CSIRO PUBLISHING

Marine and Freshwater Research
http://dx.doi.org/10.1071/MF15233

Shell shape as indicator of pollution in marine gastropods affected by imposex

M. A. Primost[A], *G. Bigatti*[A,B] *and F. Márquez*[B,C,D]

[A]Laboratorio de Reproducción y Biología Integrativa de Invertebrados Marinos (LARBIM)–Instituto de Biología de Organismos Marinos (IBIOMAR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Boulevard Brown 2915 (U9120ACD), Puerto Madryn, Argentina.
[B]Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Boulevard Brown 3100, Puerto Madryn, Argentina.
[C]Laboratorio de Ecología y Evolución de Invertebrados Marinos (LEEIM)–IBIOMAR, CONICET, Boulevard Brown 2915 (U9120ACD), Puerto Madryn, Argentina.
[D]Corresponding author. Email: fede@cenpat-conicet.gob.ar

Abstract. Tributyltin (TBT) and other pollutants are present in Patagonia, and are associated with maritime traffic and human activities. Cause–effect relationship between TBT and imposex development in females of marine gastropods is well documented. We tested whether the imposex incidence associated with TBT pollution is related to detectable shell-shape variations in the edible marine snail (*Buccinanops globulosus*). We compared shell shape of *B. globulosus* in six contiguous sites on a gradient of imposex and maritime traffic, by using geometric morphometric analysis. Our results indicated that the registered differences in shell shape are associated to imposex incidence in the harbour zone where previous works have detected TBT pollution. Gastropods from areas of high maritime traffic presented a rounded shell with a shorter spire, and a smaller relative size of the shell aperture, whereas the opposite shape (fusiform shape, elongated-spired shell and bigger relative size of the shell aperture) occurred in gastropod shells from areas of low maritime traffic. Shell variation registered here could be useful to detect TBT pollution in populations of *B. globulosus* and another neogastropod species.

Additional keywords: geometric morphometrics, marine pollution, organotin compounds, Patagonia, TBT, tributyltin.

Received 17 June 2015, accepted 23 September 2015, published online 15 December 2015

Introduction

Many chemical compounds that are toxic for the organisms have been banned after exploring their deleterious effects. The relationship between malformation of the body of marine gastropods and the presence of tributyltin (TBT) in the environment has been widely validated (Alzieu *et al.* 1986; Gibbs and Bryan 1986; Schulte-Oehlmann *et al.* 1998). In this context, TBT contained in antifouling paints was banished in France, after registering shell deformations in *Crassostrea gigas*, a bivalve with high economic values (for review see Alzieu 1986), and TBT pollution was detected as the responsible for such alterations (Waldock and Thain 1983; Alzieu *et al.* 1986; Scammell *et al.* 1991; Dyrynda 1992; Waldock *et al.* 1996). It is well known that TBT produces reproductive alterations such as imposex development in gastropods females (Gibbs and Bryan 1987) and intersex in gastropods and bivalves (Oehlmann *et al.* 1998; Chesman and Langston 2006); also other aberrations, such as chambering and a decrease in juvenile growth in bivalves (Chagot *et al.* 1990; Ruiz *et al.* 1994) and shell-shape variation in gastropods (Bigatti and Carranza 2007; Márquez *et al.* 2011), have been registered.

The geometric morphometrics (GM) is defined as the fusion of geometry and biology (Bookstein 1982), studying shape in a two- or three-dimensional space and its co-variation with other variables. This approach allows describing and comparing shapes of organisms or particular structures (Rohlf and Marcus 1993) and analysing the size and shape separately (Zelditch *et al.* 2004). During the past two decades, the use of GM became popular among, for example, biologists, anthropologists and physicians. Recently, Márquez *et al.* (2015) reviewed the principal use of this method in several marine gastropod species. The principal topics of variation studies using GM were intra- and interspecific, allometric shift, taxonomy and adaptation. Heat, water loss, wave shock and presence of predators are the principal factors influencing morphological changes in gastropod shells (Levinton 2001). The shell-shape variation was used as an indirect measurement of the effect of pollutants on the organisms. As far as we know, only two studies have focussed on shell-shape variation owing to pollution in marine gastropods.

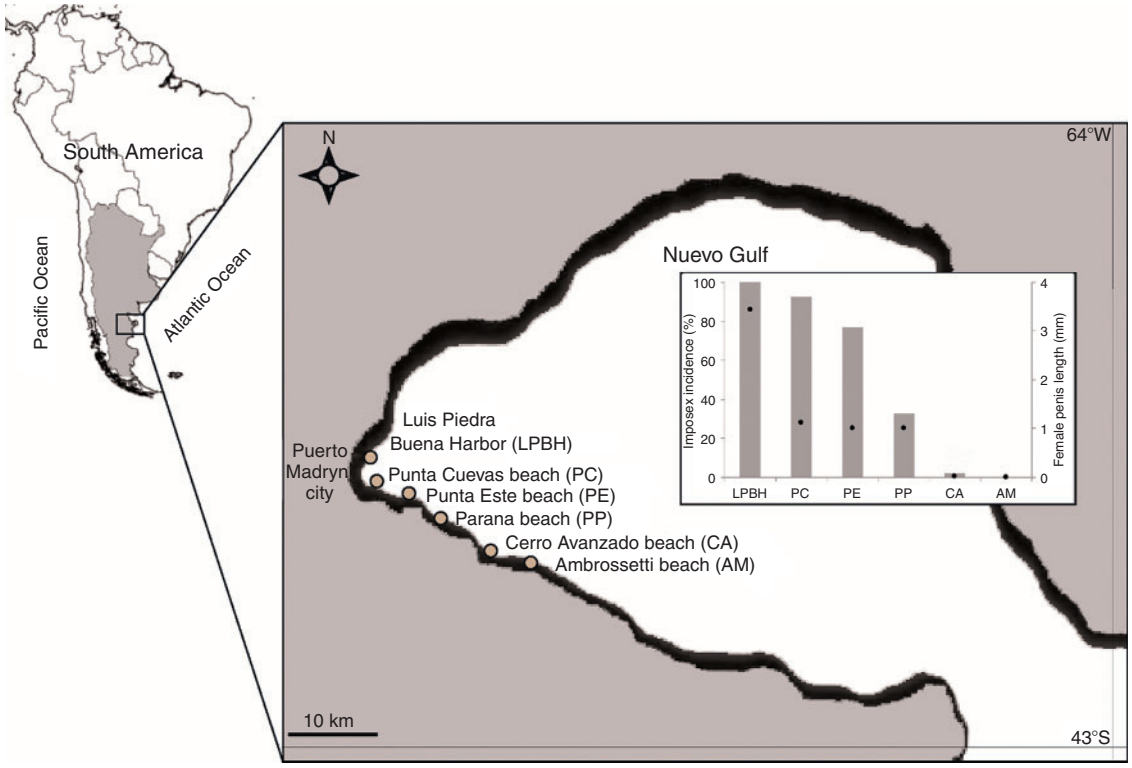

Fig. 1. Sampling sites in Nuevo Gulf and imposex parameters in *Buccinanops globulosus*. Gray bars show the imposex incidence (%) and black dots the female penis length (FPL) for each sampling site.

On one hand, Piñeira *et al.* (2008) evaluated the genetic impact of the Prestige oil spill on wild populations of *Littorina saxatilis* by using molecular microsatellites and amplified fragment length polymorphism loci and quantitative-trait genetic variation in embryo shells. On the other hand, Márquez *et al.* (2011) assessed morphometric alterations on *Odontocymbiola magellanica* inhabiting polluted and non-polluted environments, as an indicator of pollution or environmental changes. The use of shell shape to indicate pollution is important because it could allow the use of dead specimens with hard exoesqueleton presenting malformation owing to environmental contamination such as shell chambering and shell-growth anomalies (Alzieu *et al.* 1986; Chagot *et al.* 1990; Alzieu 2000).

The gastropods of the genus *Buccinanops* (d'Orbigny, 1841) are endemic of the south-western Atlantic Ocean. *B. globulosus* is present in Nuevo Gulf, inhabiting sandy- or muddy-bottom shallow waters usually up to 10-m depth (Pastorino 1993). The species lives most of the time buried in the sediment (Scarabino 1977) and reproduces by intra-capsular development without a free larval phase. Several species of the genus have been affected by imposex phenomenon (Penchaszadeh *et al.* 2001; Bigatti *et al.* 2009; Averbuj and Penchaszadeh 2010). An extensive study in Patagonia determined the imposex incidence in all harbour areas and areas of high maritime traffic (Bigatti *et al.* 2009). *B. globulosus* presented a high imposex incidence in areas of high marine traffic, and has been classified as a good indicator of the presence of TBT in the environment, with imposex development evident even at low environmental concentrations (Bigatti *et al.* 2009). This species is also an expanding resource for artisanal fisheries in northern Patagonia, with landings of up to 9 t year^{-1} (Narvarte 2006; Averbuj *et al.* 2014). Although morphometric differences between males and females, and among populations of *B. globulosus*, have been detected in Patagonia (Avaca *et al.* 2013), shell-shape alteration as a consequence of chronic exposure to toxic compounds has not been explored yet.

The aim of the present work was to explore, by GM methods, whether shell-shape variation exists in *B. globulosus* affected by imposex related to maritime traffic and TBT pollution.

Materials and methods

Sampling

The gastropods were collected from six sites with similar environmental conditions and geomorphology (Rechimont 2011; Pontones 2014; Márquez *et al.* 2015) in Nuevo Gulf, Patagonia (Fig. 1), within a restricted area of 20 km. The main difference among sampled sites was the maritime traffic that lead to different pollution of TBT and other contaminants such as polyaromatic hydrocarbons (PAHs) and trace metals (Gil *et al.* 2006; Commendatore and Esteves 2007; Massara Paletto *et al.* 2008; Torres *et al.* 2013). These sites included two areas of high marine traffic near Puerto Madryn city (harbour (LPBH) and Punta Cuevas (PC) beach). In the harbour area, ~720 vessels per year are present because of commercial and recreational activities (APPM 2013). The rest of the sampling sites were Punta Este (PE) and Parana (PP) beaches with medium maritime traffic, in which only sport boats are

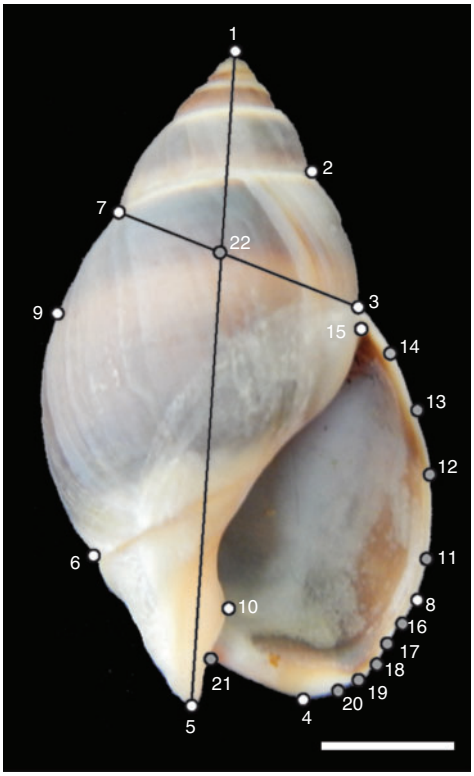

Fig. 2. Configuration of landmarks (white dots) and semi-landmarks (grey dots). Landmarks: 1, apex; 2, 7, penultimate anfract sutures; 3, 6, final lap of anfract; 4, final extreme of labium; 5, end of siphonal channel; 8, 9, tangential landmarks on lateral shell; 10, starting point of siphonal channel; and 15, starting point of shell aperture. Semi-landmarks: 11–14 and 16–21, on apertural and siphonal channel; 22, intercept between the line from 1 to 5 and the line from 3 to 7. Scale: 1 cm.

present, and two areas of low maritime traffic (Cerro Avanzado (CA) and Ambrossetti (AM) beaches), where only small boats are present occasionally because of sport activities. The gastropods were collected by SCUBA diving and using bait traps during low tides.

Morphometric data

Only adult females (23–48 mm in total length) were analysed. The individual shell form was obtained by photographs of the apertural shell view. Shells were fixed to a plasticine base to prevent the pitching and rolling effect and to match the height of the scale (Zelditch *et al.* 2004). Pitching refers to the rising and falling movement in the anterior–posterior direction along the transverse axis, and