



EVOLUTION, DEVELOPMENT AND ECOLOGY OF ANEMONEFISHES

Model Organisms for Marine Science

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15 Parental Care

Patterns, Proximate and Ultimate Causes, and Consequences

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15.1 INTRODUCTION

Parents can go to incredible lengths to improve the survival of their offspring (Royle et al. 2014). Yet, care is often costly and given at the expense of other activities, such as foraging or territory defence, resulting in stark tradeoffs (Stearns 1989). The tradeoffs involved in parenting are particularly interesting in species with biparental care, as parents must not only assess their own condition, but also the motivations of their partner. Moreover, the proximate mechanisms underlying the expression of parental care can influence whether and how parents respond to the demands of parenting; selection for parental care can result in the evolution of mechanisms and ontogenies that facilitate plasticity in parental care; however, both mechanistic and ontogenetic causes can also impose constraints on how parents to respond to their environments (Sinervo and Svensson 1998). Thus, there are several important questions concerning parental care that arise: how do parents successfully raise offspring despite the demands, tradeoffs, and constraints involved in parenting? How do parents adjust their behavior in response to environmental changes? How do parents adjust their behavior in response to the behavior of their partner? And finally, how does the parental brain manage the multiple, often competing demands involved with parenting? Answering these questions is critical to understanding how much care offspring receive from their parents, which influences offspring fate and has ecological and evolutionary consequences for future generations.

Anemonefishes are an attractive system for studying parental care in part because their tractability allows for comprehensive studies of both proximate and ultimate causes in the lab and the field. While there is interspecific

variation in anemonefish parental care (Allen 1972; Ghosh et al. 2012), the general pattern of care can be described as follows. Anemonefish live in social groups composed of a breeding pair and a small number of non-breeding subordinates (see Chapter 14). The breeding pair lay eggs together up to three times per lunar month for many years (Buston and Elith 2011; Thomas and Prakash 2015; Seymour et al. 2018; see Chapter 13). A few days before breeding, parents first clear a nest site on a hard substrate near the base of the anemone (Moyer and Bell 1976; Green and McCormick 2005; Ghosh et al. 2012). Once a clutch is laid, both parents care for the eggs for six to nine days, during which time the male provides the majority of care (Green and McCormick 2005; Ghosh et al. 2012). Direct care takes the form of mouthing or nipping eggs, fanning the clutch, and defending against intruders and egg predators (Moyer and Bell 1976; Moyer 1980; Green and McCormick 2005), all of which collectively enhance embryo survival (Moyer and Sawyers 1973; Ghosh et al. 2012). As embryos develop, parental care increases, which may reflect responsiveness to increased metabolic needs or increased reproductive value of older clutches (Green and McCormick 2005; Ghosh et al. 2012). On the night of hatching, parents provide additional care, which may serve to synchronize hatching and facilitate the transition of the benthic embryos to pelagic larvae (Moyer and Bell 1976; Ross 1978; Pacaro et al. 2022). Parental care ceases with hatching and the larvae disperse (Jones et al. 2005; see Chapter 20) before setting into another anemone (Elliott et al. 1995; see Chapter 16).

Here, we review the most recent work on parental care in anemonefishes. We focus on the two species whose parental care has been studied most extensively in the last five years: the clown anemonefish *Amphiprion percula* and its

sister taxon, the false clown anemonefish *Amphiprion ocellaris*. First, tapping into the rich literature on phenotypic plasticity and animal personalities, we consider how parents respond to changes in resource availability and social roles. Second, linking to recent theoretical advances in the study of parental care, we consider how parents respond to changes in each other's behavior (i.e., how parents negotiate care). Third, we discuss a rare and interesting form of parental care that occurs in anemonefishes as a byproduct of their social system: step-fathering. Fourth, we dive into the proximate mechanisms underlying parental care. This chapter complements chapters on reproduction (Chapter 13) and dispersal (Chapter 20), revealing the central role that parents play in the life cycle and population dynamics of anemonefishes. Our goal is to provide the reader with a review of the most recent advances in anemonefish parental care research and highlight promising future directions.

