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Imposex in the City: A Survey to Monitor the Effects of TBT Contamination in Port Curtis, Queensland

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***Imposex in the City: A Survey to Monitor the Effects of TBT
Contamination in Port Curtis, Queensland***

Project CP20

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Table of Contents

NON-TECHNICAL SUMMARY	1
INTRODUCTION	2
MATERIALS AND METHODS	4
Sample collection.....	4
Statistical analyses	7
RESULTS	7
DISCUSSION	15
ACKNOWLEDGEMENTS	18
REFERENCES	18

List of Figures

Figure 1. Sites selected for an imposex survey in Port Curtis.	5
Figure 2. The mulberry whelk, <i>Morula marginalba</i>	6
Figure 3. Penis of male <i>M. marginalba</i> (P) located behind the right tentacle (arrowed). T = teste.	6
Figure 4. Measuring the male penis of <i>M. marginalba</i> from base of penis to base of flagella.	6
Figure 5. Imposex in <i>M. marginalba</i> with penis bud (arrowed). RT = right tentacle, VD = vas deferens.	8
Figure 6. Imposex in <i>M. marginalba</i> with penis bud (arrowed). RT = right tentacle, VD = vas deferens.	8
Figure 7. Imposex in <i>M. marginalba</i> with penis bud (arrowed). RT = right tentacle.	8
Figure 8. Imposex frequency in female <i>M. marginalba</i> at ten sites (1-10) in Port Curtis in 2003.	10
Figure 9. Frequency of imposex in <i>M. marginalba</i> at ten sites in Port Curtis in 2003 in relation to the major shipping channel.	11
Figure 10. Correlation of imposex frequency against arrays of shipping intensity (Inner, Middle and Reference)	12
Figure 11. Correlation of mean shell length (male and female)(mm) against distance from harbour/open ocean interface (km).	13
Figure 12. Correlation of mean male penis size (mm) against mean male shell length (mm).	14

List of Tables

Table 1. Site locations and shipping intensity in Port Curtis.	9
Table 2. Imposex grading system for <i>M. marginalba</i>	9

Table 3. Field collection data for *M. marginalba* in Port Curtis.....9

Table 4. Univariate ANOVA comparing arcsine transformed proportion of imposex females at Inner (sites 1,3,4,6), Middle (sites 2,5,8,9) and Reference (sites 7,10) arrays. ANOVA was almost significant ($p = 0.055$)..... 10

Table 5. Pearson product moment correlations between numbers of different variables. Significant correlations ($P < 0.05$) are outlined in bold. 12

Table 6. Univariate ANOVA comparing male and female shell lengths at all sites (1-10). Although data was log10 transformed, equal variances was not achieved and therefore untransformed data are presented. An *a posteriori* Tukeys HSD multiple range test was applied to locate differences between sites with sites not significantly different being joined by a common line. Sites are arranged in ascending order of mean shell length (mm)(in parenthesis)..... 13

Table 7. Univariate ANOVA comparing male penis lengths at all sites (1-10). An *a posteriori* Tukeys HSD multiple range test was applied to locate differences between sites with sites not significantly different being joined by a common line. Sites are arranged in ascending order of mean male penis length (mm)(in parenthesis). 14

Non-technical Summary

An imposex survey of the gastropod *Morula marginalba* (mulberry whelk) in Port Curtis was conducted as a bioindicator of tributyltin (TBT) contamination. Imposex is the imposition of male sexual characteristics (notably a penis) on female marine snails and occurs due to exposure to TBT. Reproductive failure and death of affected females can occur, with the eventual decimation of entire populations of severely affected snails. TBT is predominantly used as an antifouling paint on the hulls of vessels. However, since its development in the 1960's there have been a number of deleterious impacts on non-target organisms including the imposex phenomenon in whelks, which has led to global restrictions on the use of TBT. Port Curtis is a large multi-cargo facility in which TBT has previously been identified as a contaminant of concern. Imposex occurs at extremely low concentrations of ambient TBT contamination and as an alternative to difficult and expensive chemical analysis, it is considered a sensitive bioindicator of the effects of TBT exposure.

Over 1000 whelks were collected from ten selected sites in Port Curtis. As imposex is related to shipping intensity, sites were selected in the inner harbour (wharf sites) middle harbour (adjacent to shipping channels) and outer harbour (reference sites) to establish if there were site differences in the frequency and severity of imposex. The percentage of females affected at each site and the grade of imposex based on the length of the female pseudopenis, was recorded. Shell length and length of male penes at each site were also recorded.

Up to 43% of female snails at any one site were affected by imposex in Port Curtis. The prevalence was related to shipping intensity with a decreasing gradient of the number of affected snails from inner harbour to outer harbour. No imposex was recorded at the two reference sites. The incidence reported here is low in comparison to other Australian studies where up to 100% of snails were affected. The severity of imposex (based on grade) was also not severe in comparison to other studies and indicates that the degree of imposex in this population was in the early stages. The majority of penes did not form a measurable bud, with the largest pseudopenis of 2.5mm being smaller than the average male penis of 8.9mm.

Due to the mulberry whelks' ability to recruit juveniles from unaffected areas, there should be no effect on organism abundance due to reproductive failure caused by TBT exposure. The imposex survey demonstrated a relationship between exposure to a contaminant and the production of a sub lethal biological response. Although the intensity was not considered severe the sublethal effects of TBT on other populations of marine organisms in the port are unknown. However, as there is a proposed global ban on the use of TBT by 2008, the imposex phenomena is likely to decrease in intensity over time.

Introduction

Tributyltin (TBT) is a broad spectrum, biocide used to coat the bottom of ships to prevent sea-life from attaching to the hulls causing reductions in speed and decreasing fuel economy (de Mora, 1996). Developed in the 1960s, it is one of the most effective antifouling agents in use. However, it became apparent in the 1970s that leachates of organotin compounds were having deleterious effects on non-target organisms. Although highly variable, the half-life of TBT in seawater could be as short as six hours (Batley, 2000), but it remains bioavailable in sediments with degradation rates in the order of years (Batley, 1996). Due to mobilisation of sediments, TBT contamination may present as brief pulse events (with high biological impacts), which are easily missed by periodic water quality sampling (Foale, 1993).

The harmful effects of organotin compounds were recognised in 1989 by the International Maritime Organisation (IMO), of which Australia is a member. In 1990 the IMO recommended a ban on the use of TBT on vessels less than 25m in length as well as the elimination of all paints with a leaching rate of more than four μg of TBT per day. However, in 1999 the IMO adopted a resolution calling for a global ban on the application of all organotin compounds by 2003 and a complete prohibition by 2008 (International Maritime Organisation, 2002).

The term 'imposex' was first coined by Smith (1971) to describe the imposition of male characteristics on the female intertidal mud snail, *Nassarius obsoletus* and was subsequently linked to the presence of TBT (Smith, 1981). Bryan et al. (1986, 1987) later confirmed the association of imposex with TBT through a series of laboratory and field transplant studies with the gastropod, *Nucella lapillus*. Further surveys of other prosobranch neogastropods found the imposex phenomena to be widespread within gastropod populations exposed to TBT, including some Muricids in NSW (Wilson et al., 1993) and WA (Reitsema & Spickett, 1999). Although Alzieu (2000) noted that imposex had been described in over 72 species belonging to 49 genera, more species are being identified every year. Chemical analysis of TBT can be costly and poses some difficulties. Because imposex occurs at TBT concentrations in the parts per trillion range close to analytical detection limits, it is considered to be an extremely sensitive bioindicator of TBT contamination (Gibbs et al., 1987).

