudate and necrosis of several adjacent villi often progress to involve entire gut sections. Atrophied livers with accentuated lobules may progress to marked loss of hepatic acini. Multifocal dilated attenuated renal tubules are often accompanied by casts and marked protein loosing renopathy. This study on LCHV demonstrates that it can cause significant production losses.

THE POSITIONING OF ALGAL PROTEIN IN THE FOOD AND FEED PROTEIN MARKET

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The alga food supply chain is presumed to be one of the main drivers for future supplementation of protein sources within the sustainable food system based on utilization of organic wastes. The objective of this study was to position alga based food protein in the food protein market (meat, fish, legumes, insects).

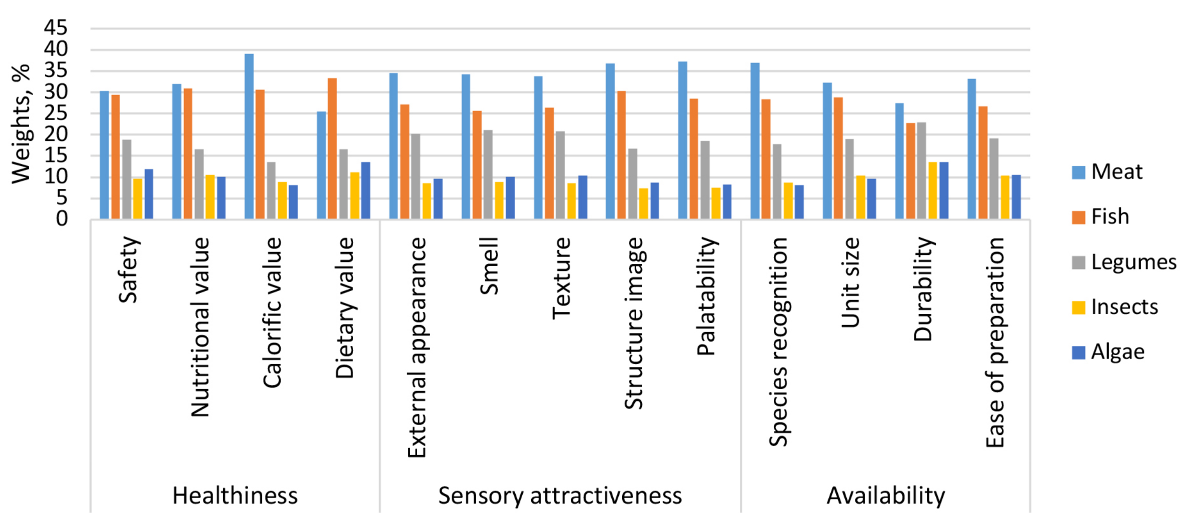
The method applied in the paper is grounded on the multi-criteria decision making method with alternatives. There were two stages of analysis (i) what is the consumer's priority on food quality criteria, and (ii) assuming the given food quality criteria what are the preferences to different sources of food protein, including algal protein. The 55 electronic questionnaires were collected from Belgium (13), Germany (5) and Poland (37).

It was showed that the most important features for consumers evaluating novel protein sources are healthfulness (55.6%), followed by sensory attractiveness (27.4%) and availability (17.0%). In the evaluation of the healthfulness criteria, consumers chose food safety as the most important category (30.5%), assigning significantly less importance to nutritional value (12.7%), dietary value (7.5%) and calorific value (4.9%).

The overall comparison of protein sources within the food quality criteria revealed the highest preference for meat followed by fish, legumes, algae and insects (Figure 1). The results of this research suggest that in the short-term perspective there are no factors that could unequivocally affect consumers' willingness to change traditional sources of protein for alternative ones, such as edible algae.

Acknowledgement. Project financially supported by a research project entitled "Sustainable up-cycling of agricultural residues: modular cascading waste conversion system"; research grant agreement No. FACCE SURPLUS/III/UpWaste/02/2020, research is supported by the National (Polish) Centre for Research and Development (NCBiR) (Project FACCE SURPLUS/III/UpWaste/02/2020).

Figure 1. The consumer's preferences for different food protein sources within selected food quality criteria.



INTERACTIONS BETWEEN SEA CUCUMBER MARICULTURE AND CAPTURE FISHERIES SECTORS IN THE PHILIPPINES

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Sandfish, *Holothuria scabra,* is a high value and heavily exploited species of tropical sea cucumber, traded primarily to China. Sandfish mariculture is regarded as a potentially economically attractive enterprise to meet market demand while rebuilding natural stocks through the release of hatchery-bred juveniles. Engaging small scale fishers in sandfish mariculture can potentially provide livelihood diversification opportunities in coastal villages. In rural Southeast Asia, sandfish mariculture and capture fisheries typically form only part of a broader livelihood portfolio that would include multiple income-generating activities, which are shifting and flexible according to seasonal and economic circumstances. Such forms of diversification are often seen as a hedge against vulnerability and against various forms of external shocks. Drawing on an ongoing program of research and development into sandfish mariculture in the Philippines, this study examines the role of sandfish within the broader livelihood portfolio of households in the coastal Philippines. Specifically, this study identifies the contributions and current status of sea cucumber capture fisheries and mariculture in the Philippines. The impacts and adaptation towards COVID-19 was also documented.

Sea cucumber capture production in the Philippines precipitously decreased from 4,023 tonnes in 1990 to 810 tonnes in 2020. Sea cucumber fishing is a highly seasonal activity due to the monsoon season in the Philippines. During peak season, traders transport up to ≥1,000 kg of dried sea cucumbers with an estimated value of PHP 888,759 monthly to buying stations in Manila. Fishers targeting mainly sea cucumbers recorded lower average monthly income than those involved in other sea cucumber related activities (Table 1). Highest average monthly income was recorded from those involved in sea cucumber processing-trading. Sea cucumber fisheries contribute between 28-65% to the household monthly income of people involved in sea cucumber fishery related activities during peak season. Beside regular fishers, the sea cucumber fishery provided employment opportunities for people displaced during the COVID-19 pandemic.

Compared to sea cucumber capture fishery, average income from sandfish mariculture was relatively low (Table 2). However, despite the relatively small income from sandfish mariculture, it is still meaningful and significant in supporting the primary income of the farmers.

The apparent increase in sandfish populations in the wild was attributed by the fishers to the sea cucumber ranch in their localities. These spillover effects were likely to have occurred by a combination of both regular dispersal of larvae from regular spawning events within the sea ranch, and from adult emigration. Results highlights the importance of effective enforcement of size limits to maximize both the ecological and economic benefits of sandfish mariculture. Additionally, results suggests that diversified livelihoods potentially provide opportunities to mitigate against systemic shocks such as the COVID-19 pandemic.

Table 1. Involvement profile of respondents from Bolinao and Infanta Northwestern, Philippines to sea cucumber fishery related activities.

Activities	Monthly income	% composition of sea cucumber to HH monthly income	% displaced due to COVID-19
Opportunistic fishing	PHP 6,586	45.2	
SC fishing	PHP 3,075	30.3	15
SC fishing/processing	PHP 6,250	27.7	5
SC processing	PHP 27,000	64.7	
SC processing/trading	PHP 45,000	33.3	5

Table 2. Income generated from the involvement in the various sandfish *Holothuria scabra* culture production systems by community partners in Bolinao, Pangasinan and Salcedo and Mercedez Eastern Samar, Philippines between 2008-2022.

Production system	Income		Source
	Bolinao	GDFI	
Floating *hapas*	~PHP 1,790	PHP 2,867	This study
Bottom set *hapas*		PHP 1,625	This study
Grow-out pens		PHP 5,600	This study
Sea ranch	PHP 25,612	PHP 42,606	Fabinyi et al., 2022
Total	PHP 27,402	PHP 52,698	

RISE OF THE EMPEROR: AQUACULTURE POTENTIAL OF *Lethrinus laticaudis*

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Species diversification in aquaculture is an increasingly important risk mitigation strategy that enables practitioners to navigate changing global scenarios. Emperor (Family: Lethrinidae) are recreationally, commercially, and culturally important fish species critically under-represented in aquaculture production. In this paper, we build on current regional diversification initiatives involving Lethrinids by exploring the aquaculture potential of grass emperor, *Lethrinus laticaudis*.

Wild *L. laticaudis* broodfish were collected in November-December 2020 and acclimated in flow-through seawater systems. More than 240 volitional spawning events without hormone intervention were recorded between one month after capture and February 2023, yielding > 51 million fertilised eggs. Spawning occurred across a range of temperatures (21 – 28 ^{o}C), salinities (24 – 38 ppt) and photoperiods (11.5 – 14.7 h). Individual fish, water quality parameters, and lunar cycle were associated drivers of spawning.

Initial larval rearing trials were characterised by poor survival and behaviour, with one attempt in 2021 producing 219 post-metamorphic juveniles. In October 2022, an approach for the commercial larviculture of Lutjanids was successfully adapted, resulting in the production of 8400 juveniles by transfer to nursery systems (50 days post hatch, dph). Larval development (Figure 1) was similar to that described previously for *L. nebulosus* and *L. lentjan*. Survival percentage and spatial productivity (2.9 juveniles/L) were each up to 10 times greater than our larval rearing attempts for *L. laticaudis* and other attempts for *Lethrinus* spp., respectively. Growth modelling of 2021 and 2022 cohorts infer marketable size is attainable following 12 months in grow-out systems.

L. laticaudis is a prolific, serial batch-spawning fish producing larvae that can be reared using simple intensive approaches, putting the species within technical reach of practitioners. Future research on seed quality, nutrition, and parametrisation of nursery and growout systems is needed to confirm compatibility with commercial aquaculture settings.

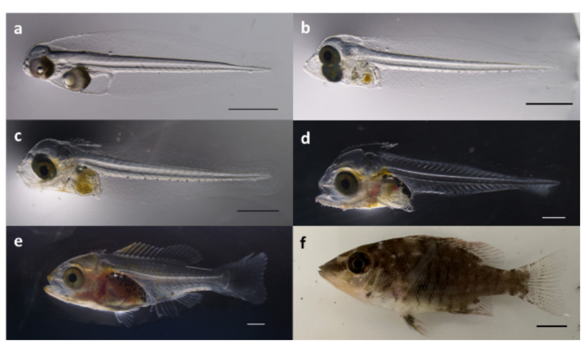

Figure 1. Larval development of *L. laticaudis* at (a) 1dph; (b) 3 dph; (c) 6 dph); (d) 12 dph; (e) 25 dph and (f) 50 dph. Scale bars: (a-d) 500 µm; (e) 1000 µm; (f) 5 mm.

EVALUATION OF GENOMIC BREEDING VALUE ACCURACY USING PROGENY POOLS ACROSS PHENOTYPIC DISTRIBUTIONS IN COMMERCIALLY REARED BLACK TIGER PRAWN *Penaeus monodon*

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Calculating the genetic merit of animals using genomic Estimated Breeding Values (gEBVs) has rapidly accelerated many terrestrial animal breeding programs. However, its adoption in aquaculture can be prohibitive due to the large amounts of resources (i.e., cost and time) required to genotype and phenotype large numbers of animals that are commonly reared together in each production cycle. Pooled genotyping is a strategy that can help circumnavigate this barrier, by reducing the resources required, while maintaining high levels of gEBV prediction accuracy. The purpose of this study is to test accuracies of gEBVs obtained from pooled records compared to individual records. The outcomes of this research will be of importance particularly for mass spawning and communal reared aquaculture species that require the ability to estimate gEBVs accurately and cost effectively to achieve industry advancement. This study used 5,273 black tiger prawn individuals (*Penaeus monodon*) genotyped and phenotyped for body weight across eight commercial grow-out ponds with overlapping families. The pooled gEBVs were calculated under two scenarios. Firstly, gEBVs were calculated based on individual progeny records and then pooled gEBVs estimated based on i) ranked phenotype and ii) full sib & half sib relationships; pool sizes consisted of 2, 5, 10, 15, 20 and 25 individuals per pool. The gEBVs were statistically derived from a single step genomic best linear unbiased prediction model (ssGBLUP). The parent gEBV accuracy pool scenario was measured by Pearson correlation coefficient between parent gEBV from pooled progeny and parent gEBV from individually genotyped progeny. The results indicated that increased pool size decreased the gEBVs accuracy of the unknown parent phenotypes. Higher correlation was achieved in pool sizes below 10 for both pool scenarios, whereas correlation further declined as pool size increased. However, the correlation was slightly lower in full sib & half sib pools. Pool sizes less than 10 produced gEBVs and predicted phenotypes that are more similar to those calculated on individual data (Table 1). Overall, this study provides the first results of genomic prediction on farm in *P. monodon* suggesting that using an optimum range pool of 10 to 15 individuals can reduce genotyping expenses (~10-fold reduction) whilst maintaining accurate estimation (~85% of individual gEBV correlation). These results could find potential applications for use in genomic selection in commercial breeding programs.

Table1. Correlation of parents gEBVs between the pooled progeny and individually genotyped progeny.

Pool scenario	Pool Size					
	2	5	10	15	20	25
Phenotype distributed	0.96	0.89	0.85	0.83	0.78	0.69
Full sib & half sib relationship	0.95	0.83	0.79	0.79	0.69	0.5

FROM INERT TRACERS TO DECISION SUPPORT: UNDERSTANDING CHEMICAL AND VIRAL DISPERSION IN AQUACULTURE USING MATHEMATICAL MODELS

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Aquaculture is a rapidly growing industry that requires the management of multiple environmental impacts and operational risks. Mathematical models can aid decision-making processes by quantifying the dispersion and concentration of various chemicals used in the treatment of pathogens as well as the pathogens themselves. Inert tracers provide a robust representation of fluid dynamics, making them an ideal tool for studying the movement and concentration of chemicals and pathogens. In two separate case studies, the TUFLOW FV 3D hydrodynamic model was used to simulate the fate and transport of bath medicine and viruses and model output compared against Environmental Quality Guidelines (EQGs).

In the first case study, we simulated the dispersion of bath medicine used to treat sea lice in a salmon farm to determine acceptable loads for minimal environmental impact. The model outputs allowed us to assess the concentration against EQGs, aiding in the comprehension of dispersion and concentration around the mixing zone and sensitive habitats (Figure 1). Additionally, we tested different management scenarios and assessed their effectiveness in reducing the dispersion and concentration of bath medicine applications.

In the second case study, we simulated inert tracers in a TUFLOW FV model to predict patterns of dispersion of viruses discharge from a series of wastewater outlets close to proposed aquaculture zones. The model outputs were used to predict the area of dispersion for concentration of the virus against EQGs. These maps were used to undertake a risk assessment for health risk for levels acceptable for shellfish production.

Both case studies demonstrate the potential of mathematical models to facilitate environmental impact assessments and mitigate risks to the health of aquaculture. Mathematical models can also optimise treatment use, such as bath medicine, by predicting dispersion and ensuring appropriate concentration. Ultimately, this improves sustainability, environmental performance, and the health and safety of farmed species. In conclusion, inert tracers can aid decision-making process, and this enables farmers, consultants and regulatory bodies to identify and mitigate environmental risks and optimise treatment use, resulting in a more sustainable and safer aquaculture industry.

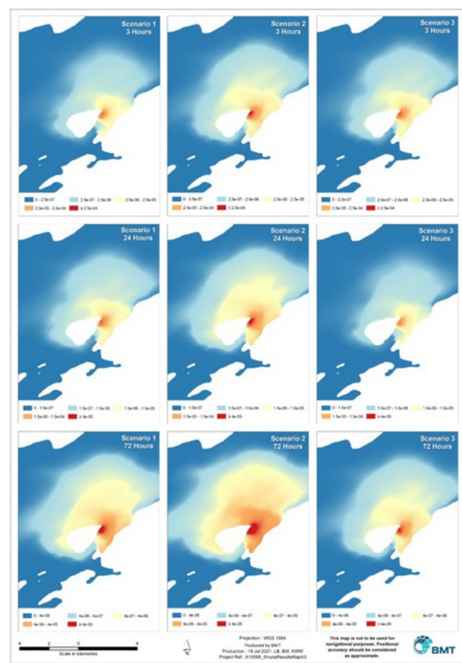

Figure 1 Bath medicine dispersion scenarios

CONSTRUCTION OF A INTEGRATED GENOMIC RESOURCE FOR GENOME WIDE INVESTIGATION OF ECONOMICALLY IMPORTANT TRAITS IN BLACK TIGER SHRIMP *Penaeus monodon*

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Black tiger shrimp (*Penaeus monodon*) are the second most cultured shrimp species globally, with over 700,000 tons produced per annum. However, like many aquaculture species the underlying drivers of economically important traits in black tiger shrimp are poorly understood, and the observed performance across individuals is highly variable. In particular, it is critical to uncover the genomic architecture of economically important traits to facilitate advanced selective breeding approaches (i.e. marker assisted selection or genomic selection). Such approaches are reliant upon a robust understanding of the placement and contribution of individual genetic markers in the prediction of an individual's performance or merit (genomic estimate breeding value).

In this study, we utilised 2,745 individuals obtained from 19 families in an Australian commercial breeding population to construct a high-density genomic map containing 5,100 SNP markers obtained using genotype by sequencing. Overall, the genome-wide distribution of the SNP markers was confirmed with 43 linkage groups identified and through comparative mapping were subsequently anchored to, and then used to correct assembly errors in the Thai reference genome and further scaffold a Australian genome assembly. By combining genotype data and phenotypic records obtained at harvest, we undertook quantitative trait mapping (QTL) and genome-wide association studies (GWAS) for the primary production traits, growth and sex. In both QTL and GWAS analysis, a single sex-associated region was identified (Fig 1.), confirming earlier studies undertaken with divergent Indian and Mozambique black tiger shrimp breeding populations and the white leg shrimp, *Litopenaeus vannamei*. Current studies of genetic drivers of growth are underway; with preliminary results indicate a highly polygenic trait architecture with moderate heritability ($h^2 \approx 0.24$). Understanding the genomic architecture of commercial traits is critical to inform genetic improvement strategies for black tiger shrimp and lays the foundation for advanced methods of selective breeding.

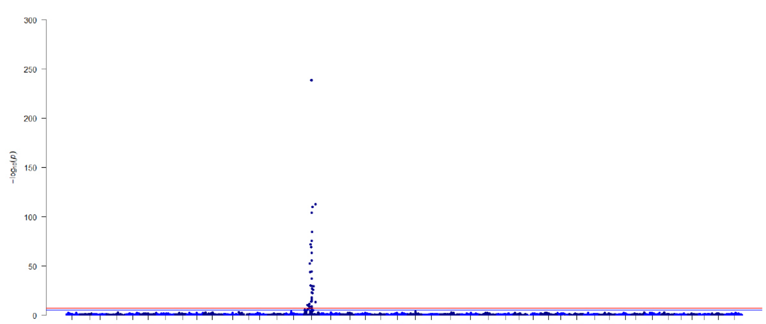

Fig 1. Manhattan plot for a GWAS of sex in *P. monodon*. Grey line indicates genome wide significance threshold.

PREDICTING DISEASE OCCURRENCE IN SHRIMP PONDS USING GENERATIVE NETWORK AND ENSEMBLE LEARNING

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Penaeus vannamei is one of the most cultured species. The global production of Penaeus *(Litopenaeus) vannamei* reached 5.8 million tonnes in 2020, contributing to 51.7% of total shrimp production. However, despite its high production, there are still many issues in this industry. One of those is the disease. The disease brings many threats to shrimp farming, such as slowing shrimp growth rate and even mortality. Previous research estimated that global production losses due to disease over the preceding 15 years amounted to approximately US$15 billion. To help the farmers in mitigating the impact of disease we tried to develop a predictive model that is able to give early warning of disease occurrence. We focused on predicting acute hepatopancreatic necrosis disease (AHPND), infectious myonecrosis virus (IMNV), and white spot disease (WS). The research used the Conditional Tabular Generative Adversarial Model (CTGAN) to synthesize the data to improve the data quality and address class imbalance issues. The synthetic data is then used as input for model development. The model algorithm consists of several engineering processes and classifications. We used Random Forest Classifier (RF) as the classifier. Applying the algorithm to 1839 cultivation data that came from 389 farms we managed to achieve F1 scores higher than 0.85 for the three diseases. However, there is a performance issue in IMNV prediction where we only get a 0.78 recall score which indicates a high false negative prediction. But despite the issue, in this research, we get a hint that the disease occurrence can be predicted based on water quality conditions.

TABLE 1. Performance of Random Forest in Predicting Diseases

Disease	Precision	Recall	F1 Score
AHPND	0.93	0.92	0.93
IMNV	1	0.78	0.88
White Spot	0.98	0.86	0.92

YOLO V8 FOR ESTIMATION OF SHRIMP BODY WEIGHT FROM IMAGES

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Figure 1. Shrimp Detection Results

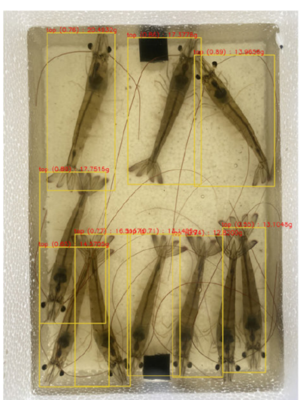
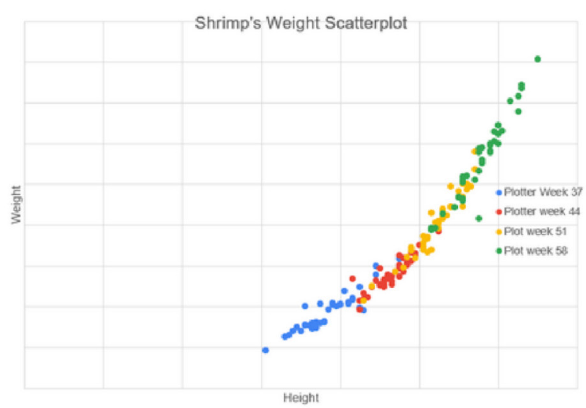


Figure 2 Shrimp Height Vs Weight Plot



BIOCHEMICAL AND MICROBIOLOGICAL VARIATIONS IN THE GONADS QUALITY OF THE EDIBLE SEA URCHINS *Echinus esculentus* HARVESTED FROM MID-NORWAY FJORDS

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Urchins' gonads are a highly prized delicacy in some countries and harvesting of these echinoderms has also been found beneficial in areas of great proliferation to allow restoration of kelp forests. Gonads quality depends on food availability and spawning cycle and the most important quality parameters are color, texture, and taste. Seasonal and geographical variations were investigated through the analysis of the biometrics, proximate composition, color, and pigments content of the Edible sea urchin *Echinus esculentus* gonads. Urchins were harvested from two different locations from Mid-Norway fjords in four seasons. These two locations are assumed to have different anthropogenic pressure. Therefore, marine pollution was also analyzed by determining the concentration of heavy metals and perfluorinated substances.

This study aimed to determine the best season and location for harvesting high quality gonads in terms of nutritional value (proteins, lipids, and fatty acids content), aspect (color and pigments content), and microbial composition. Moreover, the safety of gonads as food was also determined.

A negative correlation was found between gonad index and total body weight between seasons and locations. A statistically significant difference was noticed in the lipids content. However, no difference was reported for the other parameters of the proximate composition. In terms of fatty acid profile, a high concentration of PUFAs (33.3%-39.4%) mainly EPA ad DHA was determined followed by SFAs (22%-29.3%) especially palmitic acid (C16:0), and MUFAs (16%-17.3%).

The harvesting season had a greater impact on the microbial diversity than the geographical location. The microbial communities were mainly dominated by bacteria taxa belonging to *Shewanellaceae, Vibrionaceae* and *Yersianiceae* families in all seasons and locations.

Table1: Variations in biometrics of *Echinus esculentus*

Location	Season	Total body weight (g)	Gonad weight (g)	Diameters width (mm)	Diameters height (mm)	Gonad index
Hitra	Autumn	552.901±223.241	35.97±23.787	102.852±17.239	81.932±14.324	7.003±4.939
	Winter	741.854±244.988	35.732±21.338	97.759±12.477	80.378±11.345	4.829±2.486
	Spring	478.326±58.577	44.91±16.772	92.95±5.239	72.602±5.878	9.363±3.225
	Summer	688.261±189.447	20.265±8.399	102.276±8.253	88.723±7.498	3.088±1.431
Munkholmen	Autumn	819.496±240.92	49.488±16.826	112.439±11.014	89.896±8.192	6.249±1.986
	Winter	538.693±173.189	56.784±25.884	104.776±8.284	79.574±8.238	11.333±5.574
	Spring	829.713±185.76	43.736±34.856	113.048±6.341	91.586±8.993	5.351±4.053
	Summer	657.717±180.991	24.368±14.088	106.728±12.381	79.054±5.995	3.716±1.646

Table 2: Variations in proximate composition *Echinus esculentus*

Location	Season	Moisture content %	Ash content %	Proteins %	Lipids %
Hitra	Autumn	83.555±2.17	1.327±0.426	9.396±1.504	3.836±0.829
	Winter	85.116±2.346	2.256±0.352	8.743±3.031	3.172±0.662
	Spring	86.34±2.499	2.282±0.162	8.455±2.628	1.229±0.801
	Summer	83.611±1.1	0.969±0.456	9.261±0.476	3.284±0.46
Munkholmen	Autumn	84.469±1.336	1.364±0.174	9.71±1.016	3.277±0.807
	Winter	85.596±1.845	2.147±0.207	8.933±2.339	3.894±0.733
	Spring	85.166±1.983	2.071±0.266	10.502±2.501	3.034±1.524
	Summer	84.534±1.158	1.183±0.599	8.588±0.436	2.377±0.9

A REVIEW OF AUSTRALIA'S FISH WELFARE LAWS AND INDUSTRY PERSPECTIVES

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Australia is a federation of six sovereign States and two self-governing Territories. The Australian Commonwealth Government deals with animal welfare in the context of trade and international agreements, working with exporters to maintain international export standards. States and Territories have the primary jurisdiction for day-to-day animal welfare and enforcing animal welfare law within Australia. Hence it is at the State and Territory level that protection is afforded to animals through law.

What constitutes an "animal" under welfare laws across Australia is not consistent. In Australia it is generally accepted that terrestrial animals, including dogs, cats, birds, cows and horses, are sentient and uniformly these animals are well protected in animal welfare legislation. Protection afforded to fish, on the other hand, is not so uniform under Australia's animal welfare laws; protection afforded to crustaceans and cephalopods even less so. However, public (consumer) sentiment with respect to these animals is changing. This sentiment is driving change which is being reflected in changes to animal welfare laws in Australia.

It is critically important that those involved in the care and husbandry of fish, including aquaculturalists, commercial wild catch and recreational fishers, are aware of, and understand, the current animal welfare legislation in their jurisdiction of operation and in any changes that may be occurring.

In 2006, a major review of Australia's fish welfare arrangements was conducted. In 2022, a subsequent review was completed which included a comprehensive review of current animal welfare laws as it pertains to fish, crustaceans and cephalopods. As part of this contemporary review, four online workshops were held to present and discuss the current aquatic animal welfare legislation to fisheries sector stakeholders at the State and Territory level. Attendees at these workshops included stakeholders from government agencies, aquaculture, commercial wild harvest and recreational fishers and animal welfare advocate groups, including the Royal Society for the Prevention of Cruelty to Animals (RSPCA) and Animals Australia. Each workshop included two group discussion sessions, to capture stakeholder perspective on the information provided.

The presentation will present the findings of this major review of fish welfare laws in Australia and will also discuss the perspective of stakeholders attending the workshops. Implications of how Australia's changing animal welfare environment may impact commercial operators will also be discussed.

TILAPIA AQUACULTURE IN SOLOMON ISLANDS: STATUS, SUCCESSES, CHALLENGES AND OPPORTUNITIES

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Small-scale tilapia aquaculture has been heralded as a potential fish production source that could complement coastal fisheries in the Pacific Islands. This is given the declining coastal fisheries production where fisheries management alone will not be enough to address the future demand for fish among Pacific Islanders. In Solomon Islands, while investments have been put into developing a robust and sustainable tilapia aquaculture sector, critical gaps remain to be addressed to move beyond an emerging sector to one that is making a significant contribution to livelihoods, food security and income.

In this presentation, we trace the development of tilapia aquaculture in Solomon Islands since 2008 (post-ethnic tension) and examine how far the sector has grown. We highlight the successes, challenges, and some key opportunities that can advance the sector's tangible contribution to complementing coastal fisheries production in Solomon Islands.

We relied on project proposals, reports (project and trips), policy briefs, scientific publications, and our experiences in the field to deduce both qualitative and quantitative information on the key learnings, achievements and outcomes, and recommendations for the sector.

Results show that investments to date were driven mostly by donors through the government, non-governmental organisations, and development partners, and have been successful in contributing to increased farmers' human (increased knowledge and skills) and social capital (expanded social network through the cluster model). Importantly, the current form of tilapia aquaculture contributed minimally to food consumption and income, therefore will unlikely meaningfully complement coastal fisheries production at this stage. Persistent issues such as the limited productivity of the current tilapia species (*Oreochromis mossambicus*) leading to limited contribution to rural livelihood, food and income security remain. Hence, while progress has been made towards the introduction of a commercially viable Nile tilapia (*Oreochromis niloticus*) as an alternative option for tilapia aquaculture, the translation of production from household tilapia ponds to tangible impact on rural livelihood, food and income security remains contingent upon several key factors.

INDUSTRY-DRIVEN VOCATIONAL TRAINING PARTNERSHIPS WITH SCHOOLS

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Vocational Education and Training (VET) programs in schools require engagement with industry. Where such engagement exists, it is often driven by the school as a request for support rather than a true partnership which equally benefits employers' workforce development needs. Without true engagement by industry, VET programs in schools are therefore delivered in a school culture which does not replicate workplace conditions and environments. As a result, young people are not adequately prepared for the modern workplace and only 39% of Australian VET in Schools students gain employment in the industry in which they trained.

Drawing upon its successful engagement with the Huon Valley Trade Training Centre aquaculture program in Tasmania, the Tassal Group has established the Greater Whitsunday Aquaculture Education Partnership to grow its future prawn workforce and create positive employment opportunities for school leavers in northern Queensland.

The GWAEP is unique in that it is driven by industry in a mutually-beneficial partnership with five schools and TAFE Queensland. Tassal will provide the schools with access to its specialist staff to deliver training sessions within the schools and at its Proserpine Prawn Farm. Tassal will provide ongoing technical support to the schools in the operation of their biofloc recirculating aquaculture systems and will host an annual PICNIC (Prawn Innovations Camp – New Ideas Celebrated) where teams of students will compete to solve real production problems identified by Tassal management.

Critical to the success of the GWAEP will be Tassal's support within the schools to establish true workplace cultural environments in the training programs. Students will be treated as employees with real responsibilities, meaningful tasks, and employee/employer relationships with their training staff.

At the completion of the two-year Certificate II in Aquaculture program Tassal will commit to providing employment opportunities for graduates, thus ensuring future workforce needs are met, as well as providing meaningful futures for young people in their own community.

RECENT ADVANCES IN BARRAMUNDI SELECTIVE BREEDING

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Mainstream Aquaculture Group has operated a Barramundi Breeding Program for 17 years and introduced genomic selection as the core method of commercial trait improvement in 2018. A mix of genomic and marker assisted selection on individuals is coupled with pedigree-based methods to improve commercial traits and create optimal and sustainable breeding lines. This has enabled identification of breeding lines that are tailored/optimised to specific farming environments (particularly open pond and RAS environments). It also drives development of a unique golden-coloured barramundi breeding line.

Commercial (farming) growth rates analysed over the three-year period 2019-2022 demonstrate a 15.8% (pond farming) and 15.2% (RAS farming) increase in growth rate. Improvements both predicted and measured since the implementation of genomic selection are discussed together with practical challenges related to interpreting performance results on farms. Specifically, uncoupling the underlying impact of genetics versus operational factors that drive commercial outcomes. Data is also presented on an early growth stage experiment where wild-type and commercial barramundi are compared under similar growing conditions in RAS. This experiment highlights improved growth and lower variance achieved through domesticated and improved stocks. Challenges faced in incorporating new genetics into advanced breeding programs are discussed.

Looking to the near future, improved robustness and specific disease resistance are in focus. A program to improve the capability of barramundi stocks to cope with common parasitic (cryptocaryon, amyloodinium) and bacterial (*S. iniae*) challenges in Australian farming environments is outlined. Also outlines is work using the genomic platform to improve disease resistance to exotic regional diseases and build a breeding nucleus capable of rapid response to in-bound biosecurity threats to the Australian Barramundi Industry.

EVALUATION OF DIETARY CINNAMALDEHYDE SUPPLEMENTATION IN FEED ON THE GROWTH, AND NUTRIENT UTILIZATION OF NILE TILAPIA *Oreochromis niloticus*

Ainulyakin Hasan Imlani*, Dedi Jusadi, Muhammad Agus Suprayudi, Julie Ekasari, Ichsan Ahmad Fauzi

A study was conducted to evaluate the effects of dietary cinnamaldehyde on the carbohydrate and fat utilization, blood biochemistry, and growth of Nile tilapia *Oreochromis niloticus* at different dose treatments 0, 0.25, 0.5, 0.75, and 1.0 g kg^{-1} of feed which corresponded to T1, T2, T3,T4, and T5, respectively . Each treatment was replicated 5 times under a completely randomized design (CRD), using ANOVA with a 95% confidence interval and Duncan's test. The experimental run lasted in 60 days at the BDP-FPIK-IPB Wet Laboratoty. Based on the results, cinnamaldehyde inclusion of 0.05% showed better performance in glucose absorption, which indicated further that trans-cinnamaldehyde 99% is more active than any other ingredients. The inclusion as low as 0.25 % in Tilapia was still very efficient in lipid utilization and improving the muscle protein which is more economical from the production point of view. The fatty acid oxidation process could reduce fat deposits so as to produce low-fat meat of good quality. An appropriate inclusion level of cinnamaldehyde within the levels used in this study in tilapia diets, could also be beneficial feeding strategy used to normalize the flesh levels of beneficial omega-3 HUFAs to revert to a fish oil-based diet at an appropriate time before harvest. The inclusion level 0.5% in this study is more efficient as far as protein deposition for growth or the balance nutrient utilization is concerned, and the inclusion as low as 0.5% could be useful in improving the antioxidant activity in Nile tilapia.The digestive enzyme activities of Nile tilapia revealed that cinnamaldehyde increased the amylase activity in the intestine which could increase the absorption of glucose which increase protein sparing effect . Protein was basically used for growth in his study. Futhermore, 0.05% cinnamaldehyde inclusion increased the growth of Nile better than other treatments.Cinnamaldehyde improve lipid utilization in all treatments, but in terms of growth, these findings indicated that 0.5% is more efficient to apply in feeds. Based on SGR analysis by a polynomial orthogonal analysis at ANOVA (Sig 0.049), the optimum dosage of cinnamaldehyde inclusion (0.42 g / kg) lower the 0.5%, the use of cinnamaldehyde at this level is economically efficient lower than in the previous study that applied dose of 1 and 2 mL/kg feed.

THE BLUE $: HOW TO KEEP IT!

Katherine Hawes

Principal Solicitor, Aquarius Lawyers
AISP President
Co-Founder of Aquaculture without Frontiers Australia

Aquaculture has become a focus for venture capitalists and other types of investors who are diving into the opportunity to create the *BLUE $*.

However, the *BLUE $* in aquaculture, for both suppliers of product and services and farmers alike, is the result of passion, hard work and a belief that our industry is both viable and valuable.

In this session we will explore how to keep your hard earned *BLUE $* and potentially grow the number you already have! BLUE $ dividends are available from advances in the aquatech revolution with the use of technologies, such as, robotics, artificial technology and blockchain. However, the potential for disruption in aquaculture businesses that causes legal issues from intellectual property challenges through to contractual disputes, means that your *BLUE $'s* are at risk. Learn how to protect your BLUE capital.

From her role on the Board of the Association of International Seafood Professionals and co-founder of Aquaculture without Frontiers (Australia), Katherine is best placed to outline the legal frameworks that can optimise your commercial aquaculture business.

What will audience learn from your presentation?

Aquaculture businesses will understand:

- How to protect their *BLUE $*
- Legal issues associated with adopting new technologies
- The types of business disruption that can occur in your supply chains
- What you need to do to legally protect your assets from disruption
- How to incorporate and minimise disruption into your business operations
- If disruption occurs, and cannot be easily mediated, what you need to do to progress to a legal remedy.
- Investment agreements/funding opportunities.

COMPUTER VISION IDENTIFICATION OF INDIVIDUAL ADULT TROPICAL ROCK LOBSTERS *Panulirus ornatus* FROM DORSAL SURFACE MARKINGS

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Identification of individual animals using computer vision methods has been demonstrated for many species with unique external markings. In this study, tropical rock lobster (TRL) species *Panulirus ornatus* are shown to be uniquely identifiable from the analysis of fingerprint-like patterns between their two supra-orbital horns.

The use of an identification algorithm based on the feature detection and description method ORB delivered a superior performance with relation to speed and accuracy compared to other state of the art methods: SIFT, SURF and BRISK. Contrast limited adaptive histogram equalisation (CLAHE) and random sample consensus (RANSAC) methods were found to reduce the false match rate of the algorithm. On a sample population of 15 adult tropical rock lobster, a false match rate of 0.0% was achieved for images where the unique patterns of each individual were clearly shown. A population of 1500 lobsters was simulated using computer generated patterns.

Non-ideal image effects such as cutout, blur, and scale were found to produce a false match rate of 2.9%, compared to 25.0% for ORB without the proposed improvements.

The versatility of the algorithm was tested by confirming its ability to identify animals both after moulting and cooking (boiling). While market sized tropical rock lobster were the focus of this work. Testing of the algorithm on juveniles found that the development of their unique patterns over time increased the false match rate. The proposed identification algorithm has been deployed in a prototype mobile phone application, developed for iOS in the native Swift language. The application demonstrates the feasibility of a consumer-side autonomous, real-time identification tool, for verifying the authenticity of tropical rock lobster products. This developed technology also has potential uses for commercial aquaculture stock management, citizen science, and adaptation for other species.

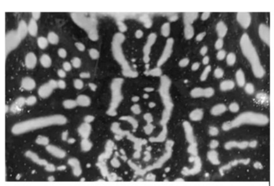

Fig 1. (left) Dorsal surface of TRL with a highlighted box between the supra-orbital horns. (top) Enlarged view of highlighted section.

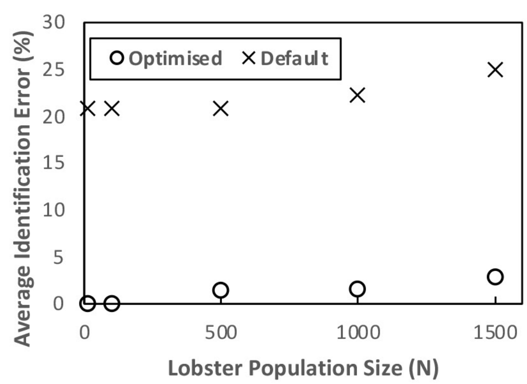

Fig 2. Average identification error (%)

DEVELOPMENT OF A NOVEL MULTIPLEX qPCR ASSAY FOR RAPID QUANTIFICATION OF HARMFUL ALGAE FOR THE SHELLFISH INDUSTRY

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Marine microbial biotoxins produced by certain phytoplankton species can accumulate at high levels in filter feeding bivalve shellfish. Monitoring programs in commercial shellfish aquaculture areas are essential to understand harmful algal blooms (HABs) and ultimately protect seafood consumers. Internationally and in Australia, the main harmful algal species that impact shellfish aquaculture are those that produce toxins associated with Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), and Amnesic Shellfish Poisoning (ASP).

We present a novel approach for the simultaneous, rapid, and precise detection of *Dinophysis* species, paralytic shellfish toxins (PSTs), and certain target *Pseudo-nitzschia* species - causative agents of DSP, PSP, and ASP, respectively. Based on multiplex qPCR assay technology, we designed specific primers targeting the ITS ribosomal region to detect *Dinophysis* species and species belonging to the *P. pseudodelicatissima* complex Clade I; while for the detection of PSTs producing species, the qPCR assay targeted the functional *sxtA* gene, responsible for PSTs biosynthesis.

To evaluate the specificity, efficiency, and sensitivity of the multiplex qPCR assay, we tested a mock community composed of artificial genetic material of the three different targets. The multiplex assay successfully amplified all three targets, with efficiencies of 96.28% for *Dinophysis* species, 94.5% for *P. pseudodelicatissima* complex Clade I, and 91.38% for the *sxtA* gene.

The following steps for this research will evaluate environmental samples as targets. Further evaluation and comparison of this multiplex qPCR method, with current monitoring methods such as microscopy and biotoxin testing of shellfish flesh, has the potential to aid in a rapid and comprehensive understanding of HAB development, and act as an early warning monitoring system for food safety.

TWO PROMISING INDIGENOUS CANDIDATES FOR DIVERSIFICATION OF AQUACULTURE IN THE PACIFIC ISLANDS: TROPICAL FRESHWATER EELS (*Anguillidae*) AND MANGROVE OYSTERS (*Crassostrea/Saccostrea)*

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Due to a lack of research on indigenous species, and the long time frames needed for domestication of new species, successful aquaculture in the Pacific Islands is dominated by a small number of species that are mainly introduced from outside the region. The aquaculture potential of tropical freshwater and marine species indigenous to the Pacific needs more research in order to increase production, increase resilience, and diversify the sector.

For this reason two aquaculture commodities, freshwater eels (*Anguilla spp.*) and mangrove oysters (*Crassostrea spp.* and *Saccostrea spp.*) are currently being trialled by the Pacific Community in collaboration with The University of the South Pacific. Glass eels collected during the peak recruitment seasons in Fiji, from January to April 2023 are being cultured in an intensive, static system to measure and compare growth rates to commercially cultured species *A. japonica* and *A. anguilla.* Mangrove oyster spat collected in Laucala Bay near Suva, Fiji, are being grown out to harvest size and sold from a pilot community farm operated by Muanaira Women's Group. The growth and survival of these oysters is encouraging for demonstration of their aquaculture potential.

SOCIAL LICENCE AND THE DEVELOPMENT OF ONSHORE AQUACULTURE IN AUSTRALIA

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Social licence is defined as an unwritten social contract that reflects the expectations of governments, non-government organisations, other industries/businesses, and the broader community on the impacts and benefits of industry practices. It comprises socio-political factors like legitimacy, credibility and trust which are relevant for the establishment of viable aquaculture industries. To date scholarly interest in the relationship between social licence and aquaculture has been predominantly concerned with the expansion of marine-based aquaculture industries both near-shore and offshore. However, this literature has not focused specifically on the relevance of social licence to the expansion of onshore aquaculture, and specifically recirculating aquaculture systems (RAS). This paper addresses this gap and reports on recent social research that investigates how social licence is relevant in the context of two Australian onshore aquaculture industries. The research comprised individual semi-structured interviews with four stakeholder groups – government agencies in those states where onshore aquaculture facilities are located; seafood as well as industry bodies/associations involved in the development of the two industries that were the focus of the research; onshore aquaculture business owners or managers, encompassing hatcheries and grow-out facilities; and relevant non-government organisations (NGOs). Through analysis of the qualitative data from these interviews, our research provides important insights into the relevance of social licence to expansion of Australian onshore aquaculture industries, as well as the main social licence priorities, drivers, challenges, and opportunities. In concluding, the paper reflects on the strategies and mechanisms that are likely to be most effective in securing social licence for onshore aquaculture industries.

GROWING AUSTRALIA'S PROTEIN PRODUCTION – DEVELOPMENT OF A NEW WHITE FLESH FISH SPECIES FOR AUSTRALIA: Pompano, *Trachinotus anak*

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Consumption of white flesh fish (WFF) in Australia is increasing, and this trend is predicted to continue. Local fish consumption, however, currently relies on 54% imported product. This poses several challenges in meeting increasing demand, including supply chain vulnerability, loss of market access, high food miles, questionable provenance, and biosecurity risks.

There is, therefore, an exciting opportunity to strengthen and diversify the Australian aquaculture WFF industry. CSIRO has strategically invested in exploring pompano culture to support domestic WFF production. Pompano is a robust, fast-growing fish with high fillet yield and excellent flesh qualities with proven commercialisation overseas. In January 2023, the pompano project achieved a major milestone with the first spawning and juvenile rearing in captivity. This now presents the opportunity and means to produce fish for commercial evaluation.

The aims of this project, however, goes beyond just new WFF species development. With increasing production, there is a rapidly growing need to develop novel, affordable farming and processing technologies to ensure sustainable growth. The current Australian WFF industries are highly successful and are increasing production with a high level of biological and economic knowledge. Developing novel technologies, that can support current production, such as waste utilisation, however, requires significant investment and access to an incredibly diverse range of capabilities, resources, and expertise. CSIRO, through this WFF project, and its diverse research capacity such as biotechnology, energy systems, and food innovation, plans to evaluate and refine technologies with the aim of finding solutions to promote cost-effective sustainable farming.

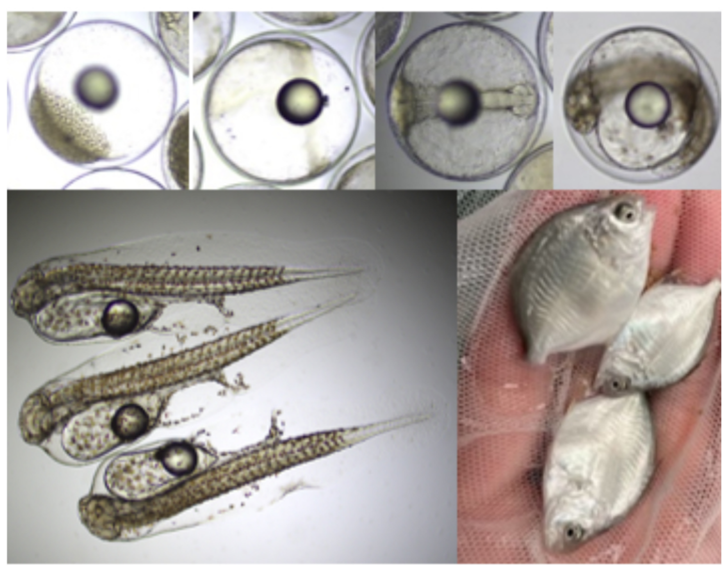

Figure 1. Pompano broodstock at the CSIRO Bribie Island research centre

Figure 2. The project has made significant advances achieving spawning and juvenile production in captivity.

THE EFFECTS OF AMOUNT OF FEED AND STOCKING DENSITY ON THE INTENSIVE PRODUCTION OF *Penaeus vannamei*

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The effects of the amount of feed delivered and stocking density were investigated in the production of white-leg shrimp *Penaeus vannamei* reared under intensive conditions. Three experiments were conducted, each over 91 days, from stocking with PL 12 to harvest, a period comparable to commercial production. All experiments were conducted in indoor tanks of 7.5 m^3 volume with 5 replicate tanks per treatment. In the first experiment, shrimp were stocked at 100 shrimp/m2 (700 shrimp per tank) and fed either 16 g or 23 g of feed per individual over the course of the experiment. In the second experiment, shrimp were stocked at 150 shrimp/m^2 and fed 17, 22 or 24 g of feed per individual. The third experiment was stocked at 150, 200, 250 and 300 shrimp/m^2 and all treatments were fed at 24 g per individual.

For Experiments 1 and 2, growth increased with increasing amount of feed provided, increasing from 16.6 g to 22 g for Experiment 1 and 16.3 g; 20.1 g and 21.5 g for the respective treatments in Experiment 2. The amount of feed provided had no affect on shrimp survival. Feed conversion ratio (FCR) increased slightly with increasing feed (1.08 to 1.13 for Experiment 1; 1.17, 1.22 and 1.25 for respective treatments in Experiment 2). Further analysis will be conducted to investigate divergence of growth trajectories and how well this matches the allometric scaling requirements of the animal for both protein and energy.

Results from Experiment 3 showed that increasing the stocking density of *P. vannamei* from 150 to 300 shrimp/m^2 had no significant effect on the growth of shrimp, with all treatments having an average final weight of approximately 20 g, and no significant effect on survival (average 85%). Our research indicates that *P. vannamei* can be successfully grown at high densities, up to 300 shrimp/m^2 and be fed to 24 g of feed per individual per 91 days under experimental conditions with little effect on survival or FCR.

GETTING AI INTO AQUACULTURE: A DIGITAL TWIN PLATFORM TO OPTIMISE GENOMIC SELECTION IN AQUACUTURE – A TEST CASE USING BARRAMUNDI *Lates calcarifer*

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In 2019, fish accounted for 17.3% of global animal protein consumption. Fisheries production has risen by 137% since 1980, whereas aquaculture production has soared by 1815%. As a result, aquaculture is expected to imminently surpass fisheries as the primary source of fish. However, the integration of genomic technologies into breeding programs has been slow, with only 10% of animals produced descending from genetically improved ones. There is a need for additional strategies to implement genomic tools into fish breeding programs to boost production.

The objective for this project is to develop a digital twin (computer simulation) platform of a Barramundi breeding program. The aim of the digital twin is to determine the highest benefit/cost ratio in the implementation of genomic selection and other genomic technologies, such as parental selection. The accuracy of the digital twin will be maximised by using real genotypes currently available through MainStream Aquaculture's breeding program.

We aim to use data from the breeding program to ensure accuracy and calibrate the digital twin. We will begin by incorporating genotype data from founders and reference populations to establish trait heritabilities and the patterns of linkage and linkage disequilibrium, which are crucial parameters affecting genomic selection accuracy. We will ensure that the digital twin is adaptable to accommodate the reproductive biology

characteristics of Barramundi. With this simulation we will be able to model maximum gains for important traits, while maintaining genetic diversity and informing the optimal breeding program for various environments.

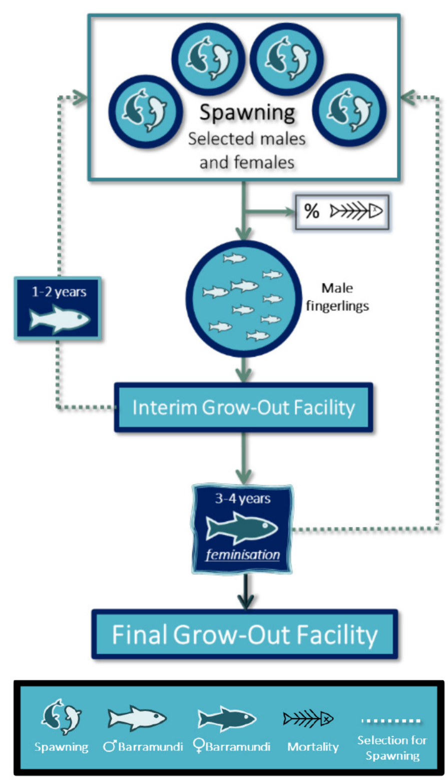

Figure 1: A simplified schematic of a digital twin simulation that hints at the complexities of a group spawning, stenothermal, protandrous fish such as Barramundi.

STREAMLINING THE ASSESSMENT AND APPROVAL PROCESS FOR TOURISM DEVELOPMENTS WHICH PROMOTE SOUTH AUSTRALIAN AQUACULTURE

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Marine-based commercial tourism activities which promote aquaculture through product tasting and education of the farming activity have existed for a number of years both within Australia and globally. These have traditionally centred around tourism activities such as vessel or wading tours which do not require physical construction or development of marine infrastructure. However, recently in South Australia two unique marine-based Pacific Oyster aquaculture tourism ventures emerged, consisting of tasting/educational fixed platforms, which required the proponents to separately seek development approval and authority to use the seabed from multiple State government agencies under different legislation. While the tourism developments have been successful, not just economically but also in promoting the aquaculture industry and its social licence, feedback from the proponents indicated that the legislative assessment and approval process was not conducive in supporting these types of developments (i.e. not streamlined; extended timeframes for approval).

The efficiency of the assessment and approval process for aquaculture tourism developments is contrary to the streamlined process under the *Aquaculture Act 2001* (the Aquaculture Act) for commercial aquaculture developments which farm aquatic organisms. The Aquaculture Act provides the State government regulatory agency, the Department of Primary Industries and Regions (PIRSA), with the capacity to regulate aquaculture development under a single dedicated legislative framework. A fundamental feature of this legislative framework is the ability to establish dedicated aquaculture zones in State waters. These are an attractive option for industry, as they remove the legislative requirement for development approval and provide a 'one-stop-shop' single government agency point of entry with PIRSA able to assess and approve their proposals.

To streamline the assessment and approval process for aquaculture tourism developments, in 2021 a Bill was passed through the Parliament of South Australia to amend the Aquaculture Act. The resulting *Aquaculture (Tourism Development) Amendment Act 2021* will now empower PIRSA to assess and approve these types of aquaculture tourism developments located within aquaculture zones in a similar efficient manner to commercial aquaculture. This legislative initiative will improve proponent confidence in the application process, encourage investment and growth in this emerging tourism industry, and promote South Australia's aquaculture industry. The process and content of the regulatory amendments made for aquaculture tourism developments will be described, along with example tourism developments.

UTILISING AQUACULTURE ZONE POLICIES TO STRENGTHEN SOUTH AUSTRALIAN MARINE AQUACULTURE DEVELOPMENT

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South Australia's aquaculture industry generates 50% of the State's seafood economic output, and was worth $200.1m (farm gate) in 2020/21. The *Aquaculture Act 2001* provides the State government regulatory agency, the Department of Primary Industries and Regions, with the capacity to regulate aquaculture development under a single dedicated legislative framework. A fundamental feature of this legislative framework is the capacity to establish dedicated statutory aquaculture zone policies in State waters. These zone policies represent a unique marine spatial planning approach for aquaculture industry access to a shared resource; providing certainty to all user groups, including fisheries, marine parks, tourism, shipping, other marine industries and Indigenous communities. The aquaculture zone policy process illustrates how dedicated legislation can effectively support strengthening growth of this industry sector in an ecologically sustainable manner.

Twelve aquaculture zone policies are prescribed in South Australia. These zone policies occupy approximately 7% of State waters. More than half (52%) of the zone policy area is allocated to aquaculture exclusion zones, where no aquaculture activity is permitted. The remaining 48% is set aside to allow aquaculture to occur, with generally only 5-10% permitted to be allocated at any one time.

The current review process for the *Aquaculture (Zones—Lower Eyre Peninsula) Policy 2013* will be detailed as a case study. This Zone Policy covers one of the most diverse and in-demand aquaculture areas in South Australia, in addition to coexisting with other marine user groups. All established aquaculture sectors (e.g. southern bluefin tuna, yellowtail kingfish, and bivalve molluscs) and emerging aquaculture sectors (e.g. algae or seaweed) are permitted within the Zone Policy. The review considered the latest science and industry developments to ensure the Zone Policy stayed relevant and appropriate, and maximised benefits to the community from the State's aquaculture resources in a sustainable manner. This included use of an updated and innovative nitrogen based carrying capacity model. The model incorporated estimates of dissolved ammonium released from supplementary fed classes of aquaculture, such as southern bluefin tuna and yellowtail kingfish, and its diffusion considering ocean flushing timescales against specific conservative water quality guideline values for the first time. Results from the modelling were used to determine maximum biomass limits for this class of aquaculture in the Zone Policy, to promote sustainable growth while allowing legislative flexibility to update biomass limits in the future. For example, robust nitrogen offset data (e.g. seaweed,) can be considered within an integrated multi-trophic aquaculture context. Policy flexibility also provides adaptability for climate change mitigation strategies (e.g. lease movement, species).

The effective and efficient process to design, consult with stakeholders, finalise and implement an aquaculture zone policy, and the opportunities for aquaculture growth will be described.

PAST RESEARCH AND BARRIERS TO COMMERCIAL AQUACULTURE UTILISING SALINE GROUNDWATER FROM SALT INTERCEPTIONS SCHEMES IN SOUTH AUSTRALIA

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In South Australia there are currently no commercial aquaculture operations which use saline groundwater although considerable past research has investigated the use of these resources for aquaculture. Substantial salinity increases occur along the course of the Murray River within South Australia. To reduce this salt input, salinity interception schemes comprised of lines of bores either side of the river operate to pipe intruding saline groundwater to land locked salt disposal basins. These schemes intercept ~ 500 tonnes of salt per day. Between 2005 and 2008 the Waikerie Inland Saline Aquaculture Research Centre (WISAC) was designed and constructed to investigate the potential use of saline groundwater from the Woolpunda and Waikerie salt interception schemes that intercept approximately 30 ML of saline groundwater per day (~350 tonnes salt day^{-1}). Water available from this scheme (350 Ls^{-1}) has relatively constant water temperature between 20-22^{o}C and a salinity of 18 - 20 gL^{-1}. Potassium concentration varied between 80-100 mgL^{-1} (36.4% – 45.5% of the level of equivalent salinity seawater).

Trials conducted at the South Australian Aquatic Science Centre showed no significant differences in growth, food conversion ratio or specific growth rate for Mulloway (*Argyrosomus japonicus*) cultured in saline groundwater, diluted seawater or seawater. Research at WISAC demonstrated that although Mulloway cultured using saline groundwater exceed growth of fish in sea cages and the wild, they did not achieve the growth rate expected for fish cultured in the optimal water temperatures provided. This was most likely due to the elevated level of dissolved CO_2. Dissolved CO_2 levels of 50-70 mgL^{-1} were present in incoming saline ground water that was reduced to an average of 10.7 $mgCO_2L^{-1}$ (SD ± 1.8 $mgCO_2L^{-1}$) by degassing prior to use. Research at WISAC showed that Mulloway grown in 6 $mgCO_2L^{-1}$ grew significantly better than fish grown in 10 $mgCO_2L^{-1}$ and 20 $mgCO_2L^{-1}$ which grew significantly better than fish cultured in 40 $mgCO_2L^{-1}$. From May 2006 until March 2008, 9.8 tonnes of Mulloway were harvested and fish and system performance data were collected.

Barriers to commercialisation of aquaculture identified include:

- High concentration of dissolved CO_2 in groundwater means that culture systems need include effective technologies to reduce CO_2 as low as possible (<10 $mgCO_2L^{-1}$).
- Water composition of groundwater from salt interception schemes limits the range of species that can be cultured without supplementation of deficient elements.
- Uncertainty of water supply. Salt interception schemes have been turned off during recent Murray River floods. Commercial ventures need system capabilities and contingency arrangements to be developed in advance of these events.
- High cost of pumping discharge water back into the interception scheme pipe. A location close to Stockyard Plain disposal basin may allow drainage into the basin.
- Stockyard Plain disposal basin has become a recognised wet land system. This may require additional water treatment prior to discharge.