15.2 PLASTICITY AND PERSONALITY OF PARENTAL CARE

15.2.1 PLASTICITY AND PERSONALITY OF PARENTAL CARE IN RESPONSE TO CHANGES IN RESOURCES

Plasticity, the capacity of individuals to respond to environmental changes by modifying traits or behaviors, is critical to our understanding of whether and how populations can persist under environmental change (West-Eberhard 2003). In the clown anemonefish *Amphiprion percula*, parental care is plastic in response to changes in resource availability, reflecting the vital role of habitat quality on reproductive success. A field study revealed that in groups that occupied large anemones and in groups supplemented with food, females laid more eggs and both males and females increased time spent tending to those eggs (Barbasch et al. 2020). Additionally, there was support for a causal pathway linking anemone size and reproductive success through effects on egg-laying by females and parental care by males (Figure 15.1; Barbasch et al. 2020). Combined these results indicate that plasticity in reproduction and parental care in response to short- and long-term changes in resource availability can generate among-group variation in embryo survival and larval production

(Box 15.1; Barbasch et al. 2020). Troublingly, *A. percula* may be particularly susceptible to environmental degradation due to the strong effects of anemone quality on reproductive success (Salles et al. 2016). Plasticity in reproduction and parental care, if adaptive, may allow parents to optimize reproductive output to take advantage of short-term increases in resource availability, while limiting investment in costly reproduction when resources are scarce. Therefore, plasticity provides some hope for the capacity for population persistence in the face of their rapidly changing environment.

BOX 15.1: CONSEQUENCES OF VARIATION IN PARENTAL CARE

Parents play a pivotal role in offspring development, but not all parents are good parents. Variation in parental care, within and among individuals, may have cross-generational consequences by influencing the *number* and *quality* of offspring that survive and reproduce (Mousseau and Fox 1998). In anemonefishes, parental care is positively related to the number of offspring produced (larval number, Figure 15.1; Barbasch et al. 2020; also Ghosh et al. 2012), and the number of offspring produced is related to recruitment success (Figure 15.1; Saenz-Agudelo et al. 2015). Whether parental care is also related to the quality of offspring produced is an outstanding question (larval quality, Figure 15.1). In anemonefishes, larvae undergo a dispersal phase after which they attempt to recruit to an anemone – this is a phase during which the chances of success may be 1 in 10,000. The distance a larva disperses and whether it can recruit are major determinants of population connectivity and structure (see Chapters 17). Dispersal distance can be related to larval size and swimming performance (Leis 2007; Nanninga and Manica 2018; Majoris et al. 2019), yet little is known about the role of parental care in generating variation in these traits (Figure 15.1). Experimental tests of parental effects on larval size, swimming ability, and local recruitment, will provide new insights into the consequences of variation in parental care for population dynamics.

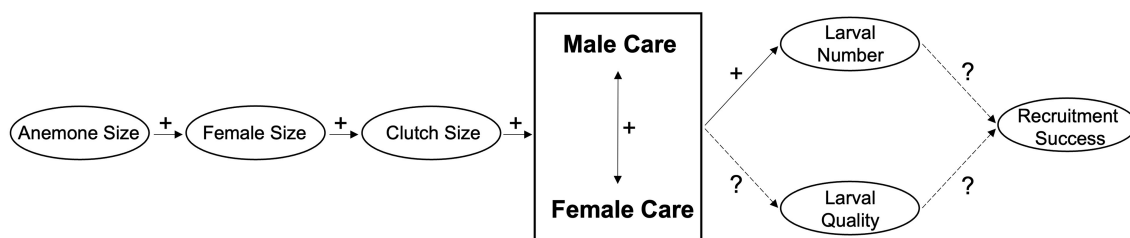


FIGURE 15.1 Parental care plays a central role in the population dynamics of anemonefishes. Hypothesized causal pathway showing the central role that parental care plays in linking anemone size to recruitment success in the clown anemonefish *Amphiprion percula* (adapted from Barbasch et al. 2020). Solid arrows represent relationships between habitat traits, fish traits, and reproduction which have empirical support in *A. percula* (see text); double-headed arrow represents a relationship between male and female care with no hypothesized causal direction, and dashed arrows represent hypothesized relationships that remain untested in *A. percula*. The (+) indicates a positive association, the (–) a negative association, and the (?) an untested, hypothesized relationship.

