A quarter century of recovery of the whelk *Thais orbita* from tributyltin pollution off Perth, Western Australia

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Abstract

Imposex is a condition in which females of dioecious marine snails develop rudimentary male characteristics. It is caused by tributyltin (TBT) used as an antifoulant in vessel paints since the late 1960s. Following the 2008 international ban on TBT, a decreasing rate of imposex has been observed worldwide. In Western Australia, imposex surveys of the whelk *Thais orbita* up to 2011 in the Perth metropolitan area suggested a decreasing trend but a significant recovery has not been confirmed. Collection of *T. orbita* from 11 sites in 2019 demonstrated a virtually complete recovery from imposex. Although it is generally accepted that male snails are not affected by TBT, the historical data set allowed confirmation that the male penis is of similar size in *T. orbita* collected at heavily affected sites and at relatively unaffected sites. Similarly, imposexed female snails had similar shell lengths to female snails at non-impacted sites.

For thousands of years vessel operators combatted the growth of biofouling on the hulls of their boats and ships. Biofouling increases drag by as much as 60%, slowing vessels and increasing energy costs (Vietti 2009). Over the centuries many methods have been used to overcome the problem, including removing the vessel from the water, cleaning the wetsides and the use of copper plates on the sides of the vessels. Widespread use of tributyltin (TBT) as an antifoulant in vessel paints started in the late 1960s. TBT was very effective in preventing fouling, but its leaching in surrounding waters caused measurable effects in gastropods in concentrations as low as 1 ng per litre (one part per trillion) (Schøyen et al. 2018). TBT has been recognised as one of the most lethal chemicals deliberately introduced into the marine environment (Goldberg 1986).

The half-life of TBT varies with environmental conditions and ranges from days to months in water while in sediments, half-lives of 360 to 775 days have been reported (Li et al. 2019). Organotins tend to accumulate in sediments, with finer sediments having higher concentrations (Antizar-Ladislao 2008). Within a few years of its introduction as an antifouling agent, adverse effects of TBT on various aquatic biota became apparent.

Imposex, a reproductive abnormality in marine snails, was first reported in *Nucella lapillus* (Linnaeus, 1758) in the United Kingdom by Blaber (1970). As early as 1974, failure of spat fall, stunted growth and anomalies in shell calcification were reported in Pacific oysters (*Magallana gigas* (Thunberg, 1793)) in Arcachon Bay on the French Atlantic coast (Alzieu 2000). As with many other chemicals, TBT is progressively concentrated in higher levels of foodwebs, and by the 1990s there were reports of high levels in fish and mammals (Suzuki *et al.* 1992; Kim *et al.* 1996; Tanabe 1999).

Imposex in dioecious marine snails is the most widespread and studied of the effects of TBT in the marine environment. Males are apparently largely unaffected, but females develop rudimentary male characteristics, including a vas deferens and/or penis. The female never becomes a functional male, but in severe cases the vagina is occluded, eggs cannot be released, causing rupture of the egg capsule, and the female dies. Even if she survives, the lack of egg release can have severe consequences to the population as fewer young emerge from egg capsules. As older individuals die, they are not replaced and the population declines (Fioroni *et al.* 1991; Oehlmann *et al.* 1991; 1996).

Imposex can be caused naturally by environmental stresses such as excreta from gull colonies (Nias *et al.* 1993) and other anthropogenic inputs such as copper, other chemicals and paints, but TBT is the leading cause both in terms of the number of species affected and the severity of the condition (Nias *et al.* 1993; Evans *et al.* 2000).

Nucella lapillus is the dominant predatory gastropod on intertidal hard substrates in the United Kingdom and Europe. Bryan *et al.* (1987) predicted that *N. lapillus* would be decimated by the year 2000. By 1991, imposex had been reported in 49 genera and 72 species worldwide (Fioroni *et al.* 1991; Oehlmann *et al.* 1991); five years later the number of affected species had risen to 120 (Oehlmann *et al.* 1996) and after 20 years, more than 260 species of gastropods affected by TBT have been reported (Laranjeiro *et al*. 2018).

Imposex has been widely reported in Australia: in *Nassarius* in Queensland (Mitchell 1989); *Dicathais orbita* (Gmelin, 1791) (as *Thais*) and *Cabestana spengleri* (Perry, 1811) in Victoria (Foale 1993); *Bedeva vinosa* (Lamarck, 1822) (as *Lepsiella*) in South Australia (Nias *et al.* 1993); and *D. orbita* and *Tenguela marginalba* (Blainville, 1832) (as *Morula*) in New South Wales (Wilson *et al.* 1993). In Western Australia the phenomenon was reported in six species of *Conus* (Kohn & Almasi (1993)), in *D. orbita* (Field 1993) and in *Morula granulata* (Gmelin, 1791) (Reitsema & Spickett 1999). Effects of TBT were also reported in the Sydney Rock Oyster, *Saccostrea glomerata* (Gould, 1850) (as *S. commercialis* (Iredale & Roughley, 1933)) (Batley *et al.* 1989) and intersex in the abalone (*Haliotis roei* Gray, 1826) (Sloan & Gagnon 2004).