FISH ECTOPARASITE MANAGEMENT ON A PHONE

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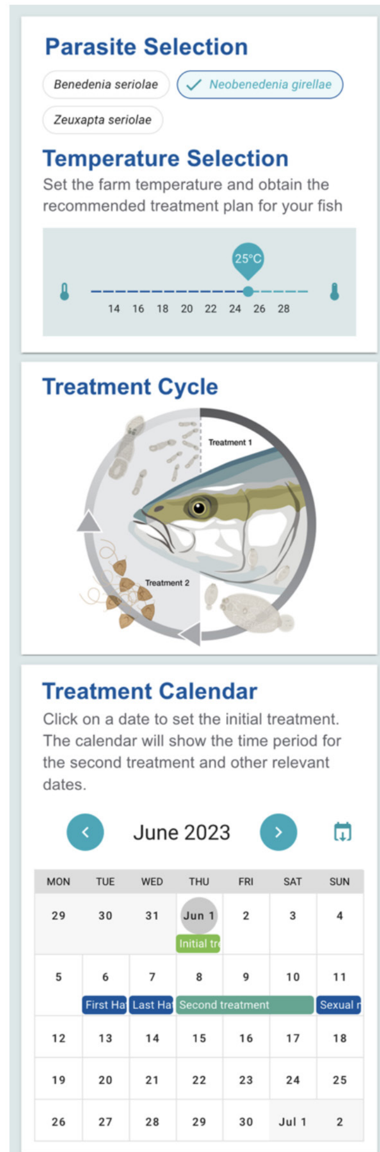
Global aquaculture of fish is impacted by numerous pathogens and parasites. While alternatives are being explored (e.g., vaccines, biological controls, exclusion), the industry relies to a large extent on medicinal baths to treat ectoparasite infestations. Medicinal baths are more effective if they are applied when they are timed to interrupt parasite life cycles (i.e., treatment intervals or therapeutic windows). Repeated use of chemotherapeutants drives the development of resistance in ectoparasites given their high fecundity and short life cycles, thus strategically timed treatments are important because they alleviate treatment frequency and reduce chemical use.



We developed the **BeNeZe** app – a free online, mobile phone compatible, interactive decision support tool that provides treatment intervals to interrupt the life cycle of ectoparasitic marine flatworms. **BeNeZe** targets management of 'skin flukes' (***Be****nedenia seriolae*, ***Ne****obenedenia girellae*), and 'gill fluke' (***Ze****uxapta seriolae*) infections in kingfish and amberjacks (*Seriola* species) but can be applied to other finfish farming enterprises (e.g., Asian seabass/barramundi, cobia, grouper, and the marine ornamental fish trade). The tool enables rapid determination of treatment intervals for two consecutive medicinal immersion or 'bathing' treatments — the first to kill adult worms attached to fish and the second to prevent maturity of new parasite recruits. The tool consolidates complex scientific information into a user-friendly format, helping farmers schedule the best time to treat parasites based on water temperature.

The BeNeZe tool, combined with a disease surveillance plan, can aid the prevention and treatment of parasite outbreaks. The tool could also be adapted for other pathogen/host combinations (e.g., management of sea-lice in salmonid farming). It can also be readily adapted to alternative on-farm decision tools related to degree-days such as net-cleaning and biofouling management.

BeNeZe provides an innovative solution to parasite management that is informed through science and provides clear guidelines for when to treat stock. The tool can be used for multi-species/concurrent infections. Ultimately, **BeNeZe** reduces treatment number and frequency, reduces infection burdens and improves fish welfare and production.

INLAND SALINE AQUACULTURE IN VICTORIA – A RETROSPECTIVE VIEW AND FUTURE OPPORTUNITIES

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Substantial amounts of groundwater with a salinity >1,000 µs/cm (brackish), and upwards of 35,000 µs/cm, is extracted especially in the north west of the state where salinities are highest. Environmental water management plans that incorporate management of salinity impacts are active in all Victorian CMAs and in some areas salt interception schemes are extracting saline groundwater to mitigate the effect of rising water tables and associated increasing salinity levels.

Previous research on inland saline aquaculture in Victoria, which occurred during the 1990s and early 2000s, focused on integration of aquaculture with existing agriculture and agroforestry industries that extracted saline groundwater and saline groundwater management projects.

As part of a Serial Biological Concentration System (SBCS) for the cost-effective management and utilisation of saline groundwaters, 12 marine, brackish and freshwater species (8 fish, 2 prawn and 2 bivalve species) were cultured in two evaporation basins with different salinities (low salinity 9-16ppt, high salinity 10-25 ppt). Species selection relied heavily on availability of seedstock at the time. Mixed results were obtained. Atlantic salmon, Australian bass and black bream survived and grew in both basins. Freshwater silver perch grew exceptionally well in the lower salinity basin but died in the higher salinity basin. Survival of oysters and prawns was low, although some Pacific oysters surveyed for over nine months.

Trials conducted in saline wastewaters from the dairy industry to reduce algal standing crop showed that brine shrimp grew well and reached sexual maturity. Commercial qualities of brine shrimp (frozen blocks and live adults for the aquarium trade) were produced in saline groundwater at a salt production facility. Yield averaged 2.5 kg/M^3.

Agricultural water has become a valuable and limited resource. In the face of an increasingly variable climate, the long-term resilience of farming communities will be dependent in part on the need for more diversified and complementary production strategies, which may include aquaculture. The proof-of-concept projects conducted earlier in Victoria demonstrated this opportunity. Further R&D may focus on, for example, seasonal farming of salmonids in winter and warmwater species in summer, micro-algae and macro-algae production, as well as evaluating new and emerging euryhaline aquaculture species.

IS LOW INPUT PRAWN QUACULTURE AN OPTION FOR INDIGENOUS COMMUNITIES IN THE NORTH OF AUSTRALIA?

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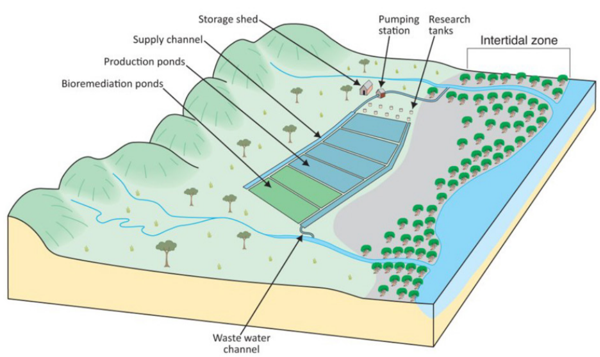
Northern Australia has millions of hectares of land potentially suitable for marine pond based aquaculture. The majority of this land is owned by Traditional owners and subject to Native Title. Traditional Owner groups have continuously occupied and managed the north for tens of thousands of years and maintain significant interests and control of natural resources, including land, water and sea country. To help realise the possibility for aquaculture and indigenous led enterprises in the North, we are exploring the viability of location appropriate farming options. A community led aquaculture operation has the potential to provide livelihood, training, and business development.

Most aquaculture operations in Australia operate at high stocking densities with a significant requirement for power, feed, access to supporting infrastructure, and expert staff. These attributes make farming profitable but likely unsuitable for remote areas of Northern Australia. The challenges of operating in this region are numerous, including environmental, biological, cultural and regulatory complexity. Non-fed (no input) species such as oysters are an attractive option for farming in remote areas, and several indigenous led enterprises are under development in the north. However, little focus has been put on the potential for fed species.

We propose location appropriate farming methods based on extensive (low input) farming. practice. Low input farming is a viable option for remote locations when species stocked at low densities have the capacity to thrive on the natural available productivity from the environment. Low input farming is being explored with Traditional Owner groups with interest and aspirations in aquaculture. We focus on the opportunity for the marine tiger prawn (*Penaeus monodon*). We describe a plan to work through the challenges in co-developing the Mapularri demonstration farm on the Tiwi Islands.



IMPROVING GROWTH AND SURVIVAL OF SANDFISH SEA CUCUMBER (*Holothuria scabra*) JUVENILES IN HAPA NURSERY SYSTEM – II

Maureen Jano [1] and Dr. Manoj R. Nair [2]

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The US Affiliated Pacific Islands of Federated States of Micronesia and the Republic of Palau have several commercially important species of sea cucumbers in their waters, including the Sandfish sea cucumber, *Holothuria scabra*. Due to their commercial importance, they have been widely exploited and are in danger of extinction. The College of Micronesia Land Grant Program has undertaken the development of Hatchery-based Sandfish sea cucumber farming technology for local community-based economic development, future commercialization, and aiming at restocking the depleted stocks in the wild. In this regard, an experiment was conducted to find out the effect of added feeding on the growth and survival of Sandfish sea cucumber, *Holothuria scabra* juveniles in Ocean Nursery Hapa Net System - II. Sandfish sea cucumber juveniles were stocked at 50 pieces per floating hapas in duplicates and fed daily a mixture of sea grass, mud, *Spirulina* sp. powder, and Milk Fish feed meal. Control animals were grazing on algae from the hapa net enclosures. The experiment was run for 45 days, at the end of which growth (length and wet weight) and survival was statistically analyzed for each treatment by measuring all surviving animals. The results showed a significant difference in the growth of Sandfish Sea cucumbers juveniles between the treatments. No significant increase in survival was observed between the treatments

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WOUND HEALING PROPERTIES AND MICRO-RNA PROFILE OF EXTRACELLULAR VESICLES (EVS) ISOLATED FROM BACTERIA (*Streptococcus parauberis*) CHALLENGED OLIVE FLOUNDER (*Paralichthys olivaceus*) PLASMA

E. H. T. Thulshan Jayathilaka*, Chamilani Nikapitiya and Mahanama De Zoysa

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The olive flounder (*Paralichthys olivaceus*) is one of the most consumed marine fishes in East and Southeast Asia. Streptococcosis is one of the major infectious diseases caused *by Streptococcus* sps. in humans, terrestrial and aquatic animals. In recent years, *Streptococcus parauberis* infections in olive flounder have increased and are severely affecting to their cultivation and economy. Extracellular vesicles (EVs) are heterogeneous group of cell-derived membranous structures with different cargos responsible for intercellular communication and homeostasis and been studied for their potentials in wound healing. MicroRNAs (miRNAs) are small, single-stranded, non-coding RNA molecules that regulate gene expression and are found in EVs. In this study, we focused on the wound healing properties of EVs derived from *S. parauberis*-infected olive flounders and their miRNA profiling for depth understanding of flounder EVs on wound healing and diverse other applications.

EVs were isolated from plasma samples of PBS-injected (PBS-Exo) and *S. parauberis*-challenged (Sp-Exo) olive flounders using ultracentrifugation method. Both EVs did not show significant *in vitro* and *in vivo* toxicity up to 400 µg/mL. The *in vitro* cell (human fibroblasts) migration assay, mRNA and protein expression results upon EVs treatment confirmed superior wound healing activity in Sp-Exo. The zebrafish larvae fin regeneration assay confirmed significantly higher tissue regeneration activity of Sp-Exo compared to PBS-Exo.

High-throughput miRNA profiling analysis of PBS-Exo and Sp-Exo illustrated that 14 known and 22 novel miRNAs were differentially expressed (DE) in Sp-Exo (log2 fold change >1 and <-1 Wald test $p < 0.05$). Pol-miR-21 and pol-miR-181 were identified as highly abundant miRNAs in Sp-Exo and are important for angiogenesis, fibroblast differentiation, and inflammatory reactions for supporting wound healing. GO enrichment and KEGG pathway analysis of DE miRNAs (hhi-miR-449, pol-miR-124, pol-miR-122 and pol-miR-203) revealed that their target genes contribute to various physiological and immunological functions, including wound healing and regeneration.

In conclusion, the miRNA profiling of Sp-Exo demonstrated potential features of wound healing activity. Further studies, such as proteomic analysis will support the development of Sp-Exo as a therapeutic agent to overcome limitations in aquaculture such as infection control and wound cure.

Acknowledgments
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SUSTAINABLE MARINE AQUACULTURE DEVELOPMENT FOR SRI LANKA

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Sri Lanka is a small island nation with a population of 22 million in a land area the size of Tasmania. It is currently in the top 10 countries for undernourishment in children, and with the current economic crisis, food production is anticipated to further decline by 40-50 % in 2022 [1]. The annual seafood consumption in Sri Lanka is 30 kg per capita with less than 10% of the aquatic food being produced by aquaculture. This is in stark contrast to other South Asian countries where aquaculture makes up 57% of the aquatic food.

Flanked by the Gulf of Mannar, Sri Lanka has access to remarkable marine resources. The development of the aquaculture sector has been hindered by several factors, including limited transport and road infrastructure, complicated licensing processes at local, provincial and federal government levels, and social tensions remaining from the 26-year civil war (1983-2009) [6]. The Sri Lankan Ministry of Fisheries has identified aquaculture as a priority development area to support the local economy and to bring employment and food security to rural areas.

The objective of this project was to develop a "road map" to support the development of sustainable marine aquaculture in Sri Lanka. Sri Lanka has large productive areas with a wide range of candidate species that are promising for aquaculture development, though have not been developed due to 30-years of civil war combined with a lack of knowledge. Partnered with Sri Lankan government agencies and universities, we undertook an expert consultative process to develop an evidenced-based consensus for country-wide aquaculture planning. To enable these markets to develop, the road maps will consider areas for improved policy and zoning, capacity building in fundamental skills (hatchery management and biosecurity), whole value chain analysis and ensuring inclusion for equitable access to resources for marine aquaculture development.

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EFFECTS OF DIETARY SUPPLEMENTATION OF SYNBIOTICS ON GROWTH PERFORMANCE, IMMUNITY, AND TEMPERATURE STRESS RESPONSES IN OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Many probiotics used in aquaculture are derived from terrestrial sources, presumably having limited ability to interact with existing microbial communities within the gut environments of aquatic animals. Host-associated probiotics (HAPs) are autochthonous bacteria inhabiting the mucosal layer of host animals, which produce beneficial substances such as digestive enzymes and physiologically active compounds. Synbiotics, the mixture of probiotics and prebiotics, have been applied in aquaculture due to its better effects than the use of probiotics. Therefore, the current study was conducted to evaluate effects of synbiotics, consisting of a single or mixture of HAPs isolated from olive flounder and fructo-oligosaccharides (FOS) as a prebiotic on growth performance, immunity, and temperature stress responses in juvenile olive flounder.

Four hundred eighty-six juveniles with an initial body weight of 7.26 ± 0.04 g (mean ± SEM) were randomly distributed into 27 rectangular tanks (18 fish per tank; $N = 3$ tanks per treatment). Three HAPs, including *Bacillus sonorensis*, *Bacillus subtilis*, and *Bacillus velezensis* and one prebiotic, FOS were supplemented in a basal diet to prepare for nine experimental diets as follows: no addition of probiotic and prebiotic as a control diet; prebiotic only; three different synbiotics with single addition of each probiotics with FOS; four different synbiotics with mixed addition of two or three probiotics with FOS. The concentration of each probiotic and FOS was 1×10^7 CFU/g and 5 g/kg basal diet, respectively. Following the 8-week growth trial, the juveniles were subjected to two temperature stress exposures: 1) lethal exposure: gradual increase in water temperature (0.5 °C increment every 30 m) until reaching 30.5 °C and 2) acute exposure: 2-h heat shock at 30 °C followed by 2-h recovery at the ambient water temperature (19.5 °C).

Results of the growth trial showed that there was no significant difference in growth performance, including weight gain, feed efficiency, and survival rate of the juvenile fed the experimental diets ($P > 0.05$). In addition, no significant difference in morphological changes such as condition factor, hepatosomatic index and viscerosomatic index as well as in plasma metabolites, including glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, glucose, total protein, triglyceride, and total cholesterol was observed among the experimental diets. Indices of immunity such as immunoglobulin M, lysozyme, and antioxidant enzyme activity levels in plasma were not affected by the experimental diets. These results indicated that there was no adverse effect of the synbiotics addition in the diets of the juvenile olive flounder.

The lethal exposure test result demonstrated that the relatively higher survival rate (16.4%) of the juveniles fed the synbiotics diets (FOS+*B. sonorensis*+*B. velezensis*, FOS+ *B. sonorensis*+*B. subtilis*+*B. velezensis*) than that (4.2%) of those fed the other diets although no significant difference was detected among the diets. Expression levels of genes involved in temperature stress responses of the juveniles responding to the acute exposure will be discussed later.

INNATE ANTI-RNA-VIRAL RESPONSES OF INTERFERON REGULATORY GENES IN FISH

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The initial sensing of infection by RNA viruses is mediated by Toll-like receptors 3,7,8 and RIG-like receptor signalling pathways. All these pathways lead to essential induction of type I interferon (IFN) via activation of interferon regulatory factor 3 and 7 (IRF3 and IRF7). These IRFs are activated by phosphorylation through kinases known as TANK binding kinase 1 (TBK1), and I-kappa-B kinase epsilon (IKKƐ). Viral hemorrhagic septicemia virus (VHSV) is an enveloped negative-sense single-stranded RNA virus that affects more than 50 marine and freshwater species. In this study, we identified AcIRF3, AcIRF7, AcTBK1, and AcIKKƐ identified from *Amphirion clarkii* and investigated the antiviral responses against (VHSV).

The genes were identified from a previously stipulated transcriptomic database, and *in silico* analysis was carried out for predicted genes and protein sequences. Spatial expressions of the gene transcripts were examined in twelve tissues in healthy yellowtail clown fishes. Four genes were cloned into pcDNA3.1$^{(+)}$ and EGFP-N1 vectors for the cell-based antiviral and sub-cellular localization assays.

According to the spatial expression analysis, all four genes are universally expressed in tested tissues and predominantly expressed in the cytoplasm of the cells. *AcTBK1, AcIRF3* and *AcIRF7* significantly induced *IFNα* expression upon VHSV infection. AcIKKƐ and AcTBK1 overexpression did not significantly change cell apoptosis, but AcIRF3 and AcIRF7 overexpression showed reduced apoptosis at 48 h of VHSV infection. However, prolonged infection (seven days of post-infection) of VHSV, AcTBK1, and AcIRF3 showed severe cytopathic effects same as the pcDNA control. AcIKKƐ and AcIRF7 showed less cytopathic effect than the pcDNA control. Therefore, AcIRF3, AcIRF7, AcTBK1, and AcIKKƐ might exert differential immune modulatory responses upon fish RNA virus infections.

DEVELOPMENT AND VALIDATION OF A QPCR-BASED DIAGNOSTIC METHOD FOR LYMPHOCYSTIS DISEASE MONITORING IN OLIVE FLOUNDER AQUACULTURE

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Lymphocystis is a viral disease caused by the lymphocystis disease virus (LCDV) which belongs to the family of Iridoviridae. It can affect over 140 species of marine and fresh water fish and cause wart-like growths on the skin, mouth, fins, and occasionally in the internal organs of infected fish. Lymphocystis is spread by fish-to-fish contact or contact with infected tissues. However, environmental stress factors due to intensive culture conditions in fish farms, such as crowding, shipping, poor water quality, poor diet and inappropriate temperatures can trigger disease outbreaks. Although lymphocystis is considered as self-limiting and usually not associated with fish death, opportunistic secondary infections of bacterial and parasitic pathogens can occur and lead to mass mortality. To date, there is no commercial vaccine available for LCDV infections. Therefore, an early diagnosis of LCDV asymptomatic carriers is considered as the key measure to prevent the transmission of virus in aquaculture system.

In this present study, we isolated LCDV strains through a monitoring survey in ten fish farms located on different geographical sites of Jeju Island, South Korea. The sequence comparison of the major capsid protein of the isolated strains revealed that these strains belonged to the same genotype and exhibited the highest phylogenetic relationship with the strain recently isolated from Japanese flounder. Based on the MCP coding sequences, we designed three sets of primers and Taqman probes for an absolute quantitative PCR assay. After evaluation of amplification efficacy, one set of primers and probes with the lowest detection limit of 100 copies of LCDV DNA was finally selected. To further validate the asssay's efficacy in lymphocystis disease monitoring, we sampled tail fin tissues from 50 fish with lymphocystis symptoms and 50 fish without symptoms in the LCDV infected tanks, and subjected them to the LCDV quantification. Compared to the fish free of LCDV infection, the lymphocystis symptomatic fish showed more than 105 times higher copies of LCDV DNA in the fin tissue, while the asymptomatic fish showed approximately 10 times higher copies. Furthermore, we also conducted a monthly LCDV monitoring of seawater in five olive flounder farms using the developed assay. The results showed a high presence of LCDV virions in the olive flounder aquaculture system, especially in the winter season (November to April). Overall, our assay has great potential as a diagnostic method for detecting the presence and spread of lymphocystis in olive flounder farms.

INTESTINAL MICROBIOTA SIGNATURES OF COMMON CARP AFTER THE INFECTION OF *Aeromonas hydrophila*

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Common carp is an important freshwater farmed fish species worldwide. Intensive rearing in aquaculture causes increasing susceptibility to various pathogens. One of the most frequently encountered pathogens is *Aeromonas hydrophila*, which has caused huge economic losses to the common carp industry. Extensive studies have been reported on the genetic mechanism underlying common carp against *A. hydrophila* at the molecular level, however, the influence of intestinal microbiota on host resistance to pathogen is less studied.

In the present study, we analyzed the composition and biodiversity of intestinal microbiota in common carp following *A. hydrophila* by utilizing 16S rRNA sequencing. Collectively, the intestinal microbiota in common carp is diverse, but dominated by four phyla, Proteobacteria, Bacteroidetes, Fusobacteria and Firmicutes. The diversity and richness of microbiota was obviously decreased after infection of *A. hydrophila*. The composition and relative abundance of gut microbiota was significantly altered upon pathogenic invasion. The relative abundance of Genera *Cetobacterium* and *Vibrio* were significantly increased in the infected groups. Further investigating the divergence of microbes between the resistant fish and susceptible fish showed that the relative abundance of *Lactococcus*, *Akkermansia* and *Vibrio* in resistant fish were significantly higher than that in both the susceptible fish and the control fish, indicating their potential correlation with the host resistance against pathogens. Our study could not only elucidate the dynamic changes in the common carp intestinal microbiota that occur in response to *A. hydrophila*, but also help us understand the underlying mechanism of intestinal microbial community in resisting pathogens and might suggest strategies for disease control in aquaculture.

BILE ACID IMPROVING GROWTH PERFORMANCE AND HEPATOPANCREASE HEALTH OF WHITE SHRIMP, *Penaeus vannamei,* UNDER NUTRITIONAL STRESS

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Bile acids as endogenous molecules synthesized from cholesterol are regarded as pleiotropic mediators that regulate multiple physiological processes and mediate the utilization for different nutrients. By virtue of their amphipathic structure, bile acids are capable of solubilizing lipids by forming micelles, thus enhancing the digestion of lipids, cholesterol and lipid-soluble vitamins, promoting lipid utilization and protecting animal liver. Besides, bile acids could also function as hormones or signaling molecules, performing pleiotropic activities by activating nuclear hormone receptors. It has been clarified that bile acids could take part in the regulation of the antioxidant defense and immune response. The objective of this present study is to study the efficacy of bile acid supplementation in diets on shrimp growth performance, and hepatopancrease health.

The study is assigned in CRD with four treatments and five replicates. Four diets with different levels of fish oil and bile acid (Shandong Longchang Animal Health Product Co.,Ltd, China) are exhibited in Table 1.

Shrimp of 1.5 gram/shrimp are randomly distribute to each cage of 2 × 1 × 1 m (totally 20 cages) installed in earth pond of 10-12 ppt saline water at 100 shrimp/cage then the density is 50 ind./m^3. During the 8-week feeding trial, shrimps are fed experimental diet 5-10% BW, three times a day and sampling every 2 weeks for observed growth performance and adjusted feed given. Hemolymph is collected at the end of 8 weeks for determined immune response. The hepatopancrease health and lipid deposition are evaluate by wet mount technique under light microscope. The results in Figure 1 shows that growth performance of white shrimp fed different fish oil diets incorporated with bile acid have trended to improvement ($p<0.1$). Feed conversion ratio shows the significantly improvement ($p<0.05$). Shrimp fed T1, T3 and T4 demonstrate the trend of better specific growth rate ($p<0.1$) and significant better FCR ($P<0.05$) than T2. The hepatopancrease health and lipid deposition presents in Figure 2 shows the improvement in shrimp fed bile acid especially on lipid deposition of T3 and T4 compares to T2 and T1, respectively. Therefore, bile acid 0.03-0.045% could promote the shrimp growth performance including hepatopancrease health under nutritional stress of energy reduction and high antinutritional factors from plant protein.

TABLE 1. Diet description of shrimp feed formula

Treatments	Fish oil	Fish meal	Soybean meal	Bile acid
T1 PC-FO2-SBM32-FM15-BA0	2	15	32	-
T2 NC1-FO1-SBM32-FM15-BA0	1	15	32	-
T3 NC1-FO1-SBM32-FM15-BA0.03	1	15	32	0.03%
T4 NC2-FO2-SBM40-FM15-BA0.045	2	15	40	0.045%

Figure 1. Growth performance of white shrimp fed different fish oil level and bile acid

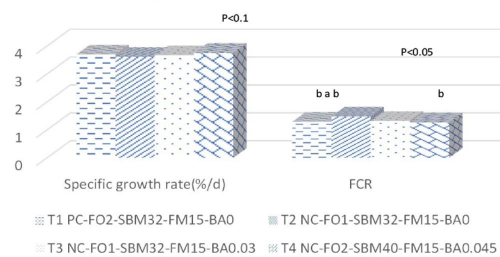
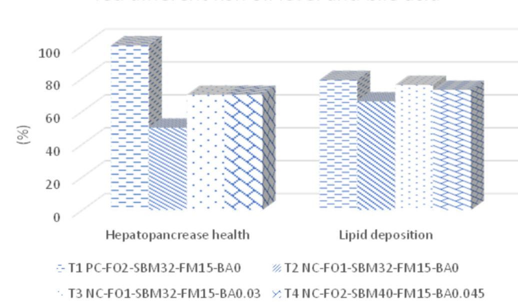


Figure 2. Hepatopancrease health of white shrimp fed different fish oil level and bile acid



MONITORING WHOLE POPULATION WELFARE STATUS IN CAGES USING HYDROACOUSTICS

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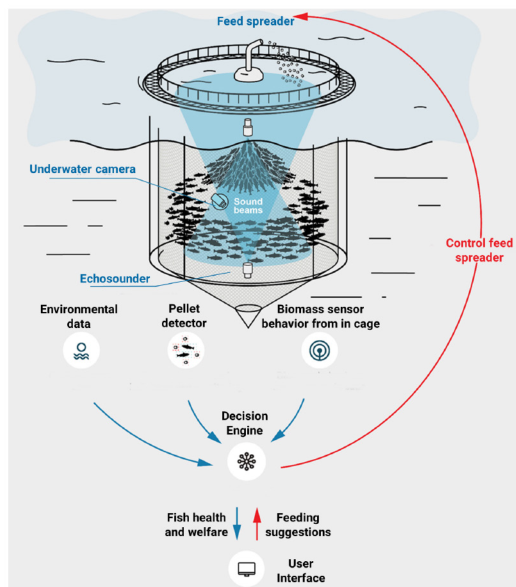
As global aquaculture expands, intensifies and becomes increasingly automated, the monitoring of health and welfare status of fish becomes increasingly challenging, particularly given the animals are out of sight underwater, and the population sizes are many times higher than in most terrestrial farming operations (Huntingford *et al.* 2018, 2023).

There is thus an increasing need to constantly monitor the welfare status of whole populations within farms, predict disease outbreaks and enable early interventions. Disease prediction of this nature was demonstrated by Maloy (2020) who used hydroacoustics to retrospectively detect behavioural indicators of pancreatic disease in Atlantic salmon, 4 weeks prior to its diagnosis by conventional methods.

The potential for such an application has led to development of a hydroacoustic system which can monitor the behavioural patterns of most of the fish population in large cages 24/7, independently of water quality and light levels. We have used this system to collect datasets covering extended periods on several commercial salmon farms, and are analysing them for behavioural signatures related to specific stressors or health events. The initial findings of these analyses will be presented. The work is part of WelfareShield, a project to develop a health & welfare monitoring system using hydroacoustic and optical sensors, appetite monitoring, AI and deep learning to provide fish farmers with important information on the status of their stocks in real time (See figure).



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GERM CELL MANIPULATION IN COMMON CARP - TOOL FOR CONSERVATION MANAGEMENT

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Surrogacy using germ stem cell transplantation into sterilized host is well suited for isogenic line production in fish or conservation of genetic resources because cryopreservation of milt has still limited use for restoration. We attempted to overcome disadvantages connected with the conventional approach for conservation of genetic resources orisogenic line production using germ cell manipulation involving cryopreservation and surrogate reproduction technology.Moreover, novel methods of uniparental inheritance induction or cultivation of germ cells in vitro were developed.

Series of experiments were performed to develop optimal procedure for cryopreservation of common carp male and female early-stage germ cells. A cryopreservation protocol for tissue of ovary using slow-rate freezing (1 °C/min) was developed using a Me2SO-based cryomedia with addition of 0.3 M trehalose. Testing of available cryoprotectants and protocols for testicular tissue resulted in the highest survival achieved using 2 M Me2SO and cooling rate of -1 °C/min. Recovery and physiological activity of cryopreserved germ cells was confirmed by transplantation into sterile goldfish when cryopreserved germ cells retained colonization rate comparable to non-cryopreserved control.Goldfish surrogates were stimulated for spawning and collected gametes were used for fertilization and genotyping using carp and goldfish specific primers.Goldfish surrogates produced viable common carp progeny as confirmed by genotyping. Both male and female gametes were obtained, even from a single double haploid donor confirming feasibility of isogenic line production.

Next aim of this study was to develop a cold shock androgenesis protocol for common by testing combination of different temperature treatments and different cold-shock durations applied shortly after gamete activation.Results of cold-shock treatment testing showed that optimal condition for egg nucleus elimination was a cold-shock at 2 °C for 60 min duration and double haploid induction performed in larger scale using 2 °C cold-shock for 60 min subsequently treated by a heat shock arresting first mitotic cleavage resulted in reduced fertilization and hatching rates for all replicates and low yield of double haploid progeny (1.09–1.28% in experimental incubation, <1% in hatchery incubation) confirmed by RADseq.

Optimization of *in vitro* germ cell culture condition for short term cell culture involved testing of basal mediaand different types of feeder cells withcontinual monitoring of mitotic proliferation.We found that germ cells cultured with hESC media and RTG2 cell line as feeder possessed significantly higher proliferation and survival.

We developed a widely applicable strategy for managing isogenic lines in common carp which is including production of homozygous donors, cryopreservation and *in vitro* culture of germ stem cells and their propagation by surrogate host.

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CANNIBALISM IN TROPICAL ROCK LOBSTER *Panulirus ornatus* JUVENILE AQUACULTURE

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Cannibalism is a limiting factor in the culture of the commercially valuable tropical rock lobster, *Panulirus ornatus.* Cannibalism is most common during the early juvenile stage when *P. ornatus* undergoes frequent moulting of the exoskeleton. Moulting is required to facilitate growth, however the process does expose the soft-bodied post-moult lobster to cannibalism. Despite the importance and impact of this behaviour, few studies have directly examined cannibalism of lobsters in culture.

This study quantifies the contribution of cannibalism to juvenile mortality and investigates pathways responsible for mediating this behaviour. A total 900 hours of time-lapse footage was recorded over 30 days, identifying and characterising moulting and cannibalism events. A 50% mortality rate was identified and attributed solely to the cannibalism of live, moulting lobsters. Over 20% of individual moulting events resulted in cannibalism of the moulting lobster. There was an increase in locomotor activity observed for up to one hour prior to cannibalism, demonstrating the pervasiveness and predetermination of this behaviour. To understand the mechanisms driving cannibalism and reduce its prevalence we investigated the role of chemoreception, particularly olfaction, in the detection of conspecific moulting cues. A behavioural assay was established using a two-current choice flume, demonstrating that lobsters display distinct preferences for moulting cues based on their moult stage and their relationship to the moulting lobster. When the olfactory organ of lobsters is functionally ablated, they no longer demonstrate preference behaviour to conspecific moulting cues in this assay, indicating olfaction is a key link in cannibal-prey recognition. Transcriptomic analysis of the olfactory organ identified several olfactory ionotropic receptor isoforms, adding to the growing genomic and transcriptomic knowledge for this key aquaculture species. These findings demonstrate the importance of conspecific chemical cues and olfaction in facilitating a behavioural response to a moulting lobster.

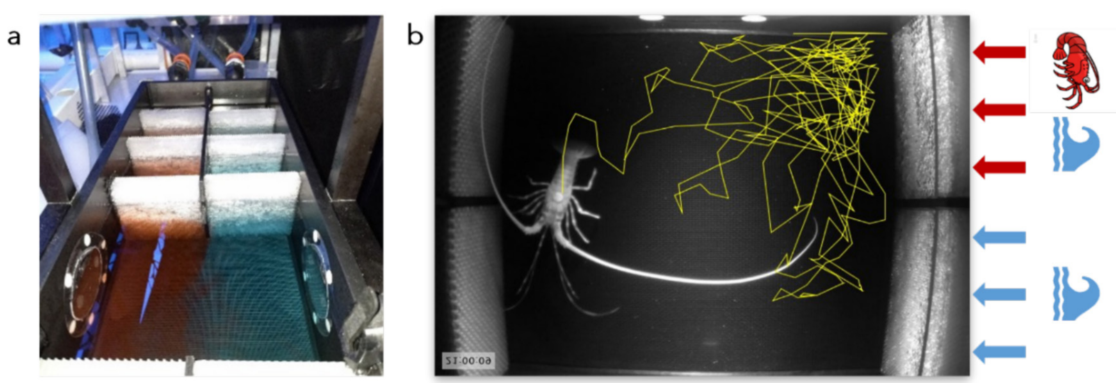

Figure. Two-current choice flume assay design. (a) Dye test demonstrating laminar flow in flume chamber allowing for behaviour response test to two cues. (b) Tracked movement of juvenile *P. ornatus* between two flume currents, one carrying conspecific moulting cues.

GENETIC PARAMETER ESTIMATES FOR GROWTH TRAITS IN BARRAMUNDI (ASIAN SEABASS; *Lates calcarifer*)

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Applied breeding in aquaculture is an area of intense activity. The Australian farmed barramundi sector now has sufficient scale to justify the development of family-based breeding. We describe genetic parameter estimates obtained using animals raised in Australia's Northern Territory. Progeny were generated by group spawning which is common practise for the species. Animals were raised under commercial pond conditions before growth was measured as harvest weight at 9 months (11,677 fish) and 15 months of age (3,068 fish). Pedigree was assigned using a SNP based genotyping tool before the dataset was used for heritability estimation. We found heritability for both traits was moderate, with growth to 9 months (WT_9; h^2 = 0.29 +/- 0.05) slightly higher than for older fish (WT_15; h^2 = 0.23 +/- 0.07). This is in line with previously published estimates for growth traits across tank and sea cage production systems. We also estimated the genetic correlation between traits ($\boldsymbol{r}_g$) using a bivariate quantitative genetic model. The age – age weight correlation was moderately positive ($\boldsymbol{r}_g$ = 0.41 +/- 0.19), suggesting the two traits have common and also distinct gene drivers. Finally, we compared estimated breeding values for broodstock drawn from different sources. This revealed the average EBV for WT_9 was highest for animals selected from within the production system when compared with broodstock from elsewhere or wild caught fish. The results offer promise for future genetic gain for growth in this important aquaculture species.

PROSPECTS USING HEART RATE BIO-LOGGER TO ASSESS THE WELFARE OF FARMED OLIVE FLOUNDER (*Paralichthys olivaceus*)

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This study was conducted to monitor the health conditions of farmed olive flounder according to water temperature. A bio-logger was inserted near the hearts of olive flounder to observe changes in heart rate in response to water temperature under two scenarios. In Experiment 1, the water temperature was raised (1°C/day) from 15 °C to 25°C. In Experiment 2, the water temperature was constant (28°C). Control fish were cultivated at 24°C. All logged heart rate measurements were graded with a data verification quality index (QI) to calibrate the electrocardiogram data. All experiments were done in duplicate. In Experiment 1, the average heart rates at 15 and 25°C were 35± 8.70 beats min^{-1} and 59 ± 20.99 beats min^{-1}, respectively, a significant ($p < 0.05$) increase of 69%. No significant changes in heart rate relative to the 15°C baseline were observed from 15°C to 22°C ($p > 0.05$), whereas a significant difference was observed at 23°C ($p < 0.05$). In Experiment 2, the heart rate of flounder at 28°C fluctuated (66–69 beats min^{-1}). In control fish, heart rate remained constant, with an average of 46 ± 1.66 beats min^{-1}. Notably, the heart rate was higher in Experiment 2 than in Experiment 1, suggesting that cardiac output increases because the oxygen requirement of olive flounder increases with water temperature conditions, especially during growth at high temperatures.

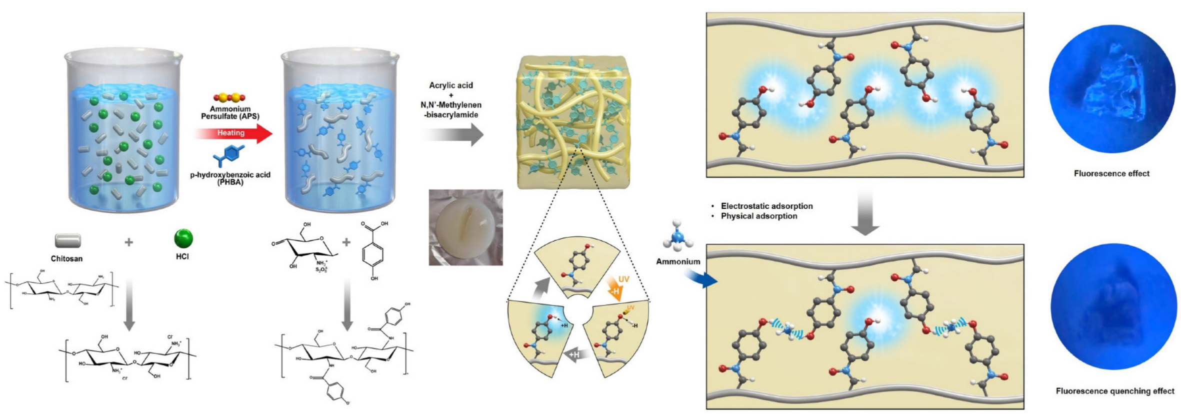

Fig. 1. Schematic diagram of chitosan-based 4-hydroxybenzoic acid hydrogel synthesis and fluorescence quenching effect depends on adsorbed ammonium concentration.

EFFECTS OF GRADED DIETARY GAMMA-AMINOBUTYRIC ACID LEVELS ON GROWTH PERFORMANCE AND STRESS RESPONSES UNDER ACUTE TEMPERATURE EXPOSURES IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Increasing water temperature associated with global warming directly or indirectly influences survival, growth, physiological activity, and immunity of cultured fish, and this environmental change can be a stressor for cold water fish species such as rainbow trout. Gamma-aminobutyric acid (GABA) is one of the free amino acids, classified as a non-essential amino acid, playing an important role as a neurotransmitter inhibitor in the nervous system. In mammals, stress-relieving and sleep-enhancing effects of GABA have been demonstrated, and a growth-promoting effect of this free amino acid has been shown in cultured fishes. Up to date, little is known about the functional role of GABA in rainbow trout. Therefore, the current study was conducted to evaluate effects of graded dietary GABA levels on growth performance and stress responses under acute temperature exposure in this species.

Two hundred twenty-five juveniles with an initial body weight averaging 18.9±0.1 g (mean±SEM) were randomly distributed into each of 15 rectangular tanks (15 fish per tank; $N = 3$ tanks per treatment). Addition of 0 (G0), 50 (G50), 100 (G100), 150 (G150), and 200 (G200) ppm GABA in a basal diet was made to prepare for the five experimental diets. Following the 8-week feeding trial, the juveniles from each treatment were abruptly exposed to 2-h heat shock at three different temperatures (20, 22, and 24 °C), respectively and recovered at ambient water temperature for 2-h.

Results of the feeding trial showed that the juveniles fed the G200 diet had a significantly higher weight gain, specific growth rate, and feed efficiency in comparison to those fed the G0 diet ($P < 0.05$), whereas no difference in survival rate, morphological changes (condition factor, hepatosomatic index, and viscerosomatic index) and plasma metabolites (glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, total protein, total cholesterol, and glucose) of the juveniles fed the experimental diets was observed.

Results of the acute temperature exposure showed that the pattern of changes in the plasma metabolites of the juveniles exposed to the different water temperature was not comparable. No significant interactive and main effects of the GABA level and temperature was detected in the juveniles exposed to 22 °C, whereas there was a significant main effect of the GABA level on plasma total cholesterol, triglyceride, total protein, and glucose levels in the juveniles exposed to 20 °C. A significant interactive effect of the GABA level and temperature on plasma total cholesterol and total protein levels in the juveniles exposed to 24 °C was detected, whereas there was a significant main effect of the temperature on plasma triglyceride level in the juveniles exposed to the same temperature.

Expression levels of genes involved in temperature stress responses in various tissues of the juveniles exposed to the different water temperature will be discussed later.

GENETIC IMPROVEMENT FOR INCREASED BODY WEIGHT IN THE THREE SPOTTED TILAPIA (*Oreochromis andersonii*) IN ZAMBIA

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The aquaculture industry in Zambia has increasingly become critical for food, income and nutrition security. However, the industry still faces major constraints including high feed costs, insufficient quality seed and slow growth among the indigenous species. In response, the Zambian government prioritized to improve the genetics of the three spotted tilapia through the Genetic Improvement Program (GIP). The main objective of the GIP is to improve the growth performance of the species targeting harvest weight and survival as the key traits of interest.

Using three founding fish populations, a complete diallel cross (Table 1) was carried out as the first step to produce the synthetic base population. The base population consisted of full sib families produced in hapas and nursed separately prior to being tagged with Passive Integrated Transponder (PIT) tags. To assess performance, the fish were grown communally to harvest size in earthen ponds which are the most used culture environment for *O. andersonii* in Zambia.

Linear mixed models were fitted to estimate variance components and narrow-sense heritability for harvest weight and individual survival at harvest. The overall survival rate at harvest was 72.5% while heritability was 0.59 ± 0.06 for harvest weight and 0.11 ± 0.02 (observed scale) or 0.19 ± 0.04 (underlying scale given an) for individual survival at harvest. The heritability estimate for harvest weight could have inflated due to the absence of maternal half-sib structure to disentangle the environmental effects common to full-sibs (called c^2) from the genetic effect. However, with a sufficiently large number (152) of families available post-harvest and a fairly high additive genetic variation, harvest weight will respond favorably to selection in the future. These results are comparable to other tilapia breeding programs and as such this work provides the basis for future assessments of selected generations for increased bodyweight at harvest for *O. andersonii* as part of the Genetic Improvement Program for the species.

Table 1: Diallel cross design to establish the Oreochromis andersonii synthetic base population (L = Luangwa River, N = NARDC (originally from the Kafue river and Kalimba farms), S=Super Upper Zambezi River).

Diallel cross design	Female parent population			
		L	N	S
Male parent population	L	LXL	LXN	LXS
	N	NXL	NXN	NXS
	S	SXL	SXN	SXS

Table 2: Heritability estimate for base population (G0) for O. andersonii.

Trait		*Va*	*Ve*	*Vp*	h^2
Harvest weight		1427.16	1003.61	2430.77	0.59 ± 0.06
Survival at harvest					
	Observed scale	0.02	0.17	0.19	0.11 ± 0.02
	Underlying scale				0.19 ± 0.04

Va = additive genetic variance, *Ve* = Residual variance, *Vp* = Total variance, h^2 = heritability

PUTTING AUSTRALIAN AQUACULTURE ON THE MAP

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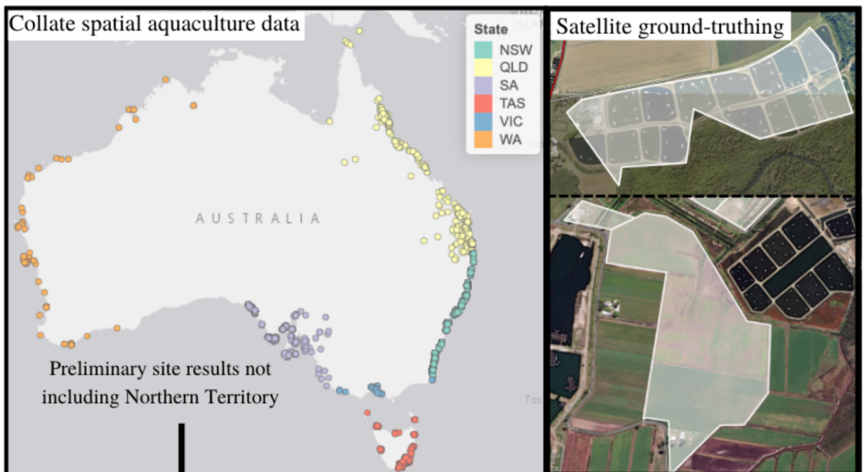
Seafood (freshwater and marine) is central to Australian culture and economy, with a value of over AUD$3.15 billion. Australia, like many countries, is transitioning from fisheries to aquaculture, which is evidenced by recent fisheries declines and a rise in aquaculture production. Australia has a national goal of doubling aquaculture value by 2027 and advancing the blue economy. Aquaculture has great potential given its environmental efficiencies compared to other food systems, however, whether this growth is environmentally sustainable is unknown, which may compromise environmental health and future aquaculture production.

A major gap for assessing the environmental impact of Australian aquaculture production is a lack of understanding of where aquaculture is produced. Here, I will discuss my recent work mapping and quantifying aquaculture's global spatial environmental footprint and will introduce my ongoing assessing opportunities and challenges for downscaling these methods to Australian aquaculture production.

Knowledge of where, how, and how much aquaculture is produced is critical for understanding impact and informing management decisions and will close critical knowledge gaps to bring Australian aquaculture in line with other major food production sectors (e.g., fisheries, agriculture).



Species	# of farms/ ponds /cages	Production (tonnes)	Pond or cage size	Pond or cage depth	Stocking rate	Yield	Harvest size	Grow out
Salmonids		66,105	11,600 m^3	-	10-15 kg/m^3	120 tonnes/cage	3-4 kg	12-18 months
Tuna		8,345	170,235 m^3	-	3 kg/m^3	-	-	-
Oysters		9,011	NA	NA	Variable	Min. 3,600/ha	29-40 or 40-67 g	2-3 years
Prawns		6,740.2	1 ha	1.5-2 m	25-40 per m^2 post-larvae	-	30-35 g	3-6 months
Barramundi		3,427	0.2-1.5 ha	2-3 m	15 kg/m^3 fingerlings	10-15 tonnes/ha (max 30)	2-3 kg	0.5-2 years

Synthesise species harvesting information to quantify production (kg) per pixel

Figure 1. General steps for mapping Australian aquaculture.

ARTIFICIAL INTELLIGENCE BASED DEEP LEARNING APPRAOCHES FOR AUTOMATIC RECOGNITION AND CLASSIFICATION OF BONE RELATED DEFORMITIES IN AQUACULTURE FISH

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Introduction
Artificial Intelligence (AI) is popularly known as the capability of a computer program to possess the intelligence comparable to human being to perform certain decision making tasks. In the real world, AI has already proven its human comparable potential in visual tasks such object recognition and classification, visual perception, speech recognition and language translation etc. Due to its ability to perform tasks that otherwise requires human level expertise, biomedical and Aquaculture communities have also started employing AI tools for their benefits. In this context, we present our work of developing AI algorithms and tools for the task of automatic recognition and classification of bone related deformities in aquaculture fish.

Methodology
In our methodology, we use a supervised learning algorithm in which labelled image data is presented to the Artificial Neural Network models during training to learn the discriminative features from the training data by itself. To implement the supervised learning approach, a deep learning based Convolutional Neural Network (CNN) model is fully trained to recognize/classify the deformities from the fish images then we finally evaluate the performance of the model on test dataset to access its applicability on new and unseen data. We use annotated fish image datasets of various modalities (Microscopy, Radiograph etc.) to train our models.

Applications
- In the context of aquaculture research, detecting bone related deformities in the early stages of the life cycle of the fish is crucial. Fish with severed deformities in the adult stage are generally rejected by potential customers thus incurs heavy economic losses to fish farmers. By detecting deformities in the early stages of the fish can potentially reduce the losses.
- To separate deformed fish with normal ones, technical expertise is required to sort them. With the use of Artificial Intelligence tools, we can make the sorting process automatic and fast which does not require manual human intervention.

THE CURRENT STATUS OF ECOLOGY, AND FISH DIVERSITY OF THE RIVER GANGES

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The Ganga River was host to one of the world's largest and most diverse fish populations, accounting for more than 20% of all freshwater species found in India. However, the development of huge bridges, dams, barrages, and hydro-projects significantly altered river flows. The fragmentation of hydrological connectivity between rivers and wetlands, riverfront development, alarming levels of pollution, extensive sand mining, and unregulated overexploitation of fish resources are the primary causes of Ganga's fish resource decline. The fish capture per kilometre length of the river has declined dramatically over time, and the species composition has altered more in favour of nonmajor carp and other species. Numerous alien fishes have also colonised the environment in favourable areas where flows have been drastically reduced as a result of water removal from the main river. Aside from changing climatic circumstances, altered hydrology has had a crucial role in transforming the river's fisheries scenario. Several studies of data collected over time revealed poor water quality at stressed locations due to the presence of heavy metals, pesticide residues in river water, and silt, which are also factors in the loss of fish species in the Ganga. Many fish species have become extinct in various parts of the river. This alteration has also had an impact on the income of riparian fishermen.

THE EFFECTS OF THE KEY ENZYME AND PRODUCTS IN OCTOPAMINERGIC SYNTHESIS PATHWAY ON IMMUNITY, PHYSIOLOGY, PATHOGEN-RESISTANCE AND ANTI-STRESS ABILITIES IN *Litopenaeus vannamei*

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Litopenaeus vannamei is one of the important cultured species. However, due to the pathogen invasion and drastic climate change, the farming industry has been impacted and induced the stress response of *L. vannamei* leading to production reduced even death. The release of biogenic amines is considered as the primary response for regulating immunity and physiology of *L. vannamei*. Our previous study had been confirmed the effects of key enzyme and products in octopaminergic biosynthesis pathway of *L. vannamei*. The octopaminergic biosynthesis pathway is tyrosine ➔ tyramine (TA) ➔ octopamine (OA), which catalyzed by tyrosine decarboxylase (TDC) and tyramine beta-hydroxylase (TBH). Both OA and TA had been evaluated to enhance the immunity and pathogen resistance ability in *L. vannamei*. *L. vannamei* revived ≤1000 pmol shrimp^{-1} OA and TA showed significant increases in total haemocyte count, differential haemocyte count, phenoloxidase activity, respiratory bursts, phagocytic activity and clearance efficiency in response to the pathogen infection. The TDC and TBH had been cloned and characterized the functions and regulation in *L. vannamei*. LvTDC and LvTBH is confirmed to involve in the neuroendocrine regulatory network, and modulate physiological and immunological response, and LvTBH gene expression is alternated under hypothermal and pathogen-challenged stresses. All results indicate that key enzyme and products in octopaminergic biosynthesis pathway are participate in physiological and immune regulation of *L. vannamei*.

DEVELOPMENT AUTOMATED ALGAE DILUTION DEVICE FOR INTELLIGENT FRY PRODUCTION SYSTEMS

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At present, the main difficulties in the hatching and growing process of fry are that the size of fry is extremely small and difficult to analyze, various complex water quality data need to be judged, and the production process of algae and live-feeding organisms is complicated. As a result, fry production is still heavily reliant on manual labor. For example, tubular rotifer filtration can efficiently produce live-feeding organisms, and use underwater visual recognition to analyze the size of fry, but the problem of algae recognition and cultivation has yet to be broken through. The main technical barrier for algae at the moment is density detection. Although the AI visual recognition system has an error rate of 3-9%, manual recognition has a range of 12-25%. However, mixed microalgae (*Picochlorum sp*. S1b) culture density can exceed 109 ind/mL, but it cannot be identified if it exceeds 105 ind/mL, and the algae sample volume is only 20cc, and the relevant dilution equipment is mostly manual and cannot be automated. The capacity of the equipment with automation is too large. As a result, this study employs solenoid valves, peristaltic pumps, and an automatic dilution device with a programmable logic controller (PLC) control system that can achieve automatic dilution 100 times with a 4.3% error rate (Figure 1). The system allows the intelligent fry production system to make accurate decisions, consistently automate algae production, and collaborate with other systems to achieve the goal of automated fish production.

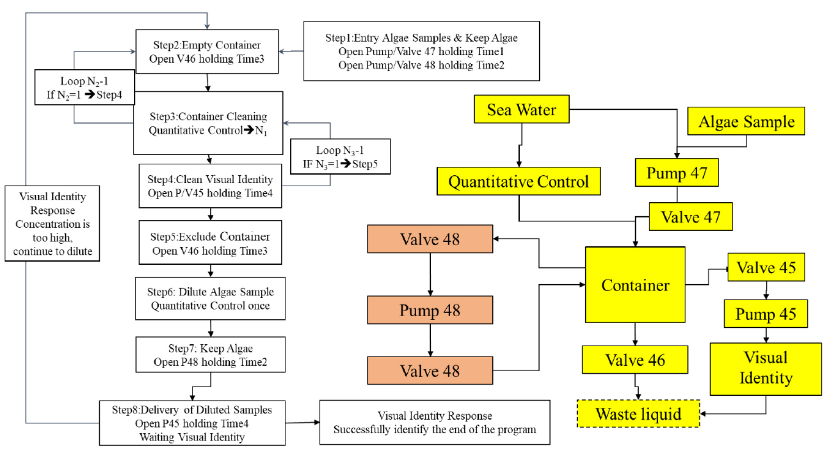

Figure 1 Automated algae dilution device design and logic diagram

CHARACTERISTICS OF THE IMPACT OF THE USE OF FEED ON TILAPIA *Oreochromis niloticus* CULTIVATION USING A DYNAMICS SYSTEM APPROACH

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The topic taken from this research was fish feed. Feed was the most important part in aquaculture. Most of the operational costs of aquaculture activities came from feed. Thus, management in feeding had to be applied. Apart from the high operational costs, the feed also had an impact on the cultivation environment. Poor management in the use of feed will have a negative impact on the cultivation process, namely the mortality rate of cultivated species increases the species immune system decrease, susceptibility to viruses. The purpose of the research was to create a dynamic system model. Which, in the model a systemized algorithm was structured that would assist cultivators in carrying out aquaculture activities and with this model, problem that occur was immediately resolved. The dynamic system model was the method used in this research. This model was used to determine the optimization of feed use based on several variables that support the process of using the feed. The result showed that from a dynamic system, a causal relationship occurs, namely the feed used during cultivation was input. Which would affect the environment directly through 2 variables namely total feces and total uneaten feed. Due to these two variables, it would result in the accumulation of organic waste. In this research, the production of *O.niloticus* was 14 kg, the amount of feed used was 10 kg and the cultivation time was 65 days. The design of this dynamic system model was expected to enable tilapia (*O.niloticus*) cultivators to develop aquaculture businesses that was more environmentally friendly, effective, and efficient.

SUPPORTING WELL-BEING THROUGH MENTAL HEALTH AND PSYCHOLOGICAL SAFETY INITIATIVES

Langton

During this interactive session, atendees will be taken on a journey to deepening understanding of well-being in the workplace. The session will be led by **Jo Marshall** who will share the story of the success of key programs in Seafood including a Culture of Safety Program at the South Australian Research and Development Institute and the National Seafood Industry Mental Health Program – Stay Afloat. The Stay Afloat Program recently received a significant boost with Australia's Federal Government commiting to a significant expansion and extension for two years. A fully immersive workshop will see participants develop knowledge of key aspects of mental health and how mental health intersects with physical and psychological safety at work. From the basics of looking after ourselves, to the role of team and leadership in creating a mentally healthy and safe workplace, through to a highly interactive introduction to the innovative world-first industry-led Sensemaking program, audience members will be engaged at every stage. Expert panellists from across the Seafood Industry will help guide participation through sharing their experiences and learnings along the way. Panellists will include:

- Tarun Richards – Owner – Humpty Doo Barramundi
- Veronica Papacosta – CEO – Seafood Industry Australia
- Katherine Winchester – CEO – Northern Territory Seafood Industry Council
- Rikki Chesson – National Workplace Health and Safety Leader – Paspaley Pearling Company
- Lauren Thornton – Extension Officer – Fisheries Research and Development Corporation
- Heidi Mumme – President – Women in Seafood Australasia

Participants will leave not only with a greater understanding of the work that is being done in Australia, but methods of evaluation, emerging technologies, and most importantly practical tools action for their own well being and safety and that of their teams.

MARKETING THE ROLE OF AQUACULTURE – HOW THE AQUACULTURE STEWARDSHIP COUNCIL IS USING ITS BRAND TO EDUCATE THE COMMUNITY ABOUT THE IMPORTANCE OF AQUACULTURE.

Duncan Leadbitter * and Chi Chi Menendez

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The Aquaculture Stewardship Council is an international standard setting body for responsible aquaculture production. Now over ten years old the ASC has grown enormously in terms of certified farms, species included and labeled products in the marketplace. The first ten years' development was devoted to the creation of standards and systems. For the past year the organization has been investing in communications and marketing such that it will rapidly become a market led organization.

The ASC was developed as a market oriented driver of responsible aquaculture production. Its standards are rooted in international norms (such as the FAO Code of Conduct for Responsible Fisheries) and elaborated via multistakeholder teams comprising industry, scientists and NGOs.

In March of 2023 there were just under 2100 certified farms globally, covering 49 species with Atlantic salmon and white-legged shrimp dominating. The certified volume was 1.9mmt and there were just under 25 000 ASC labeled products in the market place.

The move into marketing has been carefully planned and supported by surveys of consumer attitudes, reviews of available literature and supplemented by observations from outreach staff in the field.

Community misunderstanding of aquaculture, especially in some western markets, is a significant entry level issue, that is shaping the communications strategy, especially here in Australia. Whereas the ASC is keen to promote its purpose to consumers, if consumers are hesitant about purchasing farmed products then the opportunity to further engage on environmental and social best practices is difficult.

There are several elements to the new market led approach, one of which is positioning the ASC as a source of independent information on the role of aquaculture as a source of food and employment. The ASC is committed to transparency as a mechanism for trust building and it proactively seeks inputs from all stakeholders as it moves to build a support base for its mission of taking care of seafood.

EFFECTS OF DIETARY *Lctobacillus plantarum* FERMENTED LEMOM PEEL SUPPLEMENTATION ON GROWTH, NON-SPECIFIC IMMUNE RESPONSES, INTESTINAL MICROBIOTA, AND RESISTANCE TO *Vibrio alginolyticus* OF PACIFIC WHITE SHRIMP

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The aim of the present study is to estimate a functional feed additive, lemon peel fermented with *Lactobacillus plantarum* (FLP), for shrimp health. Four experimental diets containing 0%, 1%, 2%, and 3% FLP were each fed to triplicate groups of white shrimp (initial weight: 2.37±0.01 g) in a recirculation system for 8 weeks. Weight gain, feed efficiency, and survival were not significantly affected ($p<0.05$) by the dietary treatments. Total hemocyte count was higher in shrimp fed diets with 1% and 2% FLP than that in shrimp fed the 0% FLP control diet. Shrimp fed the diet with 2% FLP exhibited higher hemolymph prophenoloxidase activity than shrimp fed the control diet. Intestinal *Lactobacillus plantarum* increased but *Streptococcus equinus* decreased with the increment of dietary FLP levels. After 72-hours challenge with *Vibrio alginolyticus*, survival was higher in shrimp fed diets with 2% and 3% FLP than that in shrimp fed the control diet. Results indicate that lemon peel fermented with *L. plantarum* can be used as a functional feed additive for Pacific white shrimp. A level of 2% FLP is suggested to be included in shrimp diet to enhance non-specific growth and resistance to *V. alginolyticus*.