In addition to plasticity, individuals can exhibit “personalities”, consistent behavioral traits maintained across contexts. Plasticity might be favoured by selection if it allows for adaptive responses to environmental changes, but it is not immediately apparent why personality variation would be maintained by selection (Dingemanse and Wolf 2013; Alonzo 2015). In the clownfish *Amphiprion percula* and the false clownfish *A. ocellaris*, individuals differ from each other and are repeatable through time and across contexts in parental behaviors (DeAngelis et al. 2017; Barbasch and Buston 2018), raising the question of why this variation exists. One adaptive explanation is that personalities can reflect stable among-individual differences in state (e.g., size, age, or physiological condition) (Dingemanse and Wolf 2010), which might exist in anemonefishes due to variation among groups in habitat quality. Larger or higher-quality anemones result in resident fish that grow larger, reproduce more, and provide more care than residents of smaller or lower-quality anemones (Chausson et al. 2018; Salles et al. 2020; Barbasch et al. 2020). Additionally, personality may be due to mechanistic constraints, for example, due to pleiotropy or limited ability to express the optimal phenotype across all environments (Alonzo 2015). One important future step in understanding why personalities exist is to determine to what extent they are heritable, and if so, whether they are adaptive.

Plasticity and personality are not mutually exclusive, reflected in individual-by-environment interactions, IxE (Royle et al. 2014). It is not intuitive why this IxE variation exists – why would individuals respond differently to the same environmental changes? In *A. percula*, the average level of parental care increases in response to an increase in food availability, but individuals vary significantly in the magnitude and direction of this response (Barbasch and Buston 2018). One plausible adaptive explanation for an IxE interaction in anemonefish parental care centres around social contexts. Models reveal that consistent behavioral types may favour individuals that modify their behavior in response to their social partner, which selects for individuals that are consistent in their responses, resulting in co-existence of responsive and non-responsive individuals (Wolf et al. 2011). This model may help explain IxE variation in anemonefish, as parents must interact repeatedly to coordinate offspring care. Even if IxE variation is not heritable or adaptive, its existence can have consequences for populations, as IxE can theoretically stabilize population-level responses to environmental changes simply due to the diversity of existing responses (West-Eberhard 2003; Dingemanse and Wolf 2013).

15.2.2 PLASTICITY AND PERSONALITY OF PARENTAL CARE IN RESPONSE TO SEX CHANGE

In addition to exhibiting plasticity of care in response to changes in ecological context (resource availability), anemonefishes also exhibit plasticity of care in response to changes in social roles (across sex change). Anemonefishes are protandrous hermaphrodites and individuals have

the capacity to change sex from male to female (Chapter 12). Therefore, any average differences between males and females in parental care reflect plasticity across sex change. Although *A. percula* and *A. ocellaris* males and females differ in their average level of care, representing within-individual plasticity (DeAngelis and Rhodes 2016; Barbasch and Buston 2018), parental care may also be correlated across sex change, such that males that provide a relatively high level of care also provide a relatively high level of care as a female, representing personality across sex change.

If male and female care optima differ, cross-sex correlations, as might occur in *Amphiprion*, suggest that there may be some mechanistic constraint on the independent evolution of male and female parenting behavior (Box 15.2). One hypothesis is that constraints on plasticity in the expression of isotocin and arginine vasotocin, which have antagonistic effects on parental care and territory defence (see “Mechanisms Underlying Parental Care”), could help explain variation among individuals in parental care. Future studies exploring the behavioral and molecular mechanisms underlying how a single individual can rapidly and dramatically shift parental roles, as well as the constraints involved, will ultimately help us understand why individual variation in parental care exists.