The first report of imposex in Western Australia was provided by Kohn & Almasi (1993). The western end of Rottnest Island is 29 km west of the major population centre of Perth, and of the adjacent major shipping areas of the port of Fremantle and Cockburn Sound (Figure 1). Working on intertidal platforms at the western end of Rottnest Island in 1991, Kohn & Almasi (1993) reported that 88% of six species of *Conus* were affected by imposex, primarily at moderate levels. The detection was particularly surprising as the area is well away from shipping lanes and strong wave action would be expected to dissipate any TBT that occurred. Coincidentally, the Western Australian Environmental Protection Agency (WA EPA) surveyed sediment TBT levels in the Perth metropolitan area in 1991 (Cary *et al.* 1991; Burt & Ebell, 1995), and reported that shipping areas and small boat harbours had the highest concentrations of all sites surveyed. Maximum concentrations in sediment were in southern Cockburn Sound (1.35 $\mu g.g^{-1}$ and Careening Bay, Garden Island (0.60 $\mu g.g^{-1}$) (Figure 1). The western end of Rottnest Island, where Kohn & Almasi (1993) studied *Conus*, had <10 $\mu g.g^{-1}$, <1% of the highest concentrations in Cockburn Sound (Cary *et al.* 1991; Burt & Ebell, 1995).

There was a clear need to examine imposex levels along the Perth shoreline and in Cockburn Sound, but the *Conus* species studied by Kohn & Almasi (1993) do not occur in these areas. To overcome this, *T. orbita* was examined. The species is widespread on most rocky shores along the metropolitan coastline and also at Rottnest Island. Imposex levels at the inshore sites frequently reached 100% (Field 1993; Reitsema *et al.* 2003).

Worldwide, environmental agencies reacted to the growing problem. TBT was banned in small vessels in the United Kingdom in 1987 (Evans *et al.* 1995). The WA EPA prohibited the use of TBT in vessels <25 m in 1991 (Kohn *et al.* 1999). As vessels >25 m are regulated through the International Maritime Organization (IMO), the WA EPA had no jurisdiction over these vessels. The IMO adopted the *International Convention on the Control of Harmful Anti-Fouling Systems on Ships* in 2001, but the convention did not come into force until 17 September 2008 (IMO 2019). From that date any newly built vessels could not use TBT as an antifoulant, nor could any vessels undergoing their five-year class recertification. Thus, the IMO regulations became progressively more effective until TBT was completely prohibited in September 2013.

Given most vessels visiting Rottnest Island were recreational vessels <25 m in length, Kohn *et al.* (1999) resurveyed three *Conus* species at the west end of Rottnest in 1996 (five years after the TBT ruling in WA) and Reitsema *et al.* (2003) re-examined *T. orbita* in 1998/99. Both studies demonstrated some improvement in imposex levels as a result of the partial restrictions imposed by the WA EPA in 1991. Using data collected in 1991, 1996, 2007 and 2017, Wells *et al.* (2017) traced the recovery in *Conus* populations at the western end of Rottnest Island over a quarter of a century. By 2017 the populations had essentially recovered, with only 4% of the individuals examined having low levels of imposex.

However, imposex frequency and severity were initially greater in the inshore *T. orbita* populations where TBT concentrations were up to 100 times greater than at Rottnest Island, raising the question of whether there was similar improvement inshore. Hence, the present study was designed to examine the current rate of imposex in *T. orbita* by re-sampling nine sites along the Perth metropolitan coastline, and at two sites on Rottnest Island in 2019. Results are compared to historical data at each site. Moreover, the available data were used to examine the widely accepted – but never demonstrated - assumption that male whelks are unaffected by exposure to TBT. This assumption was verified by comparing penis sizes relative to body size in specimens collected in heavily affected locations with those collected in relatively unaffected sites. In addition, female shell length at sites heavily affected by imposex was compared to female shell length at sites with no or low imposex to assess if chronic exposure to TBT affects growth in female *T. orbita*.

Reitsema *et al.* (2003) surveyed imposex in *T. orbita* at 20 sites along the Perth metropolitan coastline and at Rottnest Island in 1993 and 1998/99. The present study was conducted from January to March 2019 at 11 of the sites previously examined by these authors. Sites were chosen to include both the metropolitan and Rottnest Island locations (Figure 1) that had previously been surveyed that were historically near point sources of TBT and other sites that were not under the influence of these point sources.

Sites where *T. orbita* were collected are shown on Figure 1. Fremantle Harbour is the major shipping port for Perth, which has a population of about 2.4 million people. Fremantle Port is at the seaward end of the Swan River, with a narrow channel about 250 m wide between

North Mole and South Mole. Woodman Point, 10 km south of Fremantle Harbour, is the northern limit of Cockburn Sound. There are major shipping areas at South Jervoise Bay (Henderson) on the eastern shore of the sound, at Kwinana on the southern shore and a Royal Australian Navy (RAN) base on Careening Bay, Garden Island adjacent to Colpoys Point. A rock causeway from Rockingham to the RAN base has only a small boat channel. This substantially limits exchange of Cockburn Sound water with the open ocean and there has been considerable environmental degradation in the sound since the building of the causeway, including TBT accumulation (DEP 1996).

The remaining sites are along the Perth metropolitan coastline. Cottesloe and Trigg are intertidal platforms 7 km and 20 km north of Fremantle port. Hillarys Boat Harbour is a major enclosed marina 25 km north of North Mole that houses several hundred private boats. The site surveyed is the rock wall on the north side outside the marina entrance. Ocean Reef Boat Harbour is a small marina 34 km north of North Mole that is used only as a launch site; no boats are retained in the marina. The site surveyed is along both the inside and outside of the rock wall on the north side of the marina. The Rottnest Island sites (Radar Reef and Armstrong Bay) are 29 km offshore from Fremantle Port but the island is heavily frequented by leisure boats.

From 2005 through 2011 regular surveys measured imposex at selected sites every two years. Imposex was determined in 2005 using Relative Penis Size Index (RPSI) as described below. However, there was concern that resampling sites every two years would deplete the populations as the snails live for at least four years (Phillips 1969). In addition, *T. orbita* were being used at some of the sites for environmental impact statements by governmental authorities, adding further pressure on the populations. An Imposex Visual Examination (IVE) method was developed in which snails were relaxed by a 60-mins immersion in 7% magnesium chloride then gently pulled out of the shell and sex determined visually. In female snails, IVE was assessed using a scale from 0 to 6, with stage 0 reflecting no imposex and stage 6 showing a fully developed penis. The snails were released live on site after they had been examined and reanimated in clean seawater. IVE was used in the 2007-2011 surveys.