MOLECULAR CHARACTERIZATION AND ANTIVIRAL, ANTIAPOPTOTIC AND IMMUNE RESPONSES OF INTERFERON-RELATED DEVELOPMENTAL REGULATOR 1 (*IFRD1*) IN RED SPOTTED GROUPER (*Epinephelus akaara*)

H.A.C.R. Hanchapola[2], D.S. Liyanage[1], W.K.M. Omeka[1], H.M.V. Udayantha[1], Gaeun Kim[2], Jeongeun Kim[2], Jihun Lee[2], Y.K. Kodagoda[2], M.A.H. Dilshan[2], D.C.G. Rodrigo[2], Sukkyoung Lee[1], Taehyug Jeong[1] and Jehee Lee[1,2,*]

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Interferons are a group of signaling proteins made and released by host cells in response to the presence of several viruses. This study focused on interferon-related developmental regulator 1 (*IFRD1*) from *Epinephelus akaara*, an immediate early gene that encodes interferon-gamma protein. According to previous reports, *IFRD1* could play a role in regulating gene activity in the cell proliferative and differentiative pathways. It also helps maintain the immune system stability against virus invasion. EaIFRD1 contains an open reading frame of 1,287 bp, encoding a predicted protein of 428 amino acids with a calculated molecular mass of 48.22 kDa. The EaIFRD1 protein contains a long interferon-related developmental regulator superfamily domain between 21 and 325 amino acids. To check the spatial expression analysis, twelve different tissues were used, and the brain has the highest level of *EaIFRD1* expression among those tissues, whereas skin tissue has the lowest level. Further, blood, spleen, and kidney tissues of *E. akaara* were used to identify the immune-related response of *EaIFRD1* against different immune stimulants. Relative mRNA expression of *EaIFRD1* in blood, liver, and spleen was significantly modulated in response to polyinosinic:polycytidylic acid (poly I:C), bacterial endotoxin lipopolysaccharides (LPS) and nervous necrosis virus (NNV). Results revealed that *EaIFRD1* expression was significantly upregulated at 6 h in the liver and spleen against the poly (I:C). All three tissues showed the highest mRNA expression 6 h after the post-infection of LPS. Furthermore, to evaluate the antiviral effect of *EaIFRD1*, we observed the expression of type □ interferon pathway-related gene expression such as; interferon-stimulated gene 15 (ISG15), Myxovirus-resistance (Mx), and Viperin (Vip) by infecting the fathead minnow (FHM) cells with viral hemorrhagic septicemia virus (VHSV). After 12 h of infection, those three genes showed a rapid increment of expression in *EaIFRD1* transfected samples compared to the control. Functional assays revealed the antiviral, antiapoptotic, and immune regulation responses of overexpressed *EaIFRD1*. Collectively, these findings indicate that *EaIFRD1* is vital for the immune system of red spotted grouper by activating the interferon pathways as it stimulates and regulates the defense responses during viral infection.

MYXOVIRUS RESISTANCE PROTEIN A (MXA) IS REQUISITE FOR SENSING AND TRAPPING VHSV: A STUDY ON MXA$^{-/-}$ ZEBRAFISH

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MxA is a crucial antiviral effector, produced in cells as a response to virus detection. VHSV is a contagious and lethal RNA virus in aquatic environments. This study addresses the role of MxA on VHSV infection to better understand the host pathogen interactions, which would be important to seek remedies for viral infection in aquaculture. In this study, first MxA ablated zebrafish were generated using the CRISPR/Cas9 method. Then fish survival and the disease symptoms were evaluated by challenging the modeled fish with VHSV intraperitoneally. From the VHSV challenged fish, internal organs were collected, RNA extracted, and the gene expression was analyzed by RT-qPCR.

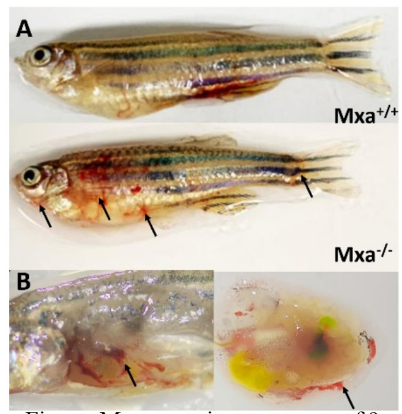
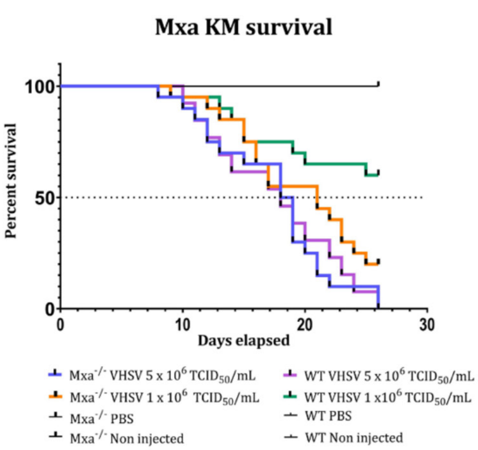
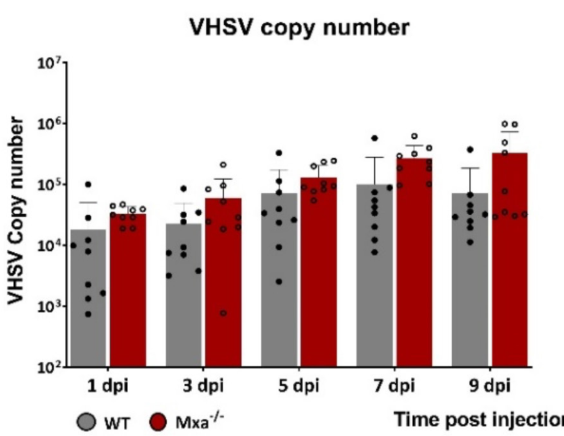


Figure: Macroscopic appearance of 9 hpi WT and Mxa$^{-/-}$ KO fish infected with VHSV concentration of 5*10^{6} TCID$_{50}$/mL. Black arrows: hemorrhages in the head mouth gill and base of the fin (A). Subserosal petechia observed in internal organs and visceral fat (B)

Comparatively higher external and internal pathological symptoms were observed in MxA KO model, which was corresponding to VHSV dose. Injection of high dose of VHSV into the MxA KO fish resulted in a 100% mortality within 26 days, whereas the WT fish reached a 65% mortality within the same period. VHSV infected MxA$^{-/-}$ fish had a significantly higher VHSV copy number compared to WT. This high virus titter in the MxA KO model indicates the reduced antiviral function towards the viral genome replication. The expression of immune-related genes including, *ifn*, *viperin*, *isg* and *irf7* were significantly modulated in the MxA KO fish compared to WT siblings, indicating the role of MxA on the gene expression regulation. The research is still ongoing, and the future work is to decipher the immune cell modulation, using the *Tg* line of MxA *Tg(mpx:mcherry)* and *Tg(mpeg:EGFP)*.





NLRC5 ABLATION IMPAIRED THE MHC CLASS-I PATHWAY IN ZEBRAFISH

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NLR Family CARD Domain Containing5 (NLRC5) was identified as a transcription factor for MHC class-I gene expression. In this study, we used zebrafish as a model to study the function of *nlrc5* under virus infections. *Nlrc5* was knocked out from the zebrafish genome by CRISPR/Cas9 mediated gene editing and the complete absence of *nlrc5* was confirmed in F2 homologs. We used the larval as well as adult zebrafish to understand the role of nlrc5. Either poly I:C or VHSV was administered intraperitoneally, organs were collected, and the gene expression was analyzed.

Immersion of the zebrafish larvae in poly I:C or VHSV (~10^8 $TCID_{50}$/mL) did not alter the immune gene expression indicating that the zebrafish larvae are well protected by physical barriers from external stimulation. Microinjection of poly I:C into 5 dpf larvae did not alter the MHC class-I gene expression pattern between the nlrc5 KO and WT. In adult zebrafish, the spleen, blood, gills, and intestine had a considerable expression of *nlrc5*. Immune stimulation indicates the downregulation of MHC class-I genes in nlrc5 KO zebrafish (**Figure A, B**). VHSV titer (**Figure C**), disease pathology, and mortality were significantly higher in the nlrc5 ablated fish compared to the WT fish.

With our findings, we suggest that the zebrafish nlrc5 gene is important for the MHC class-I gene expression in adult zebrafish. Further studies on the role of the nlrc5 on the immune cell recruitment, and the antiviral defense is in-progress to fully unveil the role of this protein.

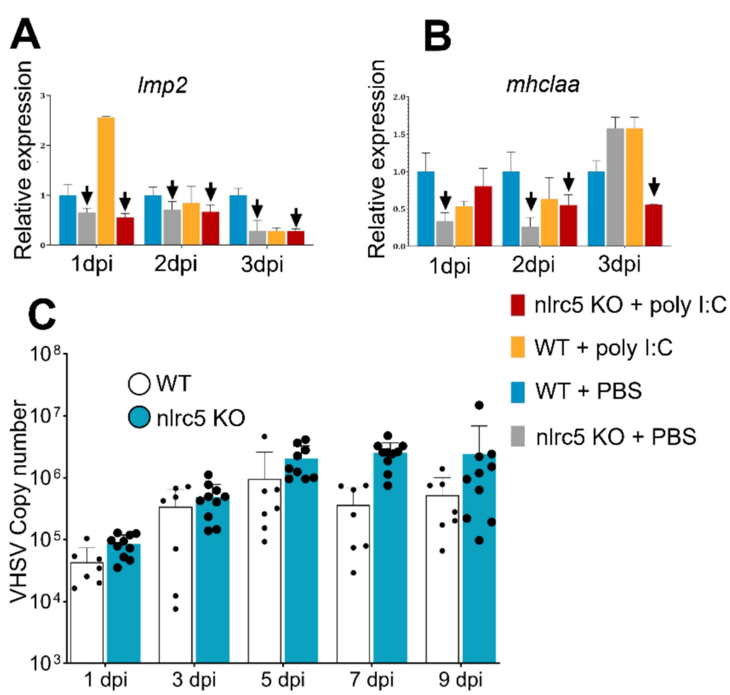

Figure. **A,B,** Expression pattern of the mhc class I genes under the poly I:C stimulation. **C,** VHSV copy number between the nlrc5 KO and WT fish after the VHSV infection.

EFFECTS OF DIETARY PROTEIN AND LIPID LEVELS ON STRESS RESPONSES OF NORTHEN SNAKEHEAD (*Channa argus*) UNDER ACUTE TEMPERATURE STRESS

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Due to the importance of major nutrients such as protein and lipid for optimal growth and physiological performances of fishes, many studies have been conducted to determine its requirement for cultured fishes. However, little is known about interactive effects of dietary protein and lipid levels on temperature stress responses in cultured fishes. Northern snakehead is a commercial important fish species in Asia but has faced with continuously increasing water temperature associated with climate change. Thus, in order to ensure the sustainability of northern snakehead aquaculture, it is important to understand how different protein and lipid levels influence physiological performance of this species in response to acute temperature exposure.

A 3 × 3 factorial experiment was designed to evaluate the relationship between interaction of dietary protein and lipid levels and physiological performance of juvenile northern snakehead responding to acute temperature stress. Four hundred five juveniles (initial body weight: 19.3 ± 0.03 g; mean ± SEM) were randomly distributed into each of 27 tanks (15 fish per tank; $N = 3$ tanks per treatment). Nine diets were prepared to contain three levels of crude protein (41, 44 and 47%) in combination with three levels of crude lipid (6, 9 and 12%). Following the 60-days of feeding trial, the juveniles from each of the nine treatments were abruptly exposed to higher water temperature at 35 □ for 2 h and were recovered at ambient water temperature for 2 h.

Results showed that a significant interactive effect of dietary protein and lipid levels on weight gain, showing the improved values as the protein increased but the lipid level decreased ($P < 0.05$). There was a main effect of both protein and lipid levels on feed efficiency and condition factor, and the overall trend of changes in those indices was comparable to that shown in weight gain. Plasma metabolites, including total protein and triglyceride levels were also significantly influenced by both dietary protein and lipid levels, whereas plasma total cholesterol and glucose levels were affected by the dietary lipid level only.

Based on results of the three-way (3 protein levels × 3 lipid level × 2 stress condition) ANOVA test, there was the interactive effect of the dietary protein level and stress condition on plasma total protein, triglyceride and total cholesterol levels, whereas neither a significant interactive effect of the two factors (lipid level and stress condition) nor a significant interactive effect of the three factors (protein level, lipid level, and stress condition) was detected.

Expression levels of genes involved in temperature stress responses in various tissues of the juveniles exposed to the acute temperature challenge will be discussed later.

EVALUATION OF GROWTH HORMONE-EXPRESSING *Chlorella vulgaris* ON GROWTH PERFORMANCE AND HEMATOLOGICAL INDICES IN OLIVE FLOUNDER (*Paralichthys Olivaceus*)

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The aim of this study was to evaluate the growth-promoting effect and safety of genetically modified *Chlorella vulgaris* expressing olive flounder (*Paralichthys olivaceus)* growth hormone(fGH) when used as a feed additive for olive flounder. At the start of the experiment, 252 juveniles with an initial body weight averaging 41.9±0.31g (mean±SEM) were randomly distributed into 21 rectangular tanks (12 fish per tank; $N = 3$ tanks per treatment). A total of seven experimental diets were prepared, including no addition of fGH (CON), 0.25 (fGH0.25), 0.5 (fGH0.5), 0.75 (fGH0.75), and 1% of fGH (fGH1.0) in a basal diet as well as 0.5 (W0.5) and 1% (W1.0) of wild-type *Chlorella vulgaris* as sham diets. The fish were fed two times (9:30 and 16:30 h) per day to apparent satiation for 8 weeks. At the end of the feeding trial, no significant difference in growth performance indices such as weight gain, specific growth rate, feed efficiency, and survival rate as well as morphological changes, including condition factor, hepatosomatic index, and viscerosomatic index among the tested diets was detected ($P > 0.05$). Plasma metabolites, including glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, glucose, total protein, triglyceride, and total cholesterol were not significantly affected by the tested diets. Antioxidant activities and immune responses as well as expression levels of genes involved in the GH/IGF-axis and nutrient uptake will be discussed later.

FEEDING PLANTS NUTRIENTS GENERATED BY FISH WASTE IN AQUAPONIC SYSTEMS POTENTIALLY COSTS UP TO FIVE TIMES MORE THAN STANDARD HYDROPONIC NUTRIENTS

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Aquaponics is the aquatic integration of an aquaculture component (e.g. usually a RAS-based fish culturing component) and a plant culturing component (e.g. usually a form of hydroponic plant culturing component) to share waste nutrients from the animal(s) cultured in the aquaculture component with the plant culturing component in a way that assists plant growth. A key argument of aquaponics is that the nutrient wastes generated by the aquaculture component are essentially provided "free of cost" to the plant culture component, because the fish (or other aquatic animal) are being cultured and sold and their associated nutrient wastes would normally be disposed of in an aquaculture-only context. However, this "free of cost" argument only exists when the animal being cultured is sold and thus, animal sales cover the costs of fish feed and any other consumables or costs of production required to operate the system. Essentially, when fish are sold from the RAS component, those sales cover the costs of the fish feed and therefore, nutrient provision to the plants in an aquaponic context are considered "free of cost".

A healthy proportion of aquaponic systems operated in a commercial context currently do not actually sell the animals cultured (e.g. the fish) and argue that the animal present is simply a "nutrient production device" for the culture of the plants, which account for the sales income from the enterprise. In this "no animal sales" aquaponic context therefore, profit generation solely sits with the sales of the plant products grown or produced. Therefore, the aquaculture component, with no animal sales present, substantially contributes to the cost of production associated with the saleable product of the aquaponic culturing system (i.e. the plants). If the vast majority of the nutrients required for plant growth and production are being generated by aquatic animal wastes (e.g. fish), and the fish are not being sold, this means the cost of nutrient provision for the plants is directly associated with the cost of the fish feed fed to the aquaponic system.

A desktop study was performed to determine the cost of supplying the nutrients required for plant growth in an aquaponic context via fish-generated wastes of the metabolism of fish feed versus the cost of direct nutrient provision to the plants via standard hydroponic nutrient solution salts. Nitrogen was selected as the representative nutrient in the study as it is the majority waste ion produced by the animal (fish) in the aquaculture component and is an essential nutrient required in plant growth that is generated and represented almost solely by fish wastes in an aquaponic context.

The study calculated the relative price of the Nitrogen produced in fish waste for a representative *Tilapia spp* fish being fed a 36% protein content fish feed commercially available on the USA market. It then calculated the relative price of the Nitrogen available in a representative, commercially available, standard hydroponic nutrient salt (Calcium Nitrate – $Ca(NO_3)_2$) commercially available on the USA market and compared the two relative prices for the Nitrogen mass required to culture a known number of standard hydroponic variety lettuce plants (based on standard plant Nitrogen uptake rates in an aquatic culture environment).

Results indicated that the Nitrogen required to culture the plants cost at least five (5) times more when supplied via a fish waste resource.

The study indicates that, if aquaponics is to be chosen as a commercial plant culturing technology, without any fish sales contributing to offsetting the costs of production, aquaponic farmers who do not sell fish are paying over five times as much for the nutrients required to culture the plants they sell than if they chose a standard hydroponic plant culturing approach. If fish sales were adopted, the potential exists for these fish sales to offset the costs of the nutrients required for plant culture and this would therefore, assist farmers to achieve nutrient costs that equal, or even better, those associated with standard hydroponic production technologies.

THE ROLE AND MECHANISMS OF ACTION OF PROBIOTICS: PERSPECTIVES AND IMPLICATIONS IN PRAWN FARMING

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The concept of probiotic administration in aquaculture seems simple: live microorganisms which when administered in adequate amounts confer health benefits to the host and its rearing environment. However, many factors need to be considered when developing the most effective administration strategy. Specific production goals require tailored probiotics solutions. Different probiotics supplements contain distinct bacterial formulations, which are often not equal in terms of mode of action and, consequently, can provide a range of benefits and different outcomes. Moreover, probiotic strains present in commercial products targeted for aquaculture can be isolated either from aquatic or terrestrial environments, which can significantly impact the optimum ability of probiotic bacteria to colonise, multiply and thrive under farming conditions. Therefore, understanding the different mechanisms by which probiotics benefit prawn production is required for reliable decision-making when implementing effective probiotic strategies.

This presentation will discuss the current global trends in probiotics use in prawn aquaculture and how this could be applied to meet some of the rising demands for enhanced animal health and system performance. For this purpose, research by the CSIRO Livestock & Aquaculture Program will be used as an example to illustrate some of the different benefits of probiotics administration, including vibrio inhibition, activation of immune response, quorum sensing regulation, secretion of antimicrobial compounds, live feed production, feed fermentation and water quality improvement.

EFFECTS OF DIETARY LYSOPHOSPHOLYPIDS TO PARTIAL REPLACE OF FISH OIL ON GROWTH PERFORMANCE AND CHOLESTEROL STATUS IN PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Phospholipids are known to be components of lipoproteins which generally transport lipids, critical enhance higher amount of dietary cholesterol from digestive tract to circulatory system and especially work as emulsifier in shrimp feed. In addition, lysophospholipids are enzymatic hydrolysis of phospholipids and have a higher function of emulsifier to promote lipid utilization in shrimps. The objective of this present study is to investigate on the effect of lysophospholipids with partial replacement of fish oil on growth performance and cholesterol status.

The study is assigned in CRD with 4 treatments and three replicates. Four diets are supplemental with different levels of fish oil and lysophospholipid (Lypotech EC) in Table 1. Healthy white shrimp with initial mean weight of 1.551±0.03 gram/head are randomly 25 shrimp distributed to each aquarium of 0.53 × 1.22 × 0.37 meters (totally 12 aquariums) and the density is 140 shrimp/m^3. During the 8-week feeding trial, the shrimp are fed 12 % of their body weight per day at first week and calculated feeding from 10 to 5% every 2 weeks until the end of experiment. All shrimp are fed 4 times a day at 08.00, 11.00, 15.00 and 19.00. Shrimp are weighed every 2 weeks and then collected hemolymph and meat sample for study cholesterol status. The results in Figure 1 show that shrimp fed diets of lysophospholipids in T3 and T4 have higher average daily gain (ADG), specific growth rate (SGR) and lower feed conversion ratio (FCR) follow by T1 which has higher growth performance compares to shrimp fed negative diet with 1% of fish oil (T2) ($P > 0.05$). The amount of cholesterol in hemolymph of shrimp fed diet with lysophospholypids (T3, T4) in Figure 2 has an increase compares to negative control diet (T2) whereas cholesterol in shrimp meat has significantly lower ($P < 0.05$). Therefore, lysophospholipids 0.03 - 0.06% in fish oil reduction diets can improve growth performance and feed utilization under the proportion of 1:16-1:33.

Table 1. Diet description of shrimp feed formula

Treatment	Diet Description
1	Positive Control with 2% Fish oil
2	Negative Control with 1 % Fish oil
3	Negative Control with 1 % Fish oil + Lypotech EC 0.03%
4	Negative Control (NC) with 1 % Fish oil + Lypotech EC 0.06%

Figure 1. Growth performance of shrimp fed diets with different level of lysophospholipids (The bar chart shows the average means of each treatment)

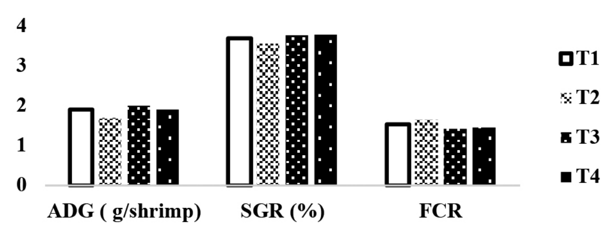
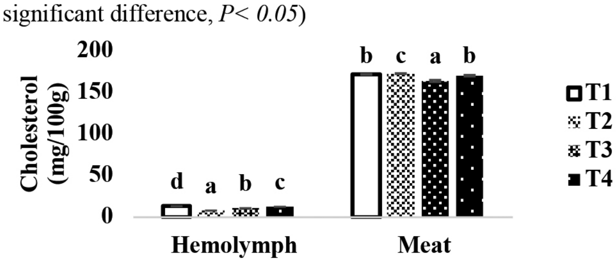


Figure 2. Cholesterol status of shrimp fed diets with different level of lysophospholipids (The bar chart shows the average means and standard deviation, alphabet characters show a significant difference, $P< 0.05$)



EFFECTS OF DIETARY METHIONINE AND TAURINE LEVELS ON GROWTH, TISSUE TAURINE CONCENTRATIONS AND TAURINE SYNTHESIS OF GIANT GROUPER, *Epinephelus lanceolatus*

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The study investigated the effects of dietary taurine (Tau) and methionine (Met) supplementation on growth, tissue taurine concentrations and taurine synthesis of giant grouper (*Epinephelus lanceolatus*) fed a diet with high plant protein. The basal diet containing soy protein concentrate (replaced 50% fishmeal protein) was supplemented with 0.25, 0.5, 1% Met or 0.1%, 0.2%, 0.3% Tau, respectively. Total of seven experimental diets were each fed to triplicate groups of fish (initial weight: 18.13 ± 0.05 g) in a recirculation system for 8 weeks. Weight gain of fish fed diets with 0.25% Met or 0.1%-0.3% Tau were higher ($p<0.05$) than that of fish fed the Met or Tau free control diet. Whole body, muscle, and hepatic Tau concentrations increased with the increment of dietary Tau levels. However, the tissue Tau concentrations were not affected by the dietary Met levels. Hepatic cysteinesulfinate decarboxylase gene expression was higher in fish fed diets with 0.25% and 0.5% Met than that in fish fed the control diet. Results indicated that dietary taurine and methionine supplementation can enhance growth performance of giant grouper fed the diet with high level of plant protein. Supplementation of methionine in diet enhances Tau synthesis gene expression but not enhances the tissue taurine concentration, suggesting that the ability of taurine synthesis in grouper is limited.

EVALUATION OF THE EFFECTS OF VARIOUS DIETARY LIPID AND CARNITINE LEVELS ON GROWTH AND BODY CHEMICAL COMPOSITIONS OF GIANT GROUPER *Epinephelus lanceolatus* FED THE PLANT-BASED DIET

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Carnitine is a key factor on metabolism of long-chain fatty acid in mitochondria of animals. It is also a limited nutrient in plant feedstuff. A 2×3 factorial designed study was conducted to evaluate the interaction of dietary lipid and carnitine levels on growth and body chemical compositions of giant grouper (*Epinephelus lanceolatus*) fed the plant-based diet. The fish meal protein was replaced by soy protein concentrate at 50% in diet. Two basal diets containing 12% and 18% lipid were each supplemented with L-carnitine at 0%, 0.03%, and 0.05%. Total of 6 experimental diets were fed to triplicate groups of juvenile grouper (initial weight: 11.09±0.14 g) in a recirculation system for 8 weeks. Weight gain, protein efficiency ratio, intraperitoneal fat content, and whole-body lipid content were significantly ($p<0.05$) affected by dietary lipid levels. These parameters were higher in fish fed the high lipid-diets than those in fish fed the low lipid-diets. However, the parameters were not influenced by the dietary carnitine supplementation. Carnitine concentrations in muscle but not in liver significantly increased by the increment of dietary carnitine levels. Regardless of dietary carnitine levels, muscle sum of n-6 fatty acids, linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) levels were higher but saturated fatty acids and C20-C22 fatty acids levels were lower in fish fed the high lipid diets. Results indicate that lipid but not carnitine in plant-based diet can improve growth and raise the tissue lipid content, C18:2 n-6 and C18:3 n-3 levels.

INVESTIGATING THE GENETIC PARAMETERS OF FILLET COLOUR IN VIETNAMESE STRIPED CATFISH, *Pangasianodon hypophthalmus,* USING HIGH-THROUGHPUT IMAGE ANALYSIS

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The production of striped catfish, *Pangasianodon hypophthalmus*, is amongst the largest aquaculture industries in Southeast Asia. Over 1.2 million tonnes per year are produced by aquaculture in Vietnam alone, with most production going to processed fillet export markets, including Europe, the USA and Australia. Consistent fillet quality is important and has a significant influence on farm-gate prices achieved by producers. For instance, undesirable fillet colour can reduce the farm-gate price by over 50% of its typical value. The most desirable colour for Vietnamese catfish fillets is white, although it is common to observe fillets with pinkish or yellowish tones. If fillet colour is under genetic control, it is possible to improve through selective breeding. This study has two main aims: 1) to develop an objective, rapid methodology for measuring fillet colour in *P. hypophthalmus*, and 2) use such measurements to estimate the genetic parameters (heritability, genetic correlations) in a commercial breeding population of *P. hypophthalmus*. To achieve this, digital images were taken of 5864 individual fillets, comprising 328 families over 4 spawning cycles. Each image was decomposed into different channels of the Red-Green-Blue (RGB) and LAB colour spaces and the average channel value was estimated for each fillet. Using the various channel values as phenotypes, heritabilities ranged from $0.18 \pm 0.03 - 0.47 \pm 0.04$ for the RGB colour space and $0.19 \pm 0.03 - 0.62 \pm 0.04$ for the LAB colour space. These preliminary results indicate genetic variation exists for fillet colour in *P. hypophthalmus* and therefore has potential to be improved by selection in ongoing breeding programs. Interpretation of digital image colour space channels in relation to the application for breeding for fillet colour is discussed. We also consider potential strategies for incorporating such an approach into routine genetic evaluation.

LABORATORY TANK CHALLENGE PROVIDES CONSISTENT GENETIC AND GENOMIC ESTIMATES OF RESISTANCE TO YERSINIOSIS IN ATLANTIC SALMON (*Salmo salar*)

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Consistent phenotyping methodologies are essential for accurate estimation of genetic variation to underpin genetic selection in breeding programs. Although some production traits (such as growth, shape and harvest quality) can be readily measured in the commercial production environment, estimation of disease survival traits can be problematic as farmers need to treat outbreaks early to optimise animal welfare and to minimise production losses. Controlled challenge in bio-secure facilities allows small test populations with known genetic structure to be tested with a pathogen. The present study was carried out to develop a challenge methodology that could be utilised to reliably produce ~70% mortality in populations of ~1,000 fish for estimation of genetic parameters of yersiniosis disease response.

Yersiniosis is a bacterial disease caused by the enterobacterium *Yersinia ruckeri*, which is endemic in cool temperate waters wherever salmonids are found. Although largely controlled by vaccination strategies in Tasmania's Atlantic salmon industry, the disease can still require treatment in freshwater hatcheries or soon after marine transfer of smolt.

Previously published studies have largely relied upon bath exposure to *Y. ruckeri* concentrations of 10^5 to 10^8 CFU/ml to cause yersiniosis in 2 – 5 g unvaccinated Atlantic salmon. We exposed ~31 g salmon to 10^6 – 10^7 CFU/ml immersion challenge in a biosecure fish challenge facility, but produced ≤ 17% mortality. At higher dose (10^7 – 10^8 CFU/ml) higher losses occurred but results were inconsistent. We then decided to run a series of preliminary intraperitoneal (IP) injection trials until we established an IP injection dose resulting in close to the targe mortality rate.

Resistance to yersiniosis was then assessed by exposing fish of known pedigree from two consecutive year classes to *Y. ruckeri* by IP injection, and recording challenge test survival. Fish showed clinical signs of yersiniosis and cumulative mortality reached 72.7- 72.9%. Pedigree analysis of binary survival (TS) and continuous days to death (DD) confirmed that mortality to yersiniosis is a heritable trait (h^2 = 0.24 and 0.29, respectively) and that consecutive year classes were highly correlated (r_g = 0.99), evidence of the development of a robust challenge model. Genomic analysis revealed the feasibility of genomic selection to improve resistance to yersiniosis in Tasmanian Atlantic salmon.

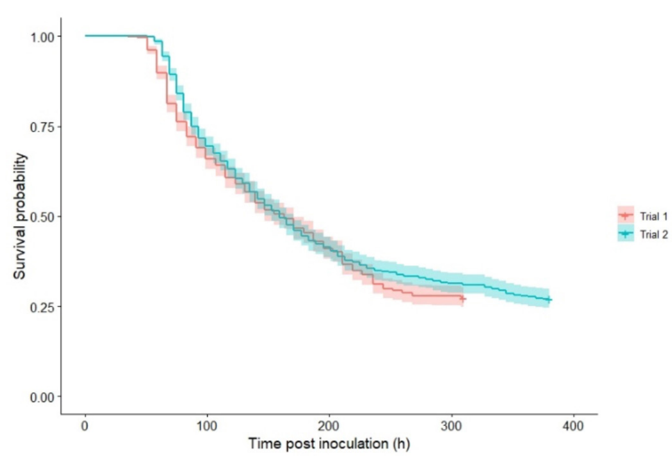

Figure 1: Kaplan-Meier mortality curves of yersiniosis challenge experiments. Trial 1 (2016 YC) was IP inoculated with a viable dose 1.95 × 105 CFU/fish. Trial 2 (2017 YC) was inoculated with a viable dose of 1.18 × 104 CFU/fish. Shaded areas indicate confidence

ESTABLISHMENT OF A FAMILY-BASED SELECTIVE BREEDING PROGRAM FOR *Crassostrea gigas* IN THE PACIFIC NORTH-WEST OF THE USA GENETIC PARAMETERS FOR PRODUCTIVITY AND CONSUMER TRAITS FOR *Crassostrea gigas* IN A COMMERCIAL BREEDING PROGRAM IN THE PACIFIC NORTH-WEST OF THE USA

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The production of the Pacific oyster, *Crassostrea gigas*, is an established industry along the west coast of the USA. Historically produced for shucked meat markets, there is a growing shift towards hatchery-produced single-seed culture of Pacific oysters for the live or half-shell market. Here we describe the development of a commercial-scale family breeding program for *C. gigas* in the US Pacific northwest. A starting point was to understand the nature of the genetic variation for this population and determine how that influences the variation in commercial traits and genetic variation was clearly explained by the additive genetic model. As part of this process, genetic parameters (heritabilities and genetic correlations) were calculated for key productivity and consumer traits. The traits under investigation were total weight, meat yield, shell shape (height and width index), hook angle and general field survivability. Between 2017 and 2021 the program successfully produced 424 full-sib families across 5 annual spawning cycles using a 2x2 mating design. Full-sib families were deployed to 12 separate field trials across 3 different sites within the Puget Sound (Bay Center, Olyview and Discovery Bay) to evaluate performance and survival. Heritabilities were calculated from variance components estimated using a mixed animal model and were as follows - Total weight: $h^2 = 0.31 \pm 0.04$; Width Index: $h^2 = 0.43 \pm 0.04$; Meat yield: $h^2 = 0.33 \pm 0.04$; Hook angle: $h^2 = 0.45 \pm 0.05$; Height index: $h^2 = 0.42 \pm 0.04$; and Survival: $h^2 = 0.15 \pm 0.02$ (underlying scale). All traits show scope for improvement through selection, however, genetic correlations (r_g) amongst traits ranged from moderately negative to moderately positive, indicating the importance of developing an appropriately weighted selection index in the future. For a given trait, r_g between sites was generally highly positive, indicating limited genotype by environment interaction across the trial environments. We highlight the importance of accounting for the origin of breeding program founders (i.e. genetic group) when estimating genetic parameters and breeding values (EBV), and demonstrate the presence of baseline genetic differences amongst founder broodstock groups, and present data showing the gains made relative to these founders.

A RELIABLE SET OF WELFARE INDICATORS FOR FARMED RAINBOW TROUT *Oncorhynchus mykiss* WITH PROVEN INTRA- AND INTER-OBSERVER RELIABILITY

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Today, more than 160 welfare indicators for farmed fish are described. In most species-specific welfare assessment systems, each indicator is assessed based on a uniform ordinal scale (mostly 3-6 scores) of identical numbers for all included indicators. Generally, each welfare indicator needs to meet the criteria of being valid, reliable, and practicable for each application to a specific species and production system. Reliability is crucial when developing and using welfare assessment systems. However, almost none of these indicators have been tested for reliability in rigorous scientific studies. We evaluated a set of 25 welfare indicators of different complexity for rainbow trout under laboratory and farm conditions. The set of indicators covered aspects of farm management, husbandry measures, resources, fish behavior, stunning and killing during slaughter, as well as fish health-related topics. Assessment systems were specified for each indicator. All indicators were statistically tested for intra- and inter-observer reliability.

The study consisted of four distinct phases: 1. A methodical review of all relevant welfare indicators and corresponding classification systems for rainbow trout; 2. Pre-Test of welfare indicators and corresponding assessment-systems according to the literature on aquaculture farms; 3. Elaboration of a survey scheme for the indicators and corresponding assessment systems regarding practicability, specific production systems, and corresponding welfare needs; 4. Training, and testing of welfare indicators under laboratory and farm conditions.

For statistical validation, we calculated relative agreement, as well as Gwet's AC1, and the Brennan-Prediger agreement coefficients. We used 0.61 as the minimum statistical benchmark for reliability in AC1 as well as in the Brennan-Prediger agreement coefficient.

After training, observers were able to reliably assess each indicator on 10 different farms and more than 200 individual fish. Indicators based on binary assessment systems generally achieved reliabilities of ≥ 0.95. Indicators based on a 3- and 4-score assessment system achieved reliabilities of ≥ 0.69. Fin condition, being the only indicator based on a 5-score assessment system, varied between 0.69 and 0.92.

In general, there was a slight negative trend between field assessment when compared to laboratory assessment. In addition, observers achieved higher reliability as they gained more experience.

In the current study, we have documented a statistically validated set of operational welfare indicators for farmed rainbow trout, based on indicator-specific assessment-systems, tested in traditional pond- as well as raceway systems.

OF RELIABILITY, COMPLEXITY, AND PERSPECTIVE: THE CASE OF FIN CONDITION AS A WELFARE INDICATOR IN FARMED FISH – RESULTS FROM A DOUBLE-BLIND STUDY ACROSS DIFFERENT STAKEHOLDER GROUPS

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Fin condition is a widely accepted welfare indicator for farmed salmonids and a variety of other farmed finfish species. To assess fin condition, several different assessment systems have been proposed. Some of these systems are fairly simple, while others are more complex and sophisticated. What these systems all have in common, is that they assess fin condition based on ordinal scales. However, little is known about the relationship between the complexity of the assessment system, and its repeatability in terms of intra- and inter-observer reliability. To obtain insight into the relationship between complexity, repeatability, and reliability, we conducted a large-scale image-based double-blind study about the fin condition in farmed rainbow trout.

Four different questionnaires, reflecting four different assessment systems (3 to 6 scores) were distributed to 499 stakeholders of the German-speaking aquaculture sector (Germany, Switzerland, Austria). Each questionnaire consisted of 25 high-resolution color images of the tail fin of farmed rainbow trout of regular marketing size. Each picture was duplicated and randomly placed twice within the questionnaires. The questionnaires could be filled electronically and an automatic return button was implemented on the last page. We used relative agreement and Gwet's AC1 agreement coefficient to calculate reliability within each participant (intra-observer) as well as between participants of the different groups (inter-observer).

A return rate of 15.9% was accomplished. Across all received questionnaires we identified four relevant groups. 1. fish farmers; 2. fish health services; 3. scientists; 4. others. Across all groups, we identified a negative relationship between the complexity of the assessment system and the ability of the participants to allocate duplicated pictures into the same category.

When analyzing participants combined, the data suggested a negative exponential relationship between complexity and reliability (Figure 01).

Two of the groups, fish health services, and scientists, were able to allocate the duplicated pictures repeatedly with statistically proven accuracy (AC1 0.67-0.87). However, the reliability of these two groups dropped below the statistical benchmark (agreement coefficient ≥0.61), when the assessment system was based on more than 4 scores.

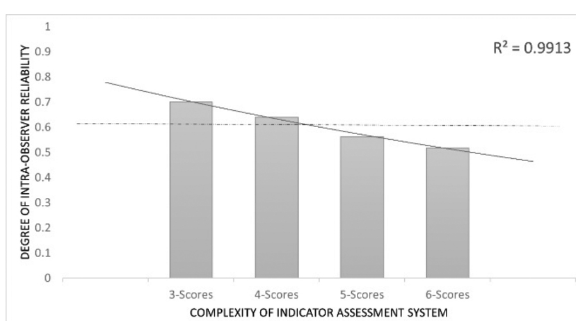

Figure 01: The negative exponential relationship between the complexity of the welfare indicator assessment system and the calculated intra-observer reliability. The dotted line shows the minimum benchmark for statistically reliable agreement of 0.61.

HIGH-THROUGHPUT PHENOTYPES AND GENETIC PARAMETERS OF CARDIAC TRAITS IN TASMANIAN ATLANTIC SALMON (*Salmo salar*)

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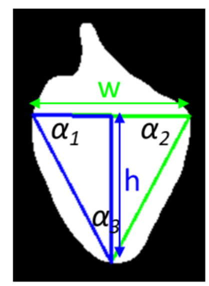
Maximising animal health and welfare is an important breeding objective for aquaculture industries. Cardiac morphology (ventricular size and shape) and epicardial fat (EF) coverage are known to be associated with the health and performance of Atlantic salmon. The degree of EF coverage in salmon is currently measured using a visual scoring system (Grade 1 to 3), which is a labour-intensive, and subjective categorical trait which lacks the precision and resolution of a truly quantitative trait. This can be limiting for statistical analyses and genetic evaluations. The aims of this study were therefore to 1) evaluate the use of image analysis to estimate EF percentage (EF%) and other cardiac morphometric traits in Atlantic salmon, and 2) estimate the genetic parameters of these cardiac traits.

Phenotypic development:

Ground truth measurements of EF% were obtained from 71 salmon hearts with a wide distribution of EF coverage using computer tomography (CT). The same 71 hearts were imaged in four planes (right and left lateral, caudal, and dorsal) using a digital SLR camera. Image processing involved background removal, contrast enhancement, and Gaussian filtering. EF% was then estimated using 1) grayscale conversion and binary thresholding, and 2) calculation of the average value of each of the red, green, and blue colour channels. Cardiac morphometric traits (heart size, ventricular height to width ratio, and internal heart angles) were estimated from a binary silhouette of the dorsal view.



Internal angles ($\alpha_{1\text{-}3}$) and ventricular width (w) and height (h)

We found that the best prediction of EF% (r = 0.80) was achieved using data from 3 images (left and right lateral and caudal views) and binary thresholding. Best results from a single image were achieved using binary thresholding applied to the dorsal view (r = 0.75).

Genetic analysis:

A categorical EF score and an image of the dorsal aspect of the heart were available for each of 1071 animals with known pedigree. Genetic parameters were estimated for heart size, EF%, EF score, ventricular height to width ratio, and the three internal heart angles (α_1, α_2 and α_3), using univariate (variance components and heritability) and bivariate (phenotypic and genetic correlations) animal models.

We found that all traits except the angle α_1 ($h^2 = 0.04 \pm 0.04$) were moderately heritable ($0.16 \pm 0.05 \leq h^2 \leq 0.37 \pm 0.07$). Encouragingly, the genetic correlation between image-predicted EF% and categorical EF score was 0.98 ± 0.03, indicating that from a breeding perspective they are the same trait. Furthermore, the heritability of the image-predicted EF% was greater than that of the manual heart fat score ($h^2 = 0.28 \pm 0.04$ and 0.32 ± 0.07, respectively), suggesting that the more quantitative phenotyping approach captures more of the genetic variation of the trait. This result coupled with the possibility of evaluating other heritable cardiac traits leads us to conclude that image analysis is a valuable high-throughput phenotyping method for measuring cardiac traits in Atlantic salmon.

MAKING WAVES: ADVANCEMENTS IN AQUACULTURE WELFARE

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Welfare is an ancient concept, although was almost exclusively applied to humans until the late 20th century. Early philosophy, science and practice highlighted a difference between humans and animals – with animals only seen as being there to serve a purpose to humans. Darwin and others started to highlight the emotions of animals, but it was another 100 years – linked to the industrialisation of animal farming – before welfare really started to be of concern to scientists and consumers. Of course, many farmers of land animals will readily tell you that for generations they have known that happy animals are more productive, but welfare in aquaculture is a very recent, but already growing, discussion. Animal welfare is understood in many ways in different countries; how actively they move, how well they feed, how healthy they look. The initial focus in aquaculture was on salmon, aiming to address issues during culture and slaughter. Considerations around health have long been tied to welfare, but wider issues are now considered. Recent work on eyestalk ablation in shrimp has helped to highlight welfare issues in other species and right along the production chain. The science demonstrates to shrimp hatcheries and producers that better fecundity and growth can be expected when shrimp don't have their eyes chopped off to stimulate reproduction.

Today we see innovators highlighting welfare in many aquaculture species, including carp and octopus. During this presentation we will discuss how these innovators are promoting technical solutions and good practice to farmers and processors. In the production environment, appropriate stocking density is a key consideration, but water quality of the growing environment is also highlighted. As buyers increasingly demand high-welfare across supply chains, how can farmers and processors access the latest information or demonstration their understanding of and compliance with those requirements? Data-driven farming is helping to open up opportunities for improvement in all aspects of aquaculture and the potential for greater transparency should also lead to better production environments, health and ultimately welfare. Training programs are provided by some retailers who are keen to ensure that farmers in their supply chains are well-informed in order to comply with demands. Other companies are providing on-line training for farmers wanting to educate and differentiate themselves. Further still, some organisations are providing equipment, e.g., electrical stunning machines, to producers and processors in order to provide real-time demonstration of the value and ease of shifting to better welfare practices.

WHAT IS THE CIRCULAR ECONOMY, HOW DOES IT RELATE TO SUSTAINABILITY AND WHY DOES AQUACULTURE HAVE A NUMBER OF ADVANTAGES IN THIS SPACE?

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Exactly who first coined the term "circular economy" is not entirely clear, but the concept has certainly been prevalent in business thinking since the early 1990s. It would seem to have its philosophical roots in the environmental movement inspired by Rachel Carson's "Silent Spring" and the "limits to growth theory" from the 1970s. The objective of the circular economy (CE) is to demonstrate the economic viability of a business whilst at the same time optimising the use of resources and minimising waste. Originally the concept was applied in relation to waste management, but as the impacts of climate change have become increasingly apparent the CE model has really taken hold as a means to measure and compare business performance, and differentiate companies and production strategies.

The need to understand emissions and manage carbon footprints has extended the application from just closed loop waste management to a more comprehensive understanding of inputs and outputs in the production cycle, and even to the broader supply chain. This has seen the application of new assessment approaches and modelling tools to quantitatively evaluate these processes and interactions (e.g. material flow analysis, life cycle analysis and product/ organisation environmental footprint analysis).

To date, most CE assessments only focus on a subset of sustainability criteria but this is improving with each iteration. Sustainability should capture not just environmental dimensions, but also social and economic responsibilities, and increasingly the ethical intention (purpose) of business is being called to account. Sustainability is more complex than just choosing eco-friendly packaging options or switching to a renewable energy source, and governance and consumer expectations are increasingly reflecting that.

The aquaculture sector has some clear advantages in that it can already demonstrate safe and sustainable circular economy practices such as:

- Reuse/ recycling of key inputs and outputs (raw and waste material).
- Sustainable alternatives to complex waste materials (e.g. packaging).
- Integrated multi-trophic aquaculture.
- Fully integrated business models and bioproducts potential.

THE INFLUENCE OF MALE FERTILITY ON SPAWNING PERFORMANCE AND LARVAL SURVIVAL DURING BARRAMUNDI *Lates calcarifer* MASS-SPAWNING EVENTS

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Barramundi (*Lates calcarifer*) has a mass-spawning reproductive strategy whereby adults synchronously release their gametes into the water column. In captivity, this spawning strategy usually results in highly skewed family sizes, hindering the effectiveness of selective breeding programs. As high inter-male variation in sperm quality was observed in previous studies, we investigated the relationship between sperm quality and fertilization success and early larval development to provide a better understanding of broodstock contribution during mass-spawning events.

To do so, we assessed the fertility of male barramundi (n = 22) from three different breeding cohorts. The physical condition of broodstock was recorded, including body weight, total length, and condition factor (K). Milt samples were collected through testicular cannulation, and semen volume, and the concentration and total count of spermatozoa determined. Sperm quality was assessed using computer-assisted sperm analysis for motility and flow cytometry for sperm integrity (i.e. Hoechst/PI viability assay and TUNEL DNA fragmentation assay). Mass-spawning events were induced using intramuscular injection of luteinizing hormone-releasing analog. Broodstock spawned on two consecutive nights after the injection. Eggs at 2.5 h and 12 h post-fertilization (hpf), and larvae at 24 h and 48 h post-hatch (hph) were collected to assess spawning success and survival. Offspring collected at 2.5 hpf and 24 hph were also genotyped using microsatellites for parentage analysis.

In this study, we observed highly skewed paternal and maternal contributions in all spawns resulting in a loss of genetic diversity and increased inbreeding rates in offspring in agreement with previous studies. However, three key insights were found. Firstly, males with higher physical conditions and higher sperm motility also had the highest level of sperm DNA damage. Secondly, sperm DNA damage correlated negatively with offspring survival rates ($r(22) = -0.67$, $P < 0.001$) for the first, but not the second, spawning night. Lastly, it was determined that male fertility accounted for up to 33% of the total variation in mass-spawning events. This study highlights the importance of male fertility to spawning outcomes and the need for advanced sperm quality assessment during the selection of broodstock. However, these results also raise further questions about male-female spawning behaviour and gamete quality. Notably, further study is required to unravel the causes of sperm DNA damage. These results also support the need to further develop advanced reproductive technology in barramundi to precisely control the breeding process for selective breeding programs.

REVEALING THE MOLECULAR DRIVERS BEHIND GOLDEN COLOURATION IN BARRAMUNDI *Lates calcarifer*: A TRANSCRIPTOME APPROACH

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Skin colouration in many aquaculture species is an important trait, particularly where rare colour phenotypes have high economic value. The wild-type skin phenotype of barramundi (*Lates calcarifer*) is typically silver and slightly darker dorsally. However, golden (xanthic) variants are very rarely but occasionally oserved. These golden variants are of interest to not only the aquarium trade, but also to aquaculture as golden barramundi exhibit lower levels of "greyness" in their flesh – a characteristic that often is a negative consumer attribute. The genetic basis of the golden variant is unknown. To understand the possible genetic and molecular mechanisms underlying this phenotype, we analyzed the transcriptomic differences between the golden and wild type colour variants.

RNA was extracted from the dorsal fin of 5 wild type (WT) and 5 golden (G) barramundi. Pair-end sequencing was undertaken on an Illumina NovaSeq S4 Lane, 300 cycles, yielding 150 bp reads. The average raw reads for the WT were 115,181,698 and 104,918,905 for the G samples. Raw reads were analyzed, cleaned, and mapped to the *L. calcarifer* genome. DEseq analysis revealed 2,409 Differentially Expressed Genes (DEG) between colour variants, with 58.57% of DEG being upregulated and 41.39% being downregulated (Figure 1A).

Gene Ontology (GO) analysis revealed that the circadian cycle, tyrosine metabolism, xanthophore associated genes and melanogenesis were within the top pathways that differed between WT and G. Additionally, analysis of normalized counts of selected genes was conducted, alongside the protein-protein interaction network between DEG. From this, a direct link between melanogenesis, the circadian cycle, and xanthophore associated genes was established, with genes known to be involved in melanogenesis and to produce proteins present in melanocyte melanosomes, playing a critical role in linking those processes. Thus it appears that these genes are implicated in the colouration of the golden variant.

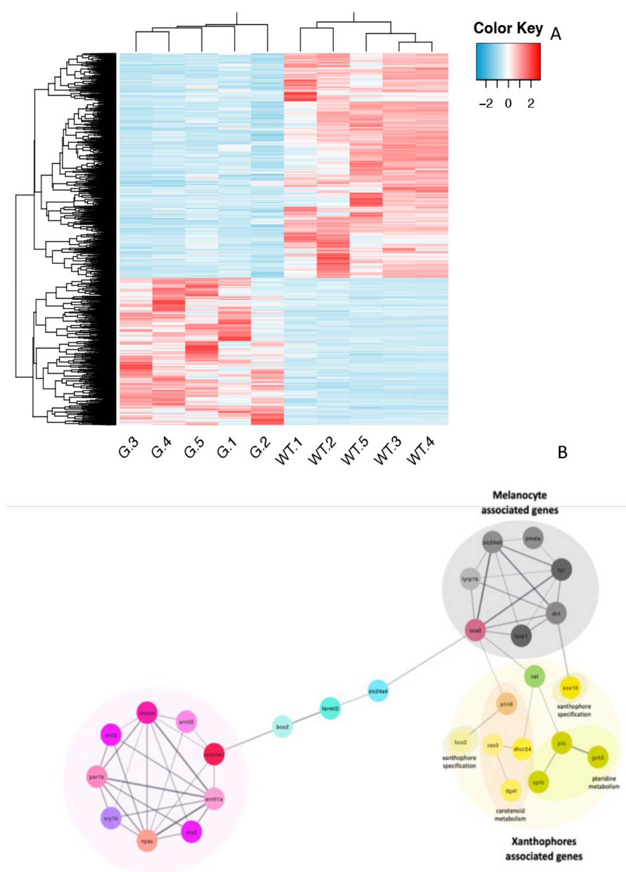

Figure 1: A: the Heatmap representing the Z-score of the Differentially Expressed Genes between wild type and golden. B: the protein-protein interaction and network between of selected Differentially Expressed Genes.

SUPPORTING WELL-BEING THROUGH MENTAL HEALTH AND PSYCHOLOGICAL SAFETY INITIATIVES

Jo Marshall

During this interactive session attendees will be taken on a journey to deepening understanding of well-being in the workplace. The session will be led by Jo Marshall who will share the story of success of key programs in Seafood including a Culture of Safety Program at the South Australian Research and Development Institute and the National Seafood Industry Mental Health Program – Stay Afloat. The Stay Afloat Program recently received a significant boost with Australia's Federal Government committing to a significant expansion and extension for two years. A fully immersive workshop will see participants develop knowledge on key aspects of mental health and how mental health intersects with physical and psychological safety at work. From the basics of looking after ourselves, to the role of team and leadership in creating a mentally healthy and safe workplace, through to a highly interactive introduction to the innovative world first industry led Sensemaking program, audience members will be engaged at every stage. Expert panellists from across the Seafood Industry will help guide participation through sharing their experiences and learnings along the way. Panellists will include:

- Taryn Richards – Owner – Humpty Doo Barramundi
- Veronica Papacosta – CEO – Seafood Industry Australia
- Katherine Winchester – CEO – Northern Territory Seafood Industry Council
- Rikki Chesson – National Workplace Health and Safety Leader – Paspaley Pearling Company
- Lauren Thornton – Extension Officer – Fisheries Research and Development Corporation
- Heidi Mumme – President – Women in Seafood Australasia

Participants will leave not only with a greater understanding of the work that is being done in Australia, but methods of evaluation, emerging technologies, and most importantly practical tools to action for their own wellbeing and safety and that of their teams.

DEVELOPING A BREEDING OBJECTIVE FOR SURVIVAL, SHELL GROWTH AND PEARL SIZE TRAITS FOR THE SILVER-LIP PEARL OYSTER, *Pinctada maxima*

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The pearling industry is economically important to northern Australia but has recently been plagued by mass-mortality events from an unknown disease agent, along with financial impacts from global downturns. There has been active research into the potential for selective breeding to increase the survival and overall profitability of the industry; however, efforts have been primarily focused on estimating genetic parameters and developing the knowledge and genomic tools useful for a genetic improvement program, rather than into definition of breeding objectives and indexes. Considering the effect of the environment, market traits, profitability and the current genetic knowledge on a silver-lip pearl oyster cohort, we developed a breeding objective and associated index that permits the selection of candidates based on commercial traits (see process in Figure 1).

The breeding goal is defined by the final product to be commercialised (e.g. larger pearls of gem quality). Since all pearls are produced from seeding a nucleus into an oyster, the size of a pearl is influenced not only by the size of the oyster, but also by the size of the nucleus seeded. Thus, selecting for large oysters at seeding time will influence pearl size. One way to achieve this is to focus on genetic improvement of growth and survival of oysters themselves. With a well-defined breeding goal, important traits can be determined via selection index theory. Using morphometric traits and the size of nucleus seeded, we developed a breeding index with an accuracy of 0.43 for individuals based on their own performance and where adding information from their relatives can increase the accuracy to 0.8.

The Australian pearling industry could benefit greatly from the implementation of advanced genetic breeding programs for silver-lip pearl oysters focusing on shell growth and survival as well as pearl quality

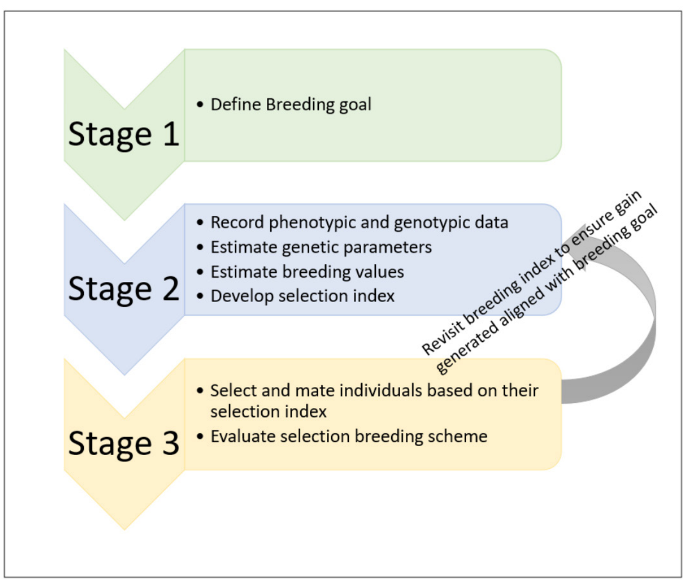

Figure 1. Adapted from Veerkamp et al. 2013

ENHANCING FARM MANAGEMENT PRACTICES: A CASE STUDY OF IMPROVING DECISION SUPPORT TOOLS FOR MANAGING THE RISK OF SEA LICE INFESTATION IN SALMON FARMS WITHIN FARM MANAGEMENT AREAS

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Aquaculture is a major industry worldwide and is a significant contributor to the global seafood supply. However, the industry is facing several challenges, one of which is sea lice infestation, which affects the health and growth of farmed fish resulting in significant economic losses for the industry and negative environmental impacts. Greater understanding of sea lice infestation rates and response to treatment will enhance farm management practices to reduce losses and costs to the industry.

The study was conducted in a salmon farming region in Scotland, where sea lice infestation is a significant problem. The goal of the study was to develop a decision support system (DSS) designed for aquaculture operators to enhance farm management practices and reduce levels of sea lice infestation (Figure 1). Data from the Scottish Environmental Protection Agency (SEPA), including sea lice monitoring data and farm management data including treatment records were incorporated into the DSS with the aim of assist farmers in making informed decisions about sea lice management. The DSS also incorporated results from a 3D hydrodynamic model (TUFLOW FV) that was coupled with a biologically responsive particle transport model (PTM) and calibrated using farm data from SEPA.

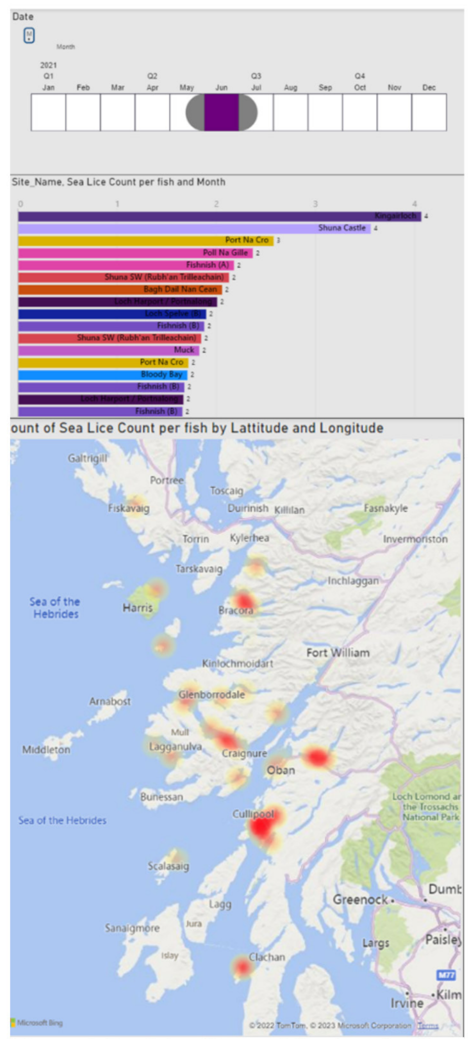

Figure 1 Decision Support System for Sea Lice Counts

The DSS was designed to provide farm managers with up-to-date information on the risk of sea lice infestation, based on environmental and farm management data within the wider domain and encompassing all farm operators. The DSS additionally provided model output data from management scenarios by adjusting the timing and use of specific treatments (both physical and chemical) and assessing the impact on predicted sea lice counts.