BOX 15.2: FUNCTION OF FEMALE CARE

In *A. percula*, male care is a strong predictor of embryo survival (Figure 15.1; Barbasch and Buston 2018; Barbasch et al. 2020); however, the function of female care is less clear. During the day, *A. percula* females spend time in proximity to the clutch (referred to as tending) but do very little mouthing and next to no fanning (Barbasch and Buston 2018). The amount of time a female spends tending is sensitive to food availability, suggesting that females may face a trade-off between tending and other activities like foraging (Barbasch and Buston 2018). If female tending does not enhance offspring survival, why do females engage in tending at all? One hypothesis is simply that we have not had the statistical power to detect an effect of female tending on embryo survival. An alternative hypothesis is that female tending has more to do with monitoring clutch development, in preparation for their involvement on the night of hatching (Pacaro 2022). Another alternative is that female tending is indicative of her monitoring her partner’s efforts, as part of a negotiation over levels of care (Barbasch et al. 2021). Finally, it’s also plausible that selection for male care is strong and selection against low levels of female care is weak, meaning that female care may be a case of intergender hitchhiking (Clint et al. 2012) in these sex-changing fish. Novel experiments manipulating levels of female tending are needed to understand the function of female care.

15.3 NEGOTIATIONS OVER CARE

In the previous section, we demonstrated that parents exhibit plasticity in response to changing ecological conditions and social roles. However, anemonefishes are biparental, and thus *A. percula* parents are also faced with variation in the social environment created by interactions with their partner. In species with biparental care, conflict arises between parents because each parent benefits from shifting the burden of care to their partner. Theory predicts that parents should respond to each other to reach a negotiated settlement over how much care each should provide (McNamara et al. 1999).

While the theory is sound, a test of a series of negotiation models in *A. percula* revealed that no current models fully explain whether and how anemonefish parents negotiate (Barbasch et al. 2021). When one parent was experimentally handicapped via fin clipping, the other parent seemingly did not respond: males tended just as much when the female was handicapped and females tended just as much when the male was handicapped. However, when one parent was handicapped, pairs did not face any consequences in terms of larval production, suggesting that parents are fully compensating in some way for changes in their partner's effort.

Apparent full compensation, as seen in *A. percula*, presents an evolutionary conundrum, because if parents are completely making up for reductions in their partner's effort, one parent should cease care altogether, resulting in uniparental care as the evolutionary outcome (McNamara et al. 1999). One explanation is that, in *A. percula*, there may not be sufficient conflict to be resolved by negotiation. Actual conflict might be low because ecological constraints and the social hierarchy within groups enforce cooperation (Chapter 14; Rueger et al. 2018; Branconi et al. 2020). However, potential conflict still exists because the non-breeding group members can rapidly replace either member of the breeding pair, thus serving as future reproductive opportunities (Buston 2004a).

The role of conflict in negotiations could be tested by manipulating the presence/absence of non-breeders. Conflict should theoretically be greater in groups with non-breeders, which represent future mates for the breeding pair, than in groups without non-breeders (Buston 2004a; Chapter 14). If non-breeders were experimentally removed, parental interests would become strongly (if not fully) aligned, and thus full compensation for a reduction in partner effort makes sense. However, when non-breeders are present and one parent is experimentally handicapped, such generosity is not predicted. Both parents have other options, in the form of non-breeders, if their partner is unable to provide sufficient care, therefore the predictions of negotiation models are expected to hold. Another potential explanation for full compensation in negotiations is that parental care was not sufficiently reduced to incite conflict between parents. This hypothesis could be tested using phenotypic engineering to manipulate parental care at the mechanistic level (Nugent

et al. 2019), for example by administering an isotocin receptor antagonist, which is known to reduce direct egg care in *A. ocellaris* males and females (DeAngelis et al. 2020; see “Mechanisms Underlying Parental Care”) and determining the consequences for parental interactions and fitness.

15.4 STEP-FATHERING AND ALLOPARENTAL CARE

Another evolutionary conundrum arises with the existence of alloparental care, parental care directed towards non-descendant offspring, because the benefits of care are accrued by distantly related or unrelated individuals (Emlen 1991; Wisenden 1999). Anemonefishes live in social groups composed of a breeding pair and a small number of non-breeders, setting the stage for alloparental care (see Chapter 14). However, unlike many other species that occur in such social groups, non-breeding subordinates do not participate in alloparental care when the parents are present (Buston 2004b). In anemonefishes, group members are unrelated and thus do not benefit from alloparental care in the traditional way via kin selection (Buston et al. 2007). Yet, in some anemonefishes, when one or both parents are removed while eggs are in the nest, the non-breeder will provide care (Yanagisawa and Ochi 1986; Phillips et al. 2020).