The penis in male neogastropods is situated behind the right tentacle (eye) with sperm being carried to it through a subsurface duct (vas deferens). Masculinization in female *Nucella lapillus* originally presents as the growth of a penis and vas deferens and proceeds in a dose-responsive fashion, with the degree of vas deferens and penis formation dependent on the ambient TBT concentration (Gibbs & Bryan, 1996). Apart from the percentage of the female population affected (imposex frequency), there are a number of indices that have been used to measure imposex. Comparisons based on penis length conveyed little idea of the contrast in mass between a small penis and a large penis. Bryan et al. (1986) therefore developed a quantitative measure of the degree of imposex in *Nucella lapillus*, based on the relationship that the weight or volume of the penis is related to the cube of its length. The relative penis size (RPS) index as it became known (Gibbs et al., 1987) for any given population is defined as the mean bulk of the female penis expressed as a percentage of the mean bulk of the male penis. The bulk of a penis is calculated by the cube of its' length.

The size of the female penis relative to that of the male was a convenient measure of the intensity of imposex in *Nucella lapillus* but gave no indication of reproductive competency. Gibbs and Bryan (1986) described three stages of imposex development; an 'early' stage involving the formation of a vas deferens and small penis, an 'intermediate' stage characterized by an enlarged female penis approaching the size of a male penis and a 'late' stage where there is blockage of the female opening preventing the release of egg capsules. In the latter stages reproductive failure and most likely premature death of the female occur. As the development of the vas deferens precedes that of the penis in *Nucella lapillus*, Gibbs et al. (1987) modified the above stages and developed the vas deferens sequence (VDS) index, which categorises six stages of imposex development. The above described indices or modified versions of them have since been accepted and used to measure imposex in other species worldwide (Liu et al., 1997, Tan, 1999, Ramon & Amor, 2001, Terlizzi et al., 2004).

The Port of Gladstone (Port Curtis) located 550km North of Brisbane is Queensland's largest multi-cargo port and the fifth largest port in Australia. It's major functions are to facilitate the export of resources from the Central Queensland region and to handle the import of raw material and the export of finished products from major industry in Gladstone. There are six wharfing complexes situated over 17km, accepting over 800 international and domestic merchant vessels per year (GPA, 2002). In addition the Port Curtis region supports a large recreational and commercial fishing and tourism industry, with a large number of smaller vessels also accessing the harbour and adjacent estuaries. Therefore TBT has the potential to be a likely contaminant of concern in Port Curtis.

The Port Curtis contaminant risk assessment team conducted an extensive systematic survey of contaminants in Port Curtis over a two-year sampling period, using a screening level ecological risk-assessment based approach (CRC Coastal Zone, in prep.). Mean water column TBT concentrations were above the trigger value of 0.006 µg Sn/L, but were much lower than concentrations in many world harbours. TBT concentrations were elevated in 13% of the 56 sediment samples analysed, but again were low compared to severely polluted harbours in other parts of the world. TBT was also found to have bioaccumulated in resident oysters, mud whelks and mud crabs from Port Curtis indicating exposure of these organisms. However, to date, risk assessment methodologies have had limited success in linking contaminant bioaccumulation with actual effects and potential risks to biota. An imposex survey of a gastropod species in Port Curtis could therefore establish links between contaminant exposure and biological effects.

Morula marginalba Blainville (1882) commonly known as the "mulberry whelk" (Wilson, 1994) and its close relative *Morula granulata* have been previously used as bioindicators of TBT contamination in Australia (Wilson et al., 1993, Reitsema & Spickett, 1999). *M. marginalba* (Order Neogastropoda, Family Muricidae) is the major carnivorous predator of macro-invertebrates in the mid-intertidal zone of rocky shores on the Australian east coast (Moran, 1985). The whelk gains access to the flesh of shelled prey by drilling a small hole using its radula. Although barnacles, limpets, snails and oysters preferentially formulate the majority of the whelks' diet, almost any species of shelled animal is potential prey (Fairweather & Underwood,

1991). As *M. marginalba* is extremely abundant in Port Curtis, it appeared to be a suitable species to determine the distribution and severity of imposex in Port Curtis.

Materials and Methods

Sample collection

Sampling of whelks (*M. marginalba*) at ten sites (Figure 1) was conducted over a two-day period in October 2003. Sites were designed in an array fashion with increasing distance from major shipping activity. Four wharf sites were selected in the inner harbour area adjacent to the main shipping channel where the most intense shipping activity occurs (Inner array). Three sites were selected in the middle harbour area equidistant from the main shipping channel (Middle array). A third site adjacent to a minor shipping channel in the north entrance was added to the Middle array. Originally three sites were chosen in the outer harbour area (Reference), however whelks were absent at one of these sites preventing its inclusion in the design. The GPS location of each site and the approximate distance to and the intensity of the nearest major shipping activity were recorded.

Where possible, at least 100 whelks were collected by hand from each site and placed on ice in zip locks bags for transport to the laboratory, where they were frozen until examined for imposex. The identity of a sub-sample of whelks was verified as *Morula marginalba* by Queensland Museum (Figure 2). After measuring shell length, a vice was used to crack open the shell allowing removal of the soft tissue. Whelks were examined under a dissecting microscope at 40X and 80X magnification. Sex was determined primarily by the presence of yellow to cream coloured ovaries for females and bright orange testes for males.

The male penis in *Morula marginalba* has a flattened T shape with a single flagellum attached (Figure 3). The penis length of male whelks from the base of the penis to the base of the flagella in a semi extended position (non-flaccid) was recorded (Figure 4). Imposex in females was determined by the presence of a penis and/or vas deferens behind the right tentacle. Two indices were used in this study when measuring imposex at each site: 1. the percentage of females affected at each site; and 2. the grade of imposex in females as described below. Penis length was difficult to measure in females due to the small size and non-uniform shape of the female penis. Observations on penis length and vas deferens development were made and a grading system developed which reflected the extent of penis development. Where possible the length of the penis in females was recorded.