Several challenges associated with predicting infestation risk in areas with high connectivity between farms could be improved with further refinement of the DSS tool to include higher frequency and refined data. Despite these challenges, the proof-of-concept model demonstrated the potential of the DSS in enhancing farm management practices and reducing the risk of sea lice infestation, and identified the need for collaboration between farmers, researchers, and regulatory authorities to address these challenges.

SUSTAINABILITY CERTIFICATIONS, GuidANCE FROM A CERTIFIERS PERSPECTIVE

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The sustainability of seafood products is becoming more important to buyers and consumers. Producers and retailers are increasingly wanting to clearly identify products that they can demonstrate are produced through legitimate harvest and production with minimal impact on the environment. Seafood certification programs like the Aquaculture Stewardship council (ASC) provide one such mechanism.

Regulators focussing on monitoring, control and surveillance have an important role to play, however, voluntary sustainability standards like ASC can have a more positive and wide-reaching impact as they are effective in the marketplace, providing direct positive reinforcement to producers.

Drawn from our group's collective experience in the seafood certification industry we will explain the assessment and certification process based on real examples at a level that will allow;

- Producers and supply chain actors to assess operational characteristics and market environments that lend themselves to the successful adoption of such schemes;
- Producers and supply chain actors to prepare their operations, staff and stakeholders, in order to most effectively and efficiently navigate the process;
- Regulators and policy makers to understand their role, and how to effectively support the industry in certification aspirations, and
- Industry and government to understand the ways to enhance the benefits and values of certification.

CLIMATE RISK, FINANCIAL DISCLOSURES AND AUSTRALIAN AQUACULTURE

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Climate change and its impacts on business and the global economy is an existential consideration for governments, regulators, investors and businesses alike. This is especially so for the aquaculture sector which faces profound risks due to a changing climate and regulatory landscape. Increasingly, innovative companies are assessing the risk of climate change on their businesses and developing strategies to both minimise impacts and to identify opportunities.

Regulators are also responding by requiring an increasing level of disclosure regarding climate change risks. Governments are reporting their National Determined Contributions to meet their Paris Agreement commitments of limiting global warming to 1.5°C. Under Australian Corporations Law, directors who ignore climate risk could be guilty of breaching their duty of care. The Australian Stock Exchange's (ASX's) 4th edition of Corporate Governance Principles and Recommendations requires companies to disclose risks associated with environmental risks that are material to them which include climate change, and if not, to explain why they have elected not to make such a disclosure.

In December 2022, the Australian Treasury released its Climate-Related Financial Disclosure Consultation Paper for public engagement on the design and implementation of standardised, internationally-aligned requirements for the disclosure of climate-related financial risks. The Paper proposed that reporting would initially align with the recommendations of the Taskforce on Climate Related Financial Disclosures ("TCFD"), later transitioning to align with the climate disclosure standards currently being developed by the International Sustainability Standards Board ("ISSB") when they become available for jurisdictional adoption.

The TCFD provides guidance on the way in which companies should identify, assess and manage climate-related risks and opportunities. The TCFD Recommendations have been endorsed by several peak financial bodies and regulators, including the Reserve Bank of Australia (RBA), the Australian Prudential Regulation Authority (APRA), the Australian Securities and Investments Commission (ASIC), the ASX Corporate Governance Committee and the Australian Accounting Standard Board.

The Australian Aquaculture sector will need to consider how to respond to these various reporting requirements and guidelines to allow sufficient time for the collection of data and the drafting of disclosures to meet the disclosure requirements.

This study provides:

- an overview of the climate-related risks and opportunities for Australian aquaculture;
- an independent high-level review of aquaculture listed companies climate change statements and disclosures; and
- a broad-brush assessment considering demand and supply-side climate risks for the sector.

With the climate disclosure reforms on the horizon, it is crucial for Australian aquaculture companies to assess their current reporting practices and frameworks to determine their readiness to disclose climate-related risks based on the TCFD framework (if not already implemented) and eventually the standards set forth by the ISSB. Additionally, it is recommended that companies and their directors and officers evaluate their internal and external audit, assurance, and verification capabilities to ensure compliance. By taking these measures, Australian aquaculture companies can effectively address climate-related risks and improve their transparency and accountability to stakeholders.

KUMUOLA: A SOURCE OF KNOWLEDGE; TRADITIONAL HAWAIIAN FISHPOND CAPTURES MAGNITUDE AND TEMPORAL PATTERN OF AN INTRODUCED MULLET INVASION, *Osteomugil engeli,* THROUGH STUDENT GENETIC BARCODING RESEARCH

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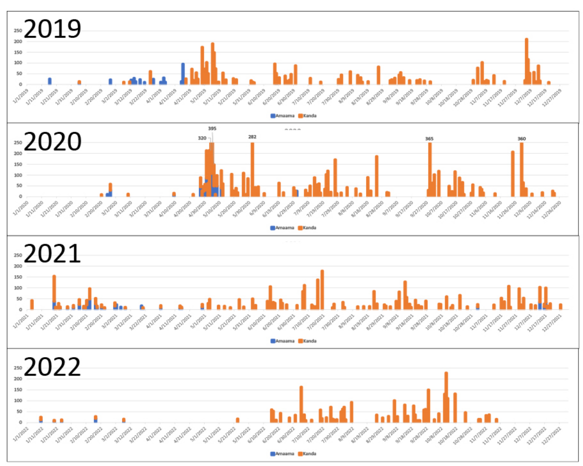
The Kumuola Marine Science Education Center (Kumuola) is a program of Kamehameha Schools Hawaiʻi that centers learning around the rehabilitation of three *loko iʻa* (traditional Hawaiian fishpond systems) in Hilo, Hawaiʻi. Loko iʻa are engineered aquaculture systems designed to capture and slow down land-derived nutrients carried by surface and subsurface waters to the shorelines as a means to promote nearshore primary productivity and support a web of primarily herbivorous fish. Between their initial construction ~800 years ago to present, these dynamic systems have faced a number of challenges to include diversion of fresh water, urbanization, invasive species introductions, land use and tenure changes, and reductions of native fish stocks. Increasing actions to rehabilitate and care for this traditional aquaculture practice is growing in Hawaiʻi through a diversity of organizations and community efforts. Understanding the contemporary functioning of loko iʻa through scientific observation and recordation is leading to new understandings of both the challenges and opportunities that theses spaces face to once again become sources of pride and protein within their community.

Figure 1. *O. engeli* and *M. cephalus* juvenile recruitment, 2019 to 2022



DNA POOLING AND LOW-DENSITY SNP GENOTYPING FOR GENETIC EVALUATION OF AQUACULTURE SPECIES

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DNA pooling and low-density SNP genotyping platforms offer promise as an effective and cost-efficient method for genetic evaluation of aquaculture species, particularly in situations where individual family rearing is not feasible or practical. This method involves combining tissue samples from multiple individuals and genotyping them as a single sample. The objective of this study was to estimate the accuracy of the genetic evaluation based on breeding values using DNA pooling on a simulated low-density SNP tool.

We tested this method using simulated data of 4,000 individuals from 40 different families, assuming a single trait with a heritability of 0.30. A 5 percent error rate in genotyping was assumed. We tested different SNP densities ranging from 100 to 500 and two different pool sizes, 10 and 20 individuals per sample. We pooled individuals by phenotype, except for the top-performing 200 individuals from the tail of the phenotypic distribution, which had individual (non-pooled) simulated genotypes. These mimics realistic scenarios in aquaculture breeding.

Our results show that the accuracy of assigning individuals genotyped in pooled samples to their family of origin ranged from 0.53 to 0.91, with the highest accuracy achieved with a pool size of 10 and 500 SNPs. We also estimated the accuracy of the estimated breeding values (EBVs) from the pooled data, which ranged from 0.69 to 0.95, with the highest accuracy achieved when the pool size was 10 with 500 SNPs. Based on these simulated data, DNA pooling coupled with low-density SNP genotyping appears an effective and economical approach for some aquaculture selective breeding programs.

POWERFUL INSIGHTS INTO NUTRITIONAL AND MOLECULAR RESPONSES IN SHRIMP AND SALMON USING PROTEOMICS AND METABOLOMICS

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Proteomics and metabolomics are powerful tools used to determine the effects that environmental stimuli exert upon aquaculture organisms and the importance of this for growth, immunity, and resilience. Here, proteomics and metabolomics were used to identify altered molecular pathways in response to diet in shrimp (haemolymph and hepatopancreas) while proteomics was used to monitor biomarkers in liver of heat stressed Atlantic salmon.

Here, hepatopancreas proteomics and haemolymph metabolomics were used to investigate the post-prandial response of black tiger shrimp (*Penaeus monodon*) fed a conventional fishmeal diet (FM); a diet supplemented with the microbial biomass Novacq^{TM} (NV); krillmeal (KM); or, fasted (FS). NV fed shrimp showed preference for energy derived from carbohydrates indicated by a strong signature of glycoconjugate metabolism and activation of the amino- and nucleotide sugar metabolic pathway. KM activated the glyoxylate and dicarboxylate pathway that denoted shrimp preference for lipidic energy. FS shrimp displayed down-regulation of oxidative phosphorylation and resorted to internal lipid reserves for energy homeostasis displaying a strong signature of autophagy.

In Atlantic salmon *Salmo salar*, a 45-peptide targeted proteomics method was used to measure biomarkers for heat stress in liver of salmon exposed to gradual temperature increases from 15°C to 20°C. Abundance of two different serpin peptides significantly increased as the temperature increased to 17°C, 19°C and 20°C compared with a control group maintained at 15°C.

Combined proteomics and metabolomics revealed heterogeneity in shrimp pathway activation for energy use and identified N-acetyl D-galactosamine as a unique feature in shrimp fed Novacq^{TM}. In salmon, two serpin peptides showed the greatest potential as heat stress biomarkers that could help monitor the effectiveness of feed additives for maintaining salmon welfare at high temperature

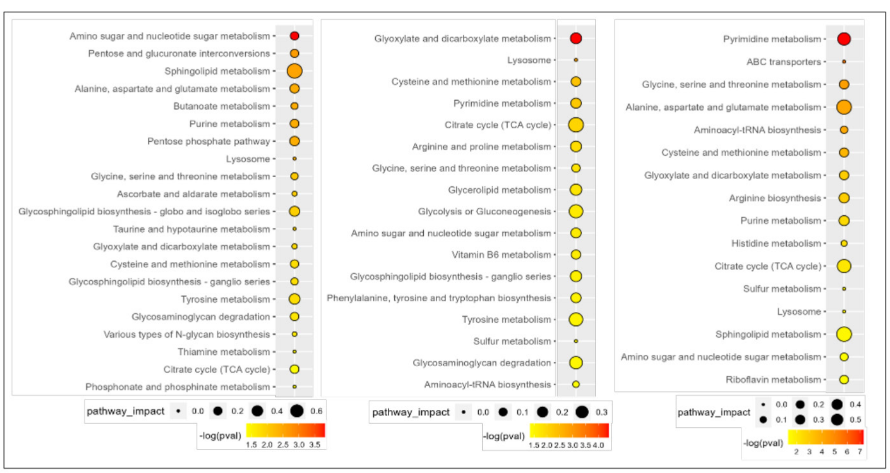

Figure 1. Stimulated pathways in shrimp fed Novacq, Krillmeal and unfed shrimp

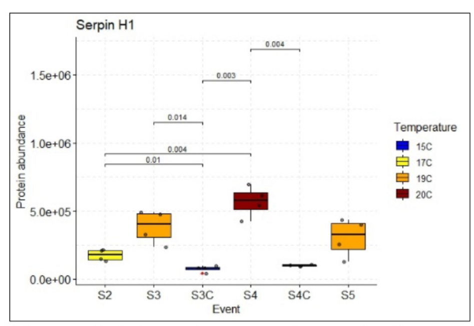

Figure 2. Biomarkers monitored in liver of heat stressed Atlantic salmon

HOW TO BREED FAT, EXPENSIVE FISH – DEVELOPING CT SCANNING FOR YELLOWTAIL KINGFISH *Seriola lalandi*

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Aquaculture is the world's fastest-growing food production system (*The State of World Fisheries and Aquaculture* 2018). This has been recognised by the Western Australian government, with substantial recent investment in the State's aquaculture industry (Department of Primary Industries, Western Australia, 2021). One aquaculture species targeted for rapid growth throughout Australia, including Western Australia, is Yellowtail Kingfish (YTK).

The novel application of CT scanning in Yellowtail Kingfish is hypothesized to allow the prediction of whole body composition (bone, muscle and fat) as well as the prediction of intramuscular fat within the fillet, which has never been done in Yellowtail Kingfish. This study is designed to determine the optimal machine settings to scan fish and verify composition using chemical methods. We hypothesize that scanning whole YTK carcass will allow accurate estimates of intramuscular fat and lean meat yield, both of which are essential for production and breeding of YTK.

The computed tomography prediction of whole fish fat with three different voltage had a low to moderate correlation with fillet fat % (intramuscular fat %) (Figure 1, R^2 = 0.397). The amount of fat in a fish bullet was estimated with a correlation of R^2 = 0.97 by computer tomography, suggested that CT scanning fish bullets is an excellent estimator to predict the fat in that bullet. Moreover, when removing fish viscera and head, there was an improvement in the estimation of fillet fat % (Figures 3), as scanning only fish bullets allows focus on fat deposits within the muscle.

FIGURE 1: Correlation between computer tomography (CT) predicted whole fish fat % and chemically extracted fillet fat %. Comparison between voltages of 100kV, 120kV and 135 kV.

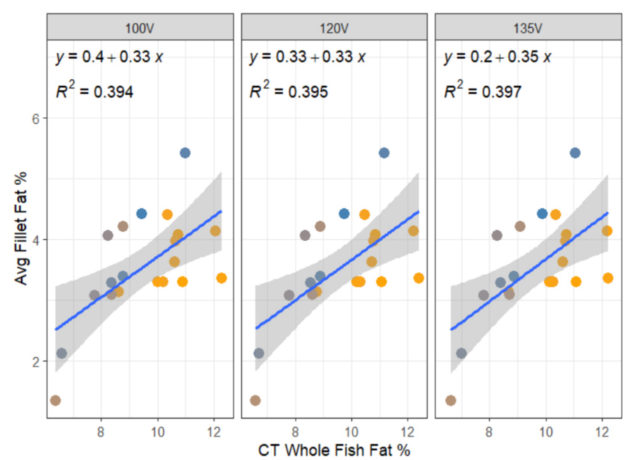
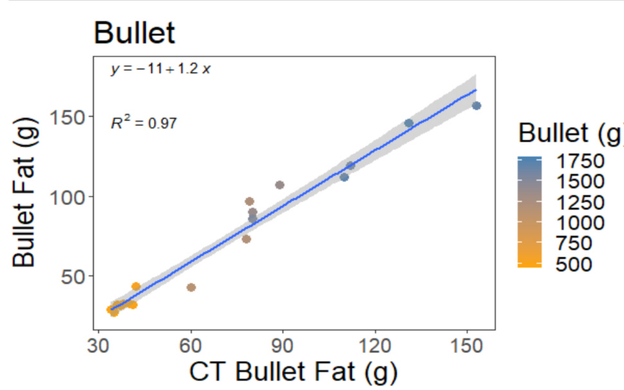


FIGURE 2: Correlation between estimated fat in a fish bullet (g), a weight of chemically extracted fat in a fish bullet



BACTERIOCIN-INFUSED SHRIMP FEED PREVENTS ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)

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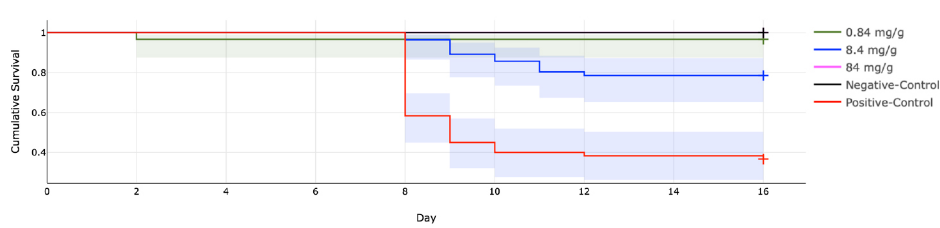
Acute Hepatopancreatic Necrosis Disease (AHPND) is a devastating bacterial infection resulting in $43 billion in global losses to the shrimp aquaculture industry since its emergence in 2009 and has resulted in decreased production in affected regions by nearly 40%. The causative agent is a strain of *Vibrio parahaemolyticus*, which produces a toxin that damages the hepatopancreas of shrimp, resulting in mass mortalities up to 100% within weeks following the transfer of post larval shrimp to grow-out ponds.

To determine if using a bacteriocin-infused feed additive would increase the survival of AHPND challenged shrimp, pathogen free juvenile shrimp *Penaeus (Litopenaeus) vannamei* were fed a commercial shrimp diet infused with a bacteriocin additive for 7 days prior to oral pathogen exposure. The bacteriocin formulation was combined with shrimp premix (Rangen Inc., Buhl, Idaho) at 0.84 mg/g, 8.4 mg/g, and 84 mg/g. The pathogen challenge was two 10 ml aliquots of 1.2×10^9 cfu/ml of a AHPND-causing *V. parahaemolyticus* strain. Fifteen 90 L aquaria, each with 20 shrimp, were then divided into (i) 3 negative controls (no bacteriocin pre-treatment/no pathogen), (ii) 3 positive controls (no pre-treatment and pathogen added), and (iii) 9 treatment tanks (bacteriocin pre-treatment and pathogen addition). The study was terminated after 16 days, with three survivors per diet group preserved for histology; mortalities were frozen at -20°C for histology. A Kaplan-Meier plot was generated and logrank test was performed (Figure 1).

The negative control group had a survival rate of 98%, while the positive control group had an expected drop in survival (35%) due to AHPND infection. The treatment groups had an average survival of 96%, with the lowest and highest concentrations yielding the highest survival rates. These results show that bacteriocin additives can be used to prevent or mitigate AHPND infection in shrimp. Incorporation rates of 0.84 mg/g and 84 mg/g were shown to produce survival rates equivalent to shrimp not challenged with AHPND. Histological examination revealed no observable impacts of the treatment. Additional assays have been done to assess the thermal stability of the bacteriocin, which shows no decreased activity after 15 min at 100C, a temperature commonly employed in creating pelleted shrimp feed. Additional assays have also been done to determine the specificity of the bacteriocin treatment, which reveals that this particular bacteriocin has high specificity towards several *Vibrio* pathogens, with limited activity against beneficial members of the shrimp gut microbiome (*data not shown*). These results suggest that, compared to treatment with conventional antibiotics, a bacteriocin treatment can more specifically eliminate shrimp pathogens while leaving the shrimp microbiome relatively intact and thus enhancing shrimp health.

Figure 1. Kaplan-Meier Survival Curve of AHPND-Challenged *Penaeus vannamei*. Colored area represents 95% C.I.



THE PACIFIC OYSTER MORTALITY SYNDROME: A POLYMICROBIAL AND MULTIFACTORIAL DISEASE

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The Pacific oyster (*Crassostreae gigas*) has been introduced from Asia to numerous countries around the world during the 20th century. *C. gigas* is the main oyster species farmed worldwide, and represents more than 98% of oyster production. The severity of disease outbreaks that affect *C. gigas*, which primarily impact juvenile oysters, has increased dramatically since 2008. The most prevalent disease, Pacific oyster mortality syndrome (POMS), has become panzootic and represents a threat to the oyster industry. Recently, major steps towards understanding POMS have been achieved through integrative molecular approaches. These studies demonstrated that infection by Ostreid herpesvirus type 1 µVar (OsHV-1 µvar) is the first critical step in the infectious process, and leads to an immunocompromised state by altering hemocyte physiology. This is followed by dysbiosis of the microbiota, which leads to a secondary colonization by opportunistic bacterial pathogens, which in turn results in oyster death (Fig. 1).

Host and environmental factors (e.g. oyster genetics and age, temperature, food availability, microbiota, …) have been shown to influence POMS permissiveness. However, the mechanisms by which these different factors control disease expression were poorly understood. Our research group develops different approaches to decipher these mechanisms and we will present our last advances.

Figure 1: POMS is a polymicrobial disease induced by a primary infection by OsHV-1, which alters hemocyte physiology. This is followed by secondary bacteremia that leads to oyster death.

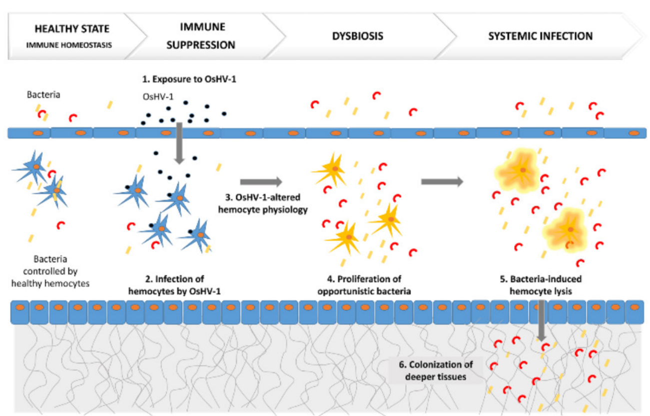
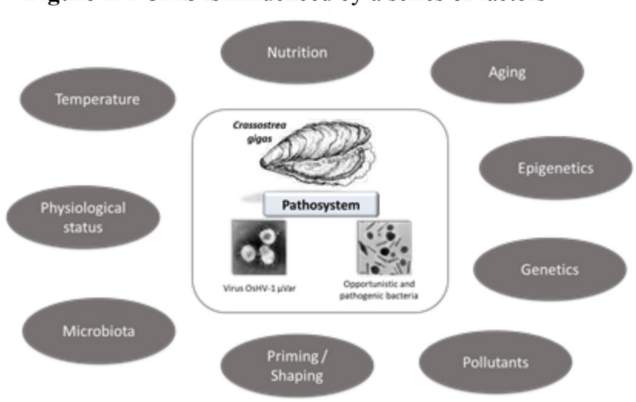


Figure 2: POMS is influenced by a series of factors



DRAFT GENOME SEQUENCES OF *Bacillus pumilus* 36R ATNSAL AND *B. safensis* 13L LOBSAL, TWO POTENTIAL CANDIDATE PROBIOTIC STRAINS FOR SHRIMP AQUACULTURE

Vida Mariel Molina Garza*, Lucio Galaviz Silva, Zinnia Judith Molina Garza, Ricardo Sánchez Díaz, José Cuauhtémoc Ibarra Gámez

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Objectives: This work aimed to isolate bacterial strains with antagonist activity against *Vibrio parahaemolyticus*, the causative agent of acute hepatopancreatic necrosis disease (VPAHPND) that was isolated from outbreaks in Mexico. Here, we report the draft genome sequences of two antagonistic strains, isolated from saline sediment in Sonora, Mexico.

Methods: Cross-streak and well diffusion tests were employed to find the bacterial strains with higher inhibitory activity against VPAHPND. The whole genomes of *B. pumilus* 36R ATNSAL and *B. safensis* 13L LOB- SAL were sequenced using Ion TorrentTM (PGM) and Illumina MiseqTM platforms, respectively. Annotation was performed using the RAST server, and the genes involved in the biosynthesis of bacterial secondary metabolites were predicted using antiSMASH.

Results: Two bacterial isolates, *B. safensis* 13L LOBSAL and *B. pumilus* 36R ATNSAL, were chosen based on their strong antagonistic profiles. The genome of 36R ATNSAL was 3.94 Mbp in length and contained 3824 genes and a total of 4116 coding sequences (CDSs); the genome of 13L LOBSAL was 3.68 Mbp and contained 3619 genes and 3688 CDSs. Twenty-eight and 32 biosynthetic gene clusters responsible for putative antimicrobial metabolite production were identified in 36R ATNSAL and 13L LOBSAL, respectively.

Conclusions: The two strains 13L LOBSAL and 36R ATNSAL showed excellent probiotic profiles in vitro. The genome sequences will help with the mining and reconstruction of metabolic pathways in Bacillus strains. Genome sequence–guided strain improvement could augment the probiotic potential of Bacillus strains for applications in shrimp aquaculture.

Table 1: Genomic data for strains 36R ATNSAL and 13L LOBSAL

Feature	Counts	
Strain	36R ATNSAL	13L LOBSAL
Total genes	4263	3808
Total CDS	4174	3718
Coding genes	3947	3659
Coding CDS	3947	3659
Genes (RNA)	89	90
rRNAs	3, 5, 6 (5S, 16S, 23S)	2, 1, 12 (5S, 16S, 23S)
tRNAs	70	70
ncRNAs	5	5
Total pseudo genes	227	59

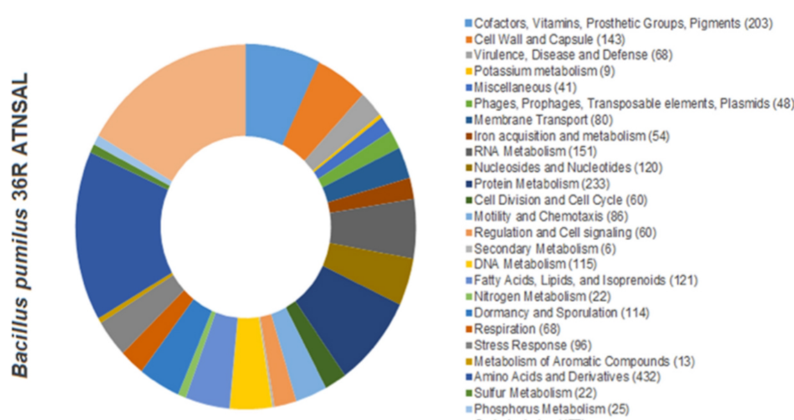

Fig 1. Schematic distribution of subsystem category, coverage, and features obtained for strain 36R.

COMBINING IN VITRO AND IN VIVO METHODS TO EXPLOIT CIRCULARITY IN FISH FARMING

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Marine fish farming is an important sector of aquaculture with most of the operations located in open seas exposed to oscillations of environmental parameters, parasites and pathogens. Ensuring robustness and fast growth are the ultimate goals under these conditions. In pursue of these goals, the use of bioactive compounds with immunostimulant and growth enhancing action is gaining appreciation while screening for such compounds asks for new approaches.

In this study, the effect of bioactive compounds, derived from by-products of olive (*Olea europea*) and *Spirulina* processing, on the growth parameters of gilthead sea bream (SBG) and European sea bass (BSS) was explored. Product 1 originated from olive pulp of pressed olives with a polyphenol content of 40.000 mg/Kg of dry product; Product 2 derived from Product 1 after extraction with 70% ethanolic solution under ultrasound to reduce the polyphenolic content to 17.000 mg/Kg of dry product; Product 3 was an enzymatically modified pomace olive oil that contained 80 ppm hydroxytyrosol; Products 4, 5 and 6 were *Spirulina* protein fraction of 3-10kDa, 10-30 kDa and >30kDa, respectively. A multi-tier approach was followed to study the effect of the bioactive compounds.

Fifteen experimental feeds were formulated with the addition of each bioactive compound in a standard feed formulation. Each feed had a different concentration of bioactive compound. The content of Kunitz Trypsin Inhibitors and Birk-Bowman Inhibitors of chymotrypsin was determined in all feeds along with the rates of dietary protein autohydrolysis and enzymatic hydrolysis *in vitro*, using digestive enzymes isolated from SBG and BSS, respectively. The species-specific appropriate levels of bioactive compounds were determined according to the results *in vitro* before the formulation of the second generation of two experimental feeds per species that were tested in a feeding trial *in vivo*. SBG and BSS of 7.45±0.26g and 9.07±0.15g, respectively were arranged in triplicate tanks per feed. Fish were fed *ad libidum* every day throughout the trial that concluded to approximately 55g final body weight.

Feed supplemented with a combination of product 1 (0.05%), product 2 (0.02%), product 3 (1%) and product 4 (0.1%) resulted in improved growth rate and feed conversion rate (FCR) in SBG. Similar results were yielded in BSS with feed supplemented with a combination of product 1 (0.2%), product 2 (0.5%), product 3 (1%) and product 4 (0.1%). The results are indicative of how by-products available in big volumes can be valorized in fish farming.

Acknowledgement: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH-CREATE-INNOVATE, Project title "*Development of new functional fish-superfood for a more efficient fish farming*" MIS 5069987.

GENE MARKERS REVEAL THE EFFECT OF PLANT-BASED SUSTAINABLE FISHFEEDS ON MUSCLE DEVELOPMENT MECHANISMS IN GILTHEAD SEABREAM (*Sparus aurata*)

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Alternative protein sources of low ecological footprint and at affordable prices for fish feed formulation are key to the sustainable development of aquaculture. Nevertheless, they often impact fish physiology and metabolism due to the presence of various endogenous antinutritional factors and phytoestrogens. The demonstrated negative effect of phytoestrogens on white muscle development and growth highlights the need for tools to screen for potential myostatic action of raw materials and fish feeds. This study combines different approaches to validate white muscle gene markers (Georgiou et al., 2016, Cell Tissue Res 363, 541) in the gilthead seabream, *Sparus aurata L.*, as indicators of compromised myogenesis when fed alternative protein sources.

Three diets were formulated - a soy-free diet (C), a 20% soybean meal diet (SBM), and a 20% soy protein concentrate diet (SPC) – and they were fed to triplicate groups of seabream of 27g average initial BW for three months. The expression levels of *mylpfa* (myosin light chain phosphorylatable, fast skeletal muscle a, associated with hypertrophy), *mylpfb* (associated with hyperplasia), and *myog* (myogenin) in white muscle were determined at the end of the experiment using Real time PCR. In a second approach, extracts of the diets were applied in primary cultures of myogenic progenitor cells from seabream for three different exposure times following the onset of differentiation. Cells were collected at the end of the exposure and the same marker genes were measured. In teleosts, muscle growth occurs through hyperplasia, which involves the recruitment of new muscle fibers, and hypertrophy, which involves a size enlargement of existing muscle fibers. The effects of SBM and SPC on hyperplasia and hypertrophy in the feeding trial were consistent with the effects recorded *in vitro*. Hyperplasia as marked by *mylpfb* expression was significantly reduced in fish fed on SBM and SPC, whereas hypertrophy as marked by *mylpfa* expression was significantly reduced only in fish fed on SBM. Myogenin expression remained unaffected. Similar results were recorded in primary cell cultures after 48h of exposure to diet extracts; both SBM and SPC were potent in downregulating *mylpfb* expression, whereas *mylpfa* expression was significantly affected only in the presence of SBM extract. To our knowledge this is the first study to demonstrate that primary cultures of myogenic progenitor cells can be used as a screening tool for potential myostatic action of fish feeds containing alternative protein sources. The validated protocol developed is time- and cost-effective while complying with the 3Rs principle.

Acknowledgement: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call SPECIAL ACTION AQUACULTURE, Project title " *FishPhytoFeed- Development of an Advanced Integrated Toolbox for in vitro high-throughput screening of quality and phyto-estrogens in feed ingredients for Mediterranean finfish*".

HOW CAN THE AQUACULTURE INDUSTRY ATTRACT AND RETAIN WOMEN?

Heidi Mumme and Sam Nowland (WISA)
Dr. Kirsten Abernethy (Executive Officer, Women in Seafood Australasia
James Garde (CEO, Seafood Maritime Training, Tasmania, Australia
Julie-Anne Kerandel (Coastal Fisheries and Aquaculture Economist, Pacific Community, New Caledonia
Tarun Richards (Business Services Manager, Humpty Doo Barramundi, Darwin, Australia
Jay R Gorospe (Postdoctoral Researcher, Marine Environment and Resources Foundation. Philippines

Previous World Aquaculture Conferences have outlined the benefits from having greater inclusivity of women and other under-represented groups in the industry. In this think-tank session, we look deeper into the barriers women face to participate and reach their full potential in aquaculture, and towards solutions – what can individuals, small and large businesses and organisations do to attract and retain women in aquaculture?

In this session we will hear about the challenges and opportunities for women in aquaculture from different perspectives. Presenters will also tell their stories from the ground - the actions they have taken in aquaculture and the lessons they have learned in their journeys towards gender equity.

These presentations will form the basis of an engaging and facilitated discussion between the panel of aquaculture leaders and the audience. We will discuss the deeper tensions for businesses and organisations to make change, opportunities for increasing the attractiveness of the aquaculture industry to women and other under-represented groups entering and thriving in the industry, and actionable solutions for the aquaculture industry in different environments in both the short and long term.

THE ROLE OF BIOSECURITY IN HIGH DENSITY SHRIMP PRODUCTION

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Shrimp production is becoming intensified globally up to high (eg 250/m2) and super intensive (eg 2,500 /m2) levels in some countries. This involves the use of plastic lined ponds, extensive water treatment, high energy and high-quality feed inputs. However, without high levels of biosecurity, these production systems are risky, difficult to control and inevitably fail. Whilst the concept of biosecurity at times seems simple to many, a biosecurity mindset in all team members and 100% compliance with biosecurity protocols 24/7 can be hard to maintain. The simplest of breaches in the biosecurity barrier within a high or super intensive farm can be the difference between success or failure.

In this study we overlayed a biosecurity mindset, biosecurity auditing system, training regime and standardized bio-secure operating procedures to super intensive L. vannamei production systems that had a track record of being high risk with low profitability, and were often found to be in an uncontrollable state. The implementation of these biosecurity processes delivered consistent de-risked reliable production outputs that when run efficiently resulted in a considerable increase in production and profitability. We will present the basic concepts and approach that can be applied to any high-density production system around the world that is challenged with unreliable, unprofitable production resulting from sub-optimal biosecurity practices.

SURVEYING OYSTER CONSUMERS, AND HOW THESE INSIGHTS CAN INFLUENCE PROMOTIONAL CAMPAIGNS

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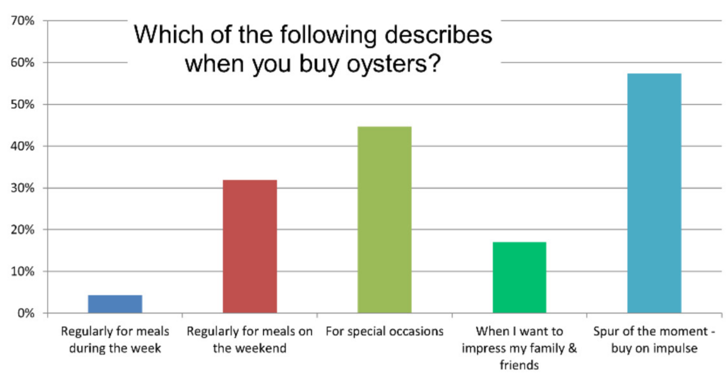
Oyster promotion in Australia is in its infancy. While some of the large, vertically integrated companies have developed promotional campaigns, these are largely confined to social media platforms, and in-person events. There has never been national effort to promote these beautiful briny bivalves.

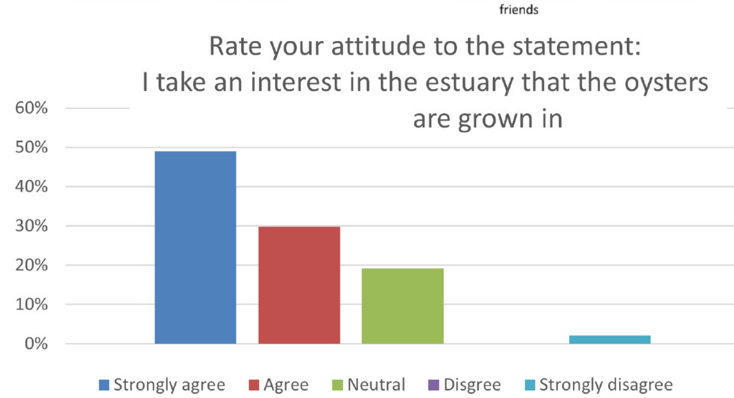
Following the black-summer bushfires of 2019-20, closely followed by Covid lockdowns, there was genuine concern about an over-supply of oysters. Many growers reported running out of gear and room on their farm to accommodate ballooning stock volumes. During this period, one in six growers in New South Wales reported disposing of oysters to make room available for juvenile stock.

Marketing advice was commissioned, and efforts made to initiate a national oyster promotional campaign. Ultimately, this advice was only taken up by one state, who has consequently invested in an oyster promotional campaign, focused on point-of-sale materials and in-person tasting events.

Through the in-person tasting events, over 500 seafood consumers were surveyed, eliciting interesting insights about oyster consumption, buying patterns, industry understanding, and the roadblocks that stand in the way of boosting oyster sales.

This presentation will focus on the results of the survey and start to pull-apart how these insights can help guide future oyster marketing initiatives.





PERFORMANCE OF SANDFISH SEA CUCUMBER (*Holothuria scabra*) JUVENILES IN AN IMTA SYSTEM WITH FORKTAIL RABBIT FISH (*Siganus argenteus*)

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The College of Micronesia Land Grant Program has undertaken the development Hatchery based Sandfish Sea cucumber, *Holothuria scabra* farming technology for local community based economic development, future commercialization and aiming at restocking the depleted stocks in the wild. In this regard, an experiment was conducted to find out the effect of polyculture on the growth, survival of Sandfish Sea cucumber, juveniles in floating Ocean Nursery Hapa Net System – II with and without Forktail Rabbitfish (*Siganus argenteus*) fingerlings in an IMTA polyculture system. The experiment aims to find out if any significant improvements in the growth and survival of juvenile Sandfish Sea cucumbers can be achieved by polyculturing them with Rabbit fish fingerlings. Sandfish sea cucumber juveniles were stocked at 20 pieces per floating hapas in duplicates as controls and a second treatment batch of 20 juveniles were grown in poly culture with 100 Rabbitfish fingerlings in duplicates. A third treatment of just 100 Rabbitfish fingerlings were grown in duplicates. The experiment was run for 30 days at the end of which time the growth (length and wet weight) and survival were tabulated for each treatment by measuring all surviving animals of fish and sea cucumbers. The Rabbitfish were fed daily a commercial feed at 5 % body weight in both the treatments while the control animals of sea cucumber juveniles would be just grazing on algae from the hapa net enclosures. Results showed a significant increase in growth of sandfish juveniles and Rabbitfish fingerlings in terms of wet weight when both were poly cultured in an IMTA set up than separately. No significant improvement in survival was noticed among the treatments. The results show promise for polyculture of early sea cucumber juveniles with pelagic fish like Rabbitfish in an IMTA setup.

SANDFISH SEA CUCUMBER (*Holothuria scabra*) FARMING TECHNOLOGY DEVELOPMENT IN US AFFILIATED MICRONESIAN ISLAND COUNTRIES

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Sea Cucumber, as one of the possible resources for economic development, has been the subject of College of Micronesia Land Grant (COM) research in Micronesia for the past several years. NIFA (USDA), the funding agency for COM researches, expects that research findings and results should be transferred, when appropriate, from the “lab” to the “field”; to the consumers/users/stakeholders/communities.

The College of Micronesia Land Grant Program has undertaken the development of Hatchery based *Holoturia scabra* or Sandfish sea cucumber farming technology for local community-based economic development, future commercialization, and aiming at restocking the depleted stocks in the wild in Micronesian Islands of Pohnpei, Yap and Palau. The project aims to develop site-specific nursery and grow-out technologies for Sandfish sea cucumber which local fishers can adopt, thereby providing them supplementary income in addition to their fishing. This project further aims to attract foreign investors to invest in the local economy through 100 % foreign-owned businesses or being part of community-based business ventures, thereby boosting the local economy. The results of these research and extension efforts and the current and future challenges faced by the program would be discussed.

BIOFOULING MARINE MACROALGAE: A BOON OR BANE?

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Marine biofouling is regarded to be problematic in mariculture incurring businesses direct costs in clearing biofouling from sea cages and reducing efficiencies. Marine biofouling on sea cages is predominantly composed of opportunistic marine macroalgae, with microphytobenthos, hydroids and other settling fauna that make up the remainder of the community. When the settlement duration is <5 months, biofouling is predominantly marine macroalgae that thrive in a high nutrient environment from intensive fin-fish farming. This study highlights the real commercial opportunity presented by biofouling macroalgal biomass when transformed into a premium liquid fertiliser in addition to nutrient trading credits (NTC) through bio-assimilation of carbon, nitrogen and phosphorus from the adjacent marine environment.

This presentation will encompass settlement of marine macroalgae on mooring lines of Southern bluefin tuna cages of a farm in Port Lincoln, South Australia. Results reveal close to 70 species of marine macroalgae that make up the assemblage with settlement ranging from 0.04 to 26.19 gDW m^{-1} (depth integrated) on the mooring lines over an 8-month settlement period. *Polysiphonia*, *Uronema*, *Vaucheria* and *Dasya* dominated the algal community on the mooring lines. Higher settlement was observed at 20-24m depth equivalent dominated by Rhodophyceae.

A scaled-up pilot was undertaken with 0.8 ha of seaweed settlement lines deployed adjacent to the tuna cages. Results reveal a very different community composition with *Sarconema* dominating (~95%) the assemblage followed by Dasya (2%) and *Claviclonium* (2%). Mean settlement of 115 gDW m^{-1} was recorded with tissue carbon, nitrogen and phosphorus content of 27%, 3% and 0.35%, respectively. Based on these preliminary outcomes, harvesting marine macroalgae from the settlement lines present a commercial opportunity ranging between \$9,600-18,000 ha^{-1} $annum^{-1}$ from premium liquid fertiliser in addition to \$1,300-3,600 ha^{-1} in nutrient trading credits.

META-ANALYSIS OF QUANTITATIVE TRAIT LOCI (QTLS) REVEALS THE GENETIC CONTROL OF REPRODUCTIVE TRAITS IN FARMED RAINBOW TROUT (*Onchorhynchus mykiss*)

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The field of association mapping studies has recently received major attention for genetic studies of quantitative traits in many important aquatic species. Access to next generation sequencing technologies, high phenotypic data and a variety of sophisticated statistical tools have enabled association mapping studies in aquatic species to be successful in identifying gene loci controlling quantitative traits. Due to the importance of association mapping method in mapping studies of the quantitative traits, the present project was prepared to explain the association mapping method and its use in rainbow trout breeding and also to perform a meta-analysis of these QTL to identify regions of the rainbow trout genome that are consistently associated with growth traits. To identify Meta-QTL (MQTL), a QTL database was developed from 1400 QTL targeted at growth traits. This project also provides some information about statistical software packages used in association mapping and then the opportunities and challenges of association mapping and post-genome wide association studies at the whole genome level discussed. For QTL mapped relative to a single marker, nucleotide sequence of the marker was retrieved from the relevant marker database. For QTL mapped relative to two flanking markers, sequences for both flanking markers were retrieved from the database. The positions of individual QTL were projected onto a consensus genetic map based on the presence of common molecular markers and a 95% confidence interval (CI) was calculated for each QTL. After positioning the individual QTL, the software 'Biomercator v2.1' was used to predict the location and CI of MQTL based on maximum likelihood. In total, 854 QTL were reported for 80 growth traits. This included 280 for average daily gain (ADG), 16 for body weight (BW), 9 for Condition factor (CF) and 7 for fork length (FL) trait QTL in rainbow trout genome. In total, 27 QTLs were detected on four linkage groups for the studied traits. That from 2 to 23/8% of the phenotypic variation (LOD) were justified. Most QTLs were detected on 13 linkage groups. In this study, for the body height traits not detected a QTL. Results revealed the existence of co-localized QTLs for studied traits, which enhance the efficiency of marker-assisted selection and developing rainbow trout breeding programs.

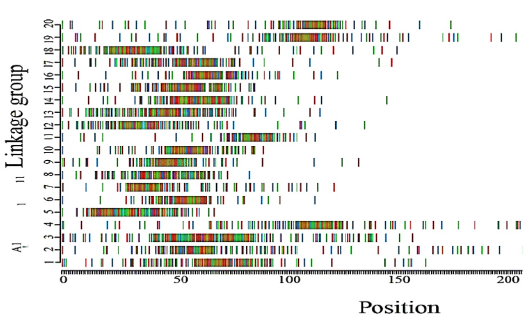

Fig. 1. Linkage distribution of multi-locus for rainbow

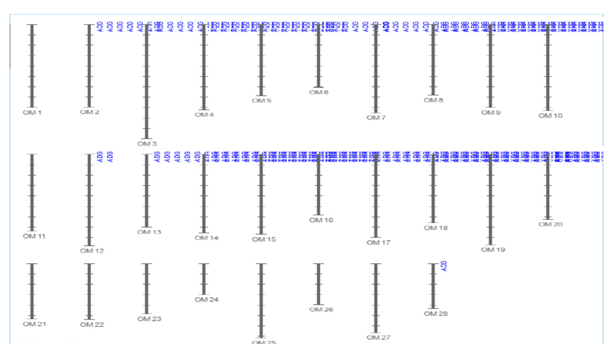

Fig. 2. Distribution of QTLs for body weight trait for the rainbow trout

EXPLORING CO-CULTURE METHODS IN CORAL AQUACULTURE

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Coral aquaculture is undergoing a massive expansion to meet the demand of emerging reef restoration activities and the ornamental trade. However, aquaculture production of corals, particularly sexually propagated individuals, still requires much development to be cost-effective at scale. As such, many novel solutions are being explored to boost production and reduce costs, one of which is co-culture. Similar to integrated aquaculture, this is the practice taking advantage of existing symbiotic relationships between animals by culturing corals with one or more organisms that can provide different services. Commonly explored applications of co-culture include the use of microherbivores to control algae growth in coral recruit grow-out, the addition of various pest and parasite predators to help control outbreaks, and the use of companion fish as a source of nutrient enrichment. While small-scale trials have been performed, and shown positive results, large-scale trials taking into account the cost-effectiveness of these methods compared to more traditional approaches have yet to be undertaken. As such, here we summarise the current state of co-culture research in coral aquaculture, and suggest avenues for future research to explore.

THE EVOLUTION OF FARM ANIMAL WELFARE: UNDERSTANDING AND EXPECTATIONS OF THE SUPPLY CHAIN FROM RETAILERS, INVESTORS, NGOS AND CONSUMERS

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The scientific understanding of farm animal welfare has advanced significantly over the last five decades. The most dominant framework The Five Freedoms[1] was first proposed over 50 years ago and helps to define the mental and physical needs of kept animals by primarily aiming for 'freedom from' negative welfare states. The 1994 Five Domains framework acknowledged how the mental state of animals is affected by four physiological "domains"[2]. More recently it has been proposed that farm animal welfare needs to go beyond avoidance of negatives toward providing an environment that actively promotes positive experiences and emotions for animals[3],[4],[5] (Figure 1), impacting the way in which we farm animals and what resources we provide them.

Alongside an increased understanding of animal welfare, we have also seen increasing pressure from NGOs, through the use of public benchmarking for food businesses, to provide increased transparency on the practices, performance and impact they are having on farm animal welfare within supply chains. While the focus so far has been on terrestrial species such as poultry and pigs kept in intensive farming systems, we have seen in recent years an increasing interest in aquaculture. This presentation will provide an overview of the key areas of focus for farm animal welfare and look at the expectations for reporting in farm animal supply chains.

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APPLICATION OF IMMUNE CHALLANGED EXTRACELLULAR VESICLES (EVS) FROM OLIVE FLOUNDER (*Paralichthys olivaceus*) PLASMA FOR FISH MEDICINE

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Extracellular vesicles (EVs) are lipid-bound membranes (ranging in diameter from ~30 to 1000 nm) secreted by cells into the extracellular space. They exist in body fluids, and tissues, and contain various biological cargo molecules, such as nucleic acids (DNA, mRNA, miRNAs), proteins, lipids, enzymes, etc. EVs can mediate intercellular communication and immune regulation, and they are considered biomarkers for disease. The wide array of functions and biomedical applications of EVs as therapeutic agents (e.g cancer therapy, wound healing, etc.) have been reported. Moreover, as a nanoscale vesicle with a lipid bilayer membrane, exosomes are considered biocompatible drug carriers released by cells. Therefore, EVs can consider as a potential biomaterial for fish medicine, but the area of utilization has not been applied with well-defined strategies or approaches. Although EVs from fish have been isolated, characterized, and investigated the composition (miRNA, protein, etc.), it still lacks sufficient experimental data for EVs used for therapeutic application in the aquaculture field (e.g. vaccine candidates, wound healing agents, etc.).

Bacteria *Edwardsiella piscicida* (Gram-negative) and *Streptococcus parauberis* (Gram-positive) are two major causative agents for diseases of Edwardsiellosis and Streptococcosis, respectively. They frequently affecting to the aquaculture industry and resulting high mortality in economically important fish including olive flounder (*Paralichthys olivaceus*). Plasma is one of the most frequently studied biological fluids in fish, and the most promising source of biomarkers. It may contain a collection of EVs from different cells. We hypothesize that EVs could be one of the efficient and promising biomaterials to discover novel proteins expressed in EVs against pathogenic bacteria and viruses. These EVs have unique proteins for specific immune activation, thus it could be used as a tool for multi-functional bioactivities, such as immunomodulatory, disease resistance, and wound healing for therapeutic uses.

Our target is to develop plasma-derived immunogenic and biocompatible EVs from immune-modulated olive flounder as a multifunctional biomaterial for application in fish medicine. Finally, we will ensure that the functionally characterized plasma-derived EVs can be applied in a wide spectrum of therapeutic applications as vaccine candidates, antimicrobial, immunomodulatory, and wound healing agents.

Acknowledgments: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) 2021R1A2C1004431.

TIGERS IN THE TOPEND: CHALLENGES AND OPPORTUNITIES FOR EXTENSIVE PRAWN FARMING IN NORTHERN AUSTRALIA

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Darwin in the Northern Territory, Australia, was once home to a small number of prawn farms established in the 1990's. Nowadays, all these farms are either laying empty or have been repurposed for farming of other aquatic species. There were likely a number of contributing factors though anecdotally a common issue was the enormous abundance of wild prawns (*Metapenaeus* spp.) and predation by birds. On the east coast of Australia, there is a well-established and highly successful tiger prawn (*Penaeus monodon*) farming industry. These farms operate using semi-intensive farming methods with stocking densities in the range or 30-50 post-larvae (PL)/m^2. As an alternative approach to farming tiger prawns in northern Australia, we are exploring extensive, low input farming that could be more location appropriate for the region and remote communities. This offers the potential to unlock large areas of the coastal regions of northern Australia to be used for aquaculture.

To investigate the potential challenges of farming tiger prawns in the north, we collaborated with a local farm south of Darwin. Here we carried out pond-based experiments using a stocking density of 3 PL/m^2, supplemental feeding at a 50% ration, chemical treatment to exclude wild prawns and have excluded birds from one pond using netting. We also performed a controlled tank-based experiment to compare different water treatment methods and their effect on wild prawn exclusion and productivity of tiger prawns. Water treatment methods included chemical treatment prior to stocking, filtration of water through 50 um mesh screen and non-treated raw water. Results showed the presence of wild prawns significantly impacts productivity of the tiger prawns through restricting growth, reducing survival and increasing FCR (Table 1). Interestingly, the presence of wild prawns did not impact total biomass and FCR in the tanks.

Findings from these first trials have confirmed some of the challenges of farming tiger prawns in northern Australia, and solutions to common competitors and predators have been developed that will likely play a role in future extensive farming models.

Table 1: Productivity data from controlled tank experiment.

Water treatment	Species	Weight gain (g)	Survival (%)	Biomass (g)	FCR
Chemical	*P. monodon*	6.91 ± 0.25 ^{A}	98.63 ± 0.94 ^{A}	1375.60 ± 63.0 ^{A}	1.13 ± 0.03 ^{A}
Raw	*P. monodon*	2.63 ± 0.39 ^{B}	63.63 ± 8.58 ^{B}	321.66 ± 32.67 ^{B}	4.02 ± 0.49 ^{B}
	**Metapenaus*	-	-	1150.03 ± 52.76 ^{A}	1.11 ± 0.02 ^{A}
Filtered	*P. monodon*	6.9 ± 0.13 ^{A}	96.75 ± 2.54 ^{A}	1290.95 ± 27.59 ^{A}	1.24 ± 0.08 ^{A}

*includes total biomass with *Metapenaeus* spp.; Superscripts that differ are significantly different ($P < 0.05$)

ESTABLISHMENT OF NILE TILAPIA (*Oreochromis niloticus*) SATELLITE HATCHERIES FOR IMPROVED FINGERLING PRODUCTION IN PAPUA NEW GUINEA

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Country-wide supply of Genetically Improved Farmed Tilapia (GIFT) strain fingerlings in Papua New Guinea (PNG) has been one of the hurdles for aquaculture expansion. When properly managed, GIFT grows quickly with good survival under pond conditions, dramatically increasing aquaculture yields. It is a hardy strain and can survive in harsh conditions with high resistance to disease. For PNG, the most important trait is the strain's ability to grow fast. Nevertheless, production yields remain low in PNG due to low quality fingerling supply, mixed sex fingerling stocking, and poor feeding practices.

Ever since its introduction to PNG, GIFT fingerling production has depended on the traditional method of production whereby, tilapia broodfish are reared in ponds with or without supplementary feed, and the fry are collected from the pond edges. Fry productivity is typically low due to incomplete harvesting, cannibalism by older fish, and reduced spawning frequency. Mixed sex fingerlings also present a challenge to farmers because of uncontrolled breeding leading to overcrowded ponds.

Over the past decade, intensive research effort has developed a hatchery technique using hapa-in-pond systems (AASP, 1996; Little et al., 1995, 1997). Fry seed is collected from females' mouths at five- and seven-day intervals for artificial incubation. The technology has recently been commercialized and proven to be economically viable (Little et al., 1997; Bhujel, 1997). As a result of its ability to produce high-quality fry predictably, this technology is now being widely adopted (Bhujel et al., 1998). NFA has adapted this technology to establish twenty-two satellite hatcheries in eighteen provinces across the country. Importantly, monosex fingerling production methods have been included to prevent in-pond breeding, thus improving fish feeding efficiency and reducing the cost of farming. This has resulted in an increase in fish farming activities as farmers can access quality fingerlings that are readily available, and reports of improved yields has attracted new farmers.

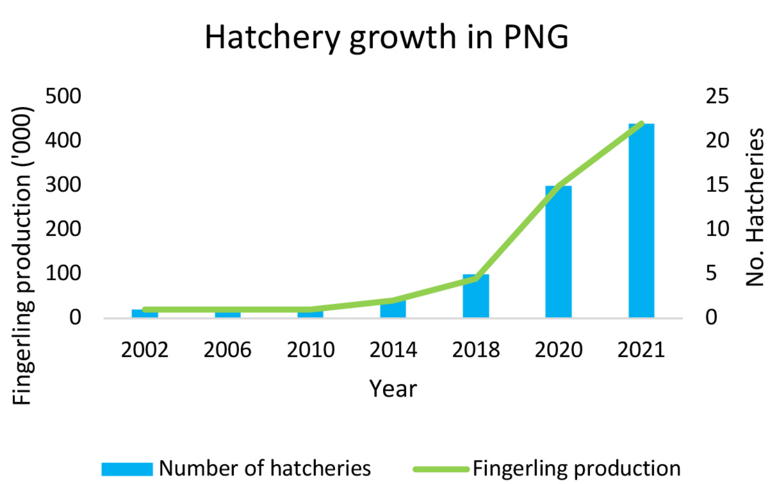

Figure 1- The number of established GIFT hatcheries in Papua New Guinea since GIFT was introduced

TWO WAY APPROACH TO AQUACULTURE DEVELOPMENT: TROPICAL ROCK OYSTER FARMING IN THE NORTHERN TERRITORY

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Yagbani Aboriginal Corporation on South Goulburn Island believe oyster cultivation is a culturally appropriate, sustainable, economic opportunity from the sea under their custodianship. Blacklip rock oysters (*Saccostrea echinata*) are a native species of tropical oyster found across the north Australian coast. This joint presentation, by Yagbani Aboriginal Corporation and the Northern Territory Governments Aquaculture and Regional Development Unit, will discuss a collaborative (two way) approach to developing tropical rock oyster aquaculture in Arnhem Land. The partnership values Aboriginal ways of knowing and doing, to work together in addressing the most significant issues confronting industry development. Investment has been long-term, for over a decade, and research focus has progressively moved through a chain of priorities; from technical/production based research, developing strong foundations for downstream research to build on. Presenters will highlight the importance of two way knowledge production and decision making, between time-tested Aboriginal knowledge and scientific enquiry, being incorporated into all aspects of oyster aquaculture. As well as a focus on the application of scientific results and what it means for people. We will discuss the future of tropical rock oyster aquaculture, as the Yagbani farm transitions out of the current research phase and into commercial production. To achieve their ultimate aim of supplying markets with a premium, native oyster, which has been sustainably grown by Aboriginal Territorians in the pristine waters of the Arafura Sea.

THE EFFECTS OF AMINO ACID BALANCE BY GLUTAMINE SUPPLEMENTATION ON THE COST OF DIGESTION AND GROWTH OF ATLANTIC SALMON *Salmo salar* L.

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Temperature is a major abiotic factor that affects fish digestion and growth and therefore can impact aquaculture production. The optimum temperature for growth for Atlantic salmon (*Salmo salar*) is around 14°C. However, during Tasmanian summers (the leading location in Australia for Atlantic salmon farming), this species can experience temperatures up to 22°C for several days. These high temperatures result in decreased feed intake, growth and when prolonged, mortality. Some evidence suggests that this reduction in feed intake could be due to limited energy available for aerobic activities such as digestion. With an increase in temperature, the minimum energy expenditure needed for metabolism maintenance increases at a greater rate than the maximum energy the animal is able to produce, thus decreasing the amount of energy available for activities such as growth or digestion. Indeed, digestion is an energetically costly process and can compete for energy allocation with other energetically demanding activities such as swimming, reproduction, and growth. Thus, it is challenging for fish to digest and grow under elevated temperatures. However, digesting and assimilating a meal and its inevitable cost is essential for growth. Hence, fine-tuning diets to provide adequate nutritional requirements and facilitate digestion at elevated temperatures to improve growth in aquaculture salmon is the focus of this study. We aimed to explore the impact of amino acid balance with the supplementation of glutamine – an important amino acid in energy metabolism and gut health - on the cost of digestion and intestine health at elevated temperatures for Tasmanian Atlantic salmon parr. Fish were fed three isoenergetic experimental diets for seven to eleven weeks at 22°C, and growth, energetic expenditure, digestion cost, amino acid plasma variation, and gut antioxidant enzymes were investigated.

FROM WASTE TO PROTEIN FOOD: A COMMERCIAL PERSPECTIVE ON USING AGRICULTURAL WASTES IN AUSTRALIA FOR BRINE SHRIMP *Artemia* PRODUCTION

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This paper will provide an overview of results obtained from screening and characterising different types of agricultural wastes in Australia and their effectiveness in producing *Artemia* under laboratory conditions, with a view to commercial application. The goal is mass production of *Artemia* biomass as live food in aquaculture, feed ingredient and possibly human nutrition, while also remediating high-volume waste, produced by the alcohol industry particularly.