Adaptive explanations for alloparental care look for benefits, including from helping relatives, acquiring breeding experience, or improving the chance of inheriting territories (Emlen and Vehrencamp 1983; Balshine-Earn et al. 1998). One hypothesis for anemonefish alloparental care is that providing care may allow non-breeders to escape punishment by the female – indeed, in *A. clarkii*, when males were experimentally removed, females were observed head-butting and nudging the non-breeder towards the clutch, and the non-breeder began caring for the eggs (Yanagisawa and Ochi 1986). Alloparental care may be a form of reciprocal altruism (Trivers 1971), such that dominants allow non-breeders access to shelter within the anemone, and non-breeders reciprocate by caring for eggs upon the death of a breeder. In this context, punishment by females may serve as retaliation against those who fail to care for her eggs. A second hypothesis is that alloparental care strengthens the pair bond and provides parenting experience for the mate replacement (Yanagisawa and Ochi 1986; Phillips et al. 2020). Indeed, breeding experience is associated with increased male care and embryo survival (Buston and Elith 2011; Phillips et al. 2020). A third hypothesis is that the non-breeder would benefit from care even with both parents removed if the larvae produced were to return to their natal anemone and become rapid mate replacements. While returns to the natal anemone are rare, they do occur (Salles et al. 2016) and may be more common when anemones are undersaturated due to the loss of both parents (Buston 2003). Discriminating among these hypotheses will require a series of carefully designed field experiments.

Non-adaptive explanations for alloparental care propose that it represents misdirected care due to the inability to

discriminate kin from non-kin (Jamieson 1989). In the lab, even when *both* biological parents are removed, *A. ocellaris* non-breeders care for eggs (Phillips et al. 2020). Thus, adaptive explanations involving strengthening the pair bond or avoiding punishment are not sufficient to explain alloparental care here (though the third adaptive hypothesis, that the non-breeders care for the eggs to rapidly produce a new mate for themselves, cannot be ruled out). Additionally, the solo non-breeder should theoretically benefit from investing in growth and development into a female, rather than gaining experience as a male (Phillips et al. 2020). The non-adaptive explanation is that caring for unrelated eggs is a side effect of selection for caring for descendant eggs and is maintained because long-term monogamy results in a low probability of a male encountering unrelated eggs.

15.5 MECHANISMS UNDERLYING PARENTAL CARE

The brain mechanisms responsible for parental care are evolutionarily ancient. All social behaviors are regulated by neuroendocrine axes which include a series of highly evolutionarily conserved interconnected sub-cortical brain areas, endocrine glands, neuro-peptides, neurotransmitters, and sex steroid hormones (Dulac et al. 2014; Rogers and Bales 2019). However, parental care is usually studied in animal models where the females are the primary caregivers of the offspring (Dulac et al. 2014). As a consequence, our understanding of paternal care at the neuroendocrine level is lacking in comparison to maternal care. While many of the substrates that regulate paternal care are likely shared with maternal care, others may differ, as males and females have sex-specific behaviors and dramatically different circulating sex steroid hormone levels that affect physiology and behavior (Ball et al. 2002; Nelson 2005). Additionally, females are primed for parenting, as changes in physiology occur during egg development, while cues for males are more subtle.

Anemonefish present an ideal model system for uncovering mechanisms of male parental (paternal) care. One of the challenges in identifying brain mechanisms of paternal care is isolating paternal care from the many other behaviors that males perform simultaneously such as territoriality and courtship (Kleszczyńska et al. 2012; O'Connell et al. 2012). In many anemonefish species, due to high dependence on host anemones for protection and consequently high social isolation from other groups, parental behavior can be examined in isolation from other confounding displays (Deangelis and Rhodes 2016). Furthermore, as described earlier, male *A. ocellaris* display alloparental care, so the entire breeding cycle can be dissociated from the display of paternal behaviors when a male that has never parented before (or is not currently parenting) is presented with eggs and they begin to display the paternal behaviors (Phillips et al. 2020). In male anemonefish, the appearance of eggs presents a rapid shift in behavior from non-parenting to parenting (Rogers and Bales 2019). This dramatic change in behavior must also be reflected within the brain.

