

Phenotypic variability associated with the occurrence of imposex in *Odontocymbiola magellanica* from Golfo Nuevo, Patagonia

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The increasing occurrence of imposex in marine gastropods has been related to their exposure to the tri-substituted organotin compounds tributyltin (TBT) and triphenyltin (TPhT), which have been widely used as antifouling agents, and has been documented for more than 200 gastropod species. *Odontocymbiola magellanica*, a large benthic neogastropod, showed 85–100% imposex near harbours with high marine traffic in Patagonia. We evaluated if, as predicted by energy allocation hypothesis, females with imposex (FWI) were in average larger and/or heavier than normal individuals, and if there were differences in shell morphometry associated with imposex, using both univariate and multivariate approaches. We detected differences in two morphometric variables, but no overall effect in shell shape, and ~10% of reduction in body weight in FWI. In addition, the multivariate index of size suggested a positive effect on FWI, in contrast with univariate analyses showing no differences in shell length. The present results demonstrate that responses to TBT vary among gastropod species and that energy allocation hypothesis may be useful only under certain conditions.

INTRODUCTION

Molluscs are effective models for the study of ecological impacts of pollutants due to their ubiquity and extreme sensitivity to anthropogenic inputs (Rittschof & McClellan-Green, 2005). In particular, the increasing occurrence of imposex in gastropods has specifically been related to the exposure of the tri-substituted organotin compounds tributyltin (TBT) and triphenyltin (TPhT), which have been widely used as antifouling agents, and has been documented for more than 200 gastropod species (Shi et al., 2005). The occurrence of imposex is an excellent indicator of the exposition to TBT, achieving lower detection limits than the analytical chemistry (Strand et al., 2006).

Odontocymbiola magellanica (Gmelin, 1791) is a large benthic neogastropod, distributed along the Atlantic and Pacific coast of South America. It has been reported from off Rio de la Plata (35°S) to the Magallanes Strait reaching the Chiloé Island in the Pacific coast of Chile (42°S). Records from the Malvinas Islands are also available (Weaver & du Pont, 1970; Gallardo & Penchaszadeh, 2001). *O. magellanica* inhabits sandy bottoms or mixed bottoms of gravel and mud or sand in Golfo Nuevo, being easily reached by SCUBA diving. In addition, it is an edible mollusc which is consumed in Patagonian coasts (Ciocco, 1999), and has been mentioned as a potential fishery resource for the artisanal fleet in Patagonia (Lasta et al., 2000; Bigatti & Ciocco, 2006). This species shows between 85% and 100% imposex near harbours with

high marine traffic in Puerto Madryn, Golfo Nuevo, while in zones with low marine traffic the imposex is null (Bigatti & Penchaszadeh, 2005).

While there is a fair amount of studies reporting the occurrence of imposex among different species, effects of exposition to TBT on shell shape and somatic growth have been seldom reported. Adverse effects on shell formation were reported for the bivalve *Crassostrea gigas* (Dyrynda, 1992). Leung et al. (2001) found differences in the growth rate of the dogwhelk *Nucella lapillus* between sites with contrasting pollution levels. Crothers (1998) mentioned effects on shell shape of *N. lapillus*, whereas Plejdrup and co-workers (2006) related genetic and morphological variation for this species, finding the largest phenotypic variation in shell size in a population of *N. lapillus* from a polluted area.

Son & Hughes (2000) suggested that if sexual allocation is depressed by imposex, a corresponding increase in somatic allocation may be expected. Since sexual and somatic functions compete for allocation from a limited energy budget (Sibly & Calow, 1986), somatic allocation in excess of maintenance requirement is expressed as body growth. The incidence of imposex can thus be expected to correlate with somatic production and shell size. According to these authors, females with imposex are predicted to (a) show a higher somatic production and (b) achieve a larger size than unaffected animals. However, this prediction is supported only by observations made on *N. lapillus*, and there is a paucity of information on other gastropods. Further testing of the above

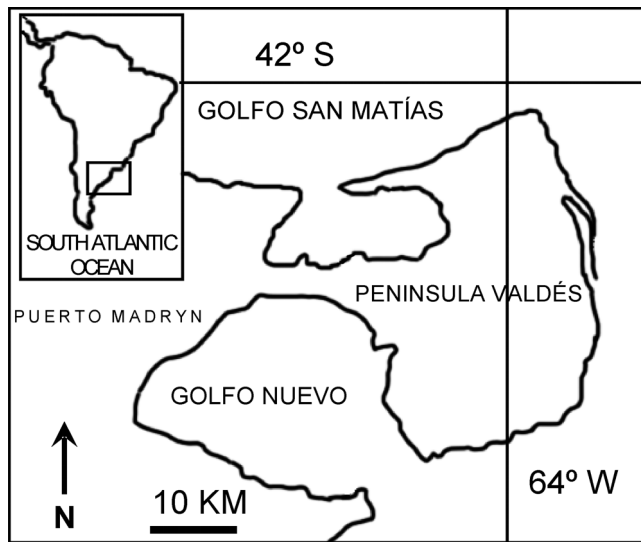


Figure 1. Map of the Patagonian coast showing the study area.

predictions in other species is thus necessary to derive general principles of the ecological effect of TBT on marine gastropods. In this paper, we tested these predictions analysing phenotypic variability in size, growth patterns, shell shape and somatic production associated with the presence of imposex in *O. magellanica* from Patagonian waters.

MATERIALS AND METHODS

Sampling

To our end, specimens of *O. magellanica* were collected from 10 stations in Golfo Nuevo, one station in Golfo San José and one station from Golfo San Matías (Argentina) during 2000–2004 (Figure 1). Animals were collected by SCUBA diving by hand. As this species lives buried in sand the juveniles were not found, so we use the mature

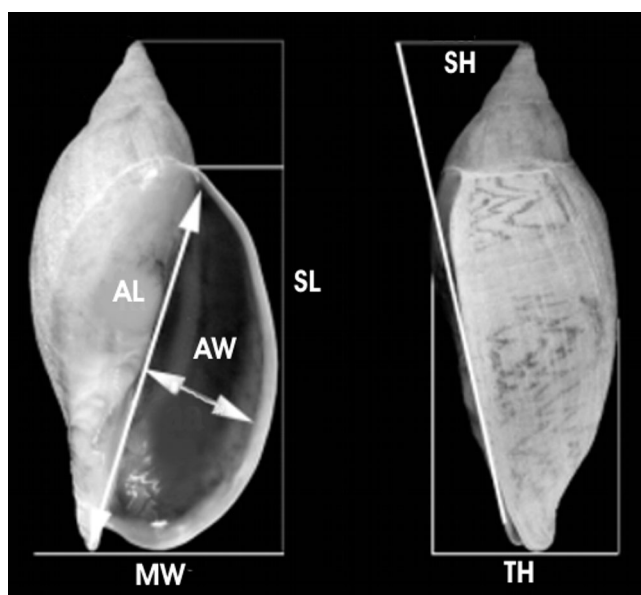


Figure 2. Measurements taken for statistical analysis: shell length (SL), maximum width (MW), aperture width (AW), aperture length (AL) spire height (SH) and total height (TH).

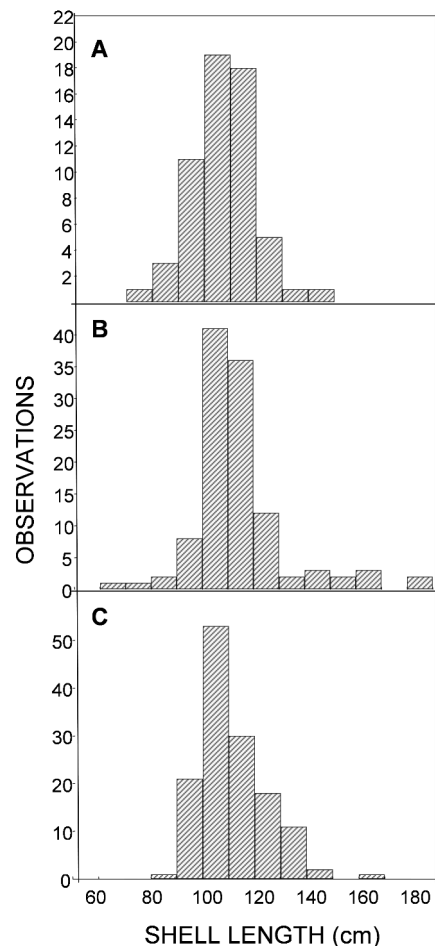


Figure 3. *Odontocymbiola magellanica*. Size-frequency distribution of FWI (A), females (B) and males (C).

individuals (i.e. > 70 mm) following Bigatti et al. (2006). Specimens were classified as males, female and females with imposex (FWI). For each specimen, we measured six shell characters to the nearest 0.1 mm using a calliper. These were shell length (SL), maximum width (MW), aperture width (AW), aperture length (AL) spire height (SH) and total height (TH; Figure 2). Body weight (without shells) was also measured in fresh individuals.

Data analyses

Differences in mean size between males, females and FWI were examined by means on a one way-analysis of variance (ANOVA) and complemented by the examination of the length frequency distributions (LFDs). However, since size cannot be measured unambiguously in any single dimension, we also adopted a multivariate approach based on PCA, which summarizes in the first two principal components most of the variation in shell morphology, with PC 1 correlated with size and PC 2 with shape (Jolicoeur, 1963; Carranza, 2005). Factor coordinates of cases for PC 1 and PC 2 were also compared by means of ANOVA.

In addition, we looked for differences in shell shape, by comparing differences in the mean of each morphometric variable relative to shell length between individuals of both sexes and FWI by means of an ANCOVA test. The same approach was utilized to detect differences among

Table 1. Summary of weight data for males, females, and females with imposex (FWI). Number of individuals, mean values, standard deviation, upper (UCL) and lower (LCL) 95% confidence limits are shown. Females and males did not differ in body weight (Tukey Unequal N HSD, $p < 0.01$).

	Mean (g)	SD	95% LCL	95% UCL	N
Females	74.13	1.72	70.73	77.52	110
Males	69.31	1.54	66.26	72.36	136
FWI	52.61	2.39	47.90	57.32	57

Table 2. Results of the multivariate allometry analysis. Allometric coefficients for the morphometric variables and Upper (UCL) and Lower (LCL) 95% confidence limits are shown. See abbreviations in text.

Measurement	Coefficient	FWI		Coefficient	Females		Coefficient	Males	
		LCL (2.5%)	UCL (97.5%)		LCL (2.5%)	UCL (97.5%)		LCL (2.5%)	UCL (97.5%)
SL	0.793	0.711	0.864	0.868	0.790	0.909	0.733	0.674	0.788
MW	1.095	1.014	1.158	1.045	0.891	1.105	1.066	0.999	1.128
AL	0.909	0.802	0.989	0.795	-3.875	1.088	0.838	0.759	0.898
AW	1.307	1.227	1.387	1.359	1.052	5.904	1.274	1.176	1.372
SH	0.935	0.696	1.282	0.924	0.783	1.401	1.072	0.864	1.277
TH	0.959	0.807	1.055	1.007	0.672	1.068	1.016	0.873	1.164

groups in somatic production (wet weight) using shell length as covariate. For the analysis, data were log-transformed in order to reach variance homogeneity and normality of residuals, and checked for statistically significant differences among groups. When the variances were not homogeneous after transformation, we followed Underwood (1997) and adjusted the critical levels to a value equal to the P -value for variance heterogeneity.

Differences in growth patterns between groups were examined by means of multivariate methods. Allometric coefficients for the six variables measured were calculated using PAST Software following Jolicoeur (1963) with extensions by Kowalewski (1993) and Kowalewski et al. (1997). To this end, data were log-transformed and subjected to PCA, and the first principal component (PC 1) was regarded as a size axis. The allometric coefficients were thus estimated by dividing the PC 1 loading for that variable by the mean PC 1 loading over all variables. Estimation of 95% confidence intervals for the allometric coefficients was made by bootstrapping specimens (2000 bootstrap replicates were made), and the 2.5 and 97.5 percentiles of the resulting set of confidence intervals of each of the parameters were calculated. Confidence limits that include 1.0 indicate isometric scaling, while those above or below 1.0 indicate positive and negative allometry, respectively. This multivariate statistical analysis is a more complete descriptor of shape variation than, and provides statistical and conceptual advantages over the standard bi-variate techniques commonly used.

RESULTS

A total of 302 specimens of *O. magellanica* were sampled between September 2000 and July 2004. These were classified as females (N=109), males (N=136) and FWI (N=57). As expected from the method used to collect the animals, shell length ranged from 70 to 188 mm.

Somatic production and size

Body weight significantly differed between groups (ANCOVA: $F_{(2,299)}=4.59$; $P < 0.05$); for the same shell length, males and females were heavier than FWI, the latter showing a 10% reduction in body weight compared with normal individuals. Post hoc test showed that females and males did not differ in body weight (Tukey Unequal N HSD, $P < 0.01$). Multivariate size (as summarized by PC 1; $F_{(2,305)}=3.18$, $P < 0.05$) but not shell length ($F_{(2,305)}=3.50$, $P < 0.03$; critical α : 0.007) differed significantly among groups, with FWI averaging higher cases scores on PC 1.

The size-frequency distribution for each group was constructed based on the shell length of specimens (Figure 3). All groups showed a continuous size structure. Males, females and FWI peaked at 100 to 110 mm; the females population reached larger sizes (up to 190 mm), whereas males reached 160 mm. No FWI grew larger than 140 mm.

Shape and growth

The principal component analysis (PCA) revealed that PC1 accounted for 74.69% of the variance of the model, while PC 2 accounted for 12.85% and PC 3 for 7.2% of the total variance. PC 1 was dominated by variance attributable to SL and MW (20% of the variance in both cases), while PC 2 was strongly influenced by variance attributable to aperture width (AW; 62%).

For all three groups, all morphometric variables were linearly related with shell length after log transformation, indicating that as the snails increase in length, all other variables increase. Non significant deviation for isometric growth was found in TH for FWI, MW and TH for females and SH, TH y MW for males. Positive allometric growth relationships were demonstrated for AW in all groups and for MW in FWI and females (Table 1).

Maximum width, aperture width and total height did not differ among groups (ANCOVA: MW $F_{(2,304)}=0.73$, $P=0.48$; AW $F_{(2,304)}=2.16$, $P=0.12$; TH $F_{(2,304)}=0.04$, $P=0.96$). Aperture length ($F_{(2,304)}=3.18$, $P=0.043$) and spire height ($F_{(2,304)}=3.229$, $P=0.04$), on the other hand, showed significant differences between groups. Overall, analysis of variance of PC 2 showed no significant differences in shape among groups ($F_{(2,305)}=1.36$, $P=0.26$).

DISCUSSION

We detected some differences in shell shape and body weight associated with the presence of imposex in the examined population of *O. magellanica*. Shell size did not increase significantly in FWI, while somatic production was depressed as observed in the mean body weight. This did not support the energy allocation hypothesis, suggesting that the trends observed in *N. lapillus* are not likely to be extrapolated to other gastropods. Son & Hughes (2000) weighed empty shells, while we weighed soft parts only. Nevertheless, as these authors recognize, shell growth is broadly correlated with somatic production in marine gastropods. However, the multivariate index of size (PC 1) showed higher average values for FWI, suggesting that there may be a positive effect on size, not reflected in an increase in shell length.

Divergences in shape were found exclusively when comparing particular shell dimensions among groups (AL and AH), but not when analysing multivariate shape (PC 2), suggesting that, in despite of the significant differences found in the univariate analysis, differences in shape attributable to TBT are negligible.

Contrary to the predictions, we did not detect that imposex becomes more frequent at size increase. The population of this species inhabiting nearest Puerto Madryn harbour in Golfo Nuevo have been exposed to TBT at least since the creation of the harbour in the middle 70's. Since the estimated age of individuals used in this study ranged from 8 to 18 years (Bigatti et al., 2006), the exposition to TBT is presumed to have been permanent since hatching: in this vein, the lack of FWI above 140 mm may be reflecting a higher mortality rate for these individuals.

Differences in body weight were detected between imposex individuals and both male and female specimens. This can be interpreted as a decrease of individual fitness associated with the exposition to pollutants. This is in agreement with the finding of Leung et al. (2001) that reported lower weights for *N. lapillus* at polluted sites. An increase in somatic production may occur at low or moderate levels of pollution, but a high degree may lead to a decrease in somatic production with sublethal and even lethal effects due to the overgrowth of the vas deferens, which blocks the vulva and prevents the egg capsules being released (Galante-Oliveira, 2006).

Some shell features, such as AL and SH differed among groups, the latter being significantly higher in FWI when compared with male and females. On the other hand, differences in growth patterns were not detected as differences in the allometric trajectories of morphometric variables, in comparison with normal male and female individuals: all variables that displayed negative or positive allometry or isometric growth in imposex individual

did the same in both normal males and females. The exception was MW, than in males did not differ from isometric growth, while being positive allometric in FWI and females. This observed differences in shell growth between imposex and normal individuals could also be attributed to hormonal disarrangements caused by the TBT exposure. Lee (1991) reported that molluscs have low cytochrome P-450 content and mixed function oxygenase activity which results in TBT accumulation, because of the low TBT metabolism rate. He also suggested that the effects observed in FWI could be related to the binding of TBT metabolites to cellular proteins and the inhibition of detoxifying enzyme systems, cytochrome P-450 systems and glutathione S-transferases, by TBT. All of the effects are hormonally regulated and TBT and hormones share common metabolic pathways. Although the differences between imposex and non imposex individuals can be attributable to hormonal disarrangements that affect the individual fitness, further studies are needed in order to ascertain the causality of this relationship and discard stochastic phenotypic plasticity not associated with contaminant effects.

The application of paintings with organotins (OTs) to ship hulls has been banned in many countries and its use on vessels of less than 25m in length is forbidden. In Argentina, the ordinance 4/98 of the Environment Protection Secretary (DPMA 1998), prohibits the application of tributyltin on sports boats (Cledón et al, 2006), but the enforcement of these laws is difficult and the effects of newly developed paintings have not been tested yet on the marine biota. This is particularly relevant, since in Puerto Madryn *O. magellanica* is habitually used for human consumption, commonly found in restaurants and sold in domestic markets (Ciocco et al., 1999). The reduction of body weight of approximately 10% could lead to economical losses if these marine gastropods are commercially exploited in the future (Bigatti and Ciocco, 2006). Concentrations of OTs in bodies of *O. magellanica* exposed to TBT must therefore be tested in order to determine the toxicity of these compounds to humans and if the differences in body weight reported in this work could be attributed to TBT contamination.

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