Wastes were characterised using different physico-chemical and biological parameters in both original form and after aerobic digestion, with or without further processing or enhancement. Quality of wastes and the *Artemia* grown on them in terms of nutrients, fatty acids, microbial community, metabolites, and heavy metal uptake was evaluated to determine which wastes have the potential for commercial use.

Distillery dunder from bioethanol or rum production using molasses are excellent food sources for large scale *Artemi*a production. The prospect of using vinasse from rum production using other types of sugar, and waste from brewery, winery and the livestock industry that require further processing or enhancement will also be discussed.

Single-cell proteins in aerobically digested waste is the key to large-scale, good-quality *Artemia* production.

COMMERCIAL VIABILITY OF A MODIFIED PARTITIONED AQUACULTURE SYSTEM (PAS) CULTURING MURRAY COD *Maccullochella peelii peelii* IN SOUTH WESTERN QUEENSLAND

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The Partitioned Aquaculture System (PAS), developed by Clemson University in 1989 was originally trialled with Channel Catfish *Ictalurus punctatus*. In the years since its inception it has been applied to a range of finfish species throughout most regions of the world. However, it does represent only an extremely small portion of total pond-based production. The general principles of a PAS have been applied and modified to a commercial finfish farm culturing Murray Cod *Maccullochella peelii peelii* in South Western Queensland, Australia. This is the only farm of its kind in the world culturing this species. Stocking densities of over 100 kilograms per cubic metre within the raceways are standard for the facility without the introduction of pure oxygen due to the high water exchange rates in the within the large (6 hectare) ponds of which the fixed concrete raceways are embedded. Production costs are minimised due to the application innovative engineering, automation and modification of existing PAS design. The farm shows commercial viability in its current form when analysing all economic factors and also clearly indicates that the all biological indicators of a heathy pond based aquaculture system can be achieved. This system has potential application to other species cultured within Australia, in particular barramundi *Lates calcarifer*

GROWTH OF *Vibrio parahaemolyticus* IN BLACKLIP ROCK OYSTERS STORED AT DIFFERENT TEMPERATURES

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Post-harvest storage conditions are informed by recommendations to keep oysters as cool as possible to limit pathogen growth, while keeping the animals alive since dead seafood may lead to rapid spoilage and adversely affect microbiological safety. In Australia, guidelines for temperate Pacific oysters (POs) (*Magallana gigas*) and Sydney Rock Oysters (SROs) (*Saccostrea glomerata*) are based on their different responses to spoilage at different temperatures, however these temperatures may not be relevant for tropical species such as Blacklip rock oysters (BROs) (*Saccostrea echinata*/ lineage J). BROs grow in warm waters which support many species of indigenous aquatic microbes including *Vibrio* spp., that are potentially pathogenic to humans. In Australia, *Vibrio* spp. are recognised as an emerging food safety risk. The objective of this study was to determine the effect of postharvest storage temperature on the growth rate of tropical *Vibrio parahaemolyticus* strains in artificially inoculated BROs to provide the necessary foundation for postharvest temperature control plans.

A mixture of four *V. parahaemolyticus* strains isolated from tropical rock oysters were injected into BROs, the oysters placed in open plastic bags and stored at 4°C, 13°C, 18°C and 25°C. Three oysters were pooled per sample, with 5 replicates per time point at each temperature. The level of *V. parahaemolyticus* was measured at different time-points in the stored oysters by plating diluted oyster homogenates on CHROMagar Vibrio. Counts were transformed to $\log_{10}$ values, and lines or curves fitted to the data. Growth rates ($\log_{10}$ CFU/h) were calculated from best fit lines at 4°C and 13°C. Specific growth rates and maximum population densities ($\log_{10}$ CFU/g) at 18°C and 25°C, data were imported into https://foodmicrowur.shinyapps.io/biogrowth/ and fitted using a modified Gompertz model.

V. parahaemolyticus did not grow at 4°C and low growth occurred at 13°C. *V. parahaemolyticus* growth at 18°C and 25°C was not significantly different, but was higher than at 13°C. Results support BRO storage at 4°C and 13°C. While these *V. parahaemolyticus* growth data provide important food safety information for the industry, other shelf-life quality characteristics will also need to be assessed to develop more definitive guidelines for BRO storage and transport to maximize product quality and safety.

POTENTIAL BIOCONVERSION OF BLACK LIP AND MAB´E PEARL OYSTER WASTE INTO AN ORGANIC FERTILIZER

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Black Lip pearl and Mab´e Pearls are emerging commercial aquaculture species in Fiji. Both species are prominently found in Fijian waters. *Pinctada margaritifera* (black lip pearl oyster) locally known as Civa, which has been cultivated into one of the major commercial species for the pearl industry. The pearl and the shell of the pearl oysters are given most of the importance in this industry, while not much thought is given to the tissue or the oyster meat itself. The by-product of the pearl industry which is majorly the oyster tissue, only 20% of which is edible and the rest (visceral mass) is discarded as waste. The current rise in the fertilizer prices provides opportunity to develop high nutrient organic fertilizer from pearl oysters that can be utilized by the local communities. This resource of the pearl is not optimally utilized in several parts of the world. While *Pteria penguin* (Mab´e Pearls) locally known as "Melamela" is a community-based project where women and children are involved in the pearl culture. This study aims to investigate the bioconversion of the waste byproducts of the black lip pearl oyster towards the development of an organic fertilizer.

Two different species of cultured pearl oysters *Pteria penguin* and *Pinctada margaritifera* were collected from different oyster culturing communities in Fiji. Mineral analysis was done to understand the biochemical composition of the oyster tissue. Six samples of each oyster species were taken and homogenized respectively. From which another three samples were taken for the different analysis. The mineral contents of the pearl oyster tissue are represented in Table 1. The results show that there is a high level of Potassium (K) in both the species of oysters followed by Nitrogen (N) and Phosphorus (P). Minor elements include Zinc (Zn), Manganese (Mn), Iron (Fe), Magnesium (Mg) and Calcium (Ca). N, P and Ca content in *P. margaritifera* were significantly lower ($P < 0.05$) than that of *P. penguin*. This results indicate that in natural occurring oysters have higher mineral content and an ideal choice for of a raw ingredient for liquid fertilizer. This study is done for the first time and has been conducted successfully. This study creates an opportunity for further research in waste bioconversion into usable products such as an organic fertilizer development and further research.

Table 1: Nutrient analysis of *P. margaritifera* and *P. penguin*(mg / 100g wet weight)

	Black Lip Pearl (PM)	**Mabe Pearl (PP)**
N	10 – 15 ± 0.50	5 - 15 ± 0.35
P	1-10 ± 0.28	20 -25 ± 0.59
K	255 - 265 ± 2.87	250 -260 ± 1.35
Zn	1 -5 ± 0.18	1 -5 ± 0.36
Mn	0.10 – 0.50 ±0.14	0.10 – 0.50 ±0.01
Fe	1- 5 ± 0.12	1 -5 ± 0.14
Mg	90 – 100 ± 0.42	90 - 100 ± 0.18
Ca	10 – 15 ± 0.06	15 - 20 ± 1.18

THE ROLE OF U.S. SOYMEAL INCLUSION IN FEEDS TO THE PERFORMANCE OF POND-RAISED *Pangasius hypophthalmus* IN TULUNGAGUNG, INDONESIA

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Pangasius farmers in Indonesia have been struggling to reach beyond its tipping point for profit. The value chains of Pangasius aquaculture industry have been inefficient due to various and complex reasons. Improving performance of fish at farm level through better feed conversion ratio (FCR) and daily growth was targeted. Despite variety of Pangasius feeds in the market, but none of them were using U.S. soymeal inclusion in the diet. USSEC Indonesia aquaculture program, in collaboration with cooperator feed mill and farmer, held a feeding demonstration project to show superior quality of U.S. soymeal in the diet for *Pangasius hypophthalmus* performance in grow-out farm.

The project was held at Sumbergempol, Tulungagung, Indonesia and was using two feed comparisons with three replicates. Six ponds averaging 245 m3 were stocked with 5,000 Pangasius fingerlings sizing 7.7. g/fish and raised to reach 700 g minimum sizes. All ponds were treated similarly in terms of feedings, water exchange, limings, and such. After 266 days, all ponds were harvested and fishes were weighted to estimate total biomass and average survival rates. Total cumulative feeds per pond were also calculated to estimate FCR. The performance of Pangasius in all demonstration ponds is provided in Table 1 below.

Table 1. Pangasius Harvest Biomass, Average Body Weight (ABW), Survival Rate (SR), and Feed Conversion Ratio (FCR)

Description	Biomass (kg)	ABW (g)	SR (%)	FCR
USSEC 1	3,716	805	92.3	1.29
USSEC 2	3,372	820	82.2	1.42
USSEC 3	3,338	879	76.0	1.43
Control 1	3,050	743	82.1	1.51
Control 2	3,150	899	70.1	1.46
Control 3	2,831	810	69.9	1.62

The project showed that Pangasius fed with USSEC-formulated diets had higher average SR (83.5%) and lower FCR (1.38) in average, compared to SR and FCR of Pangasius in control ponds (74.0% and 1.53 respectively). When analyzing the nutritional content of both feeds used in the project, there were slight differences in the composition of essential amino acids as provided in Table 2 below.

Table 2. Composition of amino acids in both USSEC-formulated and control feeds

Amino Acids	Minimum	USSEC	Control
Arginine	1.38	1.72	2.17
Histidine	0.67	0.65	0.88
Isoleucine	0.84	0.92	0.92
Leucine	1.70	1.88	1.90
Lysine	1.40	1.21	1.01
Methionine	0.55	0.29	0.26
Phenylalanine	0.86	1.28	1.75
Threonine	1.05	1.04	1.25
Tryptophan	0.24	0.29	0.28
Valine	1.11	1.13	1.17

USSEC formulated feeds slightly had more lysine and methionine compared to control feeds. Lysine has been known to improve growth, physiological function, and protein synthesis, as well as metabolism for muscle growth.

SUSTAINABLE SCALING OF AQUACULTURE IN TIMOR-LESTE: CURRENT STATUS, CHALLENGES AND FUTURE PRIORITIES

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There is a growing appreciation for the role of aquaculture in improving food and nutrition security, as well as diversifying livelihoods in Timor-Leste. The Timor-Leste government's National Aquaculture Development Strategy (NADS; 2012-30) aims to increase domestic aquaculture production to 12,000 tons and per capita fish consumption to 15 kg annually by 2030. However, the aquaculture development in the country has been constrained by the limited availability of seed (fingerlings) and feed, as well as knowledge and skills about aquaculture.

In response, WorldFish started working in partnership with Ministry of Agriculture and Fisheries (MAF) and private sectors since 2014 with the aim of supporting implementation of NADS. In the initial years (2015- 2019), the research and development efforts focussed strongly on introduction of Genetically Improved Farmed Tilapia (GIFT) in government hatchery in Ermera, and multiplication and dissemination of GIFT seed through the Public-Private Partnership (PPP) model hatchery; preparation of feed based on locally available resources (e.g., Leucaena leaves, corn meal, rice bran), *in situ* production of natural food through maintaining green water, and introduction of a Better Management Practices (BMPs) guidelines. Farmers adopting BMPs achieved fish yield averaging 4.3±1.5 t/ha. With the average production cost of USD 1.5/kg and fish sale price of USD 4.0/kg, farmers received net profit of USD 2.5/kg. Nevertheless, scaling of GIFT was challenging due to limited availability of feed ingredients in the country.

Hence, testing and validation of grow-out technologies for sustainable intensification of GIFT have been conducted in recent years (2020-2022). The technological interventions included: increasing stocking density from 3 to 5 fingerlings/m^2; feeding fish with high quality imported commercial feed pellets; and maintaining pond water green to enhance *in situ* production of natural food. By adopting the new recommendations, GIFT farmers achieved an extrapolated fish yield of 12.4±0.1 t/ha/cycle and increased net profit by 135±3% (Table1).

To facilitate scaling of GIFT, private sectors are being supported to import feed in bulk to make it available at a reasonable price, live and fresh tilapia markets have been established, and Local Service Providers (LSPs) are organised to facilitate input supply and output marketing. However, continuous technical backstopping and engagement of public and private sectors are crucial. Given the similar agro-ecological contexts, the aquaculture development model of Timor-Leste has potential for replication in the small island nations of the Asia-Pacific region.

Table 1. No. of farmers, fish stocking, and yield and net profit from the four municipalities in Timor-Leste during 2021-22.

Municipality	Total farmers	No. of ponds	Pond size (m^2)	Yield (t/ha/cycle)	Net profit (US$/pond)	Net profit (%)
Lautem	16	43	122±82^{ab}	14.6±2.1^{b}	300±264^{b}	99±51^{a}
Baucau	30	109	128±25^{b}	14.2±0.3^{b}	365±21^{c}	154±6^{b}
Ermera	33	99	80±35^{a}	11.4±0.3^{a}	158±22^{a}	97±6^{a}
Bobonaro	90	166	100±19^{ab}	10.7±0.2^{a}	270±16^{b}	166±5^{b}
Total	169	417	105±34	12.4±0.1	268±11	135±3

OXFORD NANOPORE SEQUENCING FOR RAPID IDENTIFICATION OF THE GEOGRAPHICAL ORIGIN OF FARMED MEDITERRANEAN MUSSELS (*Mytilus galloprovincialis*)

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Mytilus galloprovincialis is highly recognized as an important aquaculture species, and Spain, with a production close to 200,000 tonnes per year, is the main *M. galloprovincialis* producer in the EU. This valuable seafood product is particularly susceptible to fraudulent practices due to several factors including the high number of species involved and the complexity and globalization of the food chain. Seafood authentication includes species and geographical origin identification, and it is a major concern for consumers and seafood producers. In this sense, European Union regulation (EU) No 1169/2011 requires that consumers are appropriately informed about the food they consume.

Different analytical procedures can be used for seafood authentication and DNA-based methodologies have been revealed as the ideal approach to address the identification of species and have also proven to be useful for geographical origin verification, mainly due to the sensitivity, accuracy and DNA stability. The use of the Polymerase Chain Reaction (PCR) technique as a routine method has also enhanced the expansion of DNA-based tools in control laboratories for seafood authenticity. DNA barcoding based on Sanger sequencing has demonstrated to be a very useful authentication tool for species identification, but it results inconvenient to identify multiple Single Nucleotide Polymorphism (SNP) required for the identification of geographical origin. Instead, Next Generation Sequencing (NGS), or second-generation sequencing, may solve this issue. Unfortunately, NGS applications are slow, expensive, and need well-equipped laboratories and bioinformatic skills to analyse the large amounts of sequence data and therefore is not portable nor user-friendly at all for rapid testing. In this regard, Third-generation sequencing (also known as long-read sequencing), such as Oxford Nanopore, can be applied to solve this constraint since one of its key advantages includes portability and sequencing speed.

In a previous work, we used thousands of genome SNPs, obtained by Restriction site-Associated DNA sequencing (RAD-seq), to provide a detailed genetic structure of the *M. galloprovincialis* [1]. This genetic information was eventually refined and reduced to a panel of ten SNPs which allowed the correct origin assignment of mussels to the Atlantic or Mediterranean areas. This final panel has been successfully implemented in the MinION™, a small and portable third-generation sequencer (Oxford Nanopore Technologies), encouraging more frequent testing with a quicker turnaround time and at a lower price.

1. del Rio-Lavín, A., et al., *Population structure and geographic origin assignment of Mytilus galloprovincialis mussels using SNPs*. Aquaculture, 2022. **550**: p. 737836.

USING IOT-POWERED IN-SITU AND EARTH OBSERVATION DATA TO IMPROVE FARMERS' ANTICIPATION CAPACITIES OF ANOXIA AND HARMFUL ALGAE BLOOMS IN AQUACULTURE

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Introduction

Anoxia and harmful algae blooms (HAB) can lead to detrimental effects in the environment and in aquaculture. Anoxic events or periods of low dissolved oxygen (DO) can occur due to various factors (e.g. high water temperatures no/low currents and algae blooms). Algae can deplete the water of oxygen however some can also damage the fish gills. The IoT booming since several years allows high frequency and quality data monitoring. IoT improvement has allowed Bioceanor to develop specific predictive models. Through the example of this application in a shellfish farming sector subject to anoxic episodes, we have integrated different data sources (e.g. in situ monitoring, lab analysis and satellite images), to develop an operational tool used by shellfish farmers to anticipate dissolved oxygen concentration 48 hours in advance.

Results

All the sensors monitoring water quality in real-time (24/7, 20 min^{-1}) in a lagoon, representing a sentinel network for risk visualization in real-time. Different parameters were measured in three ways, through *in situ* sensors, locally by sampling, and by satellite.

The collected data were first qualified and processed (elimination of outliers, treatment of erroneous data, integration of time series ...) to use only qualified data for the models. The data that have been collected was firstly analysed to help identify key parameters and their variation over time before, during, and after an event of interest like anoxia or HAB. Machine learning analyses were subsequently used to create algorithms that could predict these events. Bioceanor has been able to develop and to run an algorithm that predicts DO 48h in advance with a 4% error rate, using data from IoT devices. Algorithms to predict HAB using in situ measurements and satellite, under development, yield encouraging results.

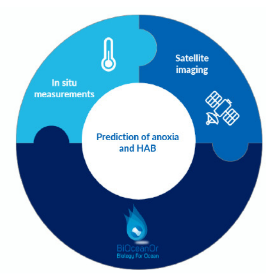

Figure 1: Bioceanor uses in situ measurements, satellite imaging and machine learning to create algorithms to predict oxygen and HAB.

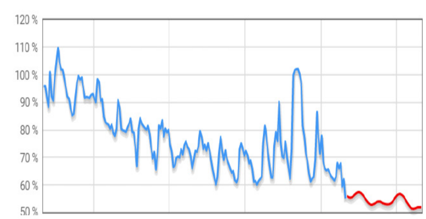

Figure 2: Real-time data (blue) and prediction (red) of dissolved oxygen.

Discussion and conclusion

The development of IoT simplifies the collection of large amounts of data, in real-time and at high frequency. It allows us to build larger and more robust data sets. Using these data with machine learning opens the world of forecasting. Several risks exist for the different industries dependent on water quality and being able to anticipate some of these events, like anoxia and HAB, can benefit aquaculture so the farmer can protect the farm and livestock. The development of this technology will be applicable all around the world and will be a benefit to many sensitive areas that are at risk. IoT development allows now massive data collection and open the era of forecasting for water quality and Aquaculture.

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF CYCLIC GMP-AMP SYNTHASE FROM RED-SPOTTED GROUPER (*Epinephelus akaara*) IN RESPONSE TO IMMUNE STIMULATION AND VIRAL INFECTION

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The cyclic GMP-AMP synthase (cGAS)-Stimulator of Interferon Genes (STING) pathway is a crucial component of innate immune system that protect host against various bacterial and viral pathogens. When cGAS senses foreign DNA or RNA in the cytoplasm of cells, it catalyzes the production of cGAMP, which can bind STING and activate the production of interferons (IFNs) and other cytokines that promote host defense. In this study, we identified a cGAS homologue (EacGAS) from red-spotted grouper. The open reading frame of EacGAS is 1509 bp in length, encoding a predicted protein of 502 amino acids. The domain prediction and phylogenetic analysis of EacGAS protein sequence was performed using online bioinformatics tools and software. To determine the spatial expression pattern of EacGAS, quantitative real-time PCR (qPCR) was conducted in 12 different tissues of the healthy, red-spotted groupers. The highest expression of EacGAS was found in the gill. Furthermore, the effects of immune stimulants on the temporal expression pattern of EacGAS was examined in the in the peripheral blood stem cells (PBSCs) of red-spotted groupers challenged with LPS, poly I:C, and nervous necrosis virus (NVV). Poly I:C challenge showed the highest induction effect on the expression of EacGAS at 24 h post-injection (h.p.i), while the LPS challenge stimulated the highest expression of EacGAS at 48 h.p.i. Under NNV challenge, the expression of EacGAS was gradually upregulated and reached peak at 48 h.pi. To further characterize the function of EacGAS, the expression of NF-κB was compared between the raw cells transfected with empty pcDNA3.1(+) vector and pcDNA3.1-EacGAS. The results revealed that the EacGAS overexpressing cells produced higher level of NF-κB, indicating NF-κB as a downstream mediator of cGAS-STING pathway in red-spotted grouper. The role of EacGAS in antiviral defense was investigated in the FHM cells transfected with pcDNA3.1-EacGAS. Compared to control cells, the EacGAS overexpressing cells showed significantly higher cell viability after VHSV infection. Taken together, our results suggest that EacGAS may act a key player in antiviral innate immune response via activation of NF-κB signal pathway in red-spotted grouper.

INLAND SALINE AQUACUTLURE IN WESTERN AUSTRALIA; PAST, PRESENT AND FUTURE?

Gavin J. Partridge

Western Australia has most of Australia's salt affected land (> 2 million hectares), predominantly in the Wheatbelt region in the south of the state. In addition to affecting agricultural land, secondary salinisation also directly affects rural infrastructure and towns. A range of different mitigation strategies have been implemented across the state to protect these various assets.

From the late 1990s through to ~2010 a significant body of work was conducted nationally investigating inland saline aquaculture (ISA) as an adaptative strategy to this environmental problem which was seen to have many potential economic, social and environmental benefits. This research was funded through a range of federal and state agencies and granting bodies.

This presentation outlines the history of those investigations in Western Australia and focusses on the fish species considered and the culture technologies which were developed and assessed. A case-study of an operating inland saline farm which was subsequently developed in the WA Wheatbelt is presented as well as an outlook for the future of inland saline aquaculture in the state.

POST-HARVEST QUALITY OF FARMED BARRAMUNDI *Lates calcarifer* IN AUSTRALIA

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Its timely to consider the product quality lessons gained from the Australian barramundi industry's first few decades of R&D. The barramundi (*Lates calcarifer*) is Northern Australia's foremost aquaculture species in volume, making market differentiation and spread into new product forms vital. To prepare for this, barramundi aquaculture research from the last few decades was examined to identify areas where further research is justified.

Feed researchers have sought to avoid the inadvertent consequences of feed ingredient substitution. Yet, sensory panels accept barramundi fed on a variety of novel ingredients. Subtle differences are reported, for example changes in fatty acid profile influence the rate of lipid oxidation (rancidity). That legacy of feed development revealed the work required to document sensory parameters – and raised questions about how future barramundi selection programs would instil new traits into fingerlings. Indeed, researchers could do more to standardise recording of flesh colour variability.

Off-flavour in freshwater fish is an issue beyond barramundi farming. It occurs in freshwater pond and recirculation aquaculture systems (RAS). Purging is most practical for RAS barramundi, but researchers also identified ways for pond farmers to avoid harvesting off-flavour fish. If there is no taint in marketed fish, then this comes at an undocumented cost to farms. If that status-quo cost is not known, it is difficult to assign cost-benefit to alternatives.

Harvest stress, such as exercise in the crowd, can alter post-mortem flesh quality of fish. Yet harvesting exercised barramundi directly into ice-slurries leaves sensory panels reporting only subtle variations in appearance, texture, or flavour. It is argued though that rapid cold shock, a practice common to many tropical farmed fish, has a levelling effect upon quality. Instantaneous stunning techniques will permit bleeding of barramundi- and a quality dividend is anticipated.

Product portioning brings customer convenience at the cost of shortened shelf life. Transport of whole, un-gutted barramundi easily gives 2-3 weeks shelf-life under tight cold-chain control. The shelf-life of chilled raw packaged fillets/portions of barramundi is less than a week- portioning must occur close to consumers. Smoking is, however, a traditional seafood preservation technique that shows great promise in tests with barramundi. Other studies indicate that consumers could also set aside their misconceptions about quality of frozen fish.

The review concluded that implementing new harvest methods and new products can build from an understanding of existing quality variability in the marketplace (especially colour and flavour). If, as expected, alternative harvest methods raise the average for flesh quality, then this should be followed through with studies of portioned and value-added samples. Understanding how harvesting steps impact processing of product will set up Australia's barramundi farming sector for a future of successful product innovation.

THE STATE-OF-THE-ART OF AQUACULTURE FEED EXTRUSION: CHALLENGES AND OPPORTUNITIES

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The manufacturing of aquafeed through extrusion has been an attractive process due to the continuous nature of production, the scalability of equipment, and the tunability of processing conditions to achieve specific product properties. In addition, extrusion processing can handle a wide range of formulation matrices, which allows the extruded feed to be tailored to specific fish species and their feeding preferences, in addition to satisfying their nutritional requirements. However, a practical limitation currently exists with regard to understanding the mechanisms governing material transformation throughout the extrusion device, which leads to difficulty in innovating, predicting, and controlling product quality. A nascent body of literature is emerging in an attempt to address inefficiencies in aquafeed product development and process optimisation, yet significant challenges remain unexplored within the literature. This study investigates the current state-of-the-art in aquafeed extrusion via a literature review, outlining the type of studies performed, the typical characterisation metrics covered, an overview of the key results, and the challenges and opportunities remaining within the field.

In the first section, the study investigates the ingredient matrices used to generate feed products, including alternative protein sources (besides fishmeal) such as insect meals, plant proteins, and microalgae products, in addition to the effect that different ingredients have on pellet quality. The second section outlines the influence of various processing parameters (e.g. barrel temperature, moisture content, screw speed) on the extrusion response variables (e.g. specific mechanical energy) and extrudate physical properties. The third section presents the subsequent impact of extrudate physical properties (e.g. texture, density, solubility) on the performance of aquaculture research trials, including feed conversion/utilization, nutrient digestibility, and animal health. Lastly, the fourth section addresses key challenges facing feed extrusion in the aquaculture industry, such as feed availability and variability, animal health and nutrition, and economic feasibility, in addition to outlining opportunities for innovation and improvement, such as digital tools for quality control and by-product utilization. Overall, this review outlines the state-of-the-art in extrusion technology for producing high-quality aquafeed, while highlighting barriers and gaps to efficient and cost-effective aquafeed manufacturing.

Figure 1: Word cloud of top 80 words mentioned within the title, keywords and abstract of literature studies relevant to the topic of aquafeed extrusion.

EVALUATING THE IMPACT OF BROODSTOCK DIET ON FLORIDA POMPANO (*Trachinotus carolinus*) EGG QUALITY AND LARVAE DEVELOPMENT

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One of the biggest challenges in commercial marine warmwater finfish production is consistent high-quality eggs, to ensure a steady supply of seedstock. A sufficiently nutritious broodstock diet is needed because a maternal diet would greatly influence the survival and development of the offspring. Florida Pompano (*Trachinotus carolinus*) is a coastal species common to Florida coasts with growing interest for commercial aquaculture production. The objective of this study was to evaluate the impact of different diets of the Florida Pompano broodstock, such as the control diet (cut-bait shrimp), commercial Breed-M and the green seaweed *Ulva*-based diet, on the quality of eggs, specifically the fertilization and hatching rate, the growth rate, the use of endogenous nutrients, and the fatty acid profiles of embryos and larvae during the endogenous and exogenous feeding period. The experimental design includes 40 fish evenly distributed in 4 tanks of 2.5 m in diameter (7.8 m^3) installed in identical Recirculating Aquaculture Systems (RAS). Broodstock in each tank were fed the same diet 4 times a day at 10 % of the fish body weight. At the time of spawning, after each diet treatment, eggs from each tank were incubated in 4 replicates. Eggs were taken for biochemical analysis and frozen at -80°C from each incubator and fresh samples were placed under the microscope for morphological measurements. It is expected that the addition of *Ulva* in the feed would provide sufficient nutrient levels for quality eggs and larvae comparable to the experimental diets and produce a similar or higher yield than the control and Breed-M diets.

ENHANCING DISEASE RESISTANCE AND PRODUCTIVITY IN PACIFIC OYSTERS THROUGH BREEDING STRATEGIES

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Pacific oysters (*Crassostrea gigas*) are one of the most widely cultivated oyster species in the world, and are farmed extensively in many regions, including Asia, Europe, and North America. In Australia, it is currently one of the largest aquaculture species after Tasmanian Atlantic salmon. Selective breeding has been an essential tool for improving the production and disease management of Pacific oysters in Australia. Australian Seafood Industries (ASI) is an industry-owned selective breeding program focused on increasing resistance to Pacific Oyster Mortality Syndrome (POMS), improving commercial traits (such as meat condition, shell shape, and growth), and increasing overall survival.

Breeding for POMS-resistant oysters began in 2012, with the deployment of existing families from the 2011-year class in New South Wales, Australia. Families specifically selected for POMS resistance were produced for the first time in November 2012 through the spawning of the 2012-year class. POMS outbreak in Tasmania (TAS) disrupted breeding in early 2016. Subsequently, the breeding goal in TAS shifted from adult (12-month-old) to spat (2–3-month-old) POMS resistance. Spat resistance and adult resistance for POMS were similar but not identical traits (0.6 correlation). Trials were conducted using two populations: TAS and South Australia (SA). Research focused on the TAS population as SA farming regions were free of POMS, and trials required exposure to the disease for in-family selection. The trials included ten-year classes (2012-2022), 1502 families, 83 field trials, and over one million individual oysters.

Adult resistance increased from 12% to 100% mean estimated breeding value (EBV), and spat resistance increased from -35% to 67% mean EBV within the TAS population. However, POMS resistance in adults remains a work in progress in SA. In 2022, TAS POMS-resistant stock was bred with SA stock after eight generations of genetic divergence caused by biosecurity barriers. This resulted in increased adult POMS resistance by 24% predicted EBV. The latest commercially available stock, exhibiting high resistance to POMS, has been released for commercial hatcheries, with farmers expected to procure it in the coming year. Further selection is necessary to produce highly resistant brood stock for both spat and adult resistance in TAS and SA. Outcomes to date indicate feasibility and the significance of selective breeding for POMS resistance in safeguarding the industry from potential losses in the future.

BUSINESS SOLUTIONS FOR SMALLHOLDER AQUACULTURE IN THE PACIFIC REGION

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There is a mixed record of sustainable outcomes from donor-funded R&D investment in aquaculture in the Pacific region. The market size and commercial viability of aquaculture enterprises, amongst other factors, place significant constraints on uptake of innovations at the scale needed and long-term viability and "strength". FUTUREFISH is a UK aquaculture company that specialises on business solutions for small-scale aquaculture. The paper provides the results of an assessment of tilapia aquaculture enterprises in the Pacific and insights from aquaculture businesses within and outside of the region on potential critical success factors and considerations for future aquaculture investment within the Pacific, with special attention to tilapia aquaculture. The research presented is part of a review being supported by the Australian Centre for International Agriculture Research (ACIAR).

BUSINESS SOLUTIONS FOR SMALLHOLDER AQUACULTURE LIVELIHOODS

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Aquaculture is growing fast, with significant innovation and investment across the sector that responds to growing market demand for fish and other aquatic foods. Innovations and investments are being made throughout the whole aquaculture system globally – from production systems through to the supply chains that deliver food from farm to plate. Aquaculture is increasingly being recognized for its potential contribution to healthy and sustainable food systems and economic development and as a low-carbon food production system. Yet, innovations and investments in aquaculture are applied unevenly, often used for higher-value commodities, and investments are not always reaching where they are needed. Crucial parts of the aquaculture ecosystem are not receiving attention – millions of small-scale actors - farmers, traders and consumers - across Africa and Asia lack access to the opportunities that aquaculture could provide for decent work, good health, and sustainably produced food. FUTUREFISH is a new aquaculture company that specialises in working with partners to develop and apply business solutions for small-scale actors in aquaculture, with a geographical focus on the Asia-Pacific and African regions. The presentation will provide early insights from our work with women entrepreneurs and small-scale fish farmers in Africa and Asia and the opportunities these insights are opening for new aquaculture business approaches that are inclusive and supportive of innovation and investment focussed on smallholder livelihoods.

AQUACULTURE NEEDS, PRIORITIES, AND FUTURE DIRECTIONS IN THE PACIFIC ISLANDS REGION

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The Pacific Islands aquaculture sector has great socio-economic value and economic potential, but remains largely under-developed. There are many reasons for this, and they are not limited only to matters of technical capacity. There are numerous challenges that continue to deny the Pacific region the long- term benefits that can be derived from the development of sustainable aquaculture. SPC members nevertheless aim to mainstream aquaculture into their economic and social development agendas, as a response to (i) the decline of coastal fisheries, (ii) population growth, (iii) increase resilience to climate change and natural disasters, and (iv) achieve post-pandemic economic recovery.

A Regional Aquaculture Assessment was conducted among SPC members in 2021-22, and a consultative Regional Aquaculture Strategy process is ongoing for completion in 2023 for endorsement by SPC members. Though not yet complete, some priority areas for actions under the Strategy are likely to include:

- Emphasis upon *Extractive* (unfed), *Restorative* or *Nature-positive* (improves the environment) and *Climate-smart* (resilient, and/or sequesters carbon) forms of aquaculture.
- Expansion of aquaculture through *under-utilized species* (bivalve shellfish, seaweeds, sea cucumbers) and *under-utilized environments* (offshore, freshwater) can play to the strengths of the Pacific region, if constraints in hatchery capacity and food safety are addressed.
- Further research on *indigenous species* with aquaculture potential, such as freshwater fishes, bivalve molluscs, sea cucumbers, and seaweeds.
- "Public money" *co-funding mechanisms* for expansion of commercial aquaculture that delivers public-good benefits should be explored: from development-partners, donors, and/or philanthropic foundations.
- Multi-sectoral approaches to explore closer *integration of blue and green economies* via Integrated Coastal Food Systems, to develop "scalable and context-specific integrated coastal food production systems that are climate resilient, nourishing, culturally relevant, and support equitable livelihoods appropriate to local contexts" (working across agro-forestry, soil and livestock management, community-based fisheries, aquaculture and other sustainable natural resource management practices, including strengthening the food- water-energy nexus).

TILAPIA IN TIMES OF WAR, CASE STUDY OF A COMPANY IN HAITI

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Taino Aqua Ferme S.A. is a company founded in Haiti in 2015 that to date has harvested 1200 tons of tilapia grown in cages in Lake Azuei, a lake with 10 ppt salinity located on the border with the Dominican Republic. Since the assassination of President Jovnel Moise in July 2021, the country has entered a phase of violence and misrule that has made it extremely difficult to operate a company, especially an aquaculture company that depends on importing feed from abroad and sells its fresh whole fish in the domestic market where there is no road safety because gangs have taken over the main roads.

The emphasis of this talk is to demonstrate with results of harvested cages that tilapia has been an ideal species to cultivate in this difficult situation of no governance, where we have reached periods of up to 33 days without feeding the cages and when we have managed to import feed, rations have been below 40% satiation.

As expected, the grow out cycle has increased on average by about 62 days (increase of 29%) and the average weight at the harvest has decreased by 122 grams on average (decrease of 20%). The pleasantly surprising and outstanding characteristic about tilapia´s biology has been that, with such extended periods without feeding and underfeeding, production efficiency indices have not deteriorated. The feed conversion ratio improved from 1.79 to 1.69 and survival from vaccination and laser count improved from 83% to 86%.

It does not make sense to underfeed, or not feed, to obtain better efficiency rates in harvest results, since the productivity of the system decreases substantially. But in times of difficulty in the operation of a company in countries where security and governance problems are likely, logistical difficulties or natural disasters, the biology of tilapia and its ability to filter natural foods and take advantage of fouling on tank or net surfaces makes it an ideal species for producing in developing countries.

EXPORING THE USE OF INNOVATIVE SUSTAINABLE PROTEINS IN MARINE FINFISH DIETS

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Each year ~16M tonnes of wild caught fish are converted into 5M tonnes of fishmeal (FM); a quantity which has been relatively constant for the past decade. Approximately 70% of this FM is used in aquafeeds. The global aquaculture industry is rapidly expanding and in 2020 production of finfish reached 55.5 M tonnes, doubled since 2010. If aquaculture is to maintain this growth in the face of a stable supply of FM, more sustainable protein ingredients must be identified to enable the expansion of the industry.

Our recent studies have investigated the use of novel alternative proteins in diets for marine yellowtail kingfish (YTK) (*Seriola lalandi*) and barramundi (*Lates calcarifer*). The proteins trialled have included black soldier fly (BSF, *Hermetia illucens*) larvae meal and single cell proteins (SCP) derived from *Methylococcus capsulatus* (StringBio Pty. Ltd.) and *Cupriavidus necator* (Kiverdi Inc.) as replacements for FM. The studies conducted in YTK have found digestibility and FCR of BSF and *Methylococcus capsulatus* proteins were equal to or better than control FM diets. However, YTK showed reduced feed intake when FM was replaced at high inclusion levels with these proteins. Improved palatability would be highly beneficial in achieving FM-free diets for YTK.

Barramundi do not appear to have the same palatability issues as YTK, and this species demonstrates the path forward for a zero FM diet. A 56-day study investigated the effects of gradual replacement of FM with SCP derived from either *Methylococcus capsulatus* (MC) or *Cupriavidus necator* (CN) on the growth performance, feed utilization and fish health in barramundi. The treatment diets included a control containing 15% FM and three diets for each SCP at inclusions of 10%, 20% and 30% (corresponding to 33%, 66 and 100% FM replacement). The feed consumption across all treatments was uniform with no effect of either SCP or inclusion level. Weight gain increased significantly in barramundi fed the diets containing CN at 30% inclusion compared to the control diet *($P > 0.05$)*. The improved growth in barramundi fed CN at 30% resulted from an improved FCR compared to the control diet (Table 1).

Table 1. Comparison of alternative proteins (BSF, black soldier fly; MC, Methylococcus capsulatus *SCP; CN,* Cupriavidus necator*) as sustainable replacements for fishmeal (FM) in diets for yellowtail kingfish (*Seriola lalandi*) and barramundi (*Lates calcarifer*). The best performing FM replacement level for SGR and FCR compared against the corresponding control diet for each trial.*

Fish species	Protein	FM replacement (%)	Control diet		FM replacement	
			SGR (%)	FCR	SGR (%)	FCR
Seriola lalandi	BSF	50	3.8	0.99	3.8	0.98
Seriola lalandi	MC	25	2.6	1.14	2.5	1.07
Lates calcarifer	MC	75	1.8	1.03	2.1	0.88
Lates calcarifer	MC & CN	100	1.6	1.2	2.2	0.96

NUTRITION BASED STRATEGIES TO IMPROVE THE YELLOWTAIL KINGFISH (S*eriola lalandi*) AQUACULTURE INDUSTRY IN AUSTRALIA

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Yellowtail Kingfish (YTK; *Seriola lalandi*) aquaculture in Australia is considered an emerging industry that presents the most significant potential for growth of the developing aquaculture industries, with some estimates predicting that Australian production of YTK could increase to 34,000 tonnes worth $440 million over the next 10 years. However to grow the industry key bottlenecks still remain particularly with regard to nutrition, feeds and feeding strategies. Here we present a synopsis of some of the key research findings of a multi-year collaborative R&D program investigating the nutritional requirements, diet development, and feeding strategies for YTK. Key outcomes include quantifying the requirements for the essential nutrients such as choline, taurine, methionine and cysteine, the comprehensive assessment of a suite of dietary raw materials to facilitate cost effective feed formulations, successfully reducing fishmeal in diets by over 70%, refining bioenergetic growth models to improve predictive accuracy and identifying feeding strategies to reduce FCR's and improve cost benefits. Taken overall, the application of the programs results will help towards the development of more cost effective, sustainable feeds and feed management strategies ultimately improving farm productivity by reducing operating costs and improving sustainability.

EVALUATION OF NANOBUBBLE TECHNOLOGY IN AQUACULTURE

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In intensive aquaculture systems oxygen supplementation is necessary to prevent hypoxia; however, oversupply can hyper-saturate systems causing gas bubble disease. Oxygenation in aquaculture systems using standard technologies is extremely inefficient; standard oxygen transfer efficiencies are estimated at between 2 and 6% per meter submergence for coarse and fine bubble diffusers, respectively, at standard conditions of 0 ppt salinity and 20 °C. Advances in the efficiency of gas–liquid phase processes has seen the emergence of nanobubble technologies producing ultrafine bubbles (⌀ <1 μm). The advantage of nanobubbles over larger micro/macrobubbles is that they are neutrally buoyant, negatively charged and can remain within the water column, potentially for weeks. Nanobubble technologies now have a demonstrated application across a broad variety of industries including wastewater treatment, biomedical engineering, gas and oil industry, agriculture, and the food industry. Surprisingly, outside of Japan, there has been little research on the application of nanobubble technology to the aquaculture sector. Nanobubble technology can potentially improve oxygen delivery systems for fish stock and water treatment in aquaculture systems, improving the nitrifying capacity of biofilters and efficiencies in fractionation units. However, there are currently no reliable studies demonstrating the efficacy of nanobubble technology, nor any assessment of the potential health impacts on fish in aquaculture systems. We have undertaken a preliminary pilot trial with encouraging results. This project investigates the efficacy of nanobubble technology for use in finfish aquaculture by assessing the health, growth and feed conversion efficiencies of fish cultured in a recirculating aquaculture system at different temperatures, salinities and stocking densities exposed to nanobubbles.

S2AQUAcoLAB: YOUR STRATEGIC PARTNER IN AQUACULTURE

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The S2AQUAcoLAB is a private non-profit institution, founded in 2021, whose mission is to elevate aquaculture to a new level by playing an interface role between academia, research and industry. The coLAB performs research in production optimization, identification of health and welfare markers, climate change adaptations and development of new products for market diversification. Its members include 1 state laboratory (IPMA), 2 higher education institutions (UAlg and IPL), 2 R&D centre (CCMAR and ARDITI), 1 municipality (CMO), 2 producer's association (Formosa and COOPAQUA) and 8 private companies (FLATLANTIC, SPAROS, NECTON, P. Espargueira, Oceano Fresco, Riasearch, SOFish, Docapesca). The S2AQUAcoLAB has 49 associated researchers with proven experience and countless scientific publications in the area and hired, until now, 20 highly qualified human resources. The ongoing research lines comprise: 1) Training of specialized human resources; 2) Optimization of the production of several marine organisms; 3) Analytical tools for the evaluation of bioindicators of health and welfare and development of *in vitro* systems; 4) Environmental monitoring and adaptation to climate change; and 6) New products, technological development and market. The S2AQUAcoLAB and its network of partners have the infrastructure, equipment and means to pursue scientific research of excellence whilst is exceptionally well-positioned to establish the link between scientific outputs and stakeholders.

The main goal of the S2AQUAcoLAB is to carry out R&D activities with a view to innovation for sustainable and intelligent aquaculture It aims to play an active role in the transfer of knowledge and technology, provide services that increase food safety, and diversify aquaculture products.

Acknowledgments: Project ALG-05-3559-FSE-000021 and Programa Interface (aviso AAC n.º 01/C05-i02/2022)

EPPO: A PORTUGUESE RESEARCH FACILITY FOR THE DEVELOPMENT OF AQUACULTURE

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The Portuguese Institute for the Ocean and Atmosphere (IPMA, I.P.) is a public research institute and act as a counselor to the national authorities on the sea and atmosphere. IPMA, I.P., possesses a strong cluster of competences for the ocean and marine resources related to research, carried out by different groups, particularly dedicated to aquaculture and fisheries.

The Aquaculture Research Station of Olhão (EPPO, figure 1) stands out for the unique experimental conditions on aquaculture at the national and international levels. This marine core facility is equipped to carry out production studies at every scale from bench-top laboratory work to a much larger semi-industrial level. EPPO has an area of about 7ha with more than 250 tanks, including an hatchery fully equipped for research and experimental production with different rearing circuits (for broodstock, larvae, juvenile production, research with live animals and recirculation systems), a support building (with rooms for trophic chain production, daily routines and biological sampling), several analytical laboratories (biochemical, histological, molecular, microbiological and fish pathology), an unit for seafood packing, an area for pre-fattening (for earthen ponds and sea cages production) and 17 earthen ponds. It holds breeders of several marine fish species (e.g. meagre, gilthead seabream, seabass, Senegalese sole and sardine among others), microalgae and invertebrates as well as the know-how on the production of these species.

Production of new species, nutrition, welfare, environmentally friendly production systems and assessment of onshore and offshore and production systems for fish grow-out are some of research lines developed at EPPO (figure 2).

Figure 1 - Aerial view of the EPPO

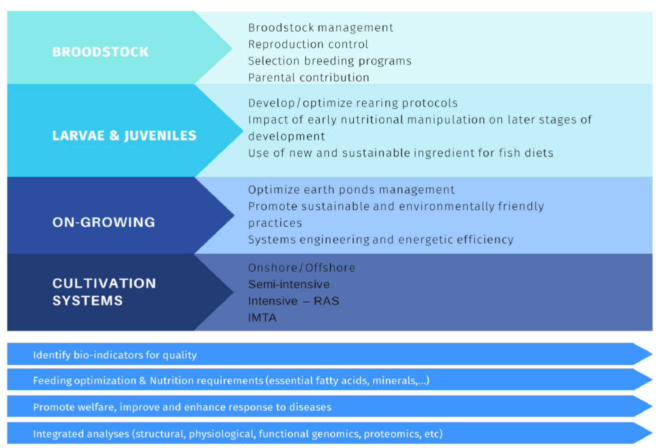

Figure 2 - On going research lines at EPPO

Acknowledgments: The research lines are partially funded by AQUARAS (Mar-02.05.01-FEAMP-0223) and ALLARVAE_069971 (ALG-01-0247) projects.

THE IMPACT OF PROBIOTICS ON GREEN WATER TECHNIQUE FOR MARINE FISH LARVAE

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Introduction

The continuous growth of world's population, led to an intensification of aquaculture, exposing fish to a wide range of stressors. Additionally, the larval phase of marine fish is the most sensitive, with common culture failure and unpredictable survival. Recent studies demonstrated that the introduction of probiotics could be a promising alternative to improve survival and overall fish health and welfare with evidence showing a better fish growth, enhanced immune response, disease prevention, and improved water quality (Chauhan & Singh, 2019). The goal of this work is to better understand the impact of the use of probiotics on larval growth, intestinal flora, and fish survival upon acute stress exposure.

Methods

As an example, work was carried out on the green water technique in which a control treatment (CTRL) was used, with live *Nannochloropsis* sp. and a group with a formulation of *Nannochloropsis* sp. and *Chlorella* sp. supplemented with probiotics (NCp). Larvae of *Argyrosomus regius* from the EPPO – Aquaculture Research Station - were maintained for 18 days after hatching (DAH) and growth, malformations, and survival were analyzed. At this point, larvae were transported for 5 hours to induce stress. Pools of larvae, from each treatment, were collected before and after stress to measure the impact of the treatments on oxidative stress by analyzing the enzymatic activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), the gene expression of markers of growth (*igf1r*), and stress response (*metII* and *cat*). Larvae were also collected for microbiological analysis (total marine bacteria and Vibrionaceae). A similar trial was performed with Gilthead Seabream (*Sparus aurata*).

Results and Discussion

The use of the new microalgae formulation containing probiotics promoted survival of 61.20 ± 15.79 % in NCp and 64.18 ± 9.31 % in the CTRL group and growth was identical in both treatments. However, the occurrence of malformations was higher in the NCp group, tending to be more severe. Throughout the trial, there were no significant differences in the number of total marine bacteria and Vibrionaceae in the digestive tract of the larvae. Through analysis of antioxidant enzymes, growth, and stress genes it was observed that the use of probiotics significantly increased the tolerance of larvae to stress.

Enzyme activity and genetic analysis suggested a positive effect on tolerance to an external factor when probiotics were added to the green water technique, overall contributing to improve their biological performance.

Overall, no deleterious effect was observed with the use of probiotics and a tendency for a better survival was observed with the supplementation, for both species, upon an acute stress.

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LONG-TERM SUSTAINED SWIMMING IMPROVES SWIMMING PERFORMANCE IN CHINOOK SALMON *Oncorhynchus tshawytscha* WITH AND WITHOUT SPINAL SCOLIOSIS

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Exercise training during pre-and post-smolt production is becoming a key component in salmon hatcheries as it is known to enhance several production-related traits in salmonids. Exercise conditions for rearing salmonids are continually being optimised and now that the salmonid industry is developing offshore, training is being considered as a tool to prepare domestic stocks for high energy environments. It is unknown if exercise can enhance traits in other understudied salmonid species and in individuals with spinal curvature (scoliosis), which is a common issue within some salmon farms. Here we exposed Chinook salmon to low (0.3 bl s^{-1}) and moderate (0.8 bl s^{-1}) tank velocities for ten to eleven months and quantified respiratory and swimming performance in individuals with and without mild scoliosis. Further, we investigated compositional changes and morphological responses at cellular and whole-body levels. Raising Chinook salmon under moderate velocities improved swimming performance in individuals with and without spinal curvature, but recovery processes in individuals with spinal curvature required higher energetic costs. Fat content was reduced in fish raised under moderate velocities, while protein content was higher in individuals with spinal curvature. Exercise regimes caused morphological changes in muscle fibres, gill, and skin. Together, the results of this study shows benefits for integrating exercise training into hatchery settings (i.e., pre- and post-smolts) to prepare stocks for offshore farming and provides evidence that some exercise-enhanced traits can be translated into individuals with spinal curvature, but concerns remain for individuals with more severe spinal curvature. Additionally, this study reveals that the product quality of fish farmed in offshore locations may differ to current product quality, and that optimising nutrient profiles for offshore feeds should be considered.

DIETARY β-GLUCAN PARTIALLY PROTECTS WHITE SHRIMP *Litopenaeus vanname* FROM ANTIBIOTICS-INDUCED SIDE EFFECTS IN INTESTINAL MICROBIAL COMPOSITION

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Antibiotics have been used worldwide to prevent, control and treat outbreaks of bacterial diseases in animal production. However, most antibiotics and their secondary metabolites cannot be absorbed and are then discharged into the aquatic environment, which might act as a severe threat to aquatic health. The present work evaluated whether dietary antibiotic-induced side effects in the shrimp could be improved by the dietary addition β-glucan in white shrimp (*Litopenaeus vannamei*) (average weight 0.37 ± 0.02 g). A 6-week feeding trial was conducted with four groups: control, 1g/kg β-glucan; 50mg/kg oxytetracycline (OTC) and 50mg/kg OTC with 1g/kg β-glucan (Mix). Parameters including growth performance, intestinal microbial composition, antioxidant capacity and non-specific immunity were determined.

The condition factor of *L. vannamei* in the Mix group was lower than those the other groups. The OTC group displayed higher SOD and CAT activities in the hepatopancreas than the β-glucan group. The MDA content in all groups showed no significant differences. The removal of low-quality reads and chimeras yielded a total of 1,690,040 clean sequences with intestinal contents and lengths ranging from 401 to 440bp (average 413 bp). Compared with the control group, the Observed_species index was significantly increased in the Mix group. The most dominant phyla in the gut of all samples were Proteobacteria, Bacteroidetes and Actinobacteria. Actinobacteria abundance was significantly greater in the OTC group compated with the groups without OTC, but the abundance of Proteobacteria and Bacteroidetes showed a totally different trend (Figure 1).

Table 1. Formulation and proximate composition of the experimental diets.

Ingredients (%)	Dietary β-(1, 3)-glucan concentration			
	Control	β- glucan	OTC	Mix
Fish meal	26	26	26	26
Soybean meal	28	28	28	28
Corn starch	23	23	23	23
Shrimp meal	4	4	4	4
Calcium dihydrogen phosphate	1.5	1.5	1.5	1.5
Vitamin premix[a]	2	2	2	2
Mineral premix[b]	2	2	2	2
Choline chloride	1	1	1	1
Fish oil	2.5	2.5	2.5	2.5
Soybean oil	2.5	2.5	2.5	2.5
Soybean lecithin	1	1	1	1
Cholesterol	0.5	0.5	0.5	0.5
Carboxymethylcellulose (CMC)	3	3	3	3
Butylated hydroxytoluene (BHT)	0.1	0.1	0.1	0.1
Microcrystalline cellulose	2.9	1.9	2.4	1.4
β-(1, 3)-Glucan[c]	0	1	0	1
Oxytetracycline[d]	0	0	0.5	0.5
Toral	100	100	100	100
Nutrient levels (%)				
Crude protein	35.2	35.5	35.3	35.5
Crude lipid	7.6	7.6	7.6	7.6
Ash	10.3	10.5	10.6	10.7
Moisture	9.2	9.2	9.2	9.2

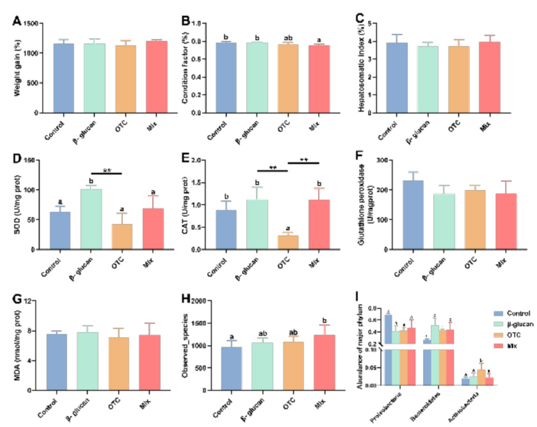

Figure 1. Effects of β-glucan and OTC on growth performance, antioxidant capacity and gut microbiota composition of *L. vannamei*. (A) Weight gain, (B) Condition factor, (C) Hepatosomatic index, (D) SOD, (E) CAT, (F) Glutathione peroxidase, (G) MDA, (H) Observed_species and (I) Abundance of phyla level.

GILL HISTOLOGY AND MICROBIOME ASSOCIATIONS ACROSS DIFFERENT HATCHERY REARED ATLANTIC SALMON

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Fish gills are in intimate contact with the aqueous environment. In intensive aquaculture production, variable physical and chemical water parameters, high stocking density, and husbandry procedures can disrupt the homeostasis of commensal and pathogenic microbiota on the gills. This has the potential to impact fish welfare and increase susceptibility to gill damage. This study investigated the relationship between microbial community profiles and gill pathology during a production cycle of Atlantic salmon in two commercial hatcheries. Relationships between gill histology, environmental conditions, and microbiome were determined using high-throughput data, including 16S rDNA amplicon sequencing data, histopathology data, and water quality parameters. Hatchery A used riverine water and operated a mixed system of recirculation aquaculture system (RAS) and flowthrough. Hatchery B used bore water and operated a RAS. Melanin deposits, hyperplastic, and inflammatory lesions were observed in the gills using histology. A higher prevalence of melanin deposits was detected and correlated to a change in beta diversity of bacterial communities in early time points (fingerling and parr stages). High abundance of *Sphaerotilus* sp., *Pseudomonas* sp., *Nitrospira* sp., *Exiguobacterium* sp., *Deinococcus* sp., and *Comamonas* sp. was correlated with a high prevalence of melanin in filaments. Bacterial diversity increased as the fish cohort transitioned from RAS to flowthrough in hatchery A. This study describes the major associations between gill histology and commensal microbiome of Atlantic salmon under commercial conditions.

Table 1. Significance of beta diversity statistic R2 (p adj) of gills with and without hyperplastic lesions, inflammation and melanin deposits from hatchery A and B.

Hatchery	Gills	Hyperplasia	Inflammation	Melanin deposits
A	With vs without	0.012 (0.478)	0.019 (0.127)	0.041 (0.006)
B	With vs without	0.036 (0.068)	0.028 (0.145)	0.092 (0.002)

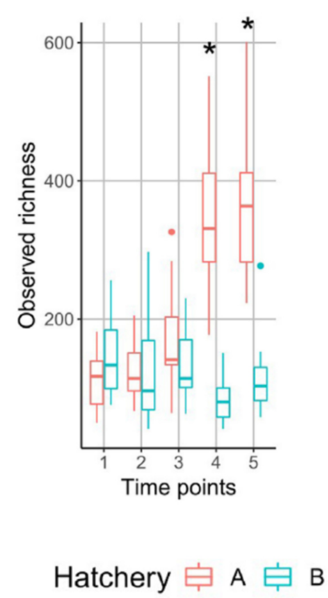

Figure 1. Alpha diversity indicator: richness of bacterial communities of gills of Atlantic salmon from two commercial hatcheries. (Quezada-Rodriguez et al., 2023)

INNOVATIONS IN REMOTE TELEMETRY WATER QUALITY MONITORING AND ITS INFLUENCE ON IMPROVED AQUACULTURE MANAGEMENT

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Water quality monitoring is a critical aspect of aquaculture management, as poor water quality can lead to disease outbreaks, reduced growth rates, and even death of farmed aquatic species. Methods used in aquaculture to monitor water quality have improved over the years, leading to more data becoming available to managers, which in turn improves management decision making. Remote monitoring of water quality through sensors connected to telemetry systems is one such innovation. Telemetry systems transmit data wirelessly to a central location, where it can be analysed and acted upon in real-time.

Jervis Bay, located in New South Wales (NSW), experiences occasional water quality issues due to rainfall runoff which adversely affects mussel farming in the area. In collaboration, the University of Technology Sydney (UTS), Department of Primary Industries (DPI) and South Coast Mariculture (SCM) deployed two telemetry buoys equipped with water quality and current profiling instrumentation. Researches from UTS will now be able to model water quality activity and farmers from SCM will be able to react to events in real-time. The telemetry systems have the potential to improve management practices and ultimately productivity of the farms.

Advancements in telemetry systems have led to the creation of the underwater acoustic modem. In earlier iterations, issues arose around signal quality between the underwater modems and base telemetry system. Point to point transmission would fail in high energy conditions. An innovative wireless underwater communication system was developed that utilises a patented mesh network with interdependent nodes, each capable of receiving and transmitting data from other nodes. The signal quality in high energy conditions were tested in a Norwegian salmon farm owned by Lerøy with remarkable results. Such a network could represent a new way to expand the capabilities of legacy telemetry systems within aquaculture industries.

Overall, the use of telemetry systems to improve management decision making is becoming common in the global aquaculture community. Technology in this space is improving with every reiteration, making it easier for managers to implement innovative systems. With improved water quality monitoring comes smarter management practices and increased productivity.

ASSESSING THE IMPACT OF ENHANCED DIETARY LEVELS OF BRINE SHRIMP (*Artemia franciscana*) CO-FED WITH MICROPARTICLE DIETS ON THE REARING OF *Litopenaeus vannamei* LARVAE

Ramena

This study aimed to determine the optimal co-feeding combination for the early larval and post-larval stages of *Litopenaeus vannamei* (Pacific white shrimp) by investigating the effects of different levels of Artemia nauplii Instar I co-fed with two commercial micro-particulate diets. Two independent larval experiments were conducted, and the performance of *L. vannamei* postlarvae was evaluated in terms of survival, growth, specific growth rate (MGR), osmotic stress test, fatty acid and amino acid composition, as well as whole-body composition. The results showed that increasing the inclusion levels of Artemia significantly improved survival during the hatchery cycle (PL15) up to a maximum level of 5,900 g of Artemia per million *L. vannamei* postlarvae produced.

Survival rates further improved with increased Artemia inclusion levels during the extended nursery life stages up to PL 35. However, biochemical analysis did not provide a clear explanation for the enhanced survival rates. Additional research is required to fully understand the underlying mechanisms behind the observed improvements. These findings contribute to our understanding of co-feeding strategies for L. *vannamei postlarvae* and emphasize the importance of further investigation in this area.