Paternal care consists not of one single behavior but a suite of behaviors (see "Introduction"). In male false clown anemonefish (*Amphiprion ocellaris*), a father robustly cares for his offspring to promote egg development, yet when potential predators arrive, he must vigorously guard his nest against predation. Hence, fathers switch parenting tactics from egg tending to egg guarding in the presence of predators as simultaneously competitive demands necessitate parents to make prudent decisions (DeAngelis et al. 2020). These decisions to display different forms of parenting are reflected by distinct mechanisms within the brain.

Two well-known neuro-peptides involved in regulating a variety of social behaviors appear to play a critical role in dynamically regulating the switch between nurturing and aggressive defence tactics. These are isotocin (oxytocin is the mammalian homolog) and arginine vasotocin (arginine vasopressin is the mammalian homolog). These neuro-peptides are small proteins produced by neurons which act as signalling molecules within the brain (O'Connell and Hofmann 2012). Cell bodies containing these neuro-peptides reside in the preoptic area of the hypothalamus while receptors are laden throughout the brain in other evolutionarily conserved brain areas involved in regulating social behavior. Oxytocin has been well described for its role in female reproduction and maternal behavior as its release at parturition serves as a catalyst for physiological and behavioral changes (Insel 2010). While less studied in males, it likely also functions to promote paternal behavior (Figure 15.2). Arginine vasopressin has been broadly implicated in male behaviors which promote reproduction but has consistently been recognized as important for aggression in teleosts (Kleszczyńska et al. 2012; O'Connell and Hofmann 2011; Yaeger et al. 2014).

In *A. ocellaris*, pharmacological blockade of arginine vasotocin (V1a) receptors and isotocin receptors affected parental behaviors in opposite ways (DeAngelis et al. 2017). As expected, a blockade of isotocin receptors reduced the amount of nipping and fanning of the eggs without altering the time spent in the nest compared to saline controls. This is consistent with other findings in teleost fishes showing that, like mammals, isotocin signalling is critical for high levels of parental care, regardless of which sex is the primary caregiver.

More intriguing was that blockade of arginine vasotocin V1a receptors actually increased the amount of direct parental care. Given the known role of arginine vasotocin signalling in aggression in teleosts, one explanation is that vigilance was blocked, which then resulted in a greater amount of time allotted to egg care. To test this hypothesis, a follow-up experiment was conducted, in which nest predators were introduced while concurrently administering either isotocin receptor or arginine vasotocin V1a receptor antagonists to males actively fathering. Here, the isotocin receptor antagonist again reduced parental care but also increased aggression. Conversely, the V1a antagonist reduced aggression while increasing direct egg care (DeAngelis et al. 2020). These results suggest that