In 2019, *Thais orbita* were collected by hand while searching intertidal platforms or snorkelling along rocky shores, for about one hour per site. At each site all available snails were measured using digital callipers. Forty snails were retained for anatomical study and frozen. An additional 30 were relaxed using magnesium chloride as described above and their imposex status determined by IVE. These animals were revived in fresh seawater and then returned to the environment. All other snails were released immediately after being measured; there was no indication of any mortality as a result of being handled. As the IVE is not comparable with RPSI, as discussed below, the 2011 survey and the additional 30 individuals in 2019 were used only to determine sex ratios, size frequency and imposex rate, but not imposex severity. Male to female ratios were compared using Chi-square test for each site, each year.

To ensure consistency in examination of reproductive structures all of the 40 frozen snails from each site were examined anatomically. The frozen snails were defrosted prior to anatomical examination. The penis of a normal male *T. orbita* is a strongly curved structure located behind the right cephalic tentacle. No attempt was made to straighten the penis; therefore, the measurement of the penis size represents the maximum width of the structure recorded using digital callipers at 0.1 mm precision. Shell length was measured from the apex to the tip of the siphonal canal using the same callipers after which the shell was cracked using a bench vice.

The penis of all individuals, both males and females (if present), was measured to determine the RPSI developed by Gibbs *et al.* (1987): RPSI = mean female penis length^3 / mean female penis length^3. Field (1993) demonstrated that cubing the measurements was not required and used the equation RPS = mean female penis length / mean female penis length. This was subsequently used by Reitsema *et al.* (2003) and is followed here. A full discussion of the method is contained in Tan (1999).

Historical data were used to test the assumption that male penis size is unaffected by chronic exposure to TBT. Because the historical data available were measured by different personnel on different years, there might have an observer bias in the measurement of the male penis size. Hence, comparison of the male reproductive structure has been done between sites within years only.

In *T. orbita*, male penis size increases with body size; therefore, the direct statistical comparison of penis size measurements between male snails collected at heavily impacted sites with penis size of males collected at sites with low imposex occurrence is inadequate. The Male Penis Size Index (MPSI) has been calculated as (penis width/shell length) x 10. Firstly, historical data were selected where male penis size was recorded. Within a year, male snail lengths were compared between sites using ANOVAs after verification of normal distribution and equal variances. Sites where male shell lengths were statistically similar but had greatly differing rates of imposex in females were compared to test the hypothesis that the penis size of males *T. orbita* was not affected by chronic exposure to TBT.

Rilov *et al.* (2000) reported that the female gastropods *Hexaplex trunculus* (Linnaeus, 1758) and *Stramonita haemastoma* (Linnaeus, 1767) were significantly larger than males of the same species at TBT-polluted sites. Recent literature also reported that in the gastropods *Leucozonia nassa* (Gmelin, 1791) and *Stramonita brasiliensis* Claremont & D. Reid, 2011, imposex-affected females have a significantly larger shell length than healthy, non-imposexed females, regardless of environmental factors (Otegui *et al*. 2019). To assess if this observation also applied to *T. orbita*, female shell length was compared between sites heavily affected by imposex and those with no or low levels of imposex. Female shell lengths were compared using the non-parametric Kruskal-Wallis test as homoscedasticity could not be achieved even with data transformation.

TBT pollution and imposex can have three effects on snail populations:

- changes in the size distributions of local populations
- alteration to the sex ratio
- induction of imposex in females

Each of these was examined in the present study.

If imposex is severe, females will either be unable to reproduce or die due to the rupture of the egg capsule. Over time the population density will decline and size frequency will be skewed towards larger individuals as there are no recruitment of young snails in the population. Table 1 shows the minimum, maximum and mean sizes of *T. orbita* at each of the 11 sites surveyed in 2005, 2011 and 2019. Reitsema *et al.* (2003) did not record these data for 1993 and 1998/99.

Except for South Mole, the 2019 survey recorded abundant populations of *T. orbita* at all sites, ranging from 43 individuals at Hillarys to 241 at Radar Reef (Table 1). Overall, there was a wide range of sizes, from 12 mm to 76 mm, with all sites except South Mole having a wide range of sizes, including small individuals. These results demonstrate that the populations were healthy, were reproducing and had no obvious population level effects due to imposex. South Mole was exceptional, with only five large individuals being collected, one of which was female.

Populations of *T. orbita* were sampled 46 times at the 11 sites between 1993 and 2019 (Table 2). There was no statistical difference between the number of males and females at 38 sites ($p > 0.05$). Female dominated at five sites ($p < 0.05$), including four sites in 2011, skewing the sex ratio for all individuals collected that year to females. Males significantly outnumbered females ($p < 0.01$) at only two stations, Colpoys Point in 1998/99 and South Jervoise Bay in 2005. Both of these stations were heavily affected by TBT during these years and had 100% of females exhibiting imposex in the years when the sex ratio favoured males.

Females at six of the seven coastal sites examined in 1993 had >80% imposex, with one site at 99% and three at 100% imposex in females. Ocean Reef (47%) and the two Rottnest Island sites of Radar Reef and Armstrong Bay (19% and 50% respectively) had lower levels than most coastal sites. Five sites examined in 1998/99 had 100% imposex and two additional coastal sites were had 93% and 95% imposex. Hillarys (81%), Ocean Reef (13%) and the two Rottnest sites (12% and 17%) were all lower than other sites examined in 1998. This follows the pattern described by Reitsema *et al.* (2003) where sites located in Fremantle and Cockburn Sound had maximal imposex rates and the percentage of female snails affected decreased with distance from these areas. The 2005 survey showed declines at Hillarys and Cottesloe, but there were still four sites with 100% imposex. By 2011 imposex along the metropolitan coastline had continued to decrease, but there was an anomalous increase at the two Rottnest Island stations. By 2019 there had been a complete recovery from imposex at all sites sampled except at Woodman Point, where a single female with imposex resulted in a 5% level. The RPSI data (Table 3) showed a similar trend, but there was more variability in the data.