COMPARATIVE PERFORMANCE OF TRIPLOID OYSTERS, PRODUCED BY CHEMICAL INDUCTION AND MATED TRIPLOID TECHNIQUES, TO THEIR DIPLOID COUNTERPARTS

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The partial sterility of triploid (3n) oysters enables year-round harvest, improved meat quality, and superior growth. Two main techniques are used to produce shellfish triploids: 1) ‘chemical induction’ method, blocking the release of polar body in embryos via chemical treatment and 2) ‘mated triploid’ method, widely used, where tetraploid (4n) males are crossed with diploid (2n) females to produce 3n offspring. There are growing concerns however that mated 3n may be more vulnerable to diseases or climate-driven stressors (e.g., temperature, salinity).

The aim of this research was to evaluate the long-term performance of 3n Pacific oysters (*Crassostrea gigas*) obtained using different induction methods to identify any potential trade-offs (e.g., impaired fitness, reproductive potential, ploidy status, or resilience to environmental stressors) from each method. Using stock of equivalent genetic background, one diploid control (2n) and three groups of triploids derived from chemical induction (3nC) or mating of 2n females with 4n males (3nT1 and 3nT2), were created in the hatchery.

Chemical induction (3nC) yielded 95% triploid larvae compared to 100% for mated triploid groups (3nT1 & 3nT2). Larval performances of mated 3n were comparable to those of 2n, whereas 3nC had lower spat yield than mated triploids.

Resistance to OsHV-1 was tested in the laboratory for each group, followed by a challenge to increasing temperatures to determine median lethal temperature (LT50). No difference in survival between groups were found, whereas thermotolerance of 2n and 3nC oysters was marginally lower than mated 3n. Remaining oysters were then deployed to multiple grow-out sites and their survival and growth assessed periodically. After 18 months, survival was high in all groups (≥ 84%) across all farm sites. We found however significant differences in live weights between groups, regardless of the farm environment (Fig. 1). Mated triploids (3nT1 and 3nT2) were on average 18% larger than 3nC, and 40% larger than their 2n counterparts.

Reproductive potential of each group was also examined; we found that mated 3n had a higher proportion of fecund animals than 3nC. This trend was exacerbated in oysters grown in warmer waters (e.g., Parengarenga). Collectively, these findings will inform stakeholders in assessing the feasibility of using tetraploidy for 3n oyster spat production moving forward.

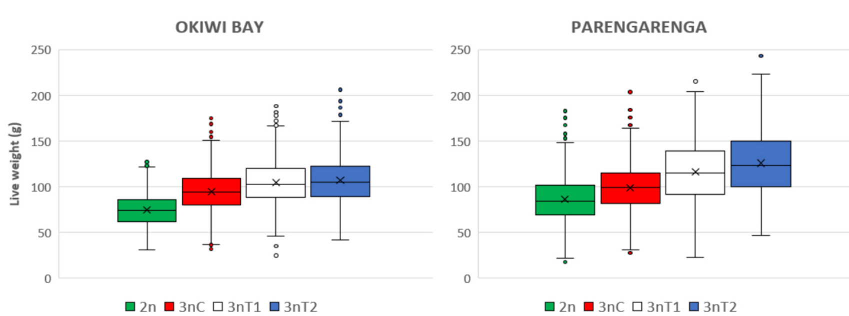

Figure 1. Final live weights of diploid (2n) chemical triploid (3nC), mated triploid using method 1 (3nT1) or method 2 (3nT2) oysters previously grown for 18 months at Okiwi Bay (Left) and Parengarenga (Right). Boxes represent mean live weights (± SD), in grams, of 10 replicated bags of ~100 individuals per group.

US EAST COAST CONSUMER SURVEY CAPTURES CLAM PREFERENCES

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In March 2022, over twelve hundred (1247) consumers in the eastern coastal United States (US) were asked about their clam consumption preferences. Of the consumers surveyed, 937 (75%) were clam consumers, and 310 did not eat clams.

Survey results showed that clam consumers tended to be of middle age, male, and have higher education and household income. The survey also suggested that those living in the northeastern US are more likely to be clam consumers than those in southeastern coastal states. Aside from Delaware, all northeastern states had more "yes" than "no" responses to eating clams.

The survey found that 79% of clam consumption occurs at restaurants, with most clams (59%) being prepared and served in the shell). The frequency of clam consumption varied between 5 and 10 times per year, with an expected value of 8.4 times. The most preferred clam size was close to a tie between the littleneck and top neck sizes.

The main factors limiting restaurant clam purchases were availability, price, and preparation method. Factors encouraging clams consumption at home included increased availability, lower prices, having the clams ready to cook or already cooked, and increased preparation knowledge.

Finally, non-consumers were asked what would encourage them to try eating clams. These non-consumers replied that sampling prepared clams, increased preparation knowledge, and a trusted local supplier would encourage them to try eating clams.

Table 1. Age of clam consumers (n=937)

Age	Percent
Under 25	12%
25 to 44 years of age	55%
45 to 64 years of age	27%
65 years or older	6%

Table 2. Where and how clams are eaten (n=937)

Where clams are eaten	Percent
Home	21%
Away from home	79%
Region of the US (Yes Responses)	**Percent**
Northeastern US States	55%
Southeastern US States	45%
How clams are prepared and served	
Clams served in the shell	59%
Clams served out of the shell	41%

Table 3. Factors limiting and encouraging clam purchases

Factors limiting restaurant purchases	Frequency
Fresh clams not available	54%
Price	44%
Not prepared the way I like	24%
Food safety concerns	23%
Factors encouraging home purchases	**Frequency**
More availability of fresh, local clams	77%
Lower prices for clams	63%
The clams are ready to cook	59%
The clams are already cooked	45%
More preparation knowledge	44%

EFFECT OF STRESS ON DIFFERENT AGE GROUPS OF CULTURED *Labeo victorianus* CAUSED BY PHYSICO-CHEMICAL WATER QUALITY PARAMETERS

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Given the growth of the aquaculture industry and concerns over negative effects of stress on fish, it is important to research how cultured fish respond to stress. The duration and intensity of stress can lead to fish mortality, disease outbreaks, poor growth performance, and reproductive failure.

This study aims to investigate the impact of physico-chemical water quality parameters on the stress response of different age groups of Labeo victorianus, a cultured fish species. Four treatments will be conducted, each consisting of 100 fish of different age groups (5g, 20g, 50g, and 100g) housed in four tanks and replicated three times. The fish will be fed high-quality feeds with a crude protein content of 30% throughout the experimental period.

Blood samples will be collected from each age group every two days, and the cortisol, glucose, sodium, and chloride ion concentrations will be analyzed. This process will continue for 10 days, with subsequent blood samples taken every two days to monitor the effects of stress. Physico-chemical parameters of each experimental set-up will also be measured by taking three water samples from each setup before extracting blood samples from the experimental fish.

The analyzed blood and water samples from each treatment will be compared to evaluate the response of different age groups of Labeo victorianus to different water quality parameters. This study will contribute to a better understanding of how fish respond to stress and may inform strategies to mitigate negative impacts on cultured fish.

IMPROVING THE AVAILABILITY OF SAFE AND EFFECTIVE VETERINARY MEDICINES TO SUPPORT AQUACULTURE GROWTH IN AUSTRALIA

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Global demand for seafood is rapidly increasing. To meet that demand, aquaculture production (122.6 million tonnes: USD 281.5 billion in 2020) is now greater than fisheries production. Over 20 times as many species are farmed in aquaculture worldwide (494 species), compared to terrestrial farming. Being a relatively young primary industry continual innovations occur which involve new species and farming methods. Consequently, the biggest limitation to aquaculture growth worldwide is new and emergent diseases.

Veterinary medicines play a valuable role in supporting aquatic animal health, welfare and production. In Australia, farmers must endeavour to use veterinary medicines that are registered or permitted by the Australian Pesticides and Veterinary Medicines Authority (APVMA). However, there are few medicines available for use in aquaculture with new permits taking several years to achieve. Reasons include market failure (i.e. low commercial incentive for pharmaceutical companies), limited funding, duplication in data collection, limited expertise and restrictive permits being applied for. This issue is highlighted in Australia's strategic plan for aquatic animal health (AQUAPLAN).

To address this critical issue, our project (FRDC 2020-094) provides national coordination to prioritise, source funding and streamline data collection to gain minor use permits (MUPs), ensuring they are available to the sectors that may require them. Objectives include 1) document an off-label use framework that provides for emergency use while ensuring safety for the animal, consumers and the environment; 2) coordinate efficient MUP applications, 3) communication and awareness on safe and effective use of veterinary medicines, and 4) determine options for ongoing national coordination after the life of the project. To date we have sought AUD 375,000 across five projects for researchers to collect data to progress the following MUPs: Toltrazuril (antiparasitic) for marine and freshwater finfish; Oxytetracycline (antibiotic) for marine and freshwater crustaceans; Chloramine-T (broad spectrum disinfectant) for marine and freshwater finfish; Trimethoprim-sulfadiazine (antimicrobial) for marine and freshwater finfish; and Isoeugenol (sedative) for marine and freshwater crustaceans. As of December 2022, 31 MUPs have been renewed or a new permit has been issued since the project commenced.

Australian aquaculture is one of the fastest growing primary industries (up to 10%/year), soon to reach AUD 2 Billion farm gate. National coordination to ensure access to safe and effective veterinary medicines will be crucial to support aquatic animal health, welfare and increasing production in an ecologically sustainable manner into the future.

ECOLOGICALLY SUSTAINABLE DEVELOPMENT OF NEW AQUACULTURE SPECIES (SEAWEED) IN SOUTH AUSTRALIA

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The South Australian aquaculture industry contributed 50% of the State's seafood economic output, worth $200 million in 2020/21. The Department of Primary Industries and Regions (PIRSA) is the agency responsible for managing South Australia's aquatic resources on behalf of the South Australian community. As part of this management, PIRSA is responsible for ensuring the ecological, social and economic sustainability as well as commercial viability of the State's aquaculture industry.

South Australia's aquaculture industry is diverse, with major sectors including Southern Bluefin Tuna, Yellowtail Kingfish, Oysters, Mussels, Abalone, freshwater finfish and crayfish, and most recently seaweed. Existing and new sectors are efficiently and effectively regulated under dedicated aquaculture legislation, the *Aquaculture Act 2001* (the Act). Subordinate legislation includes the *Aquaculture Regulations 2016*, statutory zone and standard condition policies. PIRSA provides a single point of entry into the Government assessment process for aquaculture activities, with a one-stop-shop approach to development, regulation and licensing, thereby streamlining the assessment and approval processes for the industry. Assessment of aquaculture applications undergo a rigorous Ecologically Sustainable Development (ESD) risk assessment framework. This framework ensures aquaculture is conducted in an ecologically sustainable manner, in line with the objects of the Act. A new marine lease and licence application takes on average 6-12 months to assess.

A rapidly emerging sector for South Australia's aquaculture industry is seaweed. Potential benefits of the sector include nutrient offset, Integrated Multitrophic Aquaculture (IMTA), carbon mitigation and a range of product opportunities in markets for human consumption, pharmaceuticals, nutraceuticals, bioplastics, fertilisers and livestock feed ingredients. Since 2020, PIRSA has assessed and approved 53 aquaculture licences permitted to farm seaweed. As an emerging sector, its full potential is yet to be realised with only a few of those licences trialling seaweed. From a regulatory perspective, key risks with this new sector include disease, pests and genetic contamination of local seaweed populations, primarily through stock translocations. Given the limited knowledge, a precautionary approach was required. Consequently, translocation of stock in the marine environment is regionally restricted based on recently implemented Macroalgae Management Areas. This aims to protect aquatic ecosystems and genetic diversity across the State's coastline which is home to 1800 species of seaweed – many of which are endemic – while facilitating the establishment of the sector.

GROWTH, SURVIVAL AND SHELL SHAPE IN BLACKLIP ROCK OYSTER *Saccostrea echinata/ lineage J* CULTURED UNDER DIFFERENT STOCKING DENSITIES AND TIDAL ZONE LOCATIONS IN TROPICAL AUSTRALIA

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The Blacklip rock oyster *Saccostrea echinata* has considerable potential to support new aquaculture development throughout the tropical Asia Pacific region. However, as with the tropical rock oyster industry more broadly, production remains in its infancy, and there is a lack of fundamental information relating to many aspects of the biology of *S. echinata*. Further, the suitability of farming techniques developed for bivalves in temperate growing regions remains unknown and untested in the tropics. Here, I will discuss the results of the first completed growout trial for *S. echinata*, undertaken at a pilot farm at South Goulburn Island, Northern Territory, Australia. An intertidal longline system was stocked with hatchery-produced spat (cultivated at Darwin Aquaculture Centre, Northern Territory) during February 2020. The aims of the trial were 1) to collect baseline information on survival, growth rates, and time to attain market size, and 2) to assess the effects of tidal zone location and stocking density on growth and morphometry of *S. echinata*. Oysters attained market size (70 mm DVM) in approximately 2.5 years. Shell morphology was significantly affected by stocking density, with increased abrasion due to wave action at lower densities producing a deeper, wider shell. Likewise, reduced immersion periods in the higher tidal zone resulted in reduced feeding opportunities and promoted a deeper, shorter shell. The results of this study are discussed with regard to future prospects, challenges and research opportunities for tropical rock oyster production.

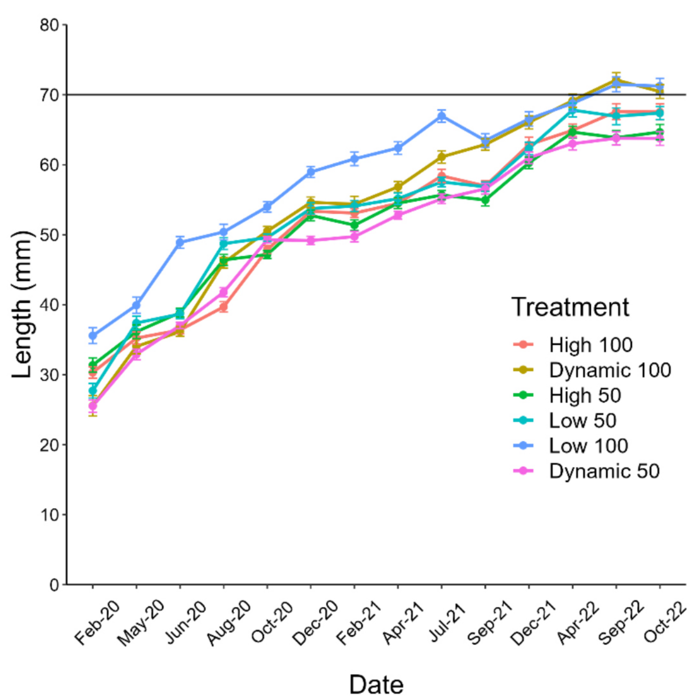

Figure 1. Growth rates (dorso-ventral axis ± se) of *S. echinata* among tidal zones (High, Low, Dynamic) and stocking densities (100% of gear manufacturer's recommendation vs 50%). Fixed horizontal line at 70 mm indicates market size.

SPATIAL GENE EXPRESSION IN ATLANTIC SALMON SKIN – DISCOVERING FUNCTIONAL GENETIC MECHANISMS GIVING RESISTANCE TO SEA LICE

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The immune response that is triggered in the skin of salmon is critical for the ability of individuals to resist infection to many bacterial and viral diseases, and to parasitisation. Some parasites, such as sea lice, can effectively suppress the immune response of host species such as Atlantic salmon, but the precise mechanisms involved are poorly understood. The mechanisms that make some species, strains and families of salmon more resistant to disease are also generally poorly understood. RNA-seq can be used to give a broad picture of how overall populations of cells in a skin sample react to infection or infestation, but bulk RNA-seq methods are unable to differentiate between the reaction of different cell types, skin layers or to compare the reaction at precise sites of infection/attachment compared to other areas in the skin. Here we describe mapping of gene expression profiles in Atlantic salmon skin using spatial transcriptomics. This technique allows us to map gene activity in two dimensions in a ~6.5mm^2 section with ~10 cell resolution. We highlight how spatial transcriptomics can complement data from single-nuclei RNA-seq (which can be used to track gene activity in different cellular populations), and how these techniques can be integrated to discover functional genetic mechanisms involved in providing resistance to sea lice.

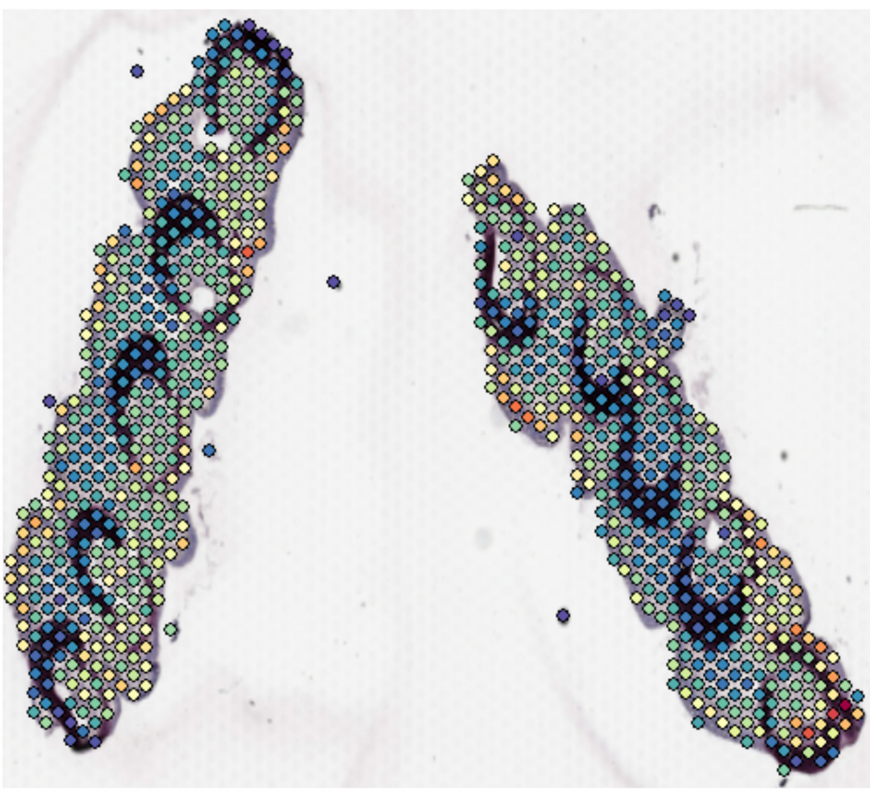

Figure 1. Section through the pectoral fins of an Atlantic salmon with coloured spots representing the number of genes expressed at that position in the tissue at the time of sampling (spectrum from zero in dark blue to 8,000 in dark red). A large number of genes are expressed near the skin surface in areas where there are many mucus glands.

EFFECTS OF FEED TYPES AND FEEDING RATION ON REPRODUCTIVE PERFORMANCE OF FEMALE BROODSTOCK *Penaeus monodon*

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A series of three feeding trials investigating different feed types (live and fresh-frozen polychaetes, fresh-frozen squid and mussel, and commercially available formulated moist feed) and feeding ration (apparent satiation vs 50% restriction) and their effects on the hepatopancreas composition and reproductive performance of females broodstock *P. monodon* were carried out at CSIRO's prawn broodstock facility at Bribie Island Research Centre.

Trial 1 compared three diets including (1) a control composed of live polychaetes and fresh-frozen squid and mussel, (2) same live and fresh-frozen ingredients supplemented with a commercial moist feed (EZ Mate), and (3) the latter supplemented with cordyceps. Trial 2 tested two feeding rations with animals housed individually fed to apparent satiation and 50% restriction. Additional control treatment consisted of animals housed in a traditional communal tank fed to apparent satiation was added for comparative purposes. Trial 3 investigated the relevance of live polychaetes by replacing them with fresh-frozen squid at 100, 50 and 25% levels.

In trial 1, dietary treatments did not affect ovarian development parameters (ovarian stage on the day of eyestalk ablation, percentage of females reaching ovarian stage 3 and 4, and stage 3 that continued developing to stage 4 in the reproductive period) and spawning parameters (percentage of females spawning, numbers of spawning per female, egg numbers per spawning event, percentage of spawnings that hatched, nauplii numbers per hatching spawning, and percentage of nauplii hatching per hatching spawning). Modest replacement of live and fresh-frozen ingredients with commercial moist feed supplemented or not with cordyceps did not reduce or enhance reproductive outcomes. Trial 2 validated the individual housing approach compared to a standard communal tank for research purposes. No significant effects of feeding ration on ovarian development were observed, likely due to the lack of eyestalk ablation and high biological variability. Trial 3 supported the hypothesis about the relevance of live polychaetes in broodstock maturation in the context of feeding at the expense of or in combination with fresh-frozen squid. Feeding 100% fresh-frozen squid impaired reproductive outcomes, while a ratio of 50% live polychaetes and 50% fresh-frozen squid corrected this effect equally performing to 100% live polychaetes in the context of females' reproductive performance.

Details of the differences or lack of them in each experiment will be discussed. Collectively, these findings provide valuable insights into the broodstock and hatchery prawn industry.

ENVIRONMENTAL MANAGEMENT OF AQUACULTURE: THE DEVELOPMENT OF 'FIT FOR PURPOSE' ENVIRONMENTAL MONITORING FOR SALMONID FARMING IN TASMANIA, AUSTRALIA

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The farming of salmonids in Australia's most valuable seafood sector, worth over $1.2b to the economy. Much of this production comes from the state of Tasmania's nearshore coastal waters. Environmental sustainability remains a central pillar to the industries ongoing success and its acceptability in the wider community. In recent years the industry has increased its production in more exposed coastal environments where the potential interaction with other sectors has raised concerns. Demonstrating that further development is done in a responsible and sustainable manner means that understanding how farming in new areas might change environmental interactions is well understood and managed to acceptable levels. An environmental monitoring program that can meet the needs of assessing environmental performance of the development will provide this understanding, allowing for appropriate regulatory responses.

Storm Bay in southeast Tasmania has been identified as a priority area for expansion. Here we will discuss the learnings and challenges of designing, implementing, and evaluating an environmental monitoring program that encompassed a range of receiving habitats after four years of data collection. Central to this was an assessment of the ecological and statistical sensitivity of the design to detect and attribute change in a system with multiple farming operations and a range of other sources of variability, both natural and anthropogenic.

THE WELFARE OF FARMED GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii*): FROM CULTURE TO THE TABLE

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The total production of freshwater prawns of the family Palaemonidae stood at 562,969 tonnes in 2020, valued at US$ 4.7 billion. This production volume and value make freshwater prawns economically important. The giant freshwater prawn (*Macrobrachium rosenbergii*) is an important species in this family with a well-developed production system and value chain. These prawns are farmed across Thailand, but the central drainage basins of the Chao Phraya River is where most of the industry is located; it mainly flourished when local laws prevented marine shrimp cultivation in interior locations, and farmers shifted their focus to prawn culture.

Sustainable aquaculture has now added the concept of welfare as a yardstick for certification and value chain improvement, particularly in Asia, where most aquaculture production occurs. Welfare issues related to *M. rosenbergii* are also similar to those in marine shrimp aquaculture. They include the artificial culture environment, high stocking density, diseases and infections, feeding and sustainability of ingredients, poor water quality, and cruel stunning/slaughter methods.

Feeding and utilizing sustainable ingredients can be handled by research into alternative feed ingredients and feeding regimes that promote better growth and welfare of the organisms under culture. The effect of improved feeding can be measured through immune response and stress markers. Poor water quality and high stocking density compromise the immunity of the species under culture conditions, while this confers a welfare advantage for freshwater prawns that are farmed at less-intensive culture conditions than marine shrimp. To overcome the disease challenge, real-time smart water quality monitoring systems can be used, and stocking density adjusted accordingly. The transportation, stunning, and killing of prawns (cold shock in ice or direct application of heat) constitute another welfare concern for this species. Advances in live transportation have been reported. Welfare indicators for this species include metrics on feed composition, environmental enrichment, stocking density and culture space, water quality, and stunning/slaughter. However, the methods that must be used to obtain data for these indicators need to be non-invasive. Some certification schemes have included these indicators in their audit process. Future research must consider reproductive welfare and vaccination against emerging diseases.

EFFECTS OF STOCKING DENSITY AND SHELTER ON THE BEHAVIOR, GROWTH, AND SURVIVAL OF *Stichopus* cf. *horrens* JUVENILES

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Stocking density is an aquaculture chronic stressor that can directly suppress the growth potential of a cultured animal. The sea cucumber *Stichopus* cf. *horrens* is a target species in the Indo-Pacific region whose culture production is emergent. Its cryptic and nocturnal nature indicate that shelters may be of importance in the rearing of early-stage juveniles, particularly under stressful conditions including stocking density.

Field and laboratory experiments were conducted to investigate the effects of stocking density on the behavior, growth, and survival of *S.* cf. *horrens* juveniles. In the field, juveniles were reared at 25 ind. tray^{-1}, 50 ind. tray^{-1}, and 100 ind. tray^{-1}, with and without shelters for 30 days. The growth and survival of the juveniles were monitored biweekly. The diel activity, location, and aggregation behavior of the juveniles at three stocking densities (i.e., 13 ind. tray^{-1}, 26 ind. tray^{-1}, and 52 ind. tray^{-1}) were also investigated in the laboratory. In both of these experiments, the equivalent stocking biomass was 4 g tray^{-1}, 8 g tray^{-1}, and 16 g tray^{-1} for the low, medium, and high-density treatments, respectively.

The results revealed that growth in length of *S.* cf. *horrens* juveniles is more significantly affected by stocking density than shelter. High-density conditions lead to significantly lower growth rates and greater size variation compared to low-density treatments, suggesting crowding stress. The occurrence of high-density aggregations at high stocking densities may be the causal mechanism of crowding stress that lead to growth suppression. The presence of shelters resulted to higher biomass increment than the unsheltered treatments. However, it was only at low density shelter treatments where a significant difference was found, indicating that the benefits of shelter may have diminished in the medium and high density treatments. Survival rate was also significantly influenced by shelter denoting that its presence may have conferred benefits.

No distinct differences were found in the diel activity of *S.* cf. *horrens* juveniles at different stocking densities. The juveniles were mostly inactive during daytime between 06:00-18:00h, and increased activity from dusk to nighttime. When inactive, a strong preference to shelter and aggregate towards the PVC undersides was observed across density treatments. This aggregation behavior may be a reflex response related to the cryptic nature of this species. Moreover, the probability encounter when shelter-seeking, which increases at high stocking densities, may have influenced aggregation densities. The results of this study highlight the importance of determining the appropriate stocking level and shelter complexity that could reduce crowding stress and optimize the growth performance and survival of *S.* cf. *horrens* juveniles.

ADDRESSING THE CHALLENGES OF INLAND FISH FARMING IN PAPUA NEW GUINEA

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Many people in Papua New Guinea (PNG) experience undernutrition and malnourishment due to a low protein diet. Staple foods, such as potatoes and rice, usually contain high glycemic carbohydrates. Stunting and wasting affects approximately 50% of children, and many adults are obese partly because of the high carbohydrate diet. Consequently, heart, vascular and metabolic diseases are more prevalent than in developed countries. Fish farming has been fostered by the PNG National Fisheries Authority (NFA) to increase access to protein and improve human health and livelihoods, mainly for rural communities where poverty is pervasive, and protein is either difficult to access or too expensive.

Over the last decade, the number of fish farms in PNG has increased from 7,000 to over 60,000 largely due to NFA's well-planned interventions; these include research into improving fish feeds and feeding strategies, improving fingerling production, and farmer training. More than 95% of PNG fish farms are low intensity, earthen pond systems that are integrated into vegetable gardens. However, inland fish farming production levels in PNG remain low compared to those of nearby Asian countries. Until recently, fish farmers struggled to produce plate size fish, and the cost of feed was a disincentive to continue farming. The low farm production levels are attributed to the environmental constraints on fish growth, principally the colder waters of the highlands, the cost and availability of quality fish feeds, the scarcity of quality fish fingerlings, and a lack of efficient fish feeding strategies and husbandry knowledge for the predominantly remote, rural fish farmers. Many fish ponds become overcrowded due to in-pond breeding of tilapia thus increasing demand for natural food and pelleted feed. The mixed sex farming also results in the fish's diversion of energy from growth to reproduction. Stunted adult fish are also sold or shared to new fish farmers as juvenile fish and this leads to crop failure and a loss of conviction for farming.

NFA, UNSW and ANSTO have worked collaboratively to address these challenges. Firstly, fingerling production has been boosted significantly by NFA by introducing monosex tilapia production practices and establishing satellite hatcheries that can provide access to fingerlings in remote areas for 18 provinces. These satellite hatcheries also act as knowledge sharing nodes. Secondly, using cutting edge technologies, UNSW, NFA and ANSTO have conducted research that has improved fish feeding practices to reduce the cost of fish farming and to better utilise local nutrient resources. Thirdly, through NFA and its partners, we have increased access to farming knowledge via training programs, the growth of a lead farmer network, and embedding advanced farmers on our research and farmer capacity building programs. Research and technical capacity building by UNSW and ANSTO has also strengthened NFA's aquaculture research and development program in PNG. Our work has been funded by NFA, UNSW (philanthropic funding), ANSTO and the Australian Centre for International Agricultural Research (ACIAR). The research and outreach activities are discussed in detail in a series of interconnected presentations at this symposium.

A CONVERSATION ON THE AUSTRALIAN SEAFOOD INDUSTRY: ITS COMMITMENT TO TRANSPARENCY AND ENVIRONMENTAL, SOCIAL & GOVERNANCE TOPICS (ESG)

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The current catchphrases in the supply chain are transparency, traceability, sustainability, and provenance. Many industries have realised the powerful edge they have over their counterparts who are not practicing these activities, but merely pay them lip service. Concurrently, there is an equally important conversation concerning ESG, and because of the global threats of politics and war there is now an overlap between these activities. In fact, recently much ado has been made about 'greenwashing' when an organisation spends time promoting themselves as environmentally friendly, when in fact they literally minimise their impact. So how do these concepts overlap and become integrated, especially in sectors where up till now action has been centred around the 3 core concepts of sustainability involving economic, environmental, and social factors?

Let us consider the Australian seafood industry, who have over the last decade have gone to great lengths to incorporate sustainability outcomes into their supply chains and have focused on connecting with consumers to build trust in their products. This has created visibility to point of origin within specific seafood sectors. Furthermore, this emphasis has given this and other agri-food industries a massive head start when it comes to this trend.

It can be uncomfortable and disruptive for an industry to move to a place of more transparency where ESG commitments are real. How does the seafood industry, and specifically the subset of this industry, aquaculture, utilise and take advantage of this opportunity? This presentation will explore the foundations of the seafood industry and how it has built out the notions of transparency in conjunction with the current ethical sentiments now resonating with the consumer.

What role does the regulator have? What technology options and opportunities are there? What role does a provenance science company have within the supply chain and how does this intersect with the need for demonstration of credentials such as sustainable? There is a need for an even and fair playing field and a form of transparency will be needed to achieve this outcome (see Fig 1). The presentation will use examples from various Australian seafood industries. This will include two aquaculture industry case studies, that have developed a transparency strategy. These industries have placed value on provenance and are committed to building high integrity supply chains to ensure that their products and promises, as well as minimising costs, get through to consumers. In the current climate there is a need to make supply chains less opaque and the seafood industries of Australia are forging this path.

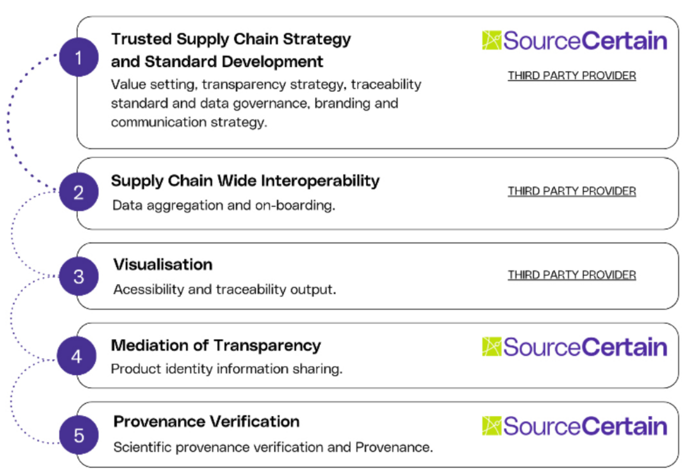

Fig.1 – Supply Chain Overview and role of provenance

THE ROLE OF SHRIMP MULTIPATH IN DISRUPTING HOW WE THINK ABOUT AND MANAGE PATHOGENS IN SHRIMP CULTURE

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The concept of pathogen presence resulting in reduced production performance of livestock and aquatic species has been embodied in text books for decades. Despite this, many Shrimp producers often don't understand and/or have not been able to quantify the true impact of multifactorial pathogen presence, load and prevalence on production. This is understandable when you consider that only until recently were the tools available for accurate multiple pathogen detection at an affordable cost and that shrimp can have any number of 13 commercially relevant (impactful) pathogens, often harbouring 3-4 of these at any one time. Establishing data points that give farmers a quantitative pathogen profile of their crop over time empowers them with data to quantify the true impact of pathogens on culture and on which to make management decisions that can change the outcome and profitability of a crop.

This study will present case studies from around the world on how the application of the Shrimp MultiPath knowledge and technology has re-defined how farmers think about pathogens, assess pathogen loading and manage pathogens throughout the production cycle. Latin America, for example are using this knowledge and technology as part of their broodstock selection program resulting in a 10-15% improvement in production and a 10% improvement in fertility. Asia-Pacific farmers utilise the technology to monitor pathogen presence and prevalence during grow-out to provide an early warning that allows simple but smarter management practices to be applied on a case-by-case basis. Finally, the power of combining accurate multiple pathogen detection with genetic selection and improvement programs will be discussed.

BLACK SOLDIER FLY *Hermetia illucens* LARVAE AND FRASS AS PROTEIN ALTERNATIVES IN BARRAMUNDI *Lates calcarifer* AQUACULTURE DIETS.

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As food insecurity becomes a growing issue, global food sectors are forced to maximise production whilst conserving the worlds remaining resources. The aquaculture sector is one of the fastest growing protein sectors. However, many sought after species are carnivorous, with high dietary protein requirements, often delivered in the form of wild caught fish (fishmeal; FM). The current demand for fishmeal is beginning to outweigh supply, prompting the increased importance of the management of fishery resources and the progression towards a more sustainable industry from a food security perspective.

Insects are a natural part of many freshwater and marine carnivorous finfish diets. Black soldier fly larvae (*Hermetia illucens*; BSF) are of particular interest as a substitute to FM as they efficiently convert organic waste (e.g., fruit and vegetable waste) into their own biomass, resulting in high protein and fat concentrations. Current literature reports the use of BSF in several aquaculture species diets, however results are variable.

The aim of this research was to evaluate the effects of BSF derived products on juvenile barramundi (*Lates calcarifer*) growth, well-being and feed utilisation. Six experimental diets contained graded ratios of BSF larvae and BSF frass as a dietary replacement. Each diet was randomly allocated to one of 24 tanks in a blocked design, with four tanks per diet and 12 fish (121.7 g ± 14.3) per tank. Fish were hand fed the experimental diets, once daily, for 84 days. At the conclusion of the trial, weight and fork length were recorded.

The results indicated that barramundi growth performance, wellbeing and feed utilisation were not impacted by inclusion of BSF derived products in any of their ratio levels. This study, therefore, demonstrates BSF suitability as a protein source.

KARNALI RIVER OF NEPAL AND LOCAL KNOWLEDGE FOR LIVELIHOODS

Mr. Thark Bahadur Shah

Nepal
Email:- tharkshah@gmail.com

1) Introduction of Karnali River
2) Bankside People Information
3) Indiginious and Local Knowledge
4) Use of Water Before and Now
5) Livelihoods in Community
6) Types of Method
7) Gender Involvement
8) Transboundry Effects from China and India
9) Culture and Climate
10) Conclusion

BEFORE IT IS TOO LATE: DANGEROUS FISH PARASITES BYPASSING AUSTRALIA'S BIOSECURITY MEASURES

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Australian aquaculture industries are becoming large internationally recognized and sustainable industries. However, Australia's aquaculture industries face numerous challenges, including threats by parasites, adversely impacting the sustainability and profitability of finfish aquaculture. In this presentation, evidence on the impact of introduced invasive species on Australia's iconic freshwater fish are provided. Despite limited resources, in the last five years research has found:

i) new parasite species in Australian aquaculture farms, previously unknown to scientists,
ii) new parasites previously not reported in Australia,
iii) host switch of invasive parasites from introduced fish to native species, and
iv) evidence of parasites spill over and spill back.

These findings suggest that many parasites still pass Australia's strict biosecurity border, successfully establish themselves in Australia and impact population of Australian native fish, many in vulnerable status. It is important to invest on research to detect the gap in our biosecurity protocols before it is too late.

This Figure shows parasite embedded in the muscle of a brown trout. This particular parasite is an introduced species commonly found in freshwater fish and is known to infect humans. Their population usually is high in areas where some agricultural practices is high.

THE ROLE OF CHEMICAL REGULATION IN THE AQUACULTURE INDUSTRY

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The APVMA is the national regulator responsible for assessing, registering, and approving agricultural and veterinary chemical products for use in Australia. The APVMA is responsible for the regulation and control of agvet chemicals up to the point of retail sale and oversees import and export. Before agvet chemical products can be legally sold, supplied, or used in Australia, the products must be evaluated and registered by the APVMA.

The APVMA approves active constituents —which are the substances in agvet chemical products that are primarily responsible for biological and other effects of a particular product — and registers end-use products. As part of the product registration process, the APVMA approves certain aspects of a product label called the relevant particulars. Veterinary chemicals regulated by the APVMA can include veterinary medicines; antibiotics and other pharmaceutical products; immunobiological products, such as vaccines and other preventative treatments for diseases; hormonal growth promotants (HGPs); and complementary animal health products.

The APVMA also considers applications for permits to authorise use of a registered agvet chemical in a manner that is different to the directions for use specified on the product label. A permit may also authorise use of an unregistered chemical under limited circumstances.

Veterinary chemicals are important tools for managing aquatic animal health and welfare; contributing to ecological sustainability by reducing disease spread to wild populations; promoting food security; and protecting human health by reducing zoonosis risks.

While there are a small number of veterinary chemicals approved for use in aquaculture under the minor use permit system, there are very few that carry a full registration.

Currently, all aquaculture species are classified as minor animal species, therefore supply and use of chemicals under minor use permits is acceptable. However, aquaculture is a rapidly expanding industry which officially has become the largest sector of the domestic seafood industry, accounting for 51% of total GVP, and 38% of total volume in 2021.

If aquaculture species are reclassified as major species, the justification for approval of uses under use permits will be no longer valid. In addition, With the ever-present chance of disease incidences and outbreaks the aquaculture industry requires ongoing access to safe and effective chemical products. This presentation will focus on the role of chemical regulation in the aquaculture industry, including the supply and use of chemicals in aquaculture and issues identified while regulating these veterinary chemicals.

A MULTICRITERIA APPROACH TO OFFSHORE AQUACULTURE DEVELOPMENT: A RARE CASE STUDY OF INTERGOVERNMENTAL COLLABORATION LEADING TO MUTUALLY BENEFICIAL OUTCOMES

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The United Arab Emirates (UAE) government has set a strategic objective to develop a competitive and sustainable aquaculture industry for Abu Dhabi, that considers the broader economic and social benefits to the region, while meeting best-practice environmental targets (Fig. 1). To support this ambitious objective, Environment Agency Abu Dhabi (EAD) commissioned a project to develop a short list of sites in the southern Arabian Gulf, supported by stakeholder engagement workshops, GIS and integrated numerical modelling. Stage 1 of the project used a multi-criteria analysis (MCA) to select suitable zones following an analysis of local social, economic and environmental values. Both the inputs and the constraints used in the MCA were identified in a workshop attended my multiple government agencies, consultants and academics. Multiple data sets were examined including optimal growing conditions, local water quality, depth, current speeds, and proximity to shipping, power station and marine park resources, to produce a 'heat map' of optimal sites under a range of scenarios.

Stage 2 of the project examined the carrying capacity of the sites using an integrated numerical model, with coupled hydrodynamic, water quality and sediment diagenesis modules. The intent of model was to determine the volume of fish that could be farmed safely, without compromising local environmental and/or social values, while supporting the regions' economic and social objectives.

The MCA used in this example is commensurate with international best practice approaches to aquaculture site selection and serves as a good template, for application to other parts of the world; particularly those lacking social and environmental governance frameworks.

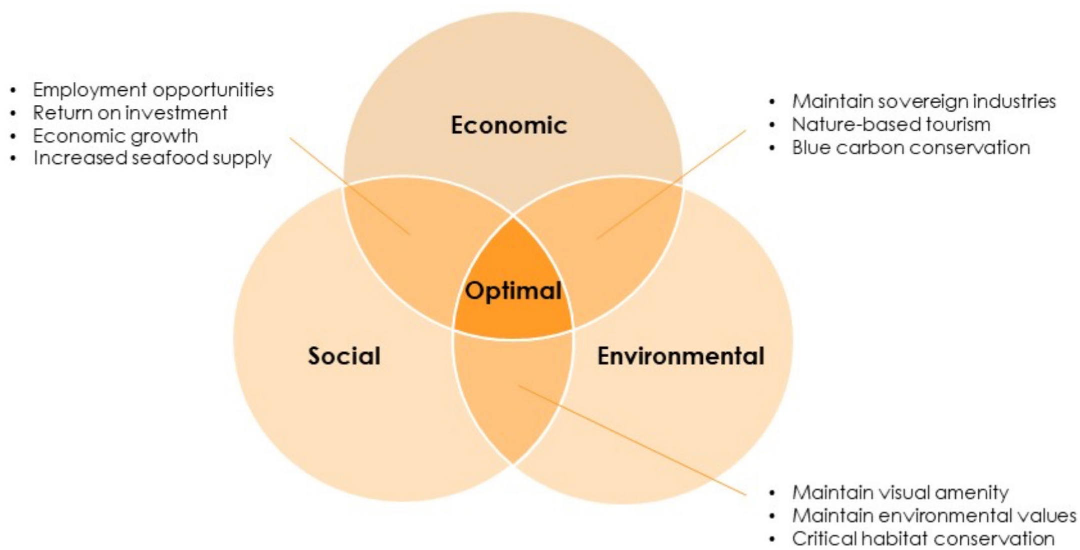

Fig 1. Factors and their interactions considered in the multi-criteria analysis

THE SCIENCE BEHIND NOVAQPRO™, A NATURAL FUNCTIONAL FEED ADDITIVE FOR PRAWNS

Cedric J. Simon*, Matt Briggs, Ha Truong, Omar Porras, Tansyn Noble, Artur Rombenso, Nick Wade, Simon Irvin, Simone Osborne, James Wynne, Richard Smullen, Ricardo Esquerra

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The aquafeed sector is becoming increasingly aware and adoptive of microbial single-cell ingredients as volumes are scaled up, their prices become more competitive, and marine products are increasingly substituted. One of such microbial ingredients now at commercial production scale is NovaqPro™, a natural microbial biomass composed principally of bacteria produced in ponds under a patented process bio-converting agrochemical inputs into a functional ingredient for shrimp feed. When strategically included in feeds at low inclusion, this microbial biomass has been demonstrated to enhance growth, feed intake, disease resilience and feed efficiency in numerous studies on tiger prawns and white-leg shrimp. Unlike most feed additives currently available, the microbial biomass is acting as a rich natural source of bioactive molecules and is not aimed at directly replacing macronutrient sources.

Over the last 6 years, Ridley Corp., the Australian licensee commercialising NovacqPro™, and CSIRO have engaged in a research alliance to delve deeper into this novel commercial product's nutritional characteristics and mode of action in prawn (Fig 1). This paper summarises the research conducted to date on NovaqPro™ and the various mechanisms by which its incorporation in a wide range of experimental and commercial formulations is benefiting prawn nutrition.

Fig.1 NovaqPro™ characteristics and mode of action in shrimp

DEVELOPMENT OF NOVEL AUSTRALIAN PLANT PROTEIN INGREDIENTS FOR AQUAFEEDS

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By 2030, Australia is aiming to generate 55% more seafood reaching approximately 150,000 tons a year via aquaculture which would require 200,000 tons of feed a year. Currently, the aquafeed industry relies on importing ingredients from overseas, mainly soy products and marine-origin fishmeal, fish oil and krill meal ingredients. The agricultural sector produces large volumes of crops (72MT in 2022-23), including grains (58MT pa), canola (8.3MT), pulses (3.7MT), and cottonseed (2.5MT pa) which dwarfs the aquafeed demands. Sourcing and developing local ingredients that are priced competitively against soy products while providing good nutrition and functionality are major challenges faced by the aquafeed and broader feed industry.

This paper presents recent advances in the development of local plant protein (mainly canola) concentrates and their impact on culture performance against imported soy products. The Aquaculture nutrition research was carried out on both Atlantic salmon, *Salmo salar* and tiger prawn, *Penaeus monodon*, aiming at an incorporation of canola products of up to 30%. The results indicate soy products can be completely substituted by canola products successfully in commercially relevant aquafeed formulations. The research highlights a great potential for local production and processing of plant protein ingredients to benefit feed manufacturers and aquaculture farmers, and the broader plant and animal protein industry through accessing new added-value and export markets.

GROWTH YIELD OF CO-CULTURED SEA GRAPES (*Caulerpa lentillifera)* WITH BLACKLIP OYSTER *Pinctada margaritifera* FROM NAMARAI VILLAGE, FIJI ISLANDS

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Marine resources have played a significant role in Fiji in recent decades and contribute to both food and income security for households, especially those residing in coastal areas. Despite a string of bad occurrences such as recurrent cyclones, climate change, and infrastructural costs, the aquaculture industry in Fiji has persevered and remained resilient. Having made a contribution to the economic development over the past years allow, further research on industry development. Therefore, it will be a good opinion to develop and rejuvenate the different aquaculture techniques in Fiji to cater for the national seafood security and improve the export trade of the marine species. The rapid growth of the human population, the looming food insecurity and declining marine and freshwater fish stocks are some reasons of food and nutrition insecurity. Seafood is a critical component of human diets, comprising one-sixth of global animal-source food consumption and plays a critical role in contributing to food security, and thus sea grapes (*Caulerpa lentillifera*) and blacklip pearl oyster (*Pinctada margaritifera*) are two species that can be co-cultured to help contribute more to the current sea food trend. Both species are edible and nutritious in Fiji and are distributed globally from the South Pacific to the western pacific. The project is implemented in Namarai village located on the eastern coast line side of Vitilevu in the tikina of Rakiraki, Fiji. The findings indicated that the water quality parameters such as the DO improved in the co-culture area as compared to the open ocean while the growth of pearl oyster was recorded to be 35.3 and 57.9mm in length and weighs between 1.59 and 2.56g over the past 4.5 months kept in co-culture. Co-culture technique is an affordable and sustainable potential solution for the world's populations facing food insecurity and is a way forward given the presence of great supporting features in Fiji, such as favorable climate, excellent water quality and abundant land resources. Additionally, this research offers to expand aquaculture to the point at which it can satiate the nation's pressing need for food security by educating people in the Pacific about co-culture and encouraging them to participate in it rather than relying on land farming.

REPLACING FISHMEAL WITH CORN FERMENTED PROTEIN WITH SOLUBLES (CFPS) IN DIETS FOR ATLANTIC SALMON (*Salmo salar*)

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A co-product from ethanol industry, distillers dried grains with soluble (DDGS) is low in lysine and contains moderate crude protein (28 - 32%) and relatively high fiber, thus limiting the inclusion level of DDGS in Atlantic salmon feed. However, through applying advanced processing techniques, DDGS can be improved to yield a corn fermented protein with solubles (CFPS) which is high in protein content. ProCap Gold (Marquis Grain Inc.) is one such product and has a protein content of 48%. Recently, our research group evaluated ProCap Gold as a complete replacer of soybean meal in the practical diet of Atlantic salmon. In that study, Atlantic salmon fed a diet replacing 100% of the soybean meal (22% of the diet) were observed to maintain maximal performance levels. From that study, came the hypothesis for the current study, that ProCap Gold can replace 100% of the fishmeal in a practical Atlantic salmon diet. Therefore, a 12-week feeding trial was conducted to evaluate this CFPS as a fishmeal (FM) replacer in the practical diets for Atlantic Salmon (*Salmo salar*) and the subsequent effects on growth, feed efficiency, intestinal morphology, and health performance. A 40% menhaden fishmeal diet was used as the Control diet, then, 25%, 50%, 75% and 100% of FM in the Control diet formulation was replaced with CFPS for diet groups Diet-2, Diet-3, Diet-4 and Diet-5, respectively. This resulted in Diet 5 containing no FM and 40% CFPS. All experimental diets were fed to satiation, twice daily to quadruplicate groups of Atlantic salmon juveniles (initial weight 12 g). After the feeding trial, final weight, specific growth rate, weight gain and survival were not significantly different among the treatment groups; however, feed conversion ratio was higher ($P<0.05$) for fish fed Diet 5. Additionally, the inclusion of CFPS did not affect hepatosomatic index and condition factor ($P>0.05$). A blood metabolic panel indicated no differences among treatment groups, and hematocrit and total hemoglobin were also similar ($P>0.05$). Effects of graded levels of CFPS on oxidative stress and intestinal histomorphology will be discussed. Taken as a whole, these results demonstrate that ProCap Gold CFPS can be used as an effective fishmeal replacement.

FARM TRIAL USING NOVAQPRO® MICROBIAL BIOMASS FOR TIGER SHRIMP *Penaeus monodon* : PERFORMANCE, PROFITABILITY AND REDUCTION OF USE OF MARINE RESOURCES

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Shrimp farming is a significant global industry which in 2021 was estimated to be worth US$ 38 billion and is expected to grow to an estimated 6m mt in 2023. However, the industry continues to face a number of challenges including higher feed costs, lower farm gate prices, and increasing demand for diets with reduced capture marine resources and lower nutrient input. Balancing these competing demands is critical to ensure the long-term profitability and sustainability of shrimp farming. The use of low-cost inputs may seem attractive, but it often negatively impacts animal health and performance, reducing yields, harvest animal quality, and water quality, which in turn affects nutrient output from the farm.

In light of these challenges, commercial-scale farm trials were conducted at Australian Prawn Farms' Ilbilbie facility with Black Tiger Shrimp (*Penaeus monodon*) to assess the performance of a diet using zero marine resources, as well as the protein and nutrient input into the farming environment and return on investment for the farm in connection with the use of the diet. The inclusion of a microbial biomass called NovaqPro® allowed for flexible formulation and the reduction of protein and marine resources in the diet it was included in.

Results from the first commercial trial showed that a diet containing NovaqPro®, and zero marine resources, performed as well as a diet containing a high level of marine resources, indicating that marine protein and oil was not necessary for commercially farmed *P. monodon*. To the knowledge of the authors, this was the first reported commercial production of *P. monodon* grown on a diet completely devoid of marine resources.

In the second trial, the use of NovaqPro® enabled the formulation of a diet for *P. monodon* that allowed for the reduction of protein and fishmeal inclusion while, as compared to the diet containing a high level of marine resources, improving growth by ~5%, increasing harvestable yield by 2.5t/ha, and increasing survival rates by ~10%. Furthermore, there was a reduction of feed conversion ratio by ~6% and total ammonia nitrogen production per tonne of biomass harvested of ~40%. Importantly, the net revenue on the ponds fed low protein diets containing NovaqPro® increased by ~16%, while the nutrient output decreased, as compared to the ponds fed a diet containing a high level of marine resources.

Overall, these trials demonstrate that the use of NovaqPro® microbial biomass can help farmers balance competing demands and reduce marine inputs, while maintaining or improving yields and profitability. By reducing reliance on marine resources and improving water quality, this approach may also contribute to the long-term sustainability of the shrimp farming industry.

THE EFFECTS OF ACUTE SALINITY STRESS ON THE SURVIVAL AND HAEMOLYMPH BIOCHEMISTRY OF JUVENILE TROPICAL ROCK LOBSTER *Panulirus ornatus*

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The tropical rock lobster, *Panulirus ornatus*, is emerging as an important species for onshore aquaculture development. Commercial grow out of lobsters from the juvenile to harvest phase are likely to be conducted in outdoor raceways or pond culture systems in tropical regions of Australia. In these areas, climate has a large impact on the prevailing environmental conditions, this includes torrential rain and high ambient air temperatures impacting pond salinity for hours to weeks. The salinity tolerance of *P. ornatus* with respect to specific moult stage survival, physiology and blood biochemistry were previously unknown.

This study investigated Lethal Concentration of salinity which caused 50% mortality (LC50) and haemolymph biochemistry of *P. ornatus* juveniles when acutely exposed for 48 h to different salinities (10, 15, 20, 25, 30, 34, 40, 45, 50, 55, and 60 ppt) at three stages of the moult cycle (post-, inter- and pre-moult). Moult stage significantly impacted the survival of lobsters at low salinity (<34 ppt), which ranged from $LC50_{48}$=12.5 ppt for post-moult lobster to $LC50_{48}$=20.0 for pre-moult (Figure 1). There was no significant effect of moult stage on high salinity (>34 ppt) tolerance of lobsters which ranged from $LC50_{48}$=50.5 - 54.5 ppt. Haemolymph osmolarity showed inter-moult lobsters to be weak hyper-regulators, with post- and pre-moult being hyper-osmoconfromers. Moult-stage significantly affected haemolymph oxyhaemocyanin and protein concentration, with pre-moults having the highest values and post-moult the lowest, with no impact of salinity treatments. At 50, and 55 ppt, oxyhaemocyanin was significantly elevated compared to 34 ppt with no effect of the moult stage. While at 55 ppt, superoxide dismutase (SOD) activity was significantly lower compared to 25, 40, 45, and 50 ppt with no effect of moult stage. Our results suggest a salinity tolerance range between 20 - 40 ppt; beyond this, lobsters experience oxidative stress due to the breakdown of osmoregulation. This study provides an improved understanding of the survival and physiological impacts of salinity change on *P. ornatus* that is essential for optimising productivity protocols for the onshore aquaculture of the species.

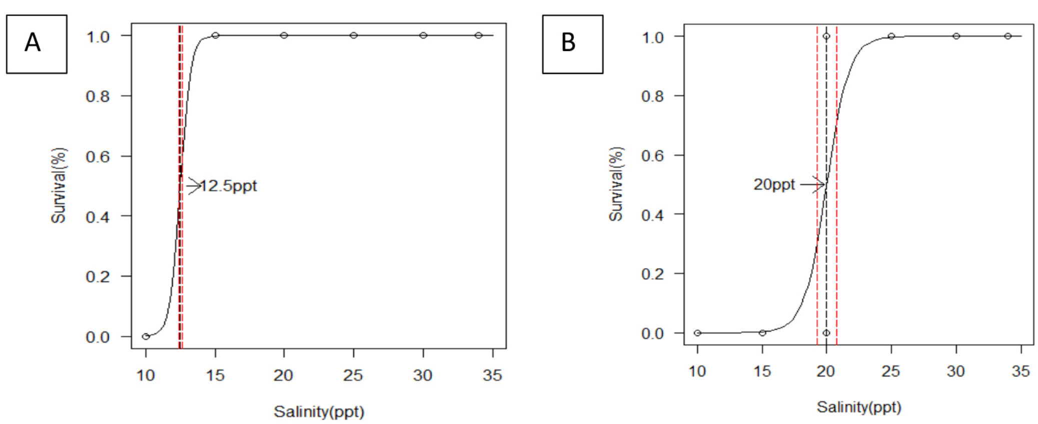

Figure 1: Survival of *P. ornatus* (%), when exposed to a range of salinities, 10-34 ppt, at different moult stages (A) post-and (B) pre- for 48 h. The Lethal Concentration of salinity which caused 50% mortality of *P. ornatus* is shown by the dotted line and two red lines represent the 95% confidence interval.

AN INDIGENOUS-LED INITIATIVE TO IMPROVE LIVELIHOODS AND SUPPORT THE *BÊCHE-DE-MER* FISHERIES IN UGAR ISLAND, TORRES STRAIT (AUSTRALIA)

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Torres Strait (TS) fisheries are very important to Traditional Inhabitants, supporting their quality of life and autonomy. Ugar Island (TS) has been historically involved in the sea cucumber (*bêche-de-mer;* BDM) fishery. The industry provides important income opportunities in a region with limited jobs and recognise the need for healthy and well managed stocks. Since 2015, the BDM fishery is 100% indigenous-owned. Aquaculture has been identified by an Indigenous entrepreneur (RGS) as a way to improve the BDM fishery in response to fishing and climate pressures. It also helps maintain lifestyles, achieve regional strategic goals and increase autonomy of Traditional Inhabitants.

The project will be the first trial to culture sea cucumbers in the region by combining traditional knowledge and science to grow them in the wild (sea ranching), rebuild or improve fishery stocks. The indigenous-led business and research partners have already identified suitable species and sea ranching areas. The next step is to establish a commercial hatchery through a three-year research project whereby techniques to culture sea cucumbers (e.g. induce spawning and rear juveniles) will be tested and refined and local staff equipped with the skills to successfully run the hatchery. The business offers an opportunity to 'close-the-gap' in disparity to non-indigenous livelihoods via building local technical capacity and generating research outputs. These outcomes will support new indigenous-led businesses and provide opportunities for other species to be cultured.

The presentation will focus on the issues involved in establishing a novel indigenous-led aquaculture venture in remote Torres Strait, including the establishment of hatchery and sea ranching areas, legislative requirements, market opportunities, capacity issues and technical challenges. The research is supported by indigenous organisations representing the interests of all fishermen in this TS fishery.

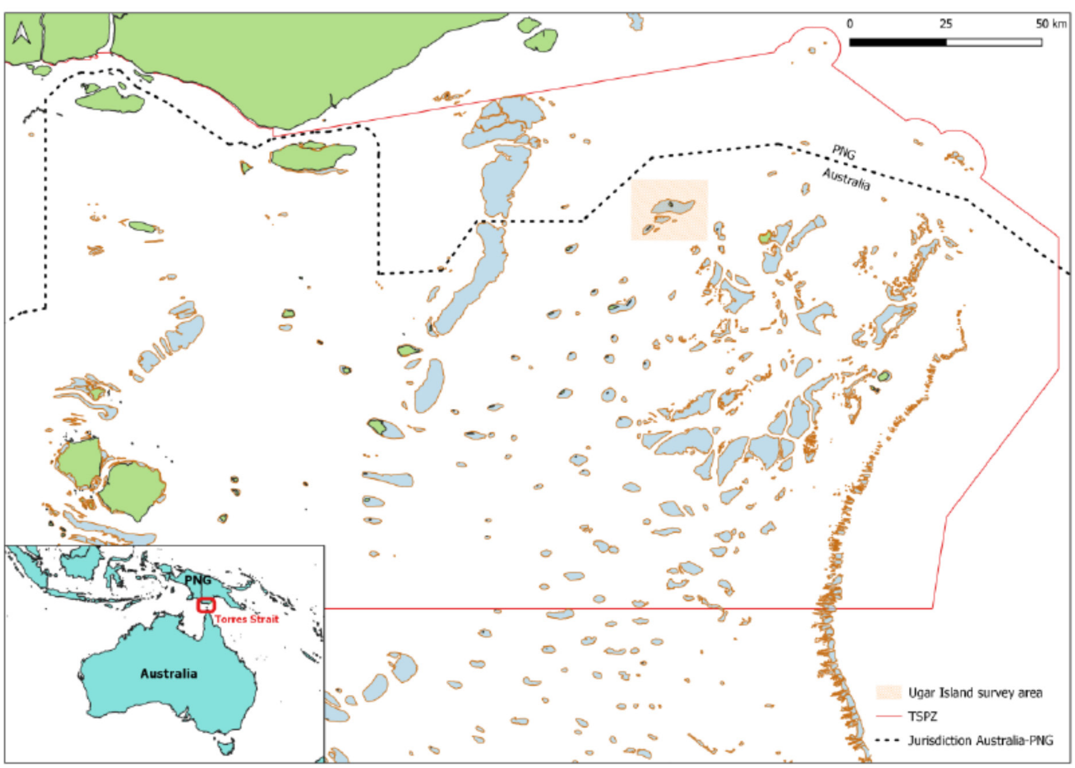

*Figure 1. Ugar Island (green square with orange fill) in Torres Strait. Source: (Murphy et al., 2020).*Murphy, N., Skewes, T., Plaganyi, E., Edgar, S., Salee, K., (2020) Ugar Island sea cucumber survey - field survey and results. AFMA project 2019/0826, Brisbane, Australia, p. 28p.