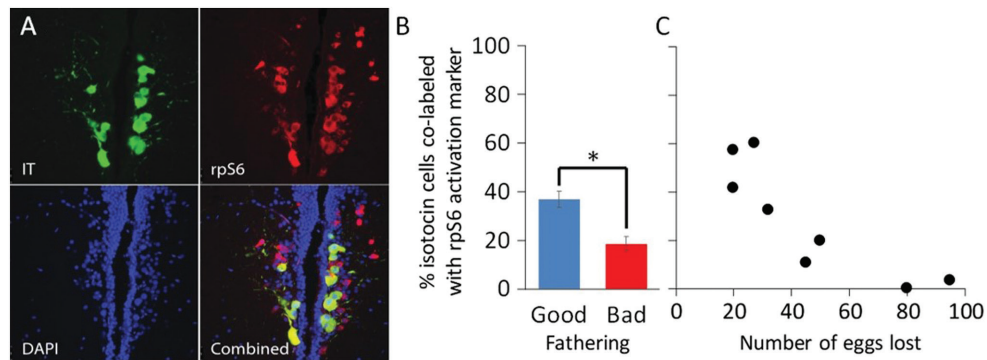


FIGURE 15.2 Activation of preoptic area isotocin neurons in good fathers. A. Representative section through the preoptic area of a fathering male *A. ocellaris* showing immunofluorescent detection of isotocin (IT, top left), the phosphorylated ribosomal protein (rpS6; top right), DAPI (bottom left), and all combined (bottom right). Performed by author. B. Percentage of IT cells co-labelled with the rpS6 activation marker in “good” and “bad” fathers (see Section 15.5) and C. as a function of the number of eggs lost. Standard errors shown. * indicates statistically significant by Fisher exact test. Methods: sexually naïve males ($n=8$) were given batches of eggs to step-father for 90 min. Total number of fans, nips, time in nest, and number of eggs at the beginning and end were recorded. The males were then euthanized by cervical transection and brains sectioned and stained for IT and rpS6. A total of 392 IT cells were identified in the eight fish and each IT cell was subsequently analyzed for co-expression of rpS6 by focusing through the cell on the z-axis. Good and bad fathers were identified by median split of a composite fathering score considering total number of paternal behaviors, time in nest, and number of eggs lost.

arginine vasotocin and isotocin signalling pathways act competitively in the regulation of two components of male parental care: as isotocin is blocked, more effort is allotted towards aggression versus egg nurturing, and vice versa when arginine vasotocin is blocked. These studies further suggest isotocin and arginine vasotocin act independently in the regulation of simultaneously occurring competitive demands of fathers providing offspring care, which can be both nurturing and aggressive in offspring defence, providing insight into how the paternal brain manages the trade-offs involved in parenting.

Given the evidence cited above that isotocin has a direct role in promoting paternal care in anemonefish, males were predicted to display a greater expression of isotocin receptors in their brains than females (since males are the primary caregivers of the eggs). Furthermore, isotocin receptor expression should be upregulated during active parental care in both sexes as compared to when they are not directly caring for eggs. As predicted, males displayed greater isotocin receptor expression compared to females, while active parents, both males and females, displayed increased isotocin receptor gene expression in the brain compared to non-parental individuals (DeAngelis et al. 2018). This supports a growing body of evidence that isotocin signalling in the brain is regulated not only by the synthesis and release of isotocin from neurons but also by the density and/or distribution of isotocin receptors in the brain. These results imply that as receptor numbers increase, the signalling efficiency of isotocin also increases, promoting parental behavior. While both parents showed increased isotocin receptor gene expression in the brain, the effect was particularly pronounced in males, consistent with the observation that males are the primary caregivers of the eggs.

Taken together, these studies suggest that the ancient evolutionarily conserved signalling pathways of vasotocin and isotocin interact with steroid hormones to regulate parental behavior in anemonefish and likely across vertebrates (DeAngelis et al. 2017, 2018, 2020). However, the brain is a complex heterogeneous organ that operates on a scale from molecules, to cells, to neural circuits. Here, we have highlighted only a small portion of the mechanisms that likely orchestrate parental care and provide insight into future promising opportunities in this model system.

15.6 CONCLUSIONS AND PROSPECTS

Anemonefishes have provided novel insights into the individual, social, ecological, physiological, and evolutionary factors that influence patterns of care. Field and laboratory studies have uncovered variation within and among individuals, populations, and species, yet little is known about *why* this variation exists and what its consequences are. Future studies focusing on proximate and ultimate explanations for this variation are now possible. Studies into the mechanisms underlying care have identified the neural pathways and brain regions regulating parental care, and emerging genomic methods (see Chapter 5) have opened the way for experimental manipulations of parental care at the mechanistic level. Targeted manipulations of parental care in both males and females present a powerful tool for answering many outstanding questions, such as whether and how parents negotiate, what the function of female care is, if any, and what the consequences of variation in care are for both parents and offspring. Parental behaviors have been well-described within some species (Green and McCormick 2005; Ghosh et al. 2012; DeAngelis et al. 2017; Barbasch and Buston 2018), but interspecific variation in care has not

been systematically studied. Our growing knowledge of the interspecific and intraspecific variation in ecology and social system as well as our understanding of phylogenetic relationships among species (Litsios et al. 2014; Rolland et al. 2018; see Chapter 3) allows for future comparative analyses of parental care. Finally, studies exploring parental effects on larval traits can help uncover the consequences of parental care for future generations.

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