To determine if chronic exposure of male *T. orbita* influenced the male penis size, historical data were examined to identify sites where male shell lengths were statistically similar. In 2005, the sites of Colpoys Point, Ocean Reef and Hillarys all had similar male shell length and 100%, 30% and 21% imposex in females respectively. However, the MPSI was significantly smaller at Hillarys than at Ocean Reef and Colpoys Point ($p < 0.001$) (Table 4).

Historical data were also available in 2007 to further test the hypothesis that male penis size was not affected by exposure to TBT. Male snails of similar shell lengths were identified at South Mole, Hillarys and Ocean Reef, while these sites had greatly different incidence of imposex with 68%, 14% and 0% imposex in 2007, respectively. Yet, the MPSI was similar at all sites ($p = 0.191$) (Table 4).

Published literature reports that female snails experiencing high levels of imposex have larger shell length relative to females with low or no imposex (Rilov et al. 2000; Otegui et al. 2019). The 2005, 2007 and 2009 historical data allowed the testing of this hypothesis for *T. orbita*. In 2005, female snails collected at Colpoys Point, South Mole and Ocean Reef were of similar shell lengths ($p = 0.106$) despite very different imposex rates at these sites (100%, 100% and 30% respectively) (Table 5).

Similarly, in 2007, female snails at Colpoys Point, South Mole and Ocean Reef had similar shell lengths ($p = 0.638$) with very different levels of imposex (91%, 68% and 0% respectively). However, in 2009, female shell lengths at Colpoys Point and South Mole were significantly smaller ($p < 0.001$) than females at Ocean Reef (with imposex levels of 80%, 68% and 18% respectively) (Table 5).

Lewis *et al.* (2011) surveyed TBT tissue levels in the mussel *Mytilus planulatus* Lamarck, 1819 (as *M. galloprovincialis planulatus*) at the naval base at Garden Island. A baseline survey was made in 1991, then a regular survey program was established. Samples were taken in and near the RAN naval base, including at Colpoys Point, twice annually from 1993 until 1998, then annually until the end of 2008. The survey overlapped three phases of TBT management by the Navy: improved vessel maintenance practices, cessation of use of TBT on small vessels and a total elimination of the use of TBT. Initial mussel TBT levels at the commencement of the study were very high, up to 2560 ng $Sn.g^{-1}$ wet weight in a mussel collected November 1991, and 3320 ng $Sn.g^{-1}$ wet weight in a mussel in January 1993. Both mussels were from the naval small ships harbour. By the end of the study most sites had levels below 200 ng $Sn.g^{-1}$ wet weight of mussel tissue.

The recovery of *T. orbita* from imposex is best illustrated at Colpoys Point, Garden Island adjacent to the RAN base. The site was one of only two during the entire study that had a statistically significant ($p< 0.05$) number of males (in 2005). By 2011 the percentage of imposex had nearly halved to 52% and in 2019 it was down to zero. Snails sampled in 2019 ranged in size from 12 mm, the smallest encountered during the 2019 sampling program, to 70 mm. The large number of small individuals made the mean size of 40.5 ± 11.23 mm, one of the lowest of any of the sites sampled in 2019, suggesting the population is growing.

The present study demonstrates a complete recovery of *T. orbita* at Rottnest Island; none of the snails sampled in 2019 had imposex, paralleling the recovery of three species of *Conus* at two of the same sites (Wells *et al.* 2017). The key question is whether the reduction in imposex also occurred at inshore metropolitan sites where initial TBT levels were up to 100 times greater in 1991 than at Rottnest Island (Cary *et al.* 1991; Burt & Ebell 1995). The answer is yes, the partial recovery in 1998/99 (Reitsema *et al.* 2003) continued in 2005 and 2011 and is now virtually complete. Only a single individual, collected at Woodman Point, of 345 females examined in 2019 had imposex. The *T. orbita* populations are healthy, as evidenced by the number of individuals collected at each site, the number of females and the number of small individuals.

The South Mole site was anomalous; only five individuals were found during the 2019 survey (three people snorkelling for one hour). The lack of snails at this site could be due either to TBT induced imposex or another environmental factor. Prior to 2019 both the North Mole and South Mole sites were on the outside of the groynes, where TBT levels would be lower. The 2019 sites were inside the moles adjacent to the shipping channel into Fremantle Port, giving a maximal potential for the development of imposex. Prevailing currents in the area are northwards and there is typically an afternoon south westerly sea breeze, particularly during summer. The North Mole site is directly across the channel about 250 m north of the South Mole site and presumably subject to the same potential exposure to TBT. The North Mole site is, however, more exposed to wave action than the South Mole site, an environmental characteristic preferred by *T. orbita*. It is interesting to note that previous surveys have collected decreasing number of *T. orbita* at the South Mole, with surveys collecting the following numbers of males/female snails: 2005 (27/24); 2007 (25/25); 2009 (11/18); and 2011 (13/14). There might have environmental factors other than TBT causing the lack of *T. orbita* at the South Mole site.

While exposure to TBT is known to induce imposex in female snails of many species, the exact underlying mechanisms by which the condition of imposex is caused is still debated (Schøyen et al. 2018). It is possible that male snails are also affected but the scientific literature has not investigated effects in male snails, stating that male snails are *apparently* not affected by exposure to TBT (e.g. Wells *et al*. 2017). To our knowledge, this is the first time that measurements of male penis size at sites where imposex is highly prevalent is compared (1) with male penis size at other sites with similar imposex levels, and (2) with male penis sizes at sites where imposex is low or absent. Our results show that the male penis size relative to shell length, is site-dependent and there are no consistent trends or indications that chronic exposure to TBT influences the male penis size in *T. orbita*.

Previous studies (Rilov et al. 2000; Otegui et al. 2019) have reported that in four species of gastropods, imposex-affected females have larger shell lengths than healthy females, regardless of environmental factors. This syndrome might be explained in terms of energetics (e.g. scope for growth, Axiak *et al.* 1995), where the unused reproductive energy in imposex-affected females is redirected to somatic growth. Comparison of *T. orbita* shell lengths in imposex-affected females to healthy females shows no consistent trends, with two years out of three showing similar shell lengths in heavily affected females relative to shell lengths in low- or no imposexed females. The third year (2009) did have smaller female shell lengths at sites where imposex was higher however, this trend was not consistent amongst years. It is concluded that in *T. orbita* the development of imposex does not translate into energy being re-invested in somatic growth as there is no consistent trend in imposex-females having a larger shell length.

In summary, Kohn and Almasi (1993) reported imposex in Western Australia for the first time using *Conus* collected in 1991 where 88% of females from the western end of Rottnest Island, 29 km offshore from Perth exhibited imposex. Along the Perth metropolitan coastline, Cary *et al.* (1991) and Burt & Ebell 1995 documented TBT concentrations in Cockburn Sound that were >100 times the concentration at Rottnest. Field (1993) examined sites in shipping areas and found 100% of females affected at numerous sites. Reitsema *et al.* (2003) found that there had been partial recovery by 1998/99 resulting from partial bans on the use of TBT implemented in 1991. Wells *et al.* (2017) documented the recovery of the *Conus* populations at the same Rottnest sites to a point where only 4% of the female *Conus* exhibited imposex. The present study demonstrates a full recovery of *T. orbita* populations in 2019, with only one individual of 345 sampled having imposex. In addition, we have provided evidence that in *T. orbita*, male penis size was not affected by exposure to TBT. Moreover, historical data sets allowed confirmation that in this species, imposex-females did not have longer shell lengths relative to healthy females.

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Declaration of competing interest

None

References

Afsar, N., Siddiqui, G. & Ayub, Z. (2015) Bioindicator *Thais carinifera* (Mollusca, Gastropoda): imposex response and consequences along the Pakistan coast during the period from 1993 to 2012. *Brazilian Journal of Oceanography* 63: 115-124.

Alzieu, C. (2000) Environmental impact of TBT: the French experience. *The Science of the Total Environment* 258: 99-102.

Antizar-Ladislao, B. (2008) Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. Environment International 34: 292–308.

Axiak, V., Vella, A.J., Micallef, D., Chircop, P. & Mintoff, B. (1995) Imposex in *Hexaplex trunculus* (Gastropoda: Muricidae): first results from biomonitoring of tributyltin contamination in the Mediterranean. Marine Biology 121:685-691.

Batley, G.E., Fuhua, C., Brockbank, C.I. & Flegg, K.J. (1989) Accumulation of tributyltin by the Sydney Rock Oyster, *Saccostrea commercialis. Australian Journal of Marine and Freshwater Research* 40: 49-54.

Blaber, S.J.M. (1970) The occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus* (L.). *Proceedings of the Malacological Society of London* 39: 231-233.

Bryan, G.W., Gibbs, P.E., Burt, G.R. & Hummerstone, L.G. (1987) The effects of tributyltin (TBT) on adult dog-whelks, *Nucella lapillus*: long-term field and laboratory experiments. *Journal of the Marine Biological Association of the United Kingdom* 67: 525-544.

Burt, J.S., & Ebell, G.F. (1995) Organic pollutants in mussels and sediments of the coastal waters off Perth, Western Australia. *Marine Pollution Bulletin* 30: 723-732.

Cary, J.L., Simpson, C.J. & Chase, S. (1991) Water quality in Cockburn Sound. *Australian Environmental Protection Authority Technical Series* 47.

DEP (1996) Southern Metropolitan Coastal Waters Study (1991-1994) Final Report. Department of Environmental Protection, Perth, Western Australia, Report 17: 1-288.

Evans, S.M., Hawkins, S.T., Porter, J. & Samosir, A.M. (1994) Recovery of dogwhelk populations on the Isle of Cumbrae, Scotland following legislation limiting the use of TBT as an antifoulant. *Marine Pollution Bulletin* 28: 15-17.

Evans, S.M., Kerrigan, E. & Palmer, N. (2000) Causes of imposex in the dogwhelk *Nucella lapillus* (L.) and its use as a biological indicator of tributyltin contamination. *Marine Pollution Bulletin* 40: 212-219.

Evans, S.M., Leksono, T. & Mckinnell, P.D. (1995) Tributyltin pollution: a diminishing problem following legislation limiting the use of TBT-based anti-fouling paints. *Marine Pollution Bulletin* 30: 14-21.

Field, S. 1993. *The use of* Thais orbita *as a bioindicator for environmental contamination by tributyltin in the Perth metropolitan area.* Unpublished postgraduate thesis, Department of Zoology, University of Western Australia.

Fioroni, P., Oehlmann, J. & Stroeben, E. (1991) The pseudohermaphroditism of prosobranchs: morphological aspects. *Zoologischer Anzeiger* 226: 1-26.

Foale, S. (1993) An evaluation of the potential of gastropod imposex as an indicator of tributyltin pollution in Port Phillip Bay. *Marine Pollution Bulletin* 26: 546-552.

Gibbs, P.E., Bryan, G.W., Pascoe, P.L. & Burt, G.R. (1987) The use of the dogwhelk *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. *Journal of the Marine Biological Association of the United Kingdom* 71: 827-838.

Goldberg, E.D. (1986) TBT: an environmental dilemma. *Environment* 28: 17-44.

IMO (2019) status of IMO treaties. International Maritime Organization, 10 April 2019. London. 550 pages.

Kim, G.B., Lee, J.S., Tanabe, S., Iwata, H., Tatsukawa, R. & Shimazaki, K. (1996) Specific accumulation and distribution of butyltin compounds in various organs and tissues of the Stellar Sea Lion (*Eumetopias jubatus*): Comparison with organochlorine accumulation pattern. *Marine Pollution Bulletin* 32: 558-563.

Kohn, A.J. & Almasi, K.N. (1993) Imposex in Australian *Conus*. *Journal of the Marine Biological Association of the United Kingdom* 73: 241-244.

Kohn, A.J., Lalli, C.M. & Wells, F.E. (1999) Imposex in *Conus* at Rottnest Island: Five years after the first report. Pages 199-209. In: Walker, D.I. & Wells, F.E. (Eds). *Seagrass and Associated Fauna of Rottnest Island, Western Australia.* Western Australian Museum, Perth.

Laranjeiro, F., Sánchez-Marín, P., Oliveira, I.B., Galante-Oliveira, S. & Barroso, C. (2018) Fifteen years of imposex and tributyltin pollution monitoring along the Portuguese coast. *Environmental Pollution* 232: 411-421.

Lewis, J.A., Baran, I.J., Carey, J.M. & Fletcher, L.E. (2011) A contaminant in decline: long-term TBT monitoring at a naval base in Western Australia. Australasian Journal of Ecotoxicology 16: 17-34.

Li Z., Yu, D., Wangbao, G., Guangjun, W., Ermeng, Y. & Jun, X. (2019) Aquatic Ecotoxicology and Water Quality Criteria of Three Organotin Compounds: A Review. *Nature Environment & Pollution Technology* 18:217-224.

Miller, K.L., Fernandes, T.F. & Read P.A. (1999) The recovery of populations of dogwhelks suffering from imposex in the Firth of Forth 1987-1997/98. *Environmental Pollution* 106:183-92.

Mitchell, R.L. (1989) *Aspects of the biology of intertidal mud-snails of the genus Nassarius (Family Nassariidae, Order Neogastropoda).* BSc(Hons) thesis, James Cook University, Townsville, Queensland.

Morton, B. (2009) Recovery from imposex by a population of the dogwhelk, *Nucella lapillus* (Gastropoda: Caenogastropoda), on the southeastern coast of England since May 2004: a 52-month study. *Marine Pollution Bulletin* 58: 1530-1538.

Nias, D., McKillup, S.C. & Edyvane, K.S. (1993) Imposex in *Lepsiella vinosa* from southern Australia. *Marine Pollution Bulletin* 26: 380-384.

Nicolaus, E.E.M. & Barry, J. (2015) Imposex in the dogwhelk (*Nucella lapillus*): 22-year monitoring around England and Wales. *Environmental Monitoring and Assessment* 187: 736-751.

Oehlmann, J., Stroeben, E. & Fioroni, P. (1991) The morphological expression of imposex in *Nucella lapillus* (Linnaeus) (Gastropoda: Muricidae). *Journal of Molluscan Studies* 57: 375-390.

Oehlmann, J., Stroeben, E., & Fioroni, P. (1996) New facts about tributyltin-induced imposex in prosobranchs: General aspects. Malacological Review Supplement 6: 149-156.

Otegui, M. B., Zamprogno, G. C., França, M. A., Daros, B. N., Albino, J., & Costa, M. B. (2019). Imposex response in shell sizes of intertidal snails in multiple environments. *Journal of Sea Research*, 147, 10-18.

Phillips, B.F. (1969) The population ecology of the whelk *Dicathais aegrota* in Western Australia. *Australian Journal of Marine and Freshwater Research* 20: 225-265.

Rees, C.M., Brady, B.A. & Fabris, G.J. (2001) Incidence of imposex in Thais orbita from Port Phillip Bay (Victoria, Australia), following 10 years of regulation on use of TBT. *Marine Pollution Bulletin* 42: 873-878.

Reitsema, T.J., Field, S. & Spickett, J.T. (2003) Surveying imposex levels in the coastal waters of Perth, Western Australia, to monitor trends in TBT contamination. Australasian Journal of Ecotoxicology 9: 87-92.

Reitsema, T.J. & Spickett, J.T. (1999) Imposex in *Morula granulata* as bioindicator of tributyltin (TBT) contamination in the Dampier Archipelago, Western Australia. *Marine Pollution Bulletin* 39: 280-284.

Rilov, G., Gasith, A., Evans, S.M. & Benayahu, Y. (2000) Unregulated use of TBT-based antifouling paints in Israel (eastern Mediterranean): high contamination and imposex levels in two species of marine gastropods. Marine Ecology Progress Series 31: 192:229-38.

Schøyen, M., Green, N.W., Hjermann, D.Ø., Tveiten, L., Beylich, B., Øxnevad, S. & Beyer, J. (2018) Levels and trends of tributyltin (TBT) and imposex in dogwhelk (*Nucella lapillus*) along the Norwegian coastline from 1991 to 2017. *Marine Environmental Research* 144:1-8.

Sloan, S. & Gagnon, M.M. (2004) Intersex in Roe's abalone (*Haliotis roei*) in Western Australia. *Marine Pollution Bulletin* 49: 1122–1126.

Souto, M.A.M. 2014. The course of TBT pollution in the world during the last decade. Master's thesis, Departamento de Biologia, Universidade de Aveiro.

Suzuki, T., Matsuda, R. & Saito, Y. (1992) Molecular species of tri-n-butyltin compounds in marine products. *Journal of Agriculture and Food Chemistry* 40: 1437-1443.

Tan, K.S. (1999) Imposex in *Thais gradata* and *Chicoreus capucinus* (Mollusca: Gastropoda: Muricidae) from the Straits of Johor: A case study using penis length, area and weight as measures of imposex severity. *Marine Pollution Bulletin* 39: 295-303.

Tanabe, S. (1999). Butyltin contamination in marine mammals – a review. *Marine Pollution Bulletin* 39: 67-72.

Tester, M., Ellis, D.V. & Thompson, J.A.J. (1996). Neogastropod imposex for monitoring recovery from marine TBT contamination. *Environmental Toxicology and Chemistry* 15: 560–567.

Vietti, P. (2009) New hull coatings cut fuel use, protect environment. *Currents* Fall 2009: 36-38. https://p2infohouse.org/ref/49/48817.pdf

Wells, F.E., Keesing, J.K. and Brearley, A. (2017) Recovery of marine *Conus* (Mollusca: Caenogastropoda) from imposex at Rottnest Island, Western Australia, over a quarter of a century. *Marine Pollution Bulletin* 123: 182-187.

Wilson, S.P., Ahsanullah, M. & Thompson, G.B. (1993) Imposex in gastropods: an indicator of tributyltin contamination in eastern Australia. *Marine Pollution Bulletin* 26: 44-48.

Table 1. Size frequency of *Thais orbita* in the Perth metropolitan area in 2019.

Location	**2019**		
	Sample size	**Mean length ±SD (mm)**	**Range (mm)**
		Rottnest Island	
Radar Reef	241	51.73 ± 11.03	18-76
Armstrong Bay	173	41.63 ± 7.46	21-61
		Metropolitan coast	
Ocean Reef	90	49.65 ± 4.60	34-62
Hillarys	43	46.86 ± 8.07	23-62
Trigg	227	38.35 ± 4.04	29-50
Cottesloe	119	41.17 ± 7.42	24-63
North Mole	88	38.44 ± 7.76	26-61
South Mole	5	60.20 ± 7.70	49-68
Woodman Point	62	56.91 ± 10.72	29-75
South Jervoise Bay	154	43.24 ± 8.47	22-62
Colpoys Point	97	40.50 ± 11.23	12-70

Table 2. Sex ratios of *Thais orbita* in the Perth metropolitan area from 1993 to 2019. Data for 1993 and 1998/99 are from Reitsema et al. (2003). NS, not significant; * significant at 0.05 level; **, significant at 0.01 level, significant at 0.001 level.

Location	1993		1998/99		2005		2011		2019	
	Males: Females	**Significance**	**Males: Females**	**Significance**	**Males: Females**	**Significance**	**Males: Females**	**Significance**	**Males: Females**	**Significance**
Rottnest Island										
Radar Reef	91:111	NS	13:17	NS	-	-	8:13	NS	37:49	NS
Armstrong Bay	49-52	NS	43:58	NS	-	-	14:29	*	13:13	NS
Metropolitan coast										
Ocean Reef	37:34	NS	41:62	*	-	-	--		42:28	NS
Hillarys	47:59	NS	56:54	NS	21:29	NS	9:7	NS	20:23	NS
Trigg	85:69	NS	41:60	NS	-	-	12:18	*	35:44	NS
Cottesloe	13:16	NS	42:58	NS	11:16	NS	11:21	NS	30:40	NS
North Mole	-	-	59:41	NS	-	-	5:14	*	40:42	NS
South Mole	106:94	NS	53:47	NS	13:14	NS	27:24	NS	-	
Woodman Point	58:56	NS	60:42	NS	27:24	NS	10:21	*	25:33	NS
South Jervoise Bay	-	-	30:20	NS	38:12	***	7:6	NS	37:33	NS
Colpoys Point	99:93	NS	63:37	**	27:23	NS	16-33	*	28:40	NS
Totals	585:584	NS	501:496	NS	137:118	NS	119:186	***	307:345	NS

Table 3. Imposex and Relative Penis Size Index of *Thais orbita* in the Perth metropolitan area and on Rottnest Island from 1993 to 2019. Data for 1993 and 1998/99 adapted from Reitsema *et al.* (2003). RPSI was not measured in 2011.

Location	Imposex (%)					RPSI			
	1993	1998/99	2005	2011	2019	1993	1998/99	2005	2019
Rottnest Island									
Radar Reef	19	12	-	46	0	1	1	-	0
Armstrong Bay	50	17	-	28	0	4	2	-	0
Metropolitan coast									
Ocean Reef	47	13	-	-	0	4	1	-	0
Hillarys	81	28	21	28*	0	11	5	2	0
Trigg	87	93	-	11	0	9	5	-	0
Cottesloe	100	95	58	41	0	13	8	11	0
North Mole	-	100	-	-	0	-	57	-	0
South Mole	100	100	100	50	0	60	47	13	0
Woodman Point	100	100	100	33	5	37	33	19	1
South Jervoise Bay	-	100	100	0*	0	-	48	60	0
Colpoys Point	99	100	100	52	0	57	63	48	0

*Based on seven females.

Table 4. Male Penis Size Index (MPSI*) in *T. orbita*. Years and sites were selected where within a year, male snails had similar shell lengths but incidence of imposex in females at these sites was significant.

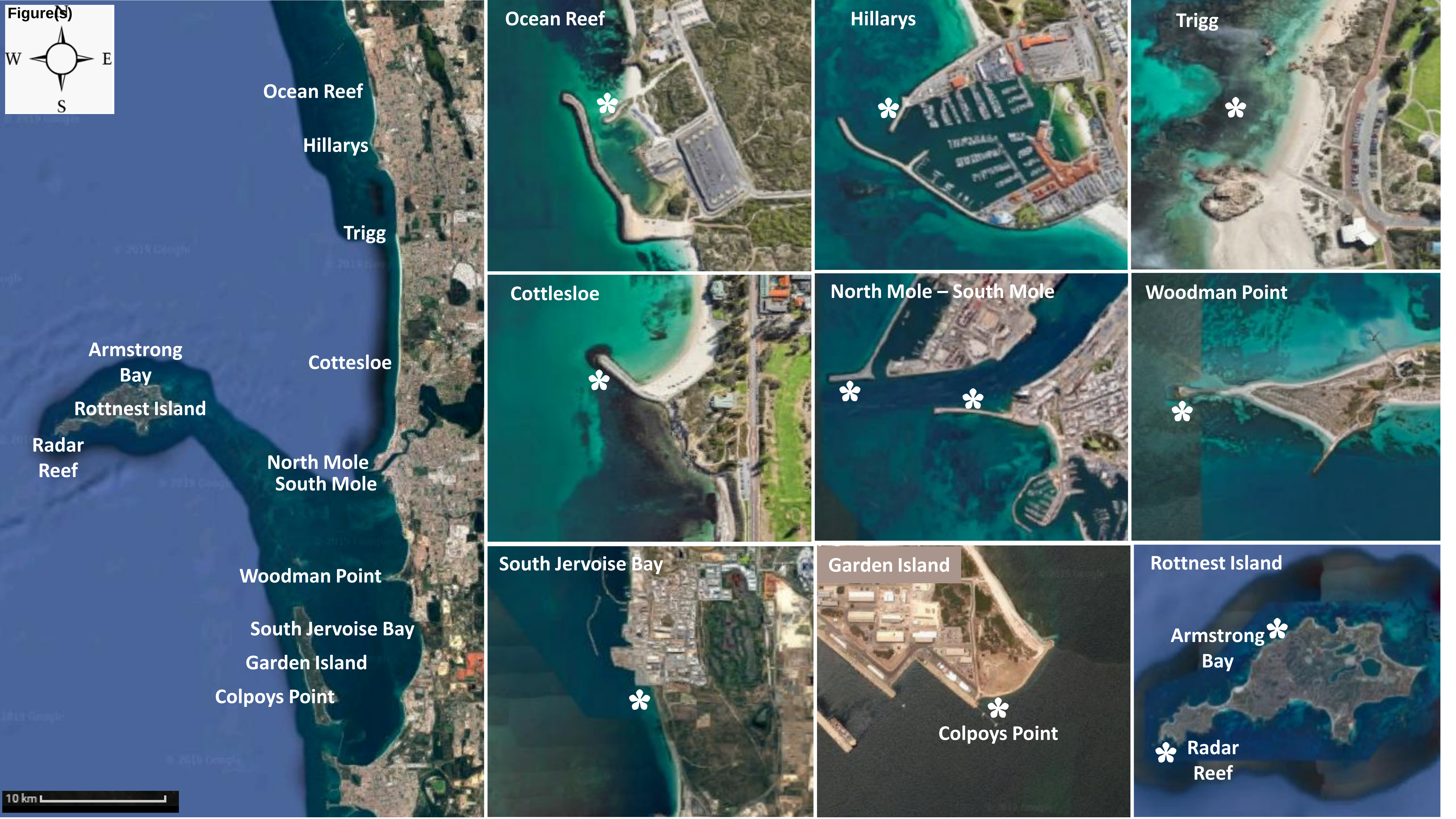
Year	Site	% imposex in females	MPSI (±SEM)	N	Statistical significance
2005	Colpoys Point	100	1.82± 0.07	27	
	Ocean Reef	30	1.79± 0.06	16	$p < 0.001$
	Hillarys	21	1.51± 0.06	21	
2007	South Mole	68	1.62± 0.04	25	
	Hillarys	14	1.73± 0.04	25	$p = 0.191$
	Ocean Reef	0	1.72 ± 0.06	19	

* MPSI calculated as (penis width/shell length) x 10

Table 5. Shell length (mm) of female *T. orbita* at sites with different levels of imposex. Different letters for shell lengths indicates a statistical difference.

Year	Site	% imposex in females	Shell Length (±SEM)	N	Statistical significance
2005	Colpoys Point	100	48.6 ± 1.01	23	
	South Mole	100	50.1 ± 2.27	24	$p = 0.106$
	Ocean Reef	30	44.2 ± 1.49	20	
2007	Colpoys Point	91	43.6 ± 1.11	22	
	South Mole	68	46.2 ± 1.81	25	$p = 0.638$
	Ocean Reef	0	46.4 ±1.53	31	
2009	Colpoys Point	80	45.7 ± 1.94[a]	20	
	South Mole	68	46.8 ± 1.66[a]	18	$p < 0.001$
	Ocean Reef	18	59.1 ± 0.71[b]	22	

Figure(s)


N
W
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Ocean Reef
Hillarys
Trigg
Armstrong Bay
Rottnest Island
Radar Reef
Cottesloe
North Mole
South Mole
Woodman Point
South Jervoise Bay
Garden Island
Colpoys Point
10 km
Ocean Reef
Hillarys
Trigg
Cottlesloe
North Mole – South Mole
Woodman Point
South Jervoise Bay
Garden Island
Colpoys Point
Rottnest Island
Armstrong Bay
Radar Reef

***Credit Author Statement**

CRediT Author statement:

Fred Wells: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration. **Monique Gagnon:** Conceptualization, Methodology, Investigation, Resources, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.