THE INFLUENCE OF TEMPERATURE ON THE FILTRATION RATE OF BLACKLIP ROCK OYSTERS (*Saccostrea echinata*) AND AN ASSESSMENT OF THEIR BIOREMEDIATORY ABILITY IN PRAWN AQUACULTURE WASTE WATER

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The Blacklip rock oyster, *S. echinata*, is an emerging species of tropical oyster displaying several characteristics that suggest it would be well-suited to bioremediatory applications. These include a large size and fast growth rate and resilience to fluctuations in temperature and salinity. In order to investigate the bioremediatory potential of Blacklip rock oysters the present study aimed to: (i) determine the influence of temperature (20, 24, 28, 32 °C) on the filtration rate of *S. echinata;* (ii) describe and quantify the uptake in total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS) and chlorophyll A, using prawn pond effluent and two levels of oyster stocking density.

The results demonstrated that higher water temperatures promote a faster filtration rate and identified an optimal performance range of 24 – 32 °C for a filtration rate of 12.68 – 15.20 L/hr/g. A maximum filtration rate of 15.20 L/hr/g was achieved at 24 °C (Fig. 1).

In addition, the high density (0.66 oysters / L) of stocked oysters resulted in significant reduction of all parameters, with TN reduced by 21%, TP reduced by 27%, TSS reduced by 99% and chlorophyll-A reduced by 39% when compared to the original effluent. Tissue analysis of 10 oysters with a mean whole weight of 75.4 g, revealed a mean of 0.09g of nitrogen per oyster. Scaling these values suggests that 1.20 kg of nitrogen is removed per tonne of harvested oysters. This study is the first to investigate the bioremediatory potential of *S. echinata* and demonstrates their potential to improve aquaculture wastewater treatment practices and bioremediation. The findings of these several studies have a direct application to this emerging industry and will hopefully assist in the future commercialisation of the species.

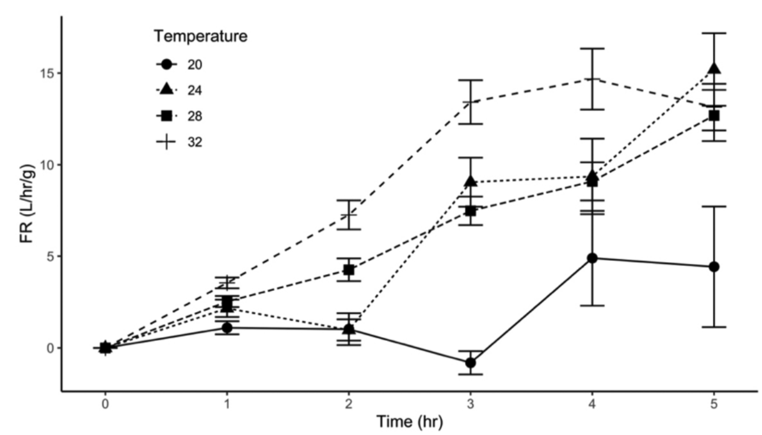

Figure 1. Oyster filtration rate (L/hr/g) ± SE across four temperatures (20, 24, 28 and 32 °C), over a five hour trial period.

UNCOVERING THE EFFECTS OF POLYETHYLENE MICROPLASTICS ON AQUATIC LIFE: A STUDY ON ASIAN SEABASS (*Lates calcarifer*, BLOCH, 1790) FRY

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Microplastics (MPs), which are water-insoluble polymeric materials with a diameter (< 5μm), have been found in aquatic environments. The emergence of microplastics as pollutants in aquatic habitats has been a serious point of concern for researchers and governments. Beside other aquatic biota, fish species are especially prone to ingesting particles of microplastics owning to their appearance. Ingestion of microplastics may influence feeding, growth and survival of fish fry. It may also expose fish to potentially hazardous chemicals. However, the potential toxic effects of microplastics continued to remain largely unidentified. Therefore, this study aimed to examine the effects of various concentrations of microplastics incorporated into the microdiet on growth, survival and intestinal histology of *L. calcarifer,* fry.

Lates calcarifer, fries with an average weight of 3.5 g, were fed diets containing 0 ppt (control), 1 ppt, 10 ppt and 100 ppt, microplastics at 5% body weight, twice a day for sixteen days. The feeding trials were conducted in 100-liter aquaria (20-fish aquarium^{-1}). Ammonia, dissolve oxygen, temperature, and pH were measured at a one-day interval. Survival of the fish were monitored every day. The length and weight of the fish were measured before and after the experiment. Three samples of intestines from each feeding treatment were collected for histopathological analysis.

The weight gain, final weight, specific growth rate and survival decreased with an increased microplastics inclusion levels from 1 to 100ppt (Table 1). However, no significant (P<0.05) effects were found among the treatments fed with dietary MPs compared with the control. The fish exposed to microplastics indicate significant (P<0.05) intestinal alterations. The morphometric evaluation of the fry fed MPs diets indicated a significant (P<0.05) decreased in villus height compared with the control. However, an increased villus thickness and diameters were observed among the dietary MPs group (Table 2).

Table 1. Growth performance of *Lates calcarifer* fed various inclusion level of microplastics

	Microplastics Concentration ppt			
Parameters	0	1	10	100
FW (g)	6.07^{a}	5.87^{ab}	5.50^{b}	5.46^{b}
WG (%)	75.91^{a}	69.22^{ab}	58.61^{b}	57.16^{b}
SGR(%/day)	3.52^{a}	3.28^{ab}	2.88^{b}	2.82^{b}
Survival (%)	76.67^{a}	61.67^{b}	55.00^{bc}	43.33^{cd}

Specific growth rate (SGR), Final weight (FW), Weight gain (WG). Means ±SEM (n=3) with different letters in the same row indicates significant different(P<0.005).

Table 2. Intestinal morphology of *Lates calcarifer* fed with Dietary Microplastics

	Microplastics Concentration ppt			
Parameters	0	1	10	100
DM (μm)	831.30^{a}	984.57^{b}	1080.62^{bc}	1238.4^{cd}
Height(μm)	466.63^{c}	365.74^{b}	241.56^{a}	225.22^{a}
Width(μm)	45.20^{a}	50.35^{b}	96.37^{c}	146.66^{bc}

DM: Diameter

Dietary MPs ingestion had significantly affected growth, survival and intestinal morphometric of *Lates calcarifer,* fry.

TACKLING WELFARE CHALLENGES IN TILAPIA FARMS IN SOUTHEAST ASIA

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Tilapia farming is a remarkable industry worldwide, with a substantial market share of approximately 9% of all finfish globally. Despite its economic importance, the rapid expansion of tilapia aquaculture, particularly in intensive culture systems, has raised concerns about its potential negative impacts on fish health, water quality, sustainability, and animal welfare. The application of animal welfare principles is essential to ensure the production of healthy animals and high-quality products, meet consumer demand for safe food, and safeguard animal welfare.

This study aimed to evaluate welfare parameters in tilapia farms in Thailand, with a focus on health, environment, behavior, and nutrition. Eight small-scale farms were assessed (Figure 1), and relative scores for the welfare indicators were compared. The study found differences in welfare behaviors during the catching process and variations in nutritional assessments, including feed amounts and quality, feed conversion rates, and K factor values. The study identified correlations between the nutritional, environmental, and health indicators, indicating opportunities to improve farming practices and enhance fish welfare. Farmers can improve their profits and provide healthier fish to consumers by improving welfare parameters, contributing to sustainable aquaculture practices. The study provides important insights for stakeholders in the aquaculture industry to promote animal welfare and sustainable practices.

Figure 1 Location of eight tilapia farms (stars) that were selected for tilapia welfare assessment in Thailand.

A NOVEL LOW-COST SPECTRAL SYSTEM TO SENSE APPROACHING ECDYSIS IN CULTURED JUVENILE TROPICAL ROCK LOBSTER *Panulirus ornatus*

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Detecting approaching ecdysis of juvenile tropical rock lobsters (*Panulirus ornatus*) is a pathway to reduce conspecific cannibalistic interactions in culture. A spectral signature created by nutrient and mineral reabsorption from the integument is a possible pre-ecdysis detection solution. Sensitive electronics in a salt-water environment coupled with the expense of a scientific grade spectrometer has promoted investigating lower-cost possibilities. A low-cost spectral system was constructed for testing and comprised of a Sony IMX-219 NOIR based camera, an AMS AS7269x 18 channel (6 near infrared channels) sensor array, and Osram SFH-4737 broadband infrared LEDs. The system was coordinated for simultaneous data collection through a Nvidia Jetson Nano with all components costing less than AUD $500. The system coordinates near simultaneous (<100 ms) collection of image and spectral data, adding an augmented layer of spectral data to the image. Lobsters near ecdysis (with developed suture lines), intermoult lobsters and post-ecdysis lobsters (following morning) were compared. We showed that IR reflectance and green channel pixel percentage are two separate effects because they did not correlate in the sample groups (Pearson's Correlation, Intermoult P>0.74, Pre-ecdysis P>0.54, Post-ecdysis P>0.81). Even so, the low-cost, low sensitivity and low-resolution components of this system were unable to detect a change on the dorsal surface indicating approaching ecdysis. However, the post-ecdysis group had significantly (P<0.01) lower infrared reflectance and significantly (P<0.01) reduced green channel pixel activation than the combined intermoult and pre-ecdysis groups. A scatterplot of the two effects shows that the post-ecdysis lobsters group together and have little overlap with the intermoult and pre-ecdysis groups. This is a promising first step in developing a low-cost spectral solution for detecting pre-ecdysis in juvenile tropical rock lobsters.

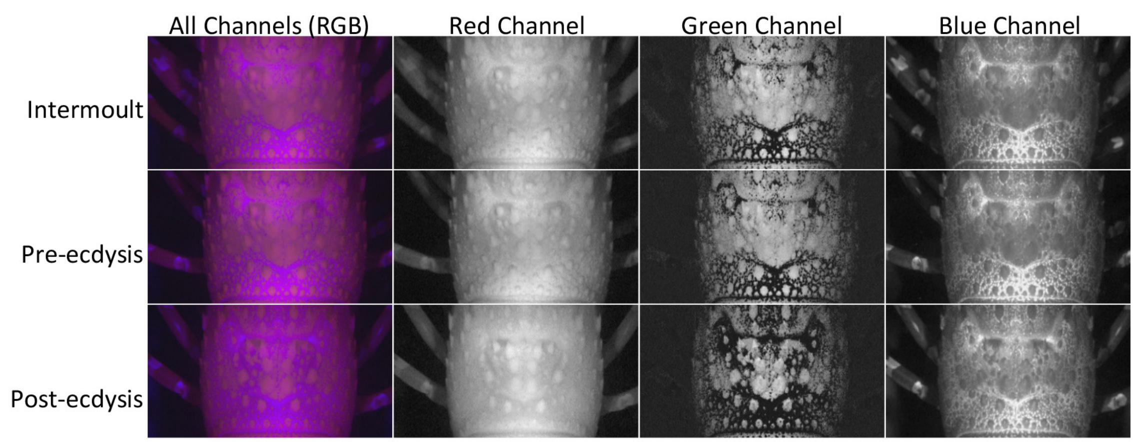

Figure 1. Close-up view of the posterior dorsal surface of *Panulirus ornatus.* Scene illumination by four SFH-4737 LEDs with image capture on a Sony IMX-219 NOIR CMOS sensor. The RGB images are broken into greyscale representations for the red, green, and blue colour channels. Moult cycle stages are shown in separate rows.

CAPTIVE SPAWNING AND REARING OF BLACK JEWFISH *Protonibea diacanthus:* A NEW CANDIDATE FOR AQUACULTURE IN NORTHERN AUSTRALIA

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Black jewfish *Protonibea diacanthus* is a commercially important fish to northern Australia's wild catch fishery. Part of the croaker family Scienidae, it has an established market for its meat and high value swim bladder. This research presents the first successful hatchery production of black jewfish in Australia and documents ontogeny of early life stages of this species. Adult black jewfish were induced to spawn on four occasions in the Northern Territory, Australia, 25-30 hours after injection with LHRHa (Syndel) during the wild black jewfish spawning season. Eggs hatched 12 hours after fertilisation (Fig 1.) and day 1 larvae measured 2.4 mm standard length (SL). Larvae mouths opened 31 hours after hatch and first feeding was observed at 57 hours. Swim bladder inflation (92%) occurred between 40 and 62 hours after hatch. Larvae were progressively fed rotifers, Otohime microdiet and *Artemia*, and metamorphosis occurred between day 20 and day 25 (average 13.5 mm SL). Tail biting cannibalism occurred in larval stages from day 17 and increased with the onset of metamorphosis, causing mortalities until day 35. Juvenile handling methods were modified over four production runs to increase survival during this stage. Initiating size grading immediately after metamorphosis and low stocking density in nursery systems is showing promise as an effective strategy for minimising cannibalism mortality. Survival from day 1 larvae to metamorphosed juvenile has increased from 1.5% to 20% over the course of four production runs, and research is continuing. Juveniles grew on average 1.3 mm per day for the first 5 months in nursery tanks, and reached average 2 kg and 51 cm total length by 14.5 months of age. Juveniles displayed salinity tolerance of 5-35ppt. Black jewfish show promise as an emerging aquaculture species due to their fast growth and ability to be cultured using existing aquaculture technology. Primary points for refinement involve management of cannibalism, increasing larval survival and improving nursery and grow-out procedures.

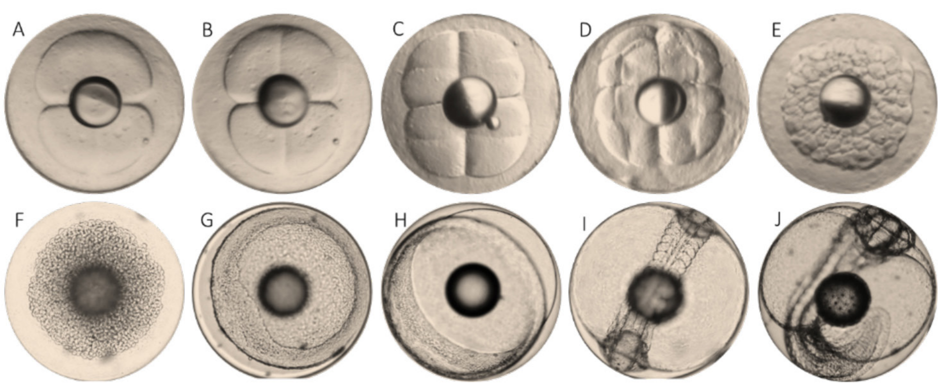

Figure 1. Embryonic development of black jewfish *Protonibea diacanthus* eggs spawned in captivity at 35ppt salinity and 30°C. A) 2 cell stage 30 minutes post fertilisation, B) 4 cell stage 45 minutes post fertilisation, C) 8 cell stage 1 hour post fertilisation (hpf), D) 16 cell stage 1.5 hpf , E) 64-128 cell stage 1.75 hpf , F) 512 cell stage 2 hpf, G) early gastrula 5 hpf, H) late gastrula 6 hpf , I) neurula stage 8 hpf, J) embryonic stage 10 hpf

CAPTIVE SPAWNING AND REARING OF BLACK JEWFISH *Protonibea diacanthus*: A NEW CANDIDATE FOR AQUACULTURE IN NORTHERN AUSTRALIA

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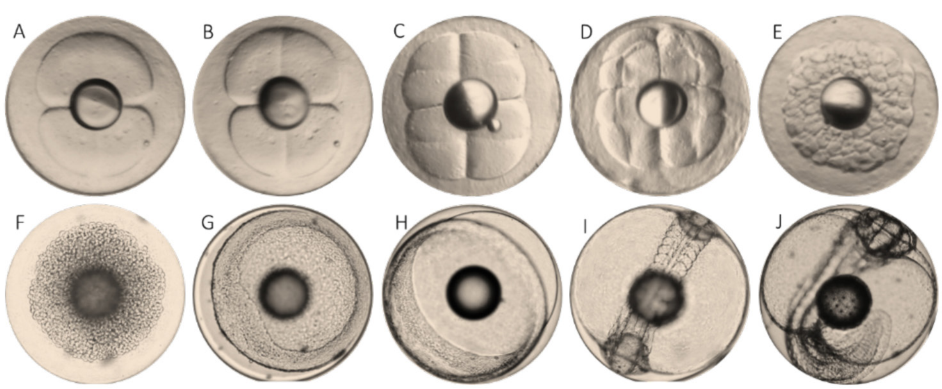

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PELLET FORMULATION WITH RECYCLED FISH GUTS AND FRUIT WASTE AS ALTERNATIVE COMMERCIAL FISH FEED

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Global consumption of seafood is predicted to increase to 182 million tonnes by 2030 (FAO 2022). Pressures to provide enough feed to supplement the growing aquaculture industry is detrimental to meet up with this production. Singapore relies heavily on imported fish feed for their aquaculture production, and feed cost attributes up to 40% of operation cost. Hence, finding alternative feed ingredients for fish feed is essential. In this study, discarded fish guts and fruit waste were utilized as alternative feed ingredients for a new formulated fish pellet.

Fish guts and fruit waste discarded by supermarkets and retailers were collected and powderised. Formulation of crude protein (cp) of the final pellets from these two feed ingredients were set at 30% cp levels, along with different proportions of wheat starch as binder for the two main ingredients. Formulated pellets were subjected to pellet solubility tests and nutrient analyses of fatty acid, protein, and amino acid profiles, against a commonly used commercial tilapia feed from Uni-president® as a controlled comparison.

Preliminary results show significant increases in the levels of fatty acids, including omega 3, 6, and 9 fatty acids, along with similar levels of crude protein were found in our formulated feed in comparison to the commercial fish feed, with all essential amino acid requirements met. Solubility tests revealed the treatment with 15% wheat starch had the least variance in terms of weight loss of a pellet before and after soaking for 120 minutes.

These results infer that our feed formulated with recycled fish guts and fruit waste could produce a sustainable substitute fish feed that provides the necessary nutrition for food fish.

Table 1: Nutritional Analysis of commercial pellet and formulated pellet

Pellet type	Unipresident®	Formulated pellet
Fatty acid		
Total saturated/100g	1.9	3.57*
PUFA	2.01	2.08
Trans Fat	0.027	0.094*
Protein		
CP/100g	31.3	28.6

Table 2: Solubility test results

Pellet type	% weight loss against time		
	30 min	**60 min**	**120 min**
Uni president®	22.5%	22.5%	20%
0% starch	25%	25%	40%*
15% starch	15%	15%	10%*
30% starch	15%	25%	27%

OVERCOMING HATCHERY BOTTLENECKS: BLACKLIP ROCK OYSTER LARVAE AND THEIR MICROBIAL HEALTH

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Blacklip Rock Oyster (BRO) aquaculture is an emerging, culturally identified industry in northern Australia. The BRO industry aims to optimise hatchery production at Darwin Aquaculture Centre (DAC), Northern Territory to produce a commercial supply of *Saccostrea echinata* (*Saccostrea* lineage J) by 2023. One bottleneck in production is repeat episodes of larval mortalities due to disease outbreaks. Little is known about the natural microbial community of BROs and their hatchery environment, as most studies focus on commercially important temperate oyster species. In 2020, a full mortality of BRO larvae occurred within the hatchery 14 days post hatch. A post-mortality investigation of BRO larvae identified *V. harveyi* as the most likely cause of the mortality due to the high relative abundance of *V. harveyi* in dead BRO larvae. A limitation of this study was that nothing was known about the bacterial and *Vibrio* communities of the larvae and their culture environment leading up to the mortality. Using molecular tools, we aim to analyse how the bacterial and *Vibrio* communities of BRO larvae and their culture environment change throughout two larval runs, particularly during mortality events. Results from this study will inform the BRO aquaculture industry of disease risk and improve hatchery management.

EFFECT OF SAUDAU (*Azadirachta indica*) EXTRACTION ON FISH LICE (*Caligus* spp) PARASITICS ON RABBIT FISH (*Siganus guttatus*)

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The research was conducted to identify the infection level of fish-lice (*Caligus* spp) on Rabbitfish (*Siganus guttatus*) which cultures in Thua Thien Hue province, and to clarify the effect of extraction from Saudau (*Azadirachta indica*) plant on the fish lice. The total of rabbit fish that were used for experiments is 120 fish, and fish were collected in 6 months at two different sites. The extraction of the Saudau plant was collected from leaf, and seed and then concentrated for experiments at ppm (0; 10; 50; 100; 500; and 1000) to evaluate the effect of extraction on fish lice. The results showed that the infection rate of fish lice on rabbit fish is 62,5% and infection intensity averaged at 19,2 lice/fish. Based on the collection data, it was clearly shown that in January, February, and March, fish lice were found much more than in other months ($p<00.05$). After fish lice were treated with the extraction from leaf and seed for 30 hours, we found that the half effective concentration (EC_{50}), and 90% effective concentration (EC_{90}) of leaf extraction are 148ppm and 928ppm, respectively. The EC_{50} and EC_{90} of seed extraction are 62ppm and 397ppm, respectively. The results from our research frankly show the potential of using extraction from Saudau leaf and seed to treat the parasite disease caused by fish lice on Rabbitfish.

CULTURE SYSTEMS FOR MANAGING CANNIBALISM IN *Panulirus ornatus*

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Following the development of larval rearing technologies pioneered at the University of Tasmania's, Institute for Marine and Antarctic Studies, commercial production of the spiny lobster *Panulirus ornatus* has commenced in northern Australia. However, low survival during juvenile culture represents a considerable bottleneck for the intensive onshore farming of this species. Mortality is primarily driven by cannibalism and can result in survival of <25% during early juvenile production. As in most crustacean species, cannibalism in *P. ornatus* typically occurs during and shortly after moulting when the animals are in the vulnerable "soft shell stage". Providing protection from conspecific attacks around this event is fundamental to reducing the incidence of cannibalism. The development of culture systems to limit the impacts of cannibalism and optimise early juvenile survival and growth is likely crucial for commercialisation of this species to reach its full potential. We have been investigating a range of options to reduce cannibalism, including examining animal behaviour, optimising nutrition, genetics, and system design.

System design includes the use of both communal and individual culture systems as avenues to resolve this bottleneck. The use of hides for shelter from conspecific attack in communal tanks has provided no significant benefits in mitigating cannibalism, despite the many configurations tested. In communal culture, we have observed stocking density to have a significant effect on juvenile survival, whereas animal size does not appear to be important. This indicates that grading by size is unlikely to be a beneficial husbandry practice. Feeding frequency can be used to optimise growth in communal culture, however with increased moulting frequency there is increased opportunity for cannibalism of conspecifics. We observed higher feed intake, driven by conspecific competition, and increased growth in communal culture compared to those cultured individually; however individual culture can provide better survival and far greater gains in biomass. Despite better outcomes for managing cannibalism to date; based on the higher operation cost of individual culture we are continuing to develop novel communal culture systems, which are showing considerable promise.

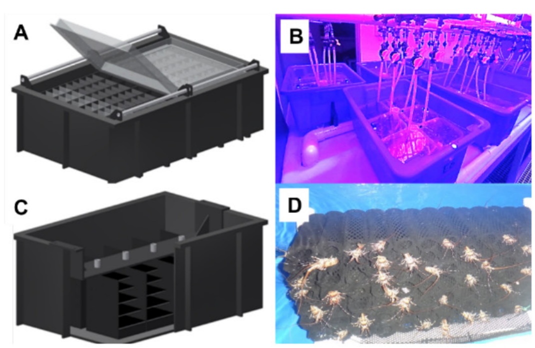

Figure 1. Examples of communal and individual culture systems: (A) rendered model of prototype individual holding system; (B) experimental testing of individual culture; (C) rendered model of communal culture tanks depicting a tiered hide system; (D) juvenile *P. ornatus* in communal culture using a mesh-tube hide system.

YEAST ARE POWERFUL MICRO-OGRANISMS TO UPCYCLE WASTE AND VALUABLE INGREDIENTS FOR AQUAFEEDS

Ha H. Truong*, Myintzu M. Hlaing, Paula C. Lima, Parveen Sangwan, Artur N. Rombenso, Carol J. Hartley, Cedric J. Simon and Pablo Juliano

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There is an opportunity to harness the power of fermentation to solve multiple problems faced by the food production industry. Food waste has become a modern-day crisis in which valuable nutrients are being lost to landfill. On the other hand, the cost to produce crops for the purposes of feeding humans and animals continues to increase. Using food and agriculture waste in animal feeds is a strategy of upcycling wastes so that these nutrients can be looped back into food production. In this context, fermentation technology is attractive for aquaculture as various less suitable substrates can be converted into high protein microbial cells which contain a more desirable nutrient profile for aquafeeds.

In a preliminary assessment, hydrolysed chickpea was used as a fermentable feedstock to grow various yeast species. Pichia species were among the best performing yeasts achieving the highest microbial growth while consuming less glucose compared to the more common *Saccharomyces cerevisiae* [1]

Pichia pastoris and *S. cerevisiae* biomass were assessed in a prawn (*Penaeus monodon*) growth and digestibility study to determine their suitability as feed ingredients. *P. pastoris* was cultured in-house while *S. cerevisiae* was purchased and assessed as two sources: human-grade Bakers yeast and animal-grade Brewers yeast. The three yeast ingredients were supplemented at 15% in a control diet (TC) containing traditional protein ingredients: fishmeal, canola meal and poultry meal. Diets were adjusted to be iso-nitrogenous and iso-energetic. A commercial diet was also assessed. Diets containing *P. pastoris* outperformed diets containing *S. cerevisiae* and performed similarly to the commercial diet for juvenile weight gain over 6 weeks (Table 1). All diets assessed were highly digestible for protein, lipid and carbohydrate (CHO).

Yeast can be a valuable protein ingredient for aquaculture diets. However, their value in producing sustainable ingredients has not been fully explored. Ongoing research will assess the economic and environmental benefits of fermenting food and agriculture waste for aquafeeds.

Hlaing et.al (2022) Agrifood waste as alternative precision-fermentation feedstock (manuscript submitted).

Table 1. Effect of dietary treatments on prawn growth and survival when fed for 6-weeks and diet apparent digestibility of nutrients as determined from prawn faeces.

				Apparent digestibility (%)		
Diet	Final weight (g)[1]	Weight gain (g)	Survival (%)	Protein	Lipid	CHO
Commercial diet	22 ± 0.28c	12.4 ± 0.31b	93.8 ± 3.61	NA	NA	NA
Control diet (TC)	20.2 ± 0.7abc	10.5 ± 0.66ab	93.8 ± 3.61	75.5 ± 2.01	73.7 ± 1.1	66.7 ± 0.74
TC + 15% Bakers yeast	18.2 ± 0.64a	8.3 ± 0.66a	96.9 ± 3.13	75 ± 2.73	74.9 ± 1.18	67.3 ± 0.98
TC + 15% Brewers yeast	18.5 ± 0.36ab	8.8 ± 0.35a	93.8 ± 3.61	78.4 ± 1.82	76.8 ± 2.25	71 ± 1.74
TC + 15% *P. pastoris*	20.8 ± 0.55bc	11.2 ± 0.55b	81.3 ± 8.07	80.3 ± 0	76.5 ± 0	70.4 ± 0
P-value[2]	<0.001	<0.001	0.219	0.285	0.368	0.173

[1]Inital weight of prawns was 9.7 ± 0.1. [2]Based on 1-way ANOVA. NA = apparent digestibility not assessed.

1

EVALUATION OF INDUCED LATE-WINTER SPAWNING ON THE GROWTH PERFORMANCE OF AUSTRALASIAN SNAPPER *Chrysophrys auratus* IN THE MARLBOROUGH SOUNDS

Nicholas P.L. Tuckey*, Sharon Ford, Warren Fantham, Annalise Runarsson, Liam Hegarty, Morgan Puklowski, Ross Jacobs, Greg Knox, Belinda Timms, Matthew J. Wylie, and Damian Moran

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New Zealand's sole commercial finfish aquaculture species is Chinook salmon (*Oncorhynchus tshawytscha*), a cold-water species at risk from increasing water temperatures. Diversification of farmed fish species can help mitigate this risk. We investigated the feasibility of manipulating the spawning of Australasian snapper (*Chrysophrys auratus*) and producing juveniles for sea pen culture in the Marlborough Sounds by early summer, when natural spawning occurs.

Water temperature and photoperiod were manipulated to induce spawning of captive broodstock in late winter. Tank water temperatures were cooled to 11°C by early July (winter). Between July and late August seawater temperature was progressively increased to 20°C, where it was held until spawning, which occurred without hormone intervention in late August. Eggs were incubated and larvae cultured in 5000-L self-cleaning tanks. A progression of live feed was used, beginning with enriched rotifers on 2 days post hatch (DPH) and progressing to *Artemia* on 20 DPH. Juveniles were weaned to commercial pellet feed (0.2–0.5 mm) by 35 DPH. At 99 DPH, 2550 juveniles under partial sedation (10 ppm Aqui-S™) with supplementary oxygen were transferred to a sea pen located in Beatrix Bay in the Marlborough Sounds. The sea pen was an 18-m polar circle sub-divided into four sub-pens, each approximately 216 m^3 in volume. Commercial pellet feed was provided at 1.5% of body mass per day, delivered during daylight hours at approximately 90-min intervals by an automated drum feeder.

The growth performance of the late-winter spawned cohort was compared with that of a cohort of juveniles produced in November 2019 and grown in 5000-L tanks under ambient conditions. At 10 months post hatch tank-cultured fish had reached a mean length of 124 ± 8 mm (± SD; n=318), compared with 163 ± 8 mm (n=257) for sea pen-cultured fish. This trial presents an early case study for farming Australasian snapper in New Zealand and highlights one potential strategy to reduce the production time of this species.

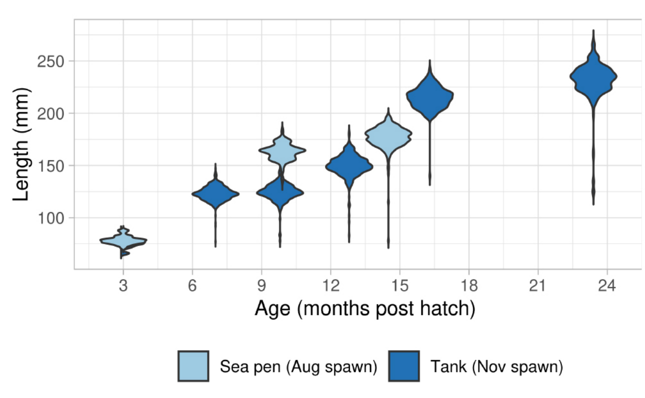

FIGURE 1. Comparative size measurements for Australasian snapper spawned in early summer (November 2019) and grown in 5000-L tanks, and a cohort spawned in late winter (August 2021) and grown in a sea pen in the Marlborough Sounds, New Zealand.

HARVEST CONDITION INFLUENCES THE PERFORMANCE OF GREENSHELL™ MUSSELS *Perna canaliculus* IN CHILLED AND REFRIGERATED SEAWATER-BASED POST-HARVEST STORAGE

Nicholas P.L. Tuckey*, Belinda A. Timms, Graham C. Fletcher, Graeme Summers, Natalí J. Delorme, Jessica A. Ericson, Norman L.C. Ragg, Poppy Miller, Reginald Wibisono, Roland Taylor, Serean L. Adams, and Leonardo N. Zamora

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Exporting live shellfish products while ensuring their quality and safety on arrival presents significant challenges. As surface transport times from Australasia to major export markets are prohibitively long air freight has so far been the only viable option. This is becoming increasingly costly and generates a significant carbon footprint. For surface shipping to be viable, more effective preservation of the 'as-harvested' shellfish properties is needed. To progress this aim, we evaluated the effects of chilled storage (6.5°C) in air, refrigerated seawater recirculated either continuously (RSW), or for 6 min per hour (Periodic RSW), on Greenshell™ mussel (GSM) post-harvest performance (i.e., physiology, product quality and food safety) for 10 days in two trials conducted in austral spring (October 2020) and summer (March 2021).

GSM interacted with seawater at 6.5°C, even when delivered periodically, as ammonia seawater concentrations increased. Mortalities across both 10-day trials were 5.5% for Chilled, 3.5% for RSW, and 2.4% for Periodic RSW. Haemolymph pH and intravalvular water weight were good measures of performance. Reduced haemolymph pH was seen in the Chilled and Periodic RSW GSM from day 2 through to day 10, and higher variance could be seen on days 4 and 10 in the summer trial. Intravalvular water weights were stable in the spring trial in all three treatments, but were lower and showed increased variance in the Chilled and Periodic RSW treaments in summer. Meat textural hardness was best preserved by RSW. For GSM inoculated with *Escherichia coli* and *Vibrio parahaemolyticus,* most probable numbers (MPN) were stable for the Chilled and Periodic RSW treatments, with reductions in MPN seen in RSW on days 7 and 10. Post-harvest seawater treatments for GSM show potential and further optimizations are plausible. We also highlight GSM condition at harvest as a factor influencing post-harvest storage outcomes.

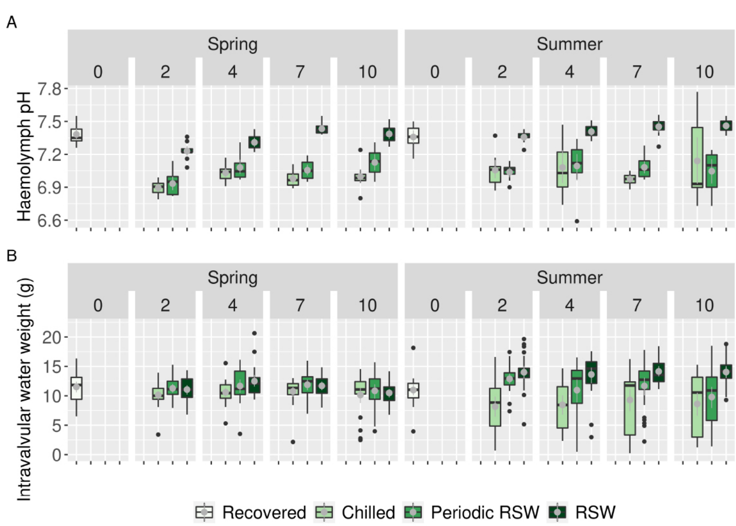

FIGURE 1. Greenshell mussel haemolymph pH (A; n=9) and intravalvular water (B; n=25) during 10 days' storage at 6.5°C exposed to air (Chilled), refrigerated seawater (RSW) and refrigerated seawater delivered for 6 minutes every hour (Periodic RSW). Trials were conducted in spring (October 2020) and summer (March 2021).

THE COSTS AND BENEFITS OF INTEGRATING MARINE BIOTOXIN RISK MANAGEMENT ACROSS MULTIPLE SPECIES IN TASMANIA, AUSTRALIA

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Aquaculture systems worldwide are impacted by algal blooms affecting food safety, market access, animal health and production. The variety of harmful algal species and toxins involved has led to the requirement for a high level of expertise and complex testing services to effectively manage risk, resulting in increasing costs to industry. Aquaculture is particularly exposed to risk, due to the confined nature of production, however wild fisheries can also be impacted.

A variety of recurrent harmful algal blooms (HABs) occur in Tasmania, impacting a broad range of user groups including commercial sectors for both wildcatch fisheries and aquaculture (combined value AUD 1.1B), recreational and Indigenous fishers. The toxins accumulate in a range of seafood in levels that could cause illness in humans, and therefore regulatory standards exist to manage the domestic and export trade. Risk to recreational and Indigenous fishers is managed through public health warnings when monitoring of commercial fisheries highlights an issue.

Currently the different Tasmanian seafood sectors are managed through separate processes and organisations, with varying levels of technical expertise available to each sector. Data sharing occurs in a highly bespoke, and in some cases *ad hoc*, manner with no easy-to-access system that can collate and display all HAB data to provide current state-wide situational awareness. Some industries benefit considerably from the monitoring activities of the aquaculture bivalve sector. The lack of a centralised system inhibits sharing costs of regulatory monitoring or leveraging of monitoring in researcher activities.

A cost benefit study is being undertaken to determine the net benefit of biotoxin risk management to the various fishing industry stakeholder groups in Tasmania. Options for integrated risk management (where resources, data and expertise are shared) are being considered, where these will account for the variable fishing activity, risk profiles, and toxin accumulation/depuration kinetics between species and fisheries. This collaborative process with stakeholders is seen as the first step towards building a collaborative program for the state.

EFFECT OF LARVAL STOCKING DENSITY ON THE POST SETTLEMENT NURSERY PRODUCTION OF SANDFISH SEA CUCUMBER *Holothuria scabra* FOR STOCK ENHANCEMENT

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The sandfish sea cucumber *H. scabra* is a candidate species for aquaculture. Despite decades of research efforts, the production of tropical sea cucumber is still hampered by the availability of seed stock for ranching and stock enhancement efforts. Increasing the stocking density in sandfish nursery systems results in slow growth and small sizes. Severe effects of density dependent growth have resulted in low productivity nursery systems compared to commercialised aquaculture species, requiring extensive nursery and growout systems. Management of stocking density at an early life stage (settlement) is a way to achieve rapid growth. Optimising stocking density to achieve fast growing healthy seedstock whilst maximising the number of juveniles produced in nursery systems will be key to the success of tropical sea cucumber aquaculture operations. The study reported here was designed to develop our understanding of appropriate pre settlement stocking rates in a plate type nursery system to increase the number of juveniles produced without compromising growth.

The number of juvenile *H. scabra* produced in a plate tank nursery system was assessed in a nursery stocking density trial. Fibreglass nursery tanks (6m x 1m x 0.45m) each containing 120, vertically oriented acrylic sheets (0.6m x 0.3m) as settlement substrate were supplied with 1µm filtered flow through seawater under ambient conditions. Fourteen-day old doliolaria stage larvae were stocked at three different stocking rates, A = 587 larvae m^{-2}, B = 352 larvae m^{-2} and C = 252 larvae m^{-2}.

After 90 days the number of juveniles produced per square metre in the A stocking density treatment group ($61 \pm 5.6m^{-2}$) was significantly greater than in the other B and C stocking rate treatments, $42 \pm 3.5m^{-2}$ and $32 \pm 2.7m^{-2}$, respectively. Growth and recovery rate (% larvae recovered as juveniles) were not significantly reduced in the high stocking group.

The trial yielded fewer than expected numbers of juveniles, however growth was rapid. Some tanks in all treatment groups were impacted by poor productivity resulting in very small stock and low biomass in those tanks.

Determining the best combination of substrate preconditioning, stocking rate and management practices to balance the trade-off between stocking rate and productivity will be vital to prevent growth being compromised to a point that impacts survival or success in sea ranching or stock enhancement operations.

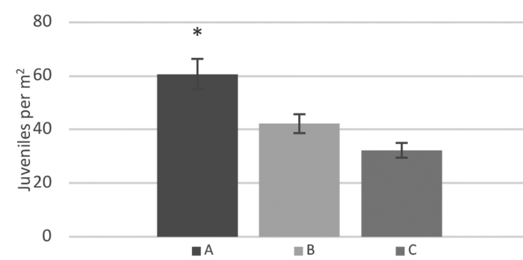

FIGURE 1: Number of juvenile *H. scabra* harvested per m^2 in a plate nursery after 90 days post settlement when stocked at A = 587 larvae m^{-2}, B = 352 larvae m^{-2} and C = 252 larvae m^{-2} larval stocking rates. Error bars are ± SE. * denotes significant difference ($P<0.05$).

TABLE 1: Juvenile *H. scabra* nursery production parameters of individual size, biomass density, recovery rate and growth rate of a plate nursery system from larvae at different stocking rates. Values are means ±SE.

Stocking density	*Individual size (g)*	*Biomass Density (gm⁻²)*	*Recovery rate (%)*	*Growth Rate (g day⁻¹)*
High	10 ± 1.2	650.2 ± 104.2	10 ± 1.0	0.12 ± 0.013
Medium	12 ± 1.1	550.7 ± 67.3	12 ± 1.0	0.14 ± 0.012
Low	15 ± 1.7	482.5 ± 61.2	13 ± 1.1	0.17 ± 0.018

OYSTER AQUACULTURE IN VIETNAM: STATUS, SUPPLY AND CHALLENGES

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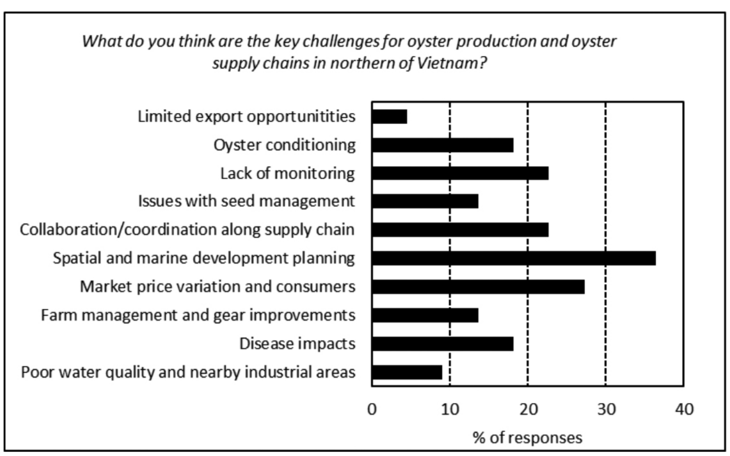
Oyster aquaculture in northern Vietnam is a new and growing industry that brings benefits to coastal communities. By understanding the oyster supply chain, the potential value can be fully explored, and socio-economic and environmental gains targeted. This study surveyed industry representatives – from farm to plate – to examine the industry status, distribution network, challenges, and opportunities. Four broad distribution network models were identified: direct-to-sale, wholesaler, cooperative/processor, and retailer. These models are quite different and comparing them is complex. A reduction in the marketing margin in any supply chain is likely to result in shared economic and/or welfare gains for pre-harvest elements, elements in the value chain, and consumers. Similarly, lower farm production and hatchery costs and increased consumer demand would result in welfare gains shared along the supply chain, although these do not necessarily translate to changes in the marketing margin. Key challenges were associated with spatial and marine development planning, lack of monitoring, collaboration/coordination along the supply chain, and market price variation and consumers. Challenges were further explored in the categories of collaboration/coordination, regulation, and export. Opportunities exist to better understand how oyster shell might be used as a by-product such as a livestock mineral supplement, in soil acidity management, and in crop and vegetable growth. Almost half of the survey respondents have oyster shell as a by-product, suggesting the potential for carbon offset schemes could also be explored, at least to the extent to which they ameliorate the carbon footprint of the industry. These opportunities could advance and value-add to oyster aquaculture in northern Vietnam.

This study was funded by Australian Centre for International Agricultural Research (ACIAR) via the project: "Blue economy: Valuing the carbon sequestration potential in oyster aquaculture" (FIS/2020/175) that is a collaboration between Research Institute for Aquaculture No.1, Vietnam, New South Wales Department of Primary Industries, and the Institute for Marine and Antarctic Studies at the University of Tasmania.



THE BLUE ECONOMY COOPERATIVE RESEARCH CENTRE: SCOPING PROJECT ON THE PATHWAY FOR SPECIES SELECTION, MULTISPECIES AND SYSTEMS INTEGRATION

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The Blue Economy Cooperative Research Centre (BECRC) brings together over 40 partners from 10 countries and will operate until the end of this decade. The overarching aim of the BECRC is to support research that develops new seafood and renewable energy systems that move production offshore safely, economically and sustainably.

Within the BECRC, there are five interconnected Research Programs that have been developed in collaboration with industry and other partners (www.blueeconmycrc.com.au). Research Program 2 (RP2) '*Seafood and Marine Products*' supports the development of offshore aquaculture systems that provide viable and sustainable growth opportunities.

There is a need to prioritise aquaculture species suitable for novel offshore multispecies systems. This Scoping Project will use a collaborative approach to first rank aquaculture species that are available in Australia and New Zealand, then rank offshore multispecies systems across regions, from temperate to tropical, and list the key challenges and opportunities. The Scoping Project will create a pathway for BECRC decision-making and support the development of future research projects by identifying priority partners and investment.

We look to engage with commercial operators to better understand the characteristics of species they grow and include in our assessment. Please contact Sarah Ugalde (sarah.ugalde@utas.edu.au)

GROWTH, MORTALITY, RECRUITMENT AND STOCK STATUS OF POTENTIAL AQUACULTURE SPECIES (*Plotosus canius*) IN THE COASTAL WATERS OF PORT DICKSON, PENINSULAR MALAYSIA

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Plotosus canius Hamilton, 1822; family plotosidae is primarily a marine catfish, but at times has been found to live in fresh or brackish waters (Riede *et al*., 2004; Ambak *et al*., 2010; Amornsakun *et al*., 2018). Literature on this fish both from national and international perspective is very scarce. Although, the status of this fish has not been assessed in the IUCN red list (IUCN, 2016), it has been declared endangered in Bangladesh and India (Mukhopadhyay, 1994; IUCN Bangladesh, 2000; Mijkherjee *et al*., 2002). Thus the present study was undertaken to examine status of *P. canius* from the coastal waters of Port Dickson, Peninsular Malaysia.

The present study has been undertaken for 12 months to investigate the population structure of *P. canius* in the coastal waters of Port Dickson, Peninsular Malaysia. The growth, mortality, recruitment and relative yield per recruit of *P. canius* were examined based on monthly length-frequency data, using the FiSAT (FAO-ICLARM Stock Assessment Tools).

The estimated total mortality (Z) value was 2.73 yr^{-1} during the study period. Natural mortality (M) and fishing mortality (F) were estimated at 1.42 yr^{-1} and 1.31 yr^{-1} respectively. The value of exploitation rate (E) obtained for *P. canius* in the study was 0.48. Higher natural mortalities (M) versus fishing mortalities obtained in the present study suggested an unbalance position in the fish stock. Two major recruitment events were observed per year and the recruitment pattern was continuous (Figure 1). The findings from the analysis of the exploitation rate (E) based on the fishing mortality estimates, and from the yield-per-recruit showed that the *P. canius* fishery in the study area is slightly below the optimum level of exploitation (Figure 2). However, more research is suggested in such direction to corroborate the present findings as management for this fish is indispensable for maximum sustainable yield.

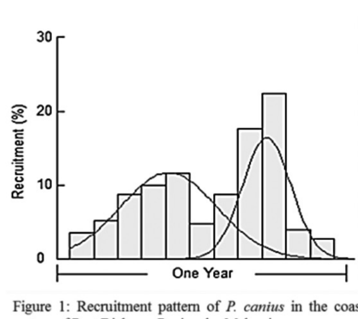

Figure 1: Recruitment pattern of *P. canius* in the coastal waters of Port Dickson, Peninsular Malaysia

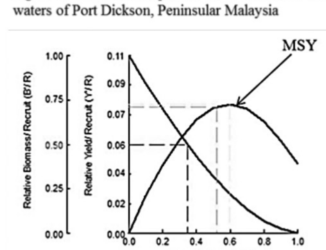

Figure 2: Yield-per-recruit and biomass-per-recruit model, showing level of yield index in *P. canius* from the coastal waters of Port Dickson, Peninsular Malaysia

SCREENING AND MONITORING OF ANTIMICROBIAL RESISTANCE IN FEED (FISHMEAL, ANIMAL AND PLANT FEEDS)

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Antimicrobial resistance (AMR) has become a major public health concern due to its impact on human and animal health. The overuse of antibiotics in aquaculture, animal and plant farming has led to the emergence and spread of AMR, which is a significant threat to food security, environmental health, and global economy. This book chapter reviews the current knowledge on the transmission of AMR between different feed sources, including plant, animal and fishmeal feed in aquaculture, and the need for screening and monitoring to prevent the spread of AMR. It also discusses the various methods for screening and monitoring AMR in fishmeal, animal and plant feeds, including the use of culture-based and molecular-based techniques. Furthermore, the chapter examines the challenges associated with the development and implementation of screening and monitoring programs in feed. Several factors contribute to the development and spread of AMR in aquaculture feed sources. These include poor management practices, the use of subtherapeutic doses of antimicrobials, and the use of antimicrobials to compensate for poor environmental conditions. Additionally, the close proximity of aquaculture facilities to each other and to wild aquatic populations can also contribute to the spread of AMR. Therefore, to address the issue of AMR in aquaculture feed sources, the chapter will discuss potential strategies to mitigate the problem. This may include improved regulation of the use of antimicrobials in aquaculture, the development of alternative treatments, and the use of vaccines and other preventive measures to reduce the need for antimicrobials. In conclusion, this chapter will provide a comprehensive review of AMR in aquaculture feed sources, highlighting the importance of this issue and the need for action to address it.

TWO DECADES OF RESEARCH AND DEVELOPMENT IN INLAND SALINE AQUACULTURE IN INDIA: PRESENT STATUS AND PROSPECTS

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In India, about 6.74 million ha area is salt-affected, of which ~ 25.36% is saline, 18.46 coastal saline, and 56.16% is sodic in nature. Besides this, 248 districts in 18 states and 6 Union Territories are also affected by groundwater salinity, which is increasing at an alarming rate causing secondary salinization. Groundwater salinity is severe in the Northwestern inland states of Haryana, Punjab, Rajasthan and Uttar Pradesh situated ~ 1500 km away from the sea coast. The quality of inland saline water (ISW) in this area varies from place to place in terms of salinity and the ionic composition of potassium (K^+), calcium (Ca^{++}) and magnesium (Mg^{++}) in contrast to seawater. Both saline soils and groundwater salinity have severely affected agriculture productivity and ultimately livelihood of the people. Two decades of research in inland saline aquaculture (ISA) in India with about a dozen of fish species namely milkfish (*Chanos chanos*), grey mullet (*Mugil cephalus*), pearl spot (*Etroplus suratensis*), tiger shrimp (*Peneaeus monodon*), Pacific whiteleg shrimp (*Penaeus vannamei*), Indian white shrimp (*Penaeus indicus*), Asian seabass (*Lates calcarifer*), silver pompano (*Trachinotus blochii*) among the sea fishes and giant freshwater prawn (*Macrobrachium rosenbegii*), common carp (*Cyprinus carpio*), striped catfish (*Pangasianodon hypophthalmus*), Nile tilapia (*Oreochromis niloticus*, GIFT) and Indian major carps revealed that the ionic imbalance in ISW in contrast to seawater follows different trends of survival and growth for fresh, brackish and marine fish species. Whereas some species sustain well in ISW without any ionic corrections or with marginal compensation of growth, the ionic amendment of ISW with fertilizer/ commercial grade chemicals was found essential for the larval rearing of giant freshwater prawn (GFP) and the farming of tiger shrimp & Pacific whiteleg shrimp. Based on our research, ICAR-CIFE has successfully demonstrated commercial production of GFP seed, and tiger shrimp & Pacific whiteleg shrimp farming in ISW with ionic amendments at its Rohtak Centre (Haryana). The major species in high and medium inland saline waters remains to be *P. vannamei*, owing to high economic return. Since maintaining K^+ in ISW requires a huge quantity of fertilizer, we are focussing our research on ionic amendments/retention through the addition of nutraceuticals in diet. The supplementation of K^+ (1% KCL) in the diet has been found to significantly minimize the requirement of K^+ in ISW and save the cost of the amendment. To further enhance performance, the supplementation of organic osmolytes such as glycine-betaine, myoinositol, taurine and alanine in the feed which crustaceans inherently accumulate while dealing with osmotic imbalances. This dietary approach will certainly bring down the operating cost and environmental impacts associated with fertilizing the culture water substantially although the maintenance of inorganic ions at minimum levels in water may be physiologically inevitable. In our initial trial, the blending of potassium (0.2 %) and taurine (0.5 %) in the diet appears best to mitigate stress and enhance the growth of tilapia (GIFT) reared in ISW and hence our future research shall be targeting the other organic supplements on suitable species for ISA.

LEGAL ASSISTANCE IS REQUIRED FOR WOMEN FISH VENDORS TO OVERCOME OBSTACLES IN THE FISH MARKET

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The rapid urbanization of Patna city in India has led to an increase in the number of fish consumers. As a result, many fish vendors have occupied various public spaces to fulfill the demand. However, due to the limited recognized marketplace, only a few vendors are able to sell their products legally. The remaining vendors, including women, use and encroach upon streets, pavements, and other public places. Young women often attempt to select new places for fish vending, and as they earn the trust of the community, the number of vendors gradually increases. While initially dominated by women, men eventually become involved and begin to cover new areas as well. When women are relocated, they start anew in a different area. The women who sell fish in the town face numerous challenges, including harassment to pay an “unofficial tax” to continue selling in certain locations, particularly for women. Legal assistance is required to provide legal security and to incorporate valid vending/hawking zones in the city/town master or development plans, including zonal, local, and layout plans, and to ensure their execution to offer street vendors. Fish sellers have the right to a hygienic place, safe drinking water availability, proper sanitation systems, waste disposal arrangements, and other essential services.

Pic: A

Pic A: Fish seller at Roadside

Pic: B

Pic B: Fish seller at Authorized market (Male dominated)

COMMERCIAL IMPLEMENTATION OF GENOMIC SELECTION IN TASMANIAN ATLANTIC SALMON: SCHEME EVOLUTION, VALIDATION, AND FURTHER DEVELOPMENTS

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The Tasmanian salmon industry is Australia's most valuable seafood sector. Salmon Enterprises of Tasmania (SALTAS) has operated a selective breeding program since 2004. Genomic information was included for the first time in the prediction of breeding values within the selective breeding program nucleus in 2016 with the scheme finalised in 2018. An efficient genotyping scheme is essential for genomic selection. Custom low and high density genotyping platforms were designed, manufactured and are deployed, allowing all individuals in every year class to be genotyped while maximizing the genetic gains and minimizing costs.

Significant increased rates of genetic gain attributed to the implementation of genomic selection in the breeding nucleus have been shown across both the primary and secondary traits. Substantial improvement in the ability to accurately select parents prior to progeny testing is observed across multiple years for all traits and the index. The resultant gains have allowed the industry to add a new primary trait without compromising the relative gains of the existing traits.

Subsequent developments and utilisation of genomics data have focussed on exploiting the potential for genomic selection to improve commercial production. A scheme was developed to allow all commercial male multipliers to be selected using genomic selection using the existing genotyping tools. This scheme also allows for the industry to produce smolt that are tailor-made for the specific environment of their marine grow-out, for example, the ability to prioritise disease resistance or thermal tolerance for fish going to certain sites. The resultant economic impacts for the industry are considerable based on the increases in genetic gain for traits achieved within the breeding program and the use of genomics for commercial production.

EFFECTS OF ASTAXANTHIN FROM *Haematococcus pluvialis* AS SUBSTITUTE FOR SYNTHETIC ASTAXANTHIN IN ATLANTIC SALMON *Salmo salar*

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In Atlantic salmon, the specific red-pink flesh color is formed by the accumulation of astaxanthin from food. While wild salmon get their pigment from their prey, farmed Atlantic salmon needs supplemented formulated diets. Nowadays, the share between synthetic- and natural sources is approximately 90:10, with an increasing interest in new natural sources. The cost of carotenoid supplements accounts for 6-10% of the total feed cost, whereas the deposition for flesh pigmentation in salmonids is less than 22%. Natural astaxanthin from the algae *Haematococcus pluvialis* contains only 3S,3'S stereoisomer, the predominant pigment in wild salmon, suggesting an improved biochemical effect compared to the synthetic form. In addition, some experimental diets containing algae-extracted astaxanthin have shown improved immune responses and stress tolerance in many aquatic species. Therefore, our study was performed to understand whether astaxanthin from *H. pluvialis* could enhance astaxanthin assimilation and potentially boost growth and immune responses.

A 108-day feeding experiment was conducted in a flow-through system with seawater taken from a depth of 250 m of eighteen 800 L-tanks, including three diets: AS (synthetic astaxanthin), AW (whole algae astaxanthin), and AX (algae-extracted astaxanthin) (3 x 6 replicates). Twenty fish (approximately 652 g) were stocked in each tank and fed twice daily. Environmental conditions were maintained in the optimal range of Atlantic salmon. Three fish were sampled from each tank on days 30, 60, and 108 to assess growth performance, visual flesh color, and astaxanthin content in Norwegian Quality Cut. At the end of the experiment, liver and pyloric caeca in each treatment (n = 4) were collected for RNA extraction and sequencing for differential expression analysis.

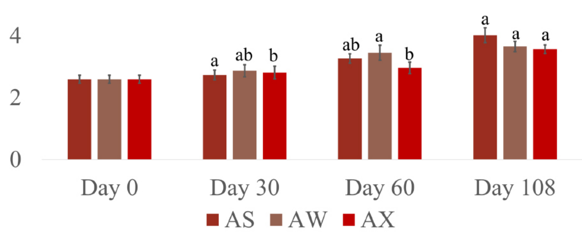

Fig. 1. Astaxanthin concentration (mg/kg) between three dietary treatments (Mean ± SD with different letters were significantly different $P < 0.05$).

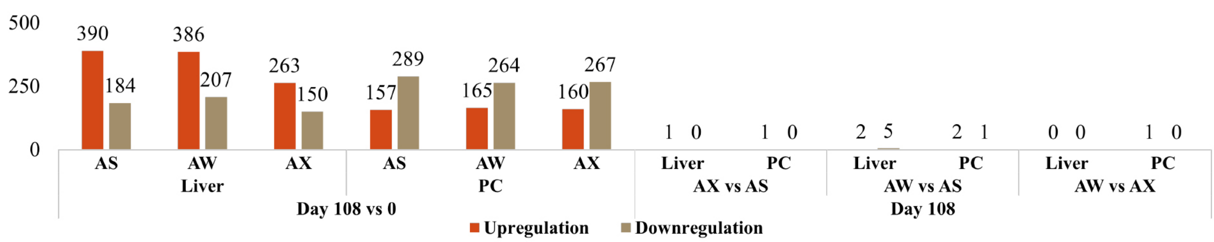

Fig. 2. The numbers of differentially expressed genes between days 0 and 108 and the three diets on day 108.

THE IMPORTANCE OF INDUSTRY ENGAGEMENT IN MIDDLE SCHOOL AQUACULTURE EDUCATION PROGRAMS

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This presentation will examine a case study of how industry engagement has supported the ongoing development of aquaculture education in a Northern Territory middle school setting resulting in positive outcomes for students, educators and local aquacultural businesses. The benefits associated with developing strong industry-classroom partnerships are many and have been well documented worldwide. By drawing on contextual examples of success it is hoped that educators, businesses, and industry experts will be encouraged to engage with schools in meaningful ways that will strengthen the future of the industry and provide rich learning experiences for students along the way.

Taminmin College is a rural high school located in Humpty Doo, Northern Territory. In 2019 Marine Technology was trialed as a year 8 elective subject due to the high number of students with a keen interest in fishing. The fast success of the subject provided the foundation to build what has become a complete pathway through middle school and on to vocational education and training in year 10 via delivery of Certificate I in Seafood - an internationally recognized vocational education course. Since its inception more than 400 students have engaged with the program resulting in real world aquaculture employment for participants.

Vital to the development of the program has been the role of local industry stakeholders. Through collaboration and the willingness of local businesses to share their knowledge and resources the program has seen students build awareness and aspirations about potential careers, link learning to real world problems, understand their future work environments and developed their future work capabilities. The capability of teachers has grown through the development of professional networks and connections outside the teaching environment resulting in an increased capacity to deliver lessons that incorporate key skill sets, modern methods and industry relevant vocabulary. Along the way students have experienced positive interactions with potential employers and built positive perceptions of local aquaculture businesses - a key element in easing the transition to work. Through industry consultation, workplace visits, career talks, mentoring, work experience, guest speakers and donations the intellectual and technological capacity of the school has grown as has access to and understanding of state-of-the-art technologies not normally accessible in a school setting.

By outlining some of the key collaborations happening at Taminmin college it is hoped that industry leaders will recognize and embrace opportunities to strengthen aquaculture education in middle school settings within their own communities. By doing so the future of the industry can only be strengthened further.

Figure 1.0: Certificate I in Seafood students visiting Monsoon Aquatics

AFRICAN AQUACULTURE: THE FUTURE FOR SMALL-SCALE FISH FARMING

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African aquaculture is rapidly growing with support from Governments, development partners and private sector. This study identifies investment and policy priorities that can advance the small scale fish farming in Africa to improve income and employment generation, rural development, and food security based on five countries. We identified five promising innovations or drivers that can transform the sub-sector in Africa. Furthermore, lessons learned that inform policy and investment priorities at the national, regional and continental levels. Scaling of aquaculture innovations through gender-responsive approach contribute to development of African agricultural sector.

FACTORS SHAPING THE MICROBIAL POPULATIONS PRESENT ON FISH AND FISH PRODUCTS

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Catfish is one of the leading finfish species produced in the U.S., with 94.6 % of total food-size fish sold after processing. The control of microbial contamination during processing is a key factor for ensuring the microbial safety, quality, and shelf life of fish products. Unfortunately, how microorganisms present in the processing environment (e.g., equipment and line workers) and fish harvesting and processing seasons shape the microbial compositions of fish and fish products remains largely uninvestigated. To address this need, this research team applied the amplicon sequencing approach to characterize microorganisms present on the intact fish skin, in fish intestine, on fish fillets, and in environmental samples collected from catfish processing plants to categorize factors determining the microbial compositions of fish and fish products based on their significance.

Three catfish processing facilities in Southeast U.S participated in this study and two visits were conducted for each plant representing the Spring and Fall harvesting and processing seasons. A total of 70 environmental swab samples were collected representing the microbial populations present on the blade of de-heading machines, conveyor belts, worker gloves, floors of the sorting, processing, and packaging areas and the exterior of bins used for holding and transferring of final fish products. In addition, swabs (n= 46 for fish and n= 46 for fillet) were used to collect microbes present in the intestine, on the skin of intact fish and on the fish fillets. DNA was extracted from swab samples using QIAGEN DNeasy PowerSoil Pro Kits, followed by library preparation and sequencing.

Figure 1A shows that fillet samples, although grouped separately from other sample types, are closer to the centroid of environmental samples than fish samples, indicting that the processing environment has a larger impact on the microbial composition of final fillet products. Both season and facility have an influence on shaping the bacterial communities of environmental samples (Figures 1B-C). Samples collected from Facility 3 formed a distinct cluster compared with other two facilities (F1 and F2).

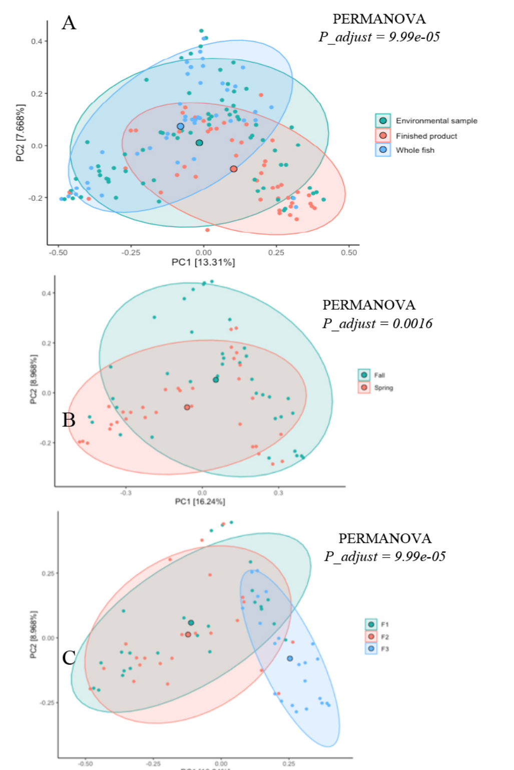

Figure 1. Principal Coordinates Analysis (PCoA) of A. all samples separated by their origins. B. environmental samples separated by season. C. environmental samples separated by processing facilities.

NAVIGATING CHALLENGES AND OPPORTUNITIES IN TILAPIA ENTERPRISE DEVELOPMENT: LESSONS FROM A FIJI AGRIBUSINESS PROJECT

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Tilapia aquaculture has existed in Fiji for over four decades, yet there remains a lack of market-oriented research and limited private sector-led action, towards a strongly supported agribusiness environment for tilapia enterprise development. Much effort has contributed to an advanced level of local technical knowledge and capacity. Industry development policies and donor initiatives have encouraged and incentivized prospective farmers to participate in tilapia aquaculture farming, only to see farmers eventually exit due to limited commercial viability. Much of the ongoing interest in tilapia is in response to rapidly declining marine resources, the need to transition towards more sustainably-sourced marine products, climate change impacts on traditional food systems, supplementing rural livelihoods, and improving local diet options. Tilapia remains an important species for food security, livelihoods and a priority for agribusiness enterprise development. Recently, the Pacific Agribusiness Research in Development Initiative (PARDI2) set out to strengthen the agribusiness environment for smallholder tilapia farmers by: 1) assisting in the establishment and development of the Tilapia Fiji Farmers Association (TFA), 2) completing a rapid assessment of the marine fish market to determine factors influencing tilapia marketing, and 3) holding live-fish market and food-service market demonstrations, with a number of café's, restaurants and resorts as buyers and TFA members as suppliers and coordinators. Key findings include: 1) most smallholder farmers produce without a market in mind; 2) tilapia remains an acceptable fish for consumers and retailers in a competitive market, but has greater potential provided quality, quantity, consistency of supply, convenience and professionalism is given more attention by producers; 3) various markets exist for tilapia and size preference is one way to differentiate them; 4) quality tilapia, consistent supply, good relationship management with chefs, innovative local cuisine development and effective marketing are some key strategies to see stronger presence of tilapia in the tourism sector; 5) building technical and enterprise support to farmer collectives can develop farmer confidence and 6) farmer collectives can play key roles in brokering stakeholder relationships and coordinating quality, consistent supply. Current agribusiness challenges remain, including: 1) lack of market-oriented mindset and actions; 2) lack of market data and its effective communication and 3) lack of production and training standards to strengthen product and market development. Opportunities that exist include: 1) the development of contextual incubator and accelerator programs for aquaculture farmers, with targeted and effective business mentoring, coaching and networking; 2) agribusiness research around farmer motivations, market opportunities, effective business models, support mechanisms for farmer collectives and 3) development of strategies towards collaborative environments for partnerships in industry strengthening and long-term sustainability.

ROE ENHANCEMENT AQUACULTURE: A SOLUTION TO CONTROLLING AN OVERABUNDANT URCHIN SPECIES IN SOUTHERN VICTORIA

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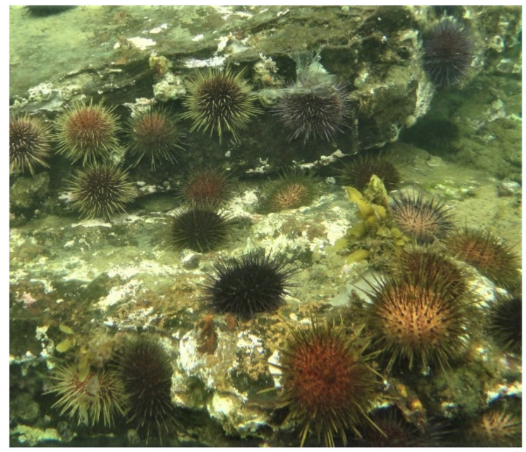
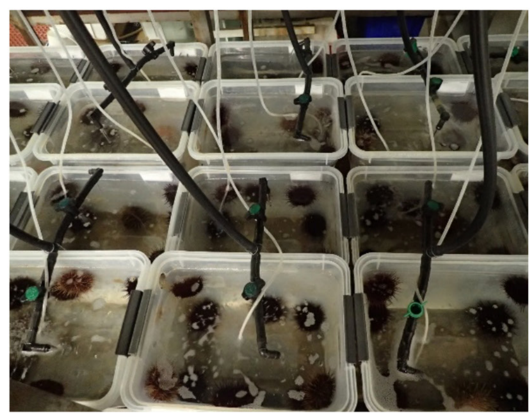
The transformation of macro-algal dominated rocky reefs into urchin barrens due to grazing pressure from overabundant sea urchins is a global phenomenon. Removing urchins from barrens by culling (smashing with hammers) can help re-establish algal cover, yet this practice is too costly for broad-scale application. Removal through harvesting urchins for their roe (gonad) by commercial fishers is also not financially feasible, as urchins in barrens are often malnourished, which results in unsalable, poor-quality roe. Creating an economic driver to remove urchins from barrens by making each urchin valuable through roe enhancement aquaculture, could be a better, more cost-effective solution.

Roe enhancement involves capturing urchins from the wild and feeding them with an urchin specific diet in an aquaculture setting to increase the quantity and quality of their roe. High quality urchin roe is a prized delicacy in the Asia-pacific region and here we demonstrate that the overabundant purple sea urchin (*Heliocidaris erythrogramma*) collected from barrens in Port Phillip Bay, Victoria, can be effectively roe enhanced in 12 weeks to create a marketable product. Furthermore, we show feed composition and culturing conditions can influence the quality and quantity of roe produced. If scaled up appropriately, the development of a roe enhancement aquaculture industry could be a cost-neutral management option for controlling overabundant purple sea urchin populations in Victoria.





DEVELOPMENT OF REMOTE METHODS FOR FACILITATING ENVIRONMENTAL BASELINES FOR AQUACULTURE

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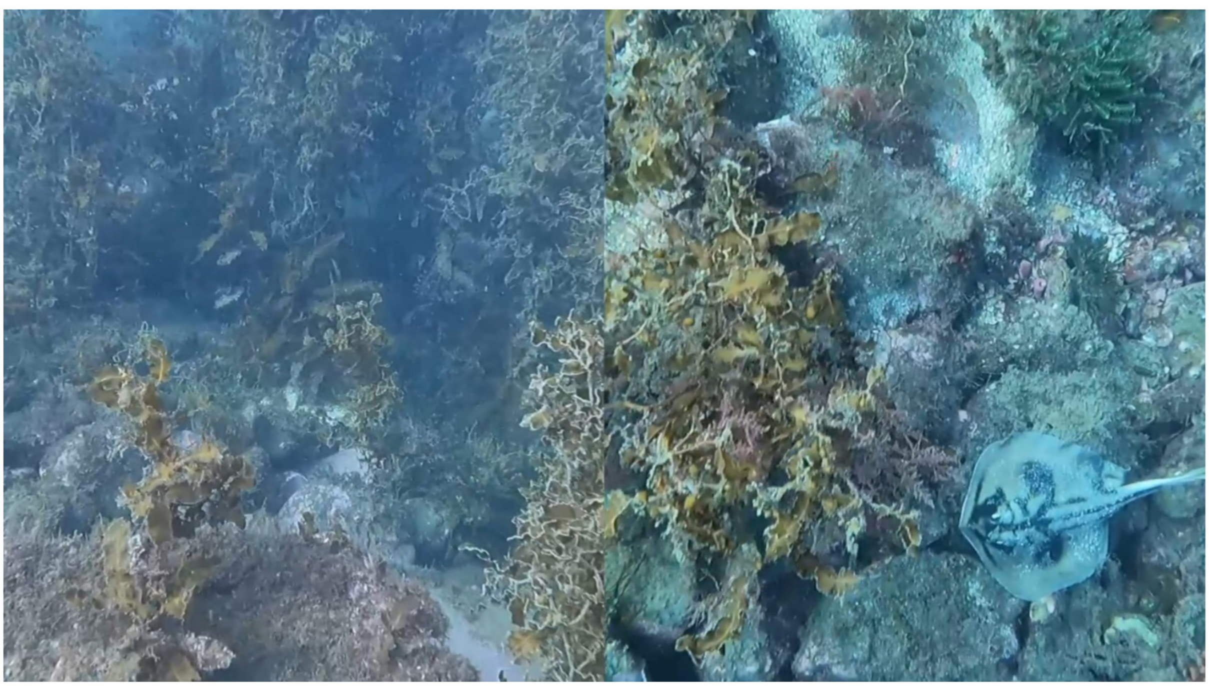
The public expectation around sustainable environmental management of established and emerging aquaculture industries has increased considerably over the past decade. In areas where aquaculture is intensive, or planned to be in the future, establishing robust baseline environmental conditions prior to any development is critical for assessing and attributing change, as well as ensuring operations are environmentally sustainable. However, what a fit-for-purpose baseline is will likely vary depending on the industry and the environmental risk in any given region. Adaptable and flexible environmental assessment methods are required for government to readily facilitate industry development. We have investigated the use of remote methods for collecting broadscale environmental information in prospective aquaculture areas. This includes towed camera systems and remotely operated vehicles (ROVs) equipped with spatially calibrated forward-facing and downward facing camera systems in the Mercury Passage, Tasmania, a region where multiple aquaculture industries are currently operating. We found that this system could collect data across multiple levels of ecological complexity and over a large spatial area. As technology advances into the future, methods such as these are likely to become integral, cost-effective components of environmental baselines.



INVERTEBRATE MARICULTURE MINI HATCHERY DESIGN AND DEVELOPMENT IN THE PACIFIC NATIONS

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The supply of invertebrate seed remains a constraint for the establishment of marine aquaculture in the Pacific Nations. While governments and partners have made significant investments in appropriate facilities and staff, productivity remains low. Without private sector development (for profit and jobs), in part caused by the lack of secure marine tenure arrangements in the Pacific Nations, the role of seed supply remains largely a public sector activity.

Communities are willing recipients of marine invertebrate seed (giant clams, sea cucumber (BDM) and oysters). But without any capital (their traditional tenures are often not recognized as a financial asset by the banking sector), this seed is mainly given without cost, making centralised public sector hatchery production unsustainable.

Developing larviculture strategies with live feed alternatives (freeze dried and microalgae pastes, greenwater microalgae cultures with copepod and artemia harvesting), along with solar powered seawater pumping solutions, might permit the absolute simplification of marine invertebrate larval techniques and facilities. This may allow their use in isolated communities in the Pacific Nations.

While capital remains a major constraint to initiating even a remote "mini" hatchery, the benefits of establishing community "marine gardens" for both improving nutrition and allowing the regeneration of invertebrate reef ecosystem components, may attract funding from a variety of sources. Operational costs may be manageable at the scale and technology proposed, although diets remain a major hurdle.

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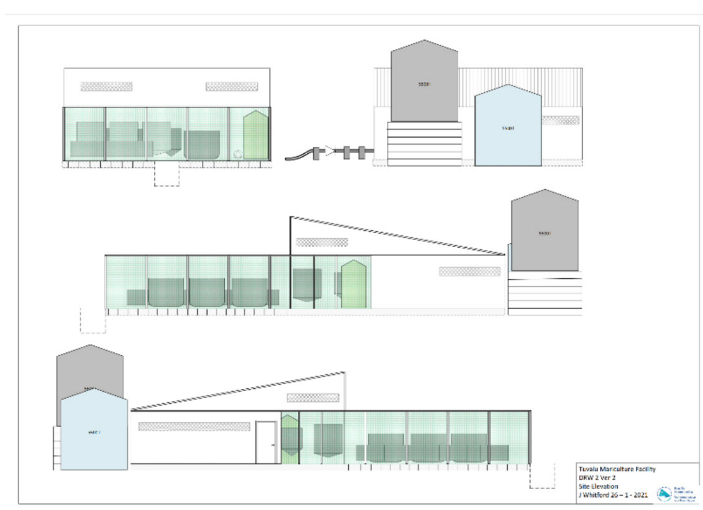

Figure 1 'Mini' Hatchery Concept Tuvalu

NEW SOUTH WALES AQUACULTURE: TRENDS, OPPORTUNITIES, AND STRENGTHS

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This presentation is about aquaculture in NSW. It will discuss the species cultivated, production outputs and trends, innovation in husbandry techniques, ecological sustainable development, regulation and policy, and growth trajectories.

NSW aquaculture contributed over $105 million in production value in 2022. This equates to a production and secondary sector output of $226 million, added value of $134 million, and $69.3 million household income. 1758 full time jobs were created in NSW.

The unique combination of temperate and sub-tropical coastal waters, and inland freshwater environments support a diverse range of seafood. Key species farmed include Sydney Rock Oysters, Black Tiger Prawns, Murray Cod, Trout, Silver Perch and Barramundi. Development of an algae industry is also on the horizon to meet growing demands for algae products.

By economic value, oyster production is the main aquaculture industry in NSW worth $55 million in 2022. Oyster aquaculture is undertaken in relatively shallow, protected estuaries across the entire coastline. This unique landscape supports thriving populations of the iconic endemic Sydney Rock Oyster, Saccostrea glomerata, only found in temperate Australian waters. Approved in 33 estuaries, there are currently over 3500 hectares of established oyster leases.

Marine water aquaculture is an emerging industry undertaken offshore to 3 nautical miles. There are currently 160ha of established marine leases in the state. Mussel production is the predominant marine waters species, while finfish and algae are emerging to meet strong demand, backed by research and development.

Existing oyster and marine lease areas have been determined in accordance with statutory planning and environmental impact assessments. They provide an investment ready platform for potential aquaculture businesses, generally acquired through public tender.

Land based aquaculture includes marine and freshwater species. Over the past five years research and development in hatchery production systems and grow-out techniques has supported significant growth in production of Black Tiger Prawns and Murray Cod. From 2018 – 2022 prawn value increased from about $3.5 million to $13.7 million, while Murray Cod jumped from $6 million to $14.8 million.

Oyster, marine and land base leases, and aquaculture permits are all administered by NSW DPI under Industry Sustainable Aquaculture Strategies. These strategies outline statutory planning policy and best practice management standards to support the sustainable growth of these industries.

SUCCESSFUL HATCHERY SETTLEMENT OF BLACKLIP ROCK OYSTER *Saccostrea echinata* SINGLE SEED SPAT, WITHOUT THE USE OF EPINEPHRINE

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The Blacklip Rock Oyster (BRO) *Saccostrea echinata* has demonstrated great potential for aquaculture across tropical Australia. This has resulted in considerable interest in farming BROs and the successful development of species-specific hatchery production processes. Now that BRO larvae can be consistently and reliably produced, the largest remaining bottleneck to large-scale spat production is the very poor rate of BRO settlement when using epinephrine as the settlement trigger.

The use of epinephrine to induce settlement is now the standard practice in Australian oyster hatcheries and, with Sydney Rock Oysters, this results in highly successful, efficient, and effective process. Unfortunately, this has not been the case for BRO larvae and their response to epinephrine is so poor that each batch of larvae must be subjected to multiple epinephrine treatments. Even after years of dedicated BRO hatchery work and ongoing refinements to the epinephrine induced settlement process, settlement rates remain low – always below 20%. It is probable that repeated exposure to epinephrine causes considerable stress to the successfully settled spat and this could reduce their general health and robustness, making the spat more prone to post settlement mortality, and further reducing spat output.

Recent trials at the Bribie Island Research Centre, using black builder's plastic as a settlement substrate, have achieved proof of concept production of robust, epinephrine-free, single seed, BRO spat. This new approach to BRO settlement now provides a viable alternative option to using epinephrine. Although refinements are still being made to the design and configuration of the plastic settlement structures, the process has proven to be simple, efficient, and successful. The details of this settlement process are presented and discussed. Further trials will be undertaken to compare and quantify the rates of settlement, and post settlement performance, of spat settled with and without epinephrine.

SKIM-SEQUENCING BASED GENOTYPING REVEALS GENETIC DIVERGENCE OF THE WILD AND DOMESTICATED BROODSTOCK POPULATIONS OF BLACK TIGER SHRIMP *Penaeus monodon* IN THE INDO-PACIFIC REGION

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The domestication of a wild-caught aquatic animal is an evolutionary process, which results in genetic discrimination at the genomic level in response to strong artificial selection. Although black tiger shrimp (*Penaeus monodon*) is one of the most commercially important aquaculture species, a systematic assessment of genetic divergence and structure of wild-caught and domesticated broodstock populations of the species is yet to be documented. In the present study, we used a combined approaches of skim sequencing (SkimSeq) based genotyping and gut metagenome analyses to investigate the genetic structure of broodstock individuals of *P. monodon* species, collected from five sampling sites across their distribution in Indo-Pacific regions. The wild-caught *P. monodon* broodstock population were collected from Malaysia (MS) and Japan (MJ), while domesticated broodstock populations were collected from Madagascar (MMD), Hawaii, HI, USA (MMO), and Thailand (MT). For the SkimSeq analysis, a total of 194,259 single nucleotide polymorphism (SNP) loci were identified, in which 4, 983 SNP loci were identified as putatively adaptive by the pcadapt approach. In both datasets, pairwise F_{ST} estimates high genetic divergence between wild and domesticated broodstock populations. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses suggested that non-synonymous mutated genes might be associated with the energy production, metabolic functions, respiration regulation and developmental rates, which likely act to promote adaptation to the strong artificial selection during the domestication process. Our metagenome results identified considerable variation in the gut microbiomes of wild and cultured shrimps, presumably reflecting differences in diet, host genetic divergence, and host-bacteria co-evolution. LEfSe analysis detected significant enrichment of beneficial/opportunistic pathogens from the gut microbiome of each host population, suggesting possible biomarkers for host health monitoring. Consistently, different spatial clustering analyses in both datasets categorized divergent genetic structure into two clusters: (1) wild-caught populations (MS and MJ), and (2) domesticated populations (MMD, MMO and MT). While wild shrimp broodstocks had higher gut microbial diversity, a pronounced divergence of core microbiota was found in both wild and cultured shrimp populations with altered community structure and predicted metabolic functions. Our results provide insights into the deterministic factors contributing to the interpopulation variation in the *P. monodon* microbiome, highlighting potential future research directions in areas such as host-bacteria co-evolution and holobiome, which may enable the assessment of host-species genomic divergence across environmental gradients.

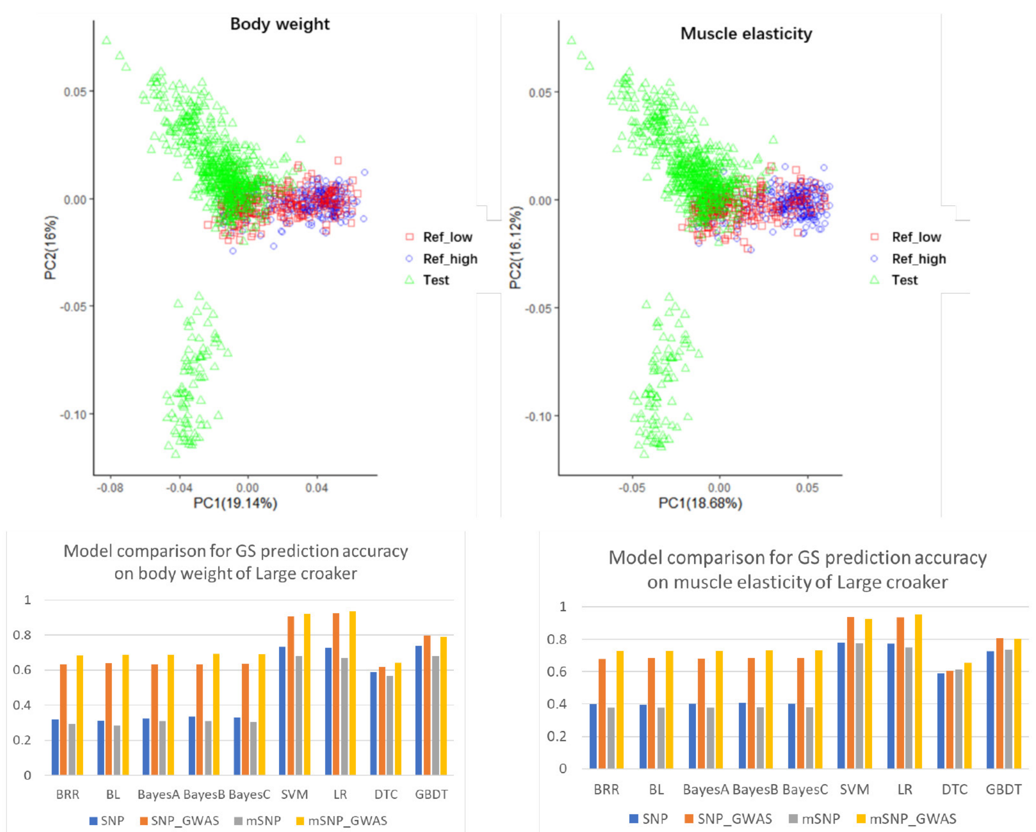
COMPARISON OF GENOMIC SELECTION MODELS ON FISH TRAIT PREDICTION ACCURACY

Jian Xu*, Jialu Fang, Shangqi Li

Background: Genomic selection (GS) is widely used in breeding for aquaculture species, and has greatly elevated the breeding efficiency. Various models for genomic selection have been applied in aquaculture research, including GBLUP, BayesA, BayesB, BayesCπ, Bayes Lasso, and several machine learning models. Optimal combinations of models and genetic variants were key to accurate prediction in breeding.

Materials and methods: In this study, the main GS models were evaluated in two traits of large yellow croaker (*Larimichthys crocea*), combining with different SNP datasets. Totally 534 samples were used as reference population for body weight trait analysis, 522 samples were used as reference population for muscle elasticity trait analysis. Moreover, 716 samples with only genotyping data were used as candidate population. All genotyping data were acquired from two resources: SNP data from probe hybridation and mSNP data from targeted sequencing.

Results: Principal component analyses were conducted on all reference and candidate populations, indicating close genetic background among different populations. GWAS for both traits were conducted considering the population structure, and associated SNPs were used as datasets as well as whole SNPs. SVM and Logistic regression model showed higher prediction accuracies than other models using mSNP-GWAS dataset, indicating the prospective of machine learning models in fish genomic breeding.



EFFICACY OF PHYTOCEE IN VIT-C PHOSPHATE REDUCTION DIETS ON THE PERFORMANCE OF WHITE SHRIMP, *Penaeus vannamei*, UNDER DENSITY STRESS CONDITION, AND DISEASE CHALLENGE AGAINST *Vibrio parahaemolyticus*

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Stress is a common factor among all animals and aquatic species which were suffer from wide variety of stresses especially management stress, nutritional stress, environmental stress. The present study was aimed to evaluate the efficacy of phytocee in Vit-C phosphate(C-PP) reduction diets on the performance of white shrimp under density stress condition and disease challenge against Vibriosis AHPND. Phytocee is phytogenic feed additive as adaptogen to mitigate and reduce stress in animals. Both phytocee and Vit-C phosphate are considered to have function on health and growth of shrimp reared in high density including immune response after pathogen challenge test.

An experiment was conducted based on a CRD, with 5 treatments and 5 replicates each. All 5 diets were supplemented different level of C-PP in combination with 0.1% Phytocee applied to selected groups as Table 1.

Shrimp with initial mean weight of 1.4 g/shrimp were randomly distributed into 240L glass aquarium (Total 25 aquariums) that contained 120L of 15 ppt seawater. Stocked 30 shrimp per aquarium (250 pcs/m^3). During 8 weeks of feeding trial, shrimp were fed experimental diets 3-5%BW 4 times a day and weighted on week 0, 4 and 8 for determined growth performance. Haemolymph samples were collected to study their immune response both under density stress condition and after challenge test by bath treatment against 6.2X10^5 CFU/ml of *Vibrio parahaemolyticus* AHPND for 12 days, mortality was recorded.

The results showed that the shrimp fed diets 0.1% phytocee in T4 and T5 had significantly higher ($p<0.05$) total production per aquarium. Moreover, shrimp fed diets 0.1% phytocee in T3, T4 and T5 had significantly higher ($p<0.05$) final weight, weight gain, average daily growth (ADG), specific growth rate (%) and better feed conversion ratio (FCR). The immune response of shrimp as total haemocyte count, hemolymph protein and phenoloxidase activity (PO) were not significantly differences ($p>0.05$). After AHPND challenging, the results found that, phenoloxidase activity of shrimp fed diets 0.1% phytocee in T3, T4 and T5 had significantly higher ($p<0.05$) than T1 and T2. The survival rate after AHPND challenging was not significantly differences ($p>0.05$). In conclusion, 0.1% phytocee showed the benefit on reducing C-PP in shrimp diets by 250-500 g/ton(25-50% reduction) without any adverse effects on growth and immune response under density stress condition and Vibriosis challenge test.

TABLE 1. Diet description of shrimp feed formula

Treatment	Diet Description
1	0.1% Phytocee without C-PP
2	0.1% Phytocee + 250 g C-PP/ton
3	0.1% Phytocee + 500 g C-PP/ton
4	0.1% Phytocee + 750 g C-PP/ton
5	0.1% Phytocee + 1000 g C-PP/ton

Figure 1. Performance of Shrimp

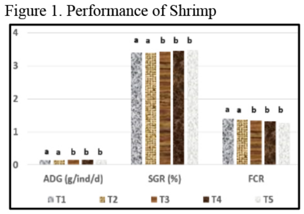


Figure 2. PO before and after challenge

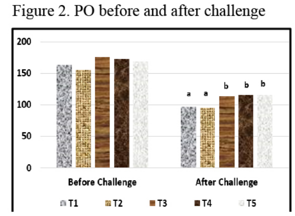
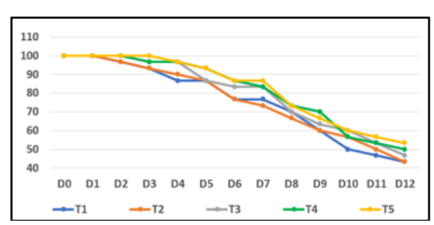


FIGURE 3. Survival of shrimp after AHPND challenging



ENERGY FORTIFICATION STRATEGY: EFFECT OF DIETARY OLEIC ACID SUPPLEMENTATION ON ADIPOGENESIS AND ENERGY DEPOSITION IN ATLANTIC SALMON *Salmo salar L.*

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Atlantic salmon are the most economically important aquaculture species in Australia. Due to climate change, water temperatures in summer exceed the upper thermal tolerance limits of this species, leading to energy loss and a slower growth. Dietary lipid, particularly triglyceride (TAG), is the main energy source for Atlantic salmon, with viscera, muscle, and whole-body representing the key reservoirs for energy storage. High inclusion of vegetable oil (VO) in formulated feeds can lead to adiposity of viscera in Atlantic salmon. This may be at least in-part due to the high oleic acid (OLA; 18:1n-9) concentrations of some vegetable oils. Thus, our experiment tested the effect of high energy and high OA supplementation in a 2x2 factorial design on the growth performance and energy deposition of juvenile Atlantic salmon. This experiment investigates the potential for OLA as a functional ingredient to achieve energy fortification prior to summer.

An 18-week feeding trial was conducted in a freshwater recirculating aquaculture system (RAS). Juvenile Atlantic salmon initially weighed 71.9 g and 14 fish were stocked to each of the experimental tanks. The RAS was maintained under a 12 h light:12 h dark light cycle at 15 □ throughout and fish were fed twice a day according to the feed ration. The experimental diets were randomly allocated to three replicate tanks and consisted of i) a commercially relevant control diet, ii) a high oleic acid diet (HL), iii) a high lipid diet and iv) a high lipid diet with added high oleic acid sunflower oil (HLHO) providing four levels of dietary OA.

The results demonstrate that Atlantic salmon fed the HL and HLHO had significantly higher fillet yield compared to the control group despite no difference in growth performance parameters between treatments. Adipose tissue index showed an increasing trend with increasing OLA included in the diet, which, at least in part can be explained by an increase in the total lipid content of the adipose tissue. Atlantic salmon fed the HO diet had the highest lipid deposition in the adipose tissue and with the highest lipid apparent digestibility coefficient (ADC). Lipid class and fatty acid data will be presented and further explain lipid metabolism in the adipose tissue towards the identification of novel feeding strategies for summer welfare.

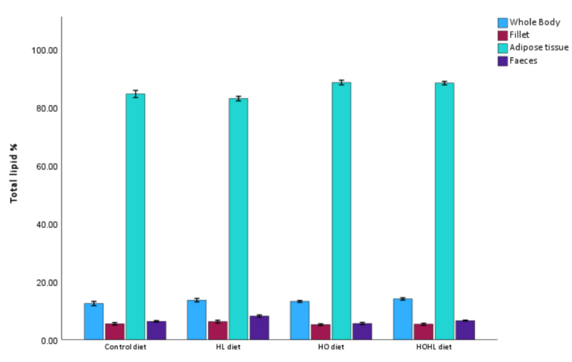

Figure 1 Total lipid content in three tissues and whole-body stores of Atlantic salmon fed with different inclusion level of OLA diets.

APPLIED WELFARE IN FINFISH AQUACULTURE

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The welfare of farmed aquatic animals continues to hold the spotlight after more than a decade of intensified research and operational changes in this area of aquaculture. Recently humane slaughter has become a focus with a need to understand insensibility indicators for aquaculture species to make an informed decision on the effectiveness of rendering the animal insensible before slaughter. This presentation will relay recent research into insensibility indicators for new species and recent trials on humane slaughter using an electro-stunning approach, quality improvements will also be discussed. Additionally, there will be some discussion on new and upcoming welfare requirements driven by certification schemes and some technology that may assist in monitoring of welfare at a population and individual level.

EFFECTS OF FISH MEAL REPLACEMENT ON GROWTH PERFORMANCE AND STRESS AND IMMUNE-RESPONSES OF OLIVE FLOUNDER (*Paralichthys olivaceus*) UNDER CHRONIC AND ACUTE TEMPERATURE STRESS

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Recently, global warming attributed to climate change is causing rapid environmental changes including high fluctuation in water temperature, resulting in mass mortality and disease outbreaks in aquaculture practices. Fish meal production is predicted to decrease over time due to reduction in capture production of fishes for use of fish meal in association with climate change. Regardless the fact that fish meal is an important ingredient for aquafeed, there is no choice but to reduce fish meal content in aquafeed for sustainable aquaculture. Therefore. The current study was conducted to understand what relationship between manipulation of fish meal content and physiological responses under chronic and acute temperature stress exists in juvenile olive flounder (*Paralichthys olivaceus*), a commercially important aquaculture fish species in Asia.

Four hundred eighty juveniles averaging 13.6±0.02g (mean±SEM) were randomly distributed into each of the 24 rectangular tanks (20 fish per tank). Each set of 12 tanks was assigned to either constant water temperature group reared at 19.5˚C(named as non-stressed group: NS) or gradually increased water temperature group reared at from 19.5˚C to 30˚C (1.5˚C increment/week) (named as thermal-stressed group: TS) throughout the trial. Each NS and TS group consisted of four fish meal replacement treatments including 60, 40, 20, 0% fish meal content in diet (2 × 4 factorial arrangement; $N = 3$ per treatment). Following the 8-week trial, fish from the NS group were exposed to acute thermal stress (2-h heat shock at 30˚C and 2-h recovery at 19.5˚C). Growth performance and stress- and immune-related measurements for stressed and non-stressed juveniles were investigated.

Results from the chronic exposure experiment showed that the TS group had significantly lower final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed efficiency (FE), condition factor, and viscerosomatic index than the NS group ($P < 0.05$). There was also a significant main effect of the fish meal replacement in both NS and TS groups showing that FBW, WG, SGR, and FE generally decreased with increasing fish meal replacement level. A significant temperature stress main effect on plasma cortisol, glucose, triglyceride, total protein, and glutamic oxaloacetic transaminase levels was observed. A significant fish meal replacement main effect on plasma total cholesterol, triglyceride, and glutathione peroxidase levels was found.

Results from the acute exposure experiment show that the overall pattern of changes in the plasmatic enzymes and metabolites were, to some extent, incomparable to those from the chronic exposure experiment.

Expression levels of genes, involved in temperature stress responses in various tissues will be discussed later.

RED TILAPIA FARMING UNDER HIGH SALINITY CONDITIONS

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This study estimated the survival, growth, and fry production of red tilapia (*Oreochromis urolepis hornorum* ♀ **× *Oreochromis*** *mossambicus* ♂) broodstock in outdoors farming conditions at various salinity levels (1‰, 25‰, 33‰, 39‰, and 42‰) at 25.3°C–39.0°C. The experiments were conducted in triplicates in 2020 using 300 broodstock per group. The survival rate, growth performance, and fry production were monitored. Our results showed that red tilapia fry production and grow-out farming were feasible under high salinity conditions; however, the various levels of salinity condition exhibited significant differences in terms of survival rate, growth performance, fry production. The results suggest that red tilapia broodstock was maintained under salinity as high as 33‰ without impairing fry production; above 33‰ was feasible for the grow-out stage, implying that the fry production in euryhaline tilapia farming would be practical in regions where freshwater acclimation is limited.

EFFECTS OF HIGH SALINITY ON GROWTH EUROPEAN SEA BASS *Dicentrarchus labrax*

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Diversifying aquaculture in Saudi Arabia with the inclusion of finfish species already reared in the Mediterranean Sea requires a sufficient understanding of the preferences and limits of cultured fish under local conditions. The trials tested four locally available commercial feeds for 84 and 80 days to determine the growth performance of fish in local high salinity 42-45‰ conditions. The average final weight was not significantly different among treatments. The feed conversion ratio was similar in all diets. The average final weight in fish showed no significant differences in final body weight, weight gain, or feed intake. The growth results appear to be in line with related research. Improvement of nutritional characteristics of the feed is expected to improve the performance of the fish.

GENERATION OF FAST GROWTH NILE TILAPIA (*Oreochromis niloticus*) BY MYOSTATIN GENE MUTATION

Linyan Zhou, You Wu

Background:

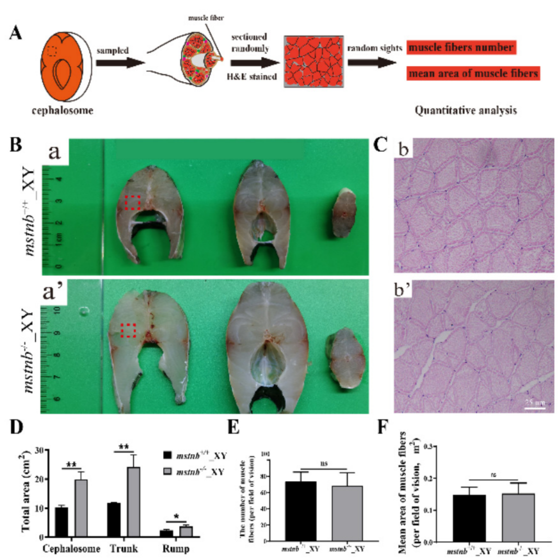
Tilapia is the second most prolific species grown in aquaculture after carp, and is widely grown in more than 100 countries. Myostatin (MSTN) has been proved to be a negative regulator of skeletal muscle growth. Mutation of MSTN gene resulted in significant increase in both body size and muscle mass in vertebrates, mostly with a species-specific effect.

Materials and methods:

In this study, the qRT-PCR, *FISH* and IF were used for gene expression detection and cellular localization. The *mstnb* homozygous mutants were obtained by CRISPR/Cas 9 gene editing technology. The feeding trial was carried out to investigate the growth performance of *mstnb*$^{-/-}$ tilapia and wild-type (WT) tilapia at 5 and 7 months after hatching (mah). The relative quantitative of muscle fibers was analyzed by image J software.

Results:

Mstnb is abundant in the basement membrane of skeletal muscle, while *mstna* is dominantly expressed in the brain. Compared with the WT fish, the *mstnb*$^{-/-}$ mutants showed a typical double-muscle phenotype with increased muscle mass, sticking out between head and dorsal fin, from 5 mah. Morphological observation revealed an excessive proliferation of white muscle fibers at 7 mah. The average body weights, body heights and body widths of the *mstnb*$^{-/-}$ fish were 49.45%, 32.74 % and 37.21% higher than those of the *mstnb*$^{+/+}$ fish at 5 mah. The growth performance parameters of the *mstnb*$^{-/-}$ fish, including weight gain rate (1.99 times), condition factor (1.77 times) and specific growth rate (1.23 times), were significantly higher than the *mstnb*$^{+/+}$ fish under laboratory conditions at 5 mah. In addition, the parameters of WGR, CF, SGR and FE were significantly increased in *mstnb*$^{-/-}$ Nile tilapia than those of *mstnb*$^{+/+}$ Nile tilapia and GIFT tilapia for a 90-day feeding trial under the wild natural environment.



THE ACTIVATION OF THE GROWTH-IGF1 AXIS, BUT NOT APPETITE, IS RELATED TO HIGH GROWTH PERFORMANCE OF THE MALABAR GROUPER *Epinephelus malabaricus* JUVENILES UNDER ISOSMOTIC CONDITIONS

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Salinity refers to the concentration of dissolved salt within water which is a determining factor to influence fish growth. This study aimed to evaluate the effect of selected salinity on the osmoregulation and the growth performance of the juvenile Malabar grouper *Epinephelus malabaricus,* a high commercial value species in Asian market, and to determine appropriate salinity for maximizing its production. Fish were reared under conditions of temperature at 26^{o}C and photoperiod (LD = 14:10) with different salinities (5, 11, 22, and 34 ‰) for 8 weeks. Salinity had little impact on the plasma Na^+ concentration, although the transcript levels of *Na□/K□-ATPaseα* in the gill was significantly lower in fish reared at 11 ‰. Low oxygen consumption was seen in fish reared at 11‰. Feed conversion ratio (FCR) was lower in fish at 5 and 11‰ than at 22 and 34‰. However, specific growth rate (SGR) was higher in fish at 11‰. These results suggest that rearing fish at 11 ‰ could decrease energy consumption in respiration and improve food conversion efficiency. When fish were reared at 11 ‰, the transcript levels of growth hormone (*GH*) in the pituitary and its receptor (*GHR*) and insulin-like growth factor I (*IGF-I*) in the liver were upregulated, suggesting that the growth axis is stimulated in low salinity conditions. On the other hand, there was little difference in the transcript levels of neuropeptide Y (*NPY*) and pro-opimelanocortin (*POMC*) in the brain of fish reared at any salinities, suggesting that salinity had low impact on alternation of appetite. It is concluded that high growth performance is obtained when fish are reared at 11‰ by activating the GH-IGF system, but not the appetite system, of the Malabar grouper juveniles.

BIOACCUMULATION AND HEALTH RISK ASSESSMENT OF TOXIC ELEMENTS IN MUSCLES OF DIFFERENT FISH SPECIES FROM DIFFERENT AREAS OF ASIA

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Freshwater bodies are being contaminated by heavy metal pollution. The major source of contamination arise from human activities such as industrial development, agricultural practices and combustion of fossil fuel that release various hazardous heavy metals causing deleterious impacts on human and aquatic life. This review paper highlights the contamination of muscles of three commercially important fishes (*Catla catla, Cyprinus carpio, Ctenopharyngodon idella*) of carp family with heavy metals such as Zn, Pb, Hg, Cu and Cr. The statistically significant differences ($P>0.05$) among values. Heavy metals concentration in fish muscles were assessed and their relationship with the fish habitat was explored.it was observed that continuous metal discharge into fish habitat leads to their bioaccumulation in various fish tissues. It was also found that the parts of freshwater which are present near the industrial areas are highly contaminated and they impose potential fish and human health risks. The occurrence of heavy metals in selected specie generally ranked in order Zn > Cu > Cr > Pb > Hg. The presence of heavy metals also causes several social, environmental and health issues. Therefore the wastewater must be treated before discharge into freshwater bodies to decrease the hazardous effects of heavy metals on aquatic life and human beings. This review article aims to study concentration of level of fish muscles concerning heavy metals.

Carp species due to easy access to breeding, food efficiency and rapid growth could be the best choice for aquaculture over worldwide (Tokur *et al.,* 2006).

Catla catla is a surface feeder mostly feed on zooplankton, small insects, phytoplankton and small crustaceans (Saleem *et al.,* 2022). *C.idella* commonly known as grass carp is an edible freshwater fish of Asia (Aslam *et al.,* 2022). *Cyprinus carpio* (common carp) habitat is usually weedy areas with muddy bottom. They feed on zooplankton like copepods, rotifers.

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Table 1: Bioaccumulation of heavy metals in *Catla catla*

Heavy Metals	**Concentration (ppm)**	**References**
Pb	4.09	(Saleem *et al.*, 2022) (Table 2)
Pb	3.51	(Dutta *et al.*, 2022) (Table 4)
Pb	0.57	(Hossain *et al.*, 2022) (Table 2)
Cr	1.34	(Dutta *et al.*, 2022) (Table 4)
Cr	1.05	(Saleem *et al.*, 2022) (Table 2)
Cr	BDL	(Hossain *et al.*, 2022) (Table 2)
Cu	33.92	(Hossain *et al.*, 2022) (Table 2)
Cu	1.57	(Saleem *et al.*, 2022) (Table 2)
Cu	-	(Dutta *et al.*, 2022) (Table 4)
Zn	110	(Saleem *et al.*, 2022) (Table 2)
Zn	105.05	(Hossain *et al.*, 2022) (Table 2)
Zn	-	(Dutta *et al.*, 2022) (Table 4)
Hg	2.58	(Hossain *et al.*, 2022) (Table 2)
Hg	0.73	(Dutta *et al.*, 2022) (Table 3)
Hg	0.33	(Saleem *et al.*, 2022) (Table 2)

Mean , median and standard deviation of heavy metals in *Catla catla*

Heavy Metals	**Mean**	**Median**	**SD**
Pb	2.723333	3.51	1.887256
Cr	1.195	1.195	0.705006
Cu	17.745	17.745	19.1466
Zn	107.525	107.525	62.1289
Hg	1.213333	0.73	1.200347

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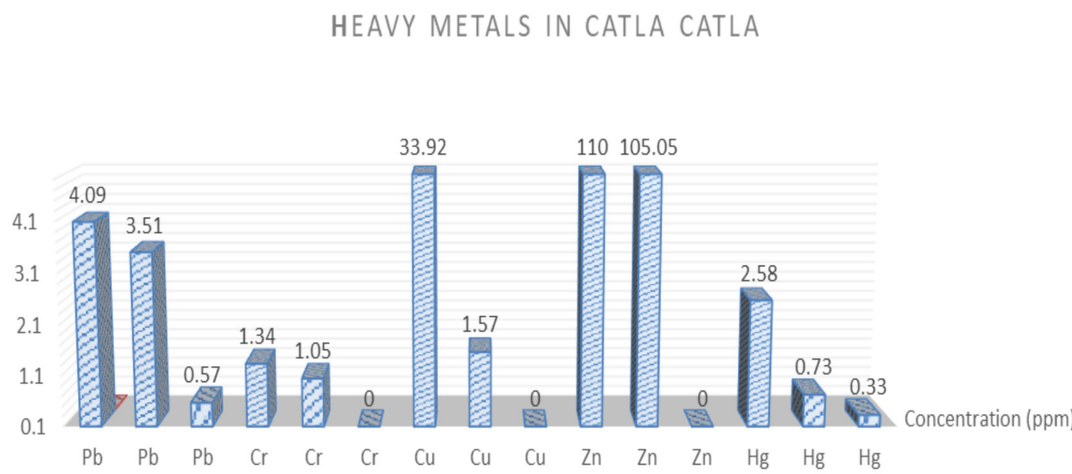

Figure 1: Concentration of heavy metals (ppm) in muscles of *Catla catla*

Table 2: Bioaccumulation of Heavy metals in Grass Carp

Heavy Metals	Concentration (ppm)	References
Pb	14.33	(Akter *et al*., 2020) (Table 3)
Pb	0.53	(Hossain *et al*., 2022) (Table 2)
Pb	0.13	(Ali *et al*., 2022) (Table 2)
Cr	13.23	(Akter *et al*., 2020) (Table 3)
Cr	7.69	(Hossain *et al*., 2022) (Table 2)
Cr	-	(Ali *et al*., 2022) (Table 2)
Cu	48.59	(Hossain *et al*., 2022) (Table 2)
Cu	9.86	(Akter *et al*., 2020) (Table 3)
Cu	-	(Ali *et al*., 2022) (Table 2)
Zn	116.82	(Hossain *et al*., 2022) (Table 2)
Zn	28.98	(Akter *et al*., 2020) (Table 3)
Zn	3.13	(Ali *et al*., 2022) (Table 2)
Hg	1.38	(Hossain *et al*., 2022) (Table 2)
Hg	0.017	(Ali *et al*., 2022) (Table 2)
Hg	-	(Akter *et al*., 2020) (Table 3)

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Mean, Median and standard deviation of Heavy metals in Grass carp

Heavy Metals	**Mean**	**Median**	**SD**
Pb	4.996667	0.53	8.085378
Cr	10.46	10.46	6.644052
Cu	29.225	29.225	25.68469
Zn	49.64333	28.98	59.59517
Hg	0.6985	0.6985	0.791882

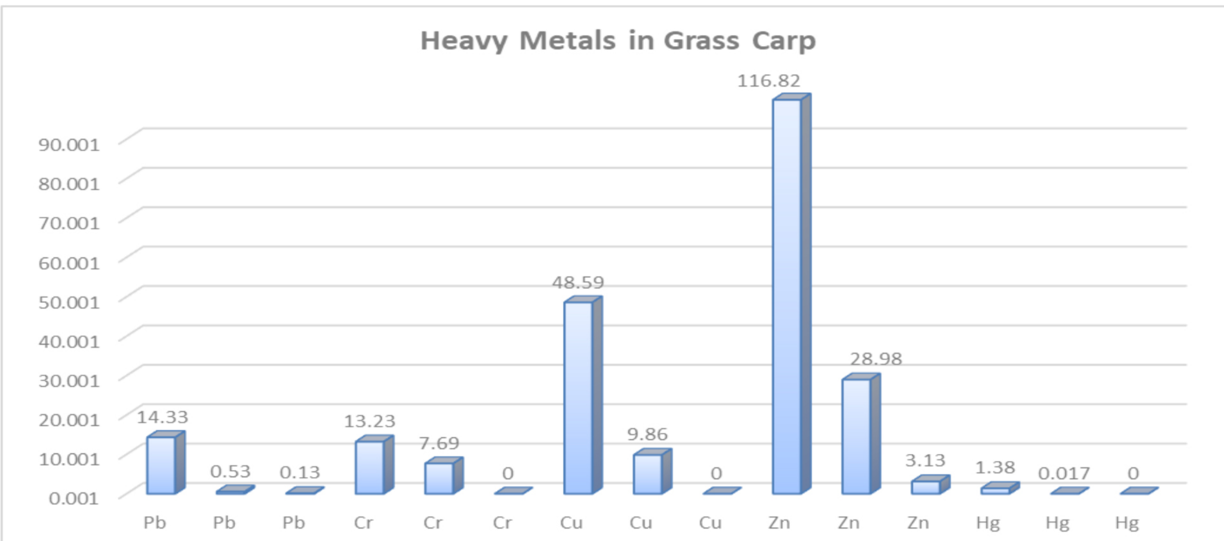

Figure 2: Concentration of heavy metals (ppm) in muscles of *C. Idella*

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Table 3: Bioaccumulation of Heavy metals in Common carp

Heavy Metals	**Concentration (ppm)**	**References**
Pb	81.9	(Zolfaghari 2018) (Table 1)
Pb	0.1	(Ali *et al*., 2022) (Table 2)
Pb	0.009	(Hao *et al*., 2022) (Table 2)
Cr	203.7	(Mansouri *et al*., 2017) (Table 1)
Cr	0.836	(Hao *et al*., 2022) (Table 2)
Cr	-	(Ali *et al*., 2022) (Table 2)
Cu	441	(Mansouri *et al*., 2017) (Table 1)
Cu	0.673	(Hao *et al*., 2022) (Table 2)
Cu	-	(Ali *et al*., 2022) (Table 2)
Zn	766	(Mansouri *et al*., 2017) (Table 1)
Zn	33.103	(Hao *et al*., 2022) (Table 2)
Zn	3.11	(Ali *et al*., 2022) (Table 2)
Hg	58.5	(Zolfaghari 2018) (Table 1)
Hg	0.011	(Hao *et al*., 2022) (Table 2)
Hg	0.002	(Ali *et al*., 2022) (Table 2)

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Mean, Median and standard deviation of Heavy metals in Common carp

Heavy Metals	**Mean**	**Median**	**SD**
Pb	27.33633333	0.1	47.25354
Cr	102.268	102.268	117.3657
Cu	220.8365	220.8365	254.4174
Zn	267.4043333	33.103	432.0569
Hg	19.50433333	0.011	33.77124

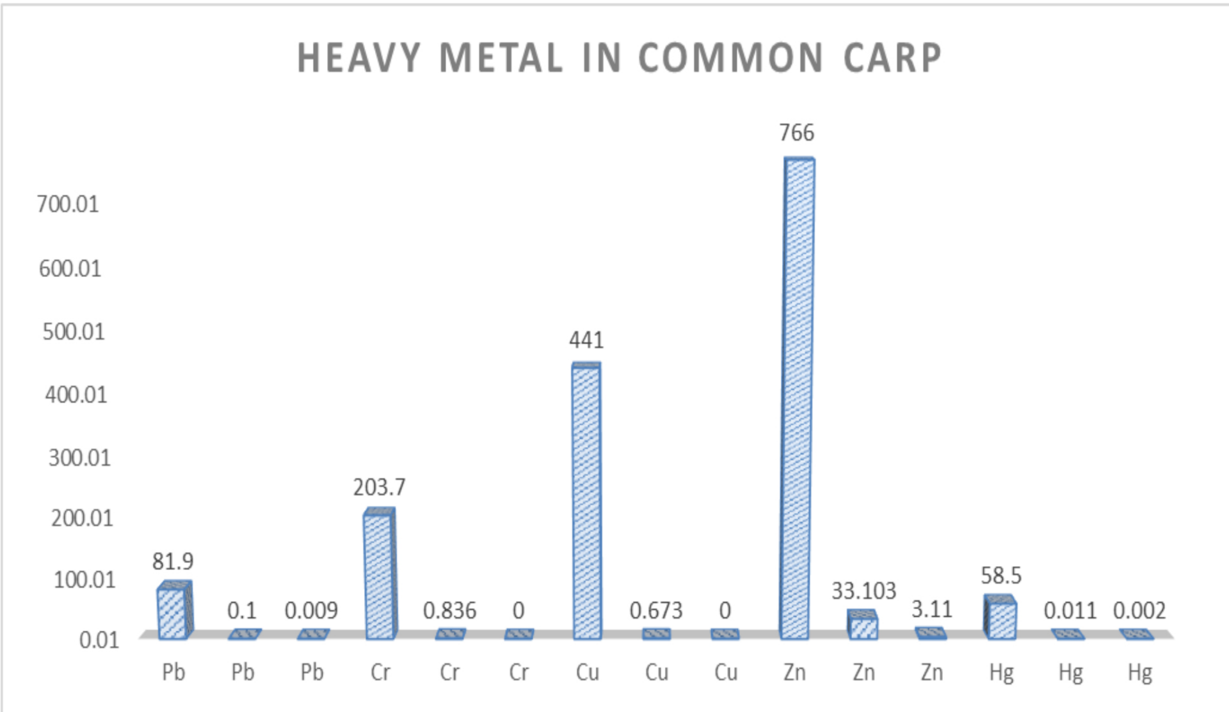

Figure 3: Concentration of heavy metals (ppm) in muscles of *C. carpio*

ADDENDUM

FOOD FOR THOUGHT: A CIRCULAR PRODUCTION MODEL TO DRIVE SUSTAINABLE AQUACULTURE

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Aquaculture will be vital in sustainably meeting global food and nutrition demands as our population grows. A changing climate has reduced the availability of common feed ingredients and introduces environmental challenges (e.g. increasing temperatures) for fish, which constrains future industry growth. With more food and more people comes an increase in waste production and growing concerns about managing challenging and volatile fisheries waste streams. Bio-converting fisheries wastes into a range of valuable products via insect farming offers a tool to develop a circular aquaculture production model. Black soldier fly farming can provide a novel solution for growing fisheries wastes and the resulting larvae provide valuable ingredients for aquafeeds.

There has been a large body of work done assessing the use of black soldier fly in diets for rainbow trout (*Oncorhynchus mykiss)*. Despite the large body of work, there is no definitive answer as to the optimal use or inclusion limits for commercial production conditions. A literature review (*Chapter 2*) highlighted four key knowledge gaps which pose barriers for industry acceptance of BSF in aqua feeds: 1) trial duration, 2) fish life stages, 3) BSF meal quality and 4) the role of chitin. These knowledge gaps provided the main objectives for this thesis and were addressed by evaluating the growth performance and nutritional quality of BSF larvae and evaluating the growth performance, health and quality of rainbow trout fed BSF diets across the juvenile, adult and brood stock life stages. Black soldier fly provide a viable and highly efficient solution for growing fisheries waste streams, with the benefit of improving BSF growth which increases product yield for downstream feed use. Nevertheless, long-term feeding of BSF through all trout life stages did reduce fish growth performance. Differences in growth performance were driven by BSF quality and fishmeal replacement levels. Supplementing a BSF diet with amino acids increased fish growth performance to levels similar to a fishmeal-based control diet. Furthermore, this is the first study to directly assess the role of chitin in rainbow trout diets. The presence of chitin in the diets was not responsible for differences in the growth performance of fish fed BSF. The results suggest that precise analysis of BSF meal quality before dietary formulations will allow for its successful inclusion in rainbow trout feeds. This thesis shows that BSF provides a viable pathway to transition towards a circular production model for more sustainable rainbow trout aquaculture.