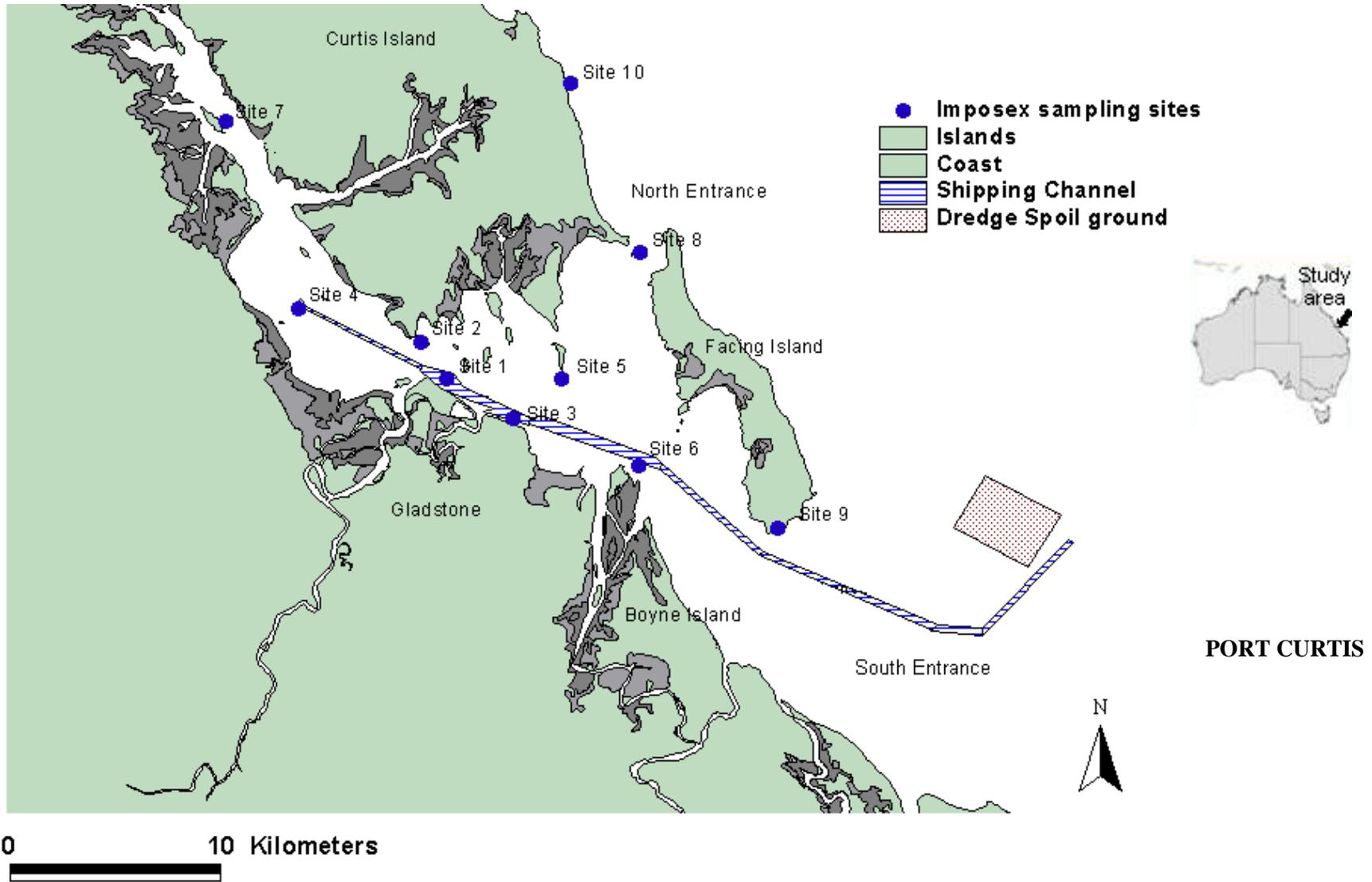


Figure 1. Sites selected for an imposex survey in Port Curtis.



Figure 2. The mulberry whelk, *Morula marginalba*.

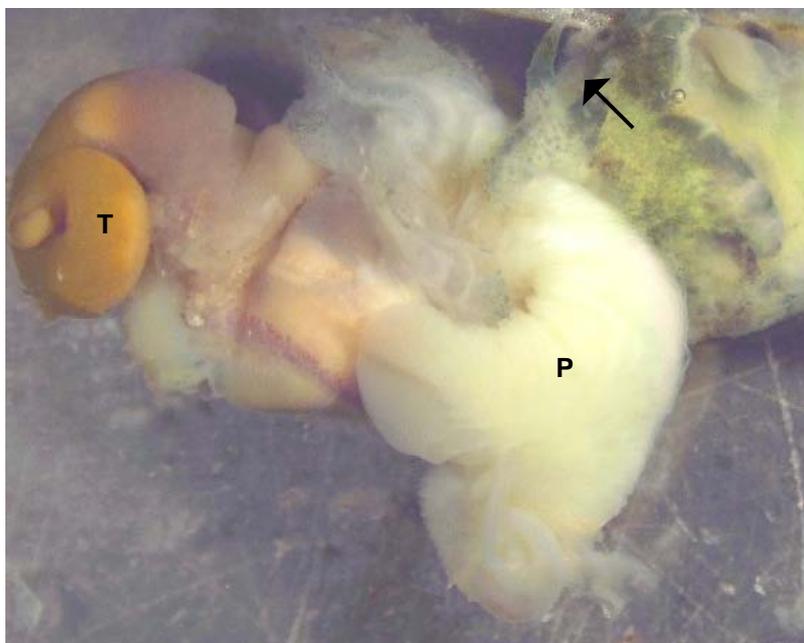


Figure 3. Penis of male *M. marginalba* (P) located behind the right tentacle (arrowed). T = teste.



Figure 4. Measuring the male penis of *M. marginalba* from base of penis to base of flagella.

Statistical analyses

In the first instance the Chi-square goodness of fit statistic was used to determine if there was a difference in the proportion of imposex females between sites. Secondly, sites were grouped into the three arrays (Inner, sites 1,3,4 and 6; Middle, sites 2,5,8 and 9 and Reference, sites 7 and 10). Prior to analysis data were arcsine transformed. The mean proportion of imposex for each array was used as a replicate to test for differences between arrays in a Univariate Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure with Type III sums of squares, as recommended for unbalanced designs. Tukeys HSD multiple range test was used to locate differences between levels where a significant main effect was recorded. Univariate ANOVA using the GLM procedure and subsequent Tukeys range test was also performed to test for differences in shell length between sites and male penis length between sites. Despite \log_{10} transformation of data, homogeneity of variances could not be achieved for shell length. As ANOVA is a fairly robust procedure (Zar, 1999), results of untransformed data are presented.

Pearson product moment correlation was performed between male shell length and male penis length; imposex frequency and grade of imposex; imposex frequency and female shell length; grade of imposex and female shell length; imposex frequency and distance from major shipping channel; imposex frequency and array of shipping intensity; distance from harbour/ocean interface (defined as the sea bottom, oceanic drop off point) and male shell length, and male shell length and male penis length, to determine if relationships exist between the pairs of variables. Results of significant relationships were plotted.

Results

The arrays to which sites were assigned according to the type, tonnage and frequency of vessels, at or in the vicinity of that site are shown in Table 1. A number of female *M. marginalba* were found to exhibit the imposex phenomena (Figure 5, 6, and 7) and the extent of the condition graded according to the grading system outlined in Table 2. There was a difference among sites in the proportion of females with imposex (Chi-squared (χ^2) = 101.2, df = 9, $p < 0.0001$). Subsequent combinations of Chi-squared analyses pair-wise and group-wise determined that Sites 1,2,5,7,8,9 and 10 had the lowest incidence of imposex and were not significantly different from each other. There were a significantly higher proportion of females with imposex at Sites 3 and 4, which were not significantly higher than Site 1. Site 6 had the highest incidence of imposex (Chi-squared (χ^2) = 17.10, df = 2, $p < 0.0001$)(Figure 8). No imposex was found at the Reference sites. A summary of the field collection data is tabulated in Table 3.

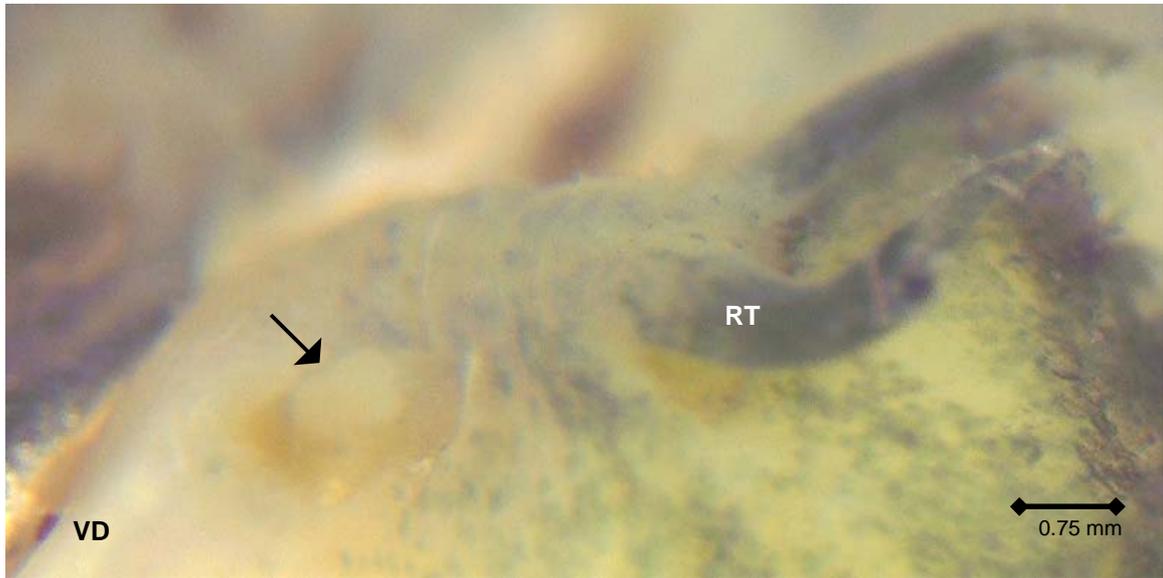


Figure 5. Imposex in *M. marginalba* with penis bud (arrowed). RT = right tentacle, VD = vas deferens.

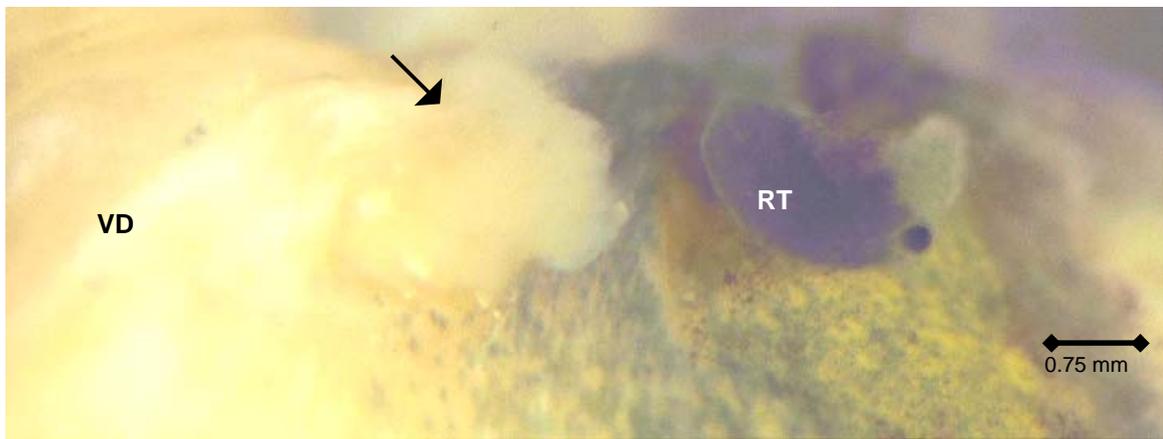


Figure 6. Imposex in *M. marginalba* with penis bud (arrowed). RT = right tentacle, VD = vas deferens.

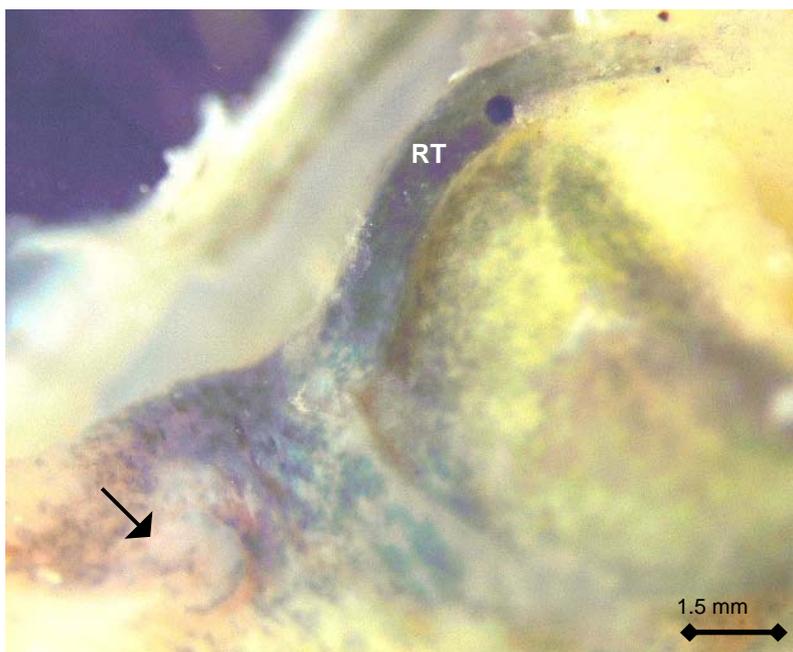


Figure 7. Imposex in *M. marginalba* with penis bud (arrowed). RT = right tentacle.

Table 1. Site locations and shipping intensity in Port Curtis.

Site	Location	GPS	Array	Shipping intensity/type
1	Clinton Coal wharf (CCW)	23° 49 100 151° 14 224	Inner	Major large vessels
2	Tide Island	23° 48 153 151° 13 596	Middle	Minor all vessels
3	Tug berth	20° 50 102 151° 15 936	Inner	Major large vessels
4	QCL berth	23° 47 369 151° 10 440	Inner	Major large vessels
5	Quoin Is.	23° 49 144 151° 17 144	Middle	Minor all vessels
6	BSL berth	23° 51 288 151° 19 143	Inner	Major large vessels
7	Worthington Is.	23° 42 626 151° 08 590	Reference	Occasional small vessels
8	Rat Island	23° 45 913 151° 19 167	Middle	Minor small/medium vessels
9	Gatcombe Head	25° 52 890 151° 22 656	Middle	Minor all vessels
10	Blackhead	23° 41 646 151° 17 396	Reference	Occasional small vessels

Table 2. Imposex grading system for *M. marginalba*.

Grade	Female penis description
1	<0.5 mm raised discoloured area up to a bump (with or without vas deferens) but unable to be measured
2	0.5 mm - <1.0 mm
3	1.0 mm - <2.0 mm
4	2.0 mm and greater

Table 3. Field collection data for *M. marginalba* in Port Curtis.

Site	Location	Number Examined	% females	% imposex	Mean grade imposex	Mean shell length (mm ± SD)	Mean male penis length (mm ± SD)
1	Clinton Coal wharf	100	55	9	1	22 ± 1.3	10.4 ± 1.4
2	Tide Island	100	60	2	1	26 ± 2.4	10.4 ± 1.0
3	Tug berth	100	69	17	1.2	20 ± 1.1	7.8 ± 1.2
4	QCL berth	100	65	17	1.1	29 ± 2.4	11.0 ± 0.7
5	Quoin Is.	100	63	3	1	21 ± 1.7	8.7 ± 1.3
6	BSL berth	91	86	43	2.4	19 ± 3.4	6.0 ± 0.9
7	Worthington Is.	50	62	0	0	30 ± 2.7	10.6 ± 1.1
8	Rat Island	100	51	4	2.5	18 ± 1.8	7.4 ± 1.3
9	Gatcombe Head	100	57	5	2	16 ± 1.4	7.9 ± 1.4
10	Blackhead	100	66	0	0	17 ± 1.4	8.7 ± 1.2

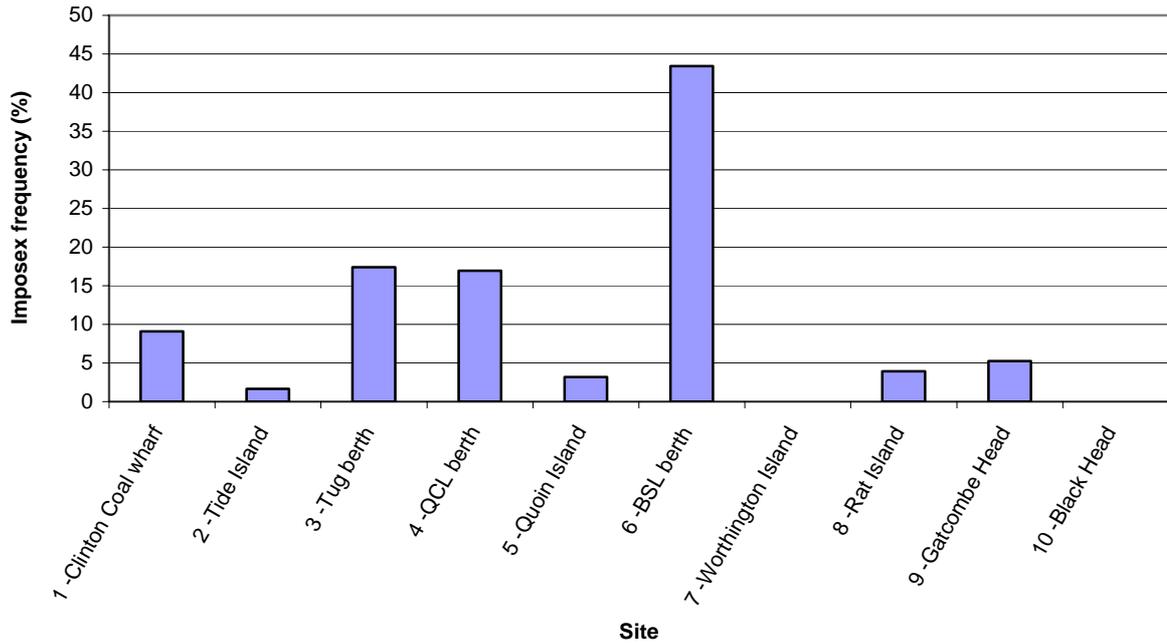


Figure 8. Imposex frequency in female *M. marginalba* at ten sites (1-10) in Port Curtis in 2003.

Sites were grouped according to increasing distance from the majority of shipping intensity: Inner (all wharf sites 1,3,4 and 6), Middle (sites 2,5,8 and 9) and Reference (sites 7 and 10). Univariate ANOVA using the GLM procedure was performed to determine if there was a significant difference between the three groups. The difference between groups was almost significant ($p = 0.055$), with an obvious gradient of decreasing incidence of imposex from inner to outer harbour sites (Table 4)(Figure 9). There was also a significant positive relationship between imposex frequency and the array of shipping intensity (Table 5, Figure 10), but not between imposex frequency and distance to the major shipping channel.

Table 4. Univariate ANOVA comparing arcsine transformed proportion of imposex females at Inner (sites 1,3,4,6), Middle (sites 2,5,8,9) and Reference (sites 7,10) arrays. ANOVA was almost significant ($p = 0.055$).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.561E-02	2	4.781E-02	4.501	0.055
Intercept	6.571E-02	1	6.571E-02	6.186	0.042
Site	9.561E-02	2	4.781E-02	4.501	0.055
Error	7.435E-02	7	1.062E-02		
Total	.275	10			
Corrected Total	.170	9			

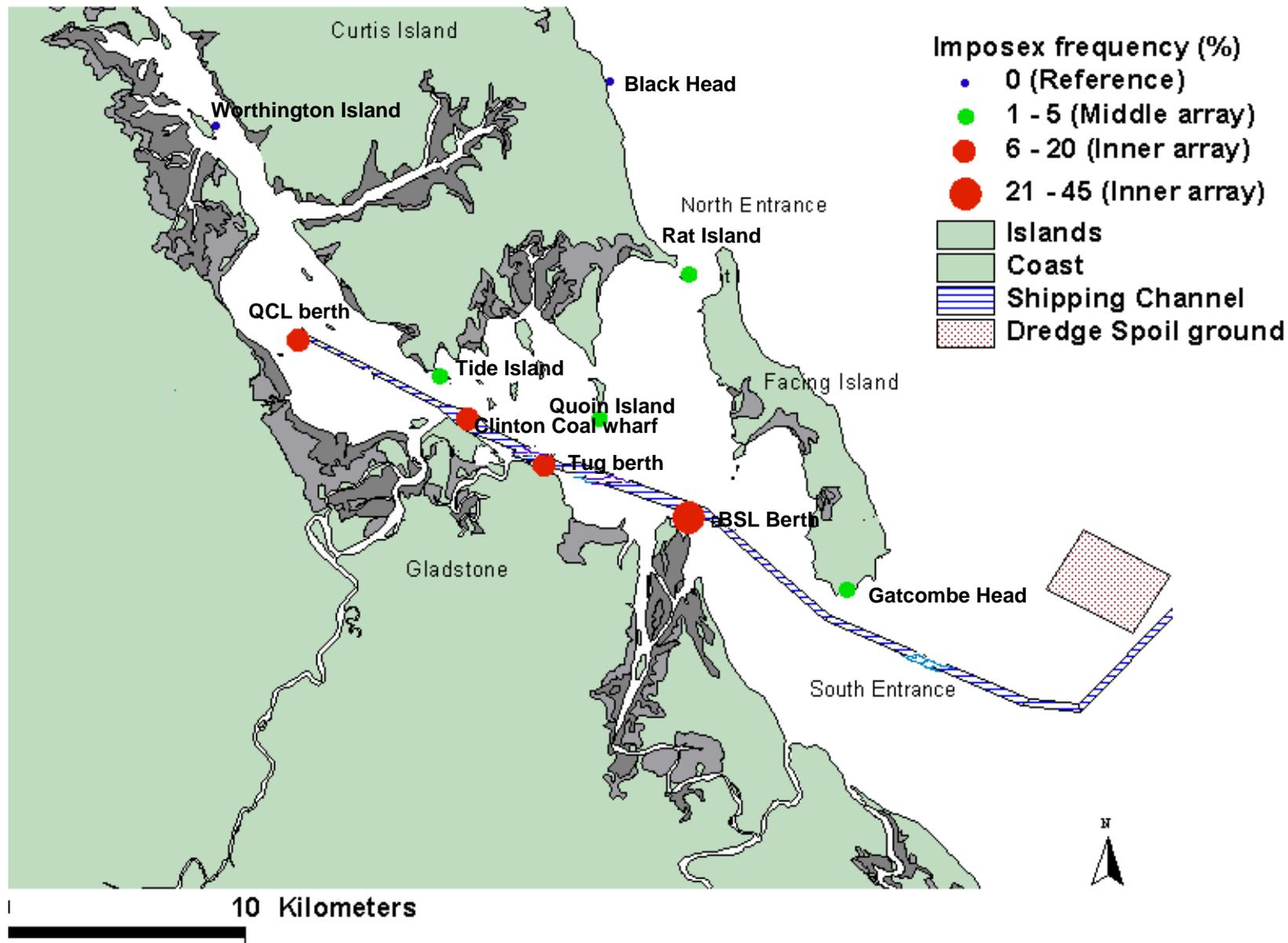


Figure 9. Frequency of imposex in *M. marginalba* at ten sites in Port Curtis in 2003 in relation to the major shipping channel.

In the majority of cases the grade of imposex was not severe. The highest grade of imposex was seen at Site 6 (BSL wharf) where the highest imposex (43%) was also recorded. The largest female penis measured at this site was 2.5 mm. Although only two and three snails were affected at sites 8 and 9 respectively, the mean grade of imposex was fairly high (2.5 and 2.0 respectively). There was no significant correlation between imposex frequency and grade of imposex, or female shell length (Table 5). Nor was there a significant relationship between grade of imposex and female shell length (Table 5).

Table 5. Pearson product moment correlations between numbers of different variables. Significant correlations ($P < 0.05$) are outlined in bold.

Variable	Correlation coefficient (r)	p
Imposex frequency (%) vs. Grade of imposex	0.530	0.115
Imposex frequency (%) vs. Female shell length	-0.148	0.684
Grade of imposex vs. Female shell length	-0.474	0.166
Imposex frequency (%) vs. Distance to major shipping channel	-0.482	0.158
Imposex frequency (%) vs. Array of shipping intensity	-0.705	0.023
Male and female shell length vs. Distance to harbour/open ocean interface	0.922	<0.0001
Male shell length vs. Male penis length	0.820	0.004

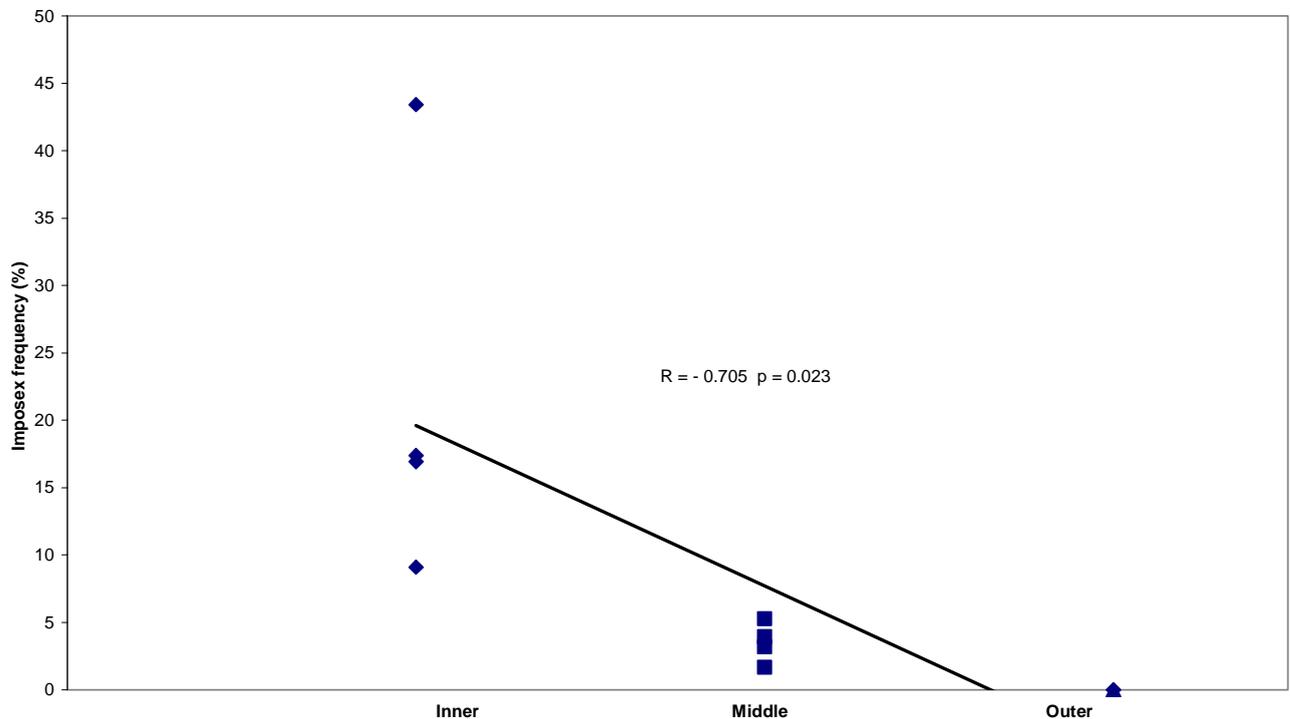


Figure 10. Correlation of imposex frequency against arrays of shipping intensity (Inner, Middle and Reference)

There was a highly significant difference in shell length between sites with subsequent post hoc tests demonstrating an obvious gradient of increasing shell size from oceanic to the more estuarine sites (Table 6)(Figure 11). Shell length was highly correlated with distance from the harbour/open ocean interface (Table 5)(Figure 11). There was also a significant difference in male penis size between sites (Table 7), which was related to male shell length (Table 5)(Figure 12).

Table 6. Univariate ANOVA comparing male and female shell lengths at all sites (1-10). Although data was log10 transformed, equal variances was not achieved and therefore untransformed data are presented. An *a posteriori* Tukeys HSD multiple range test was applied to locate differences between sites with sites not significantly different being joined by a common line. Sites are arranged in ascending order of mean shell length (mm)(in parenthesis).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	18382.27	9	2042.48	489.64	0.000
Intercept	421130.63	1	421130.63	100956.84	0.000
Site	18382.27	9	2042.48	489.64	0.000
Error	3883.57	931	4.17		
Total	445138.00	941			
Corrected Total	22265.84	940			

Tukeys HSD Multiple Range Test										
Site	9	10	8	6	3	5	1	2	4	7
	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(26)	(29)	(30)

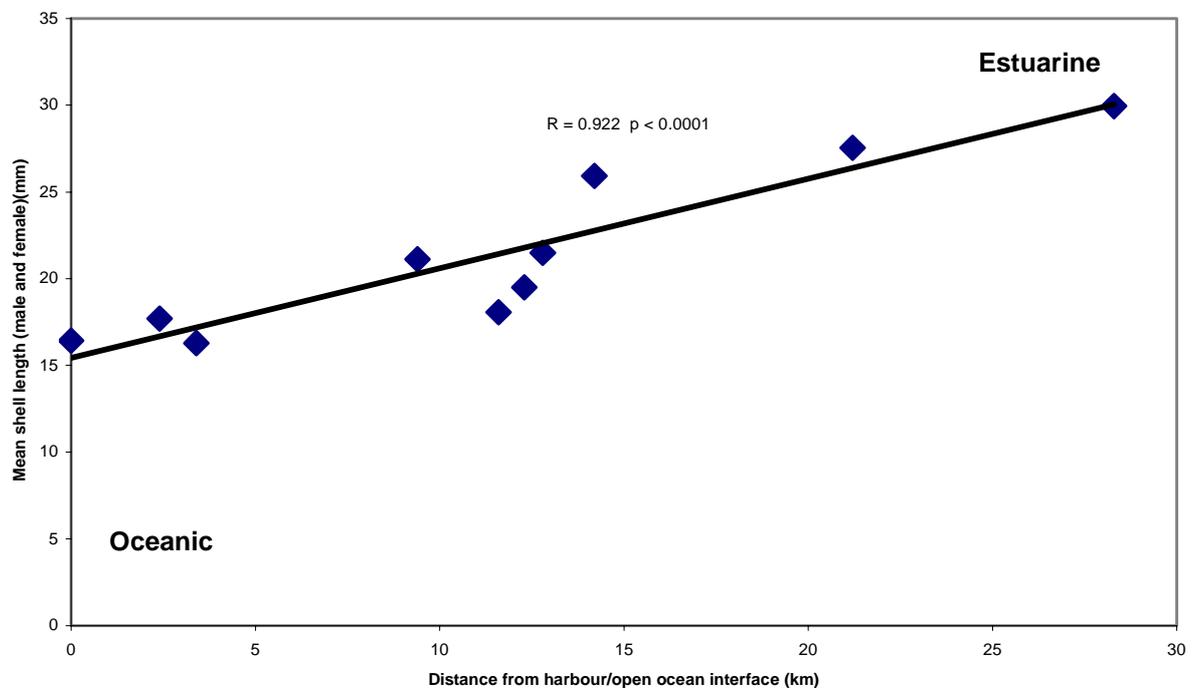


Figure 11. Correlation of mean shell length (male and female)(mm) against distance from harbour/open ocean interface (km).

Table 7. Univariate ANOVA comparing male penis lengths at all sites (1-10). An *a posteriori* Tukeys HSD multiple range test was applied to locate differences between sites with sites not significantly different being joined by a common line. Sites are arranged in ascending order of mean male penis length (mm)(in parenthesis).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	709.27	9	78.81	54.80	0.000
Intercept	24033.87	1	24033.87	16711.45	0.000
Site	709.27	9	78.81	54.80	0.000
Error	486.10	338	1.44		
Total	29069.25	348			
Corrected Total	1195.37	347			

Tukeys HSD Multiple Range Test										
Site	6	8	3	9	10	5	2	1	7	4
	(6.0)	(7.4)	(7.8)	(7.9)	(8.7)	(8.7)	(10.4)	(10.4)	(10.6)	(11.0)

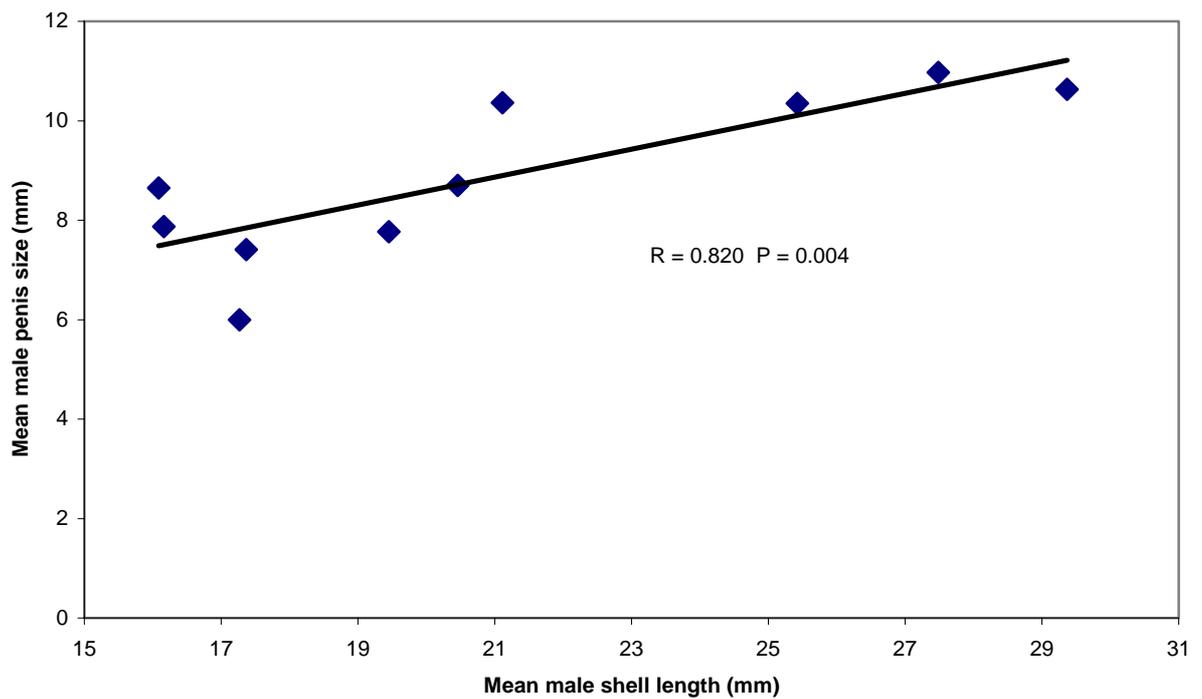


Figure 12. Correlation of mean male penis size (mm) against mean male shell length (mm).

Discussion

The results of this study clearly show that *M. marginalba* in Port Curtis are affected by imposex and that the incidence is related to shipping intensity. A significant positive relationship was established between imposex frequency the shipping intensity array to which sites were assigned. Wilson et al. (1993) found a similar result for this species on the east coast of NSW, where there was a correlation between the amount of boating activity (high, medium and low) and the degree to which the population were affected. Reitsema and Spickett (1999) also noted a correlation between distance to nearest vessel activity and imposex in *M. granulata*, a very close relative of *M. marginalba*. The relationship has been supported by the majority of imposex surveys conducted world wide, especially those undertaken prior to or shortly after 1990, when TBT use was banned on vessels <25m (Bryan et al., 1987, Foale, 1993, Gibbs & Bryan, 1996, Reitsema & Spickett, 1999).

Although there was a gradient of decreasing imposex from inner to outer harbour sites (ANOVA was almost significant at the 5% level), the imposex frequency did not relate to number of vessels or tonnage at individual wharf sites. The highest imposex frequency (43%) was found at BSL berth (Site 6), which averaged 39 vessels totalling 473 000 tonnes per year for the last ten years. Clinton Coal wharf (Site 1) averaged 295 vessels totalling 25 837 528 tonnes per year for the last ten years, yet the imposex frequency was only 9%. The average number of vessels at each of the major three wharves (CCW, BSL and QCL) has steadily increased, nearly doubling over the last ten years (Gladstone Port Authority, pers. comm.). However, the discrepancy in the imposex relationship could be related to the specificity of operations of individual wharves. There are no imports at CCW, which only exports coal. BSL imports liquid pitch and petroleum coke but only exports aluminium (CRC Coastal Zone, in prep.). The country of origin of vessels and that country's compliance with the IMO's recommendations on TBT use would influence the imposex/shipping intensity relationship.

Although leaching of antifouling paint on vessels at wharves is likely to be a major source of organotin pollution, shipyard activities such as hull painting, slipways and paint removal offer an alternate source. A large number of non-merchant vessels also access other parts of the harbour including the northern harbour entrance. Therefore it is not surprising to find imposex at sites other than commercial moorings, indicating the widespread contamination of TBT. This could explain the relatively high (4%) imposex frequency at Rat Island (Site 8) adjacent to the northern entrance, which serves as a passage to trawlers, island ferry services and supply barges > 25m in length, that are still legally able to use TBT.

Some subsequent re-surveying studies (post TBT ban) in Australia have noted an overall trend for decline in either imposex frequency and/or severity in major ports and coast sites since the introduction of the ban (Gibson & Wilson, 2003, Reitsema et al., 2003). The majority of recovery in terms of imposex expression appeared to be at more oceanic sites where the predominant vessel activity consisted of vessels < 25m in length, indicating the effectiveness of the legislation restricting TBT use to vessels over 25m. However, there was a general decline in the severity of imposex (RPSI) at the more commercial sites (vessels >25m) despite an overall increase in vessel tonnage. Reitsema et al. (2003) suggested that as the life expectancy of the species he surveyed (*T. orbita*) could be up to 10 years, some animals in the second survey could

have been alive at the time of the original survey, therefore confounding resurvey results. Combined with the irreversibility of the condition (Foale, 1993, Gibbs & Bryan, 1996, Oehlmann et al., 1998), this would make for slow recovery of populations. Perhaps an imposex survey of Port Curtis in the early 90's would have produced different results.

The incidence reported here (0-43%) is low in comparison to previous studies carried out in Australian waters of this and other species in which up to 100% of females were affected (Foale, 1993, Wilson et al., 1993, Reitsema & Spickett, 1999, Gibson & Wilson, 2003). Port Curtis is a fairly young harbour in comparison to some of the other ports surveyed, which may explain the lower prevalence. A higher frequency would most likely have occurred in the Gladstone Marina where some of the highest water and sediment TBT concentrations have been previously found (CRC Coastal Zone, in prep.). Unfortunately no whelks were available to sample in this location. Reitsema and Spickett (1999) found a similar frequency of imposex (0-57%), for *M. granulata* in a survey of the Dampier Archipelago, Western Australia, the largest tonnage port in Australia.

Generally, however, the intensity of imposex appears to be much lower in *Morula* species than other species assessed (Wilson et al., 1993, Liu et al., 1997). Species differences in the sensitivity to (Wilson et al., 1993, Tan, 1999) and the bioaccumulation of (Liu et al., 1997) TBT have been demonstrated in other surveys. Liu et al. (1997) found that the imposex symptom was much more severe in *Thais* species than *Morula* despite similar organotin burdens and suggested a genus-specific susceptibility to organotin pollution with the ranking order of *Nucella*, *Thais* and *Morula*. Differences in habitat (e.g., high-shore versus low-shore species), diet and physiology have been suggested for interspecific differences in imposex (Tan, 1999).

The majority (58%) of penes did not form a bud, but were small raised areas deemed too difficult to measure, indicating the degree of imposex was in the very early stages. The largest penis in this study was 2.5 mm, which is similar to the findings of Reitsema and Spickett (1999) for *M. granulata*. However, the pseudopenis size in this study was small compared to the average male penis length ($8.9\text{mm} \pm 1.9\text{ SD}$). There was a significant difference in shell length between sites with smaller snails being located closer to more oceanic sites. Moran et al. (1984) demonstrated that differences in growth and longevity within a single site are caused by differences in diet. That is, the availability of suitable or favoured prey will affect the growth rate and survival of whelks at a particular site. Male penis size was related to male shell length and therefore there was also a significant difference in male penis size between sites. The relationship between penis size and shell size has been observed in male whelks in other studies (Gibbs et al., 1987, Tan, 1999). Tan (1999) noted that due to this dependency RPS indices could potentially be under- or over-estimated depending on the mean shell size of a population sampled. As female pseudopenis length was not related to shell size, the author suggested that this might be a more accurate measure of imposex than RPSI.

Wilson et al. (1993) calculated the degree of imposex in this species using a modified version (penis area) of the RPS index and found the indices to correlate well with the percentage of females affected at each site. The index relies on an accurate measurement of the male penis and was originally based on the uncomplicated

structure of the male penis of *N. lapillus*. The coiled, flagellated, elastic penis of many tropical species makes measurement of penis area difficult and time consuming. Due to time constraints and considering the degree of imposex was in the very early stages, RPS index was not attempted in this study. Vas Deferens Sequence (VDS) was found not to be a reliable index of imposex in *Thais orbita* in Australia (Foale, 1993), and so was also not applied in this study. No correlation was established between the percentage of females affected and grade of imposex used in this study. Perhaps use of the RPS index may have elucidated a stronger relationship. However, Reitsema and Spickett (1999) in their survey of *M. granulata* concluded that given the complexities of other indices such as RPS index and VDS, incidence of occurrence was a suitable index to measure early stages of imposex in Morula.

The decline of *N. Lapillus* in populations severely affected by imposex has been attributed to a reduction in recruitment caused by a decrease in reproductive capacity (Bryan et al., 1986). This gastropod does not have a planktonic larval stage and apart from the small number of juveniles that may reach other sites via pieces of floating debris, maintenance of a population relies solely on its ability to reproduce. *M. marginalba*, however, has a long term planktonic larval stage (Underwood, 1974), allowing recruitment of individuals from other locations. Therefore complete decimation of this species at severely affected sites is unlikely. It has been suggested that imposex develops due to an accumulation of testosterone (Gibbs & Bryan, 1996). Although the severity of imposex in this study was in the early stages and reproductive failure was unlikely to have occurred in Morula, the potential of a sublethal effect of TBT on the efficiency of reproduction in this and other Port Curtis marine organisms is unknown.

Reproductive failure has been linked to TBT in other invertebrates. The French Pacific oyster (*Crassostrea gigas*), farming industry was almost decimated in the late 1970s due to failed spatfall and shell malformations caused by TBT (Alzieu et al., 1989, Alzieu, 1991). Other adverse biological effects of TBT have included genitalia malformations in the male gastropod *Bolinus brandaris* (Ramon & Amor, 2001), immunosuppression in the cultivated clam, *Tapes philippinarum* (Cima et al., 1999) and cytoskeletal alterations in the colonial ascidian, *Botryllus schlosseri* (Cima & Ballarin, 2000). Although TBT compounds are known to produce a variety of pathological conditions, none has been demonstrated to rival the sensitivity of the imposex response in gastropods (Gibbs & Bryan, 1996).

In conclusion imposex was found to be present in *M. marginalba* collected from Port Curtis demonstrating a relationship between exposure to a contaminant and the production of a sub lethal, biological response. Although related to shipping intensity, the frequency and grade of the imposex condition were not severe in comparison to other port surveys. Due to the ability of this species to recruit juveniles from unaffected locations, conservation of the species is highly likely despite the effects of imposex. Other more TBT sensitive species such as *Thais* and those with non-planktonic larval stages may have shown different results. Globally, the condition is likely to slowly improve with the introduction of further restrictions on the use of TBT. Further surveys sometime after the abolition of TBT in 2008, should produce promising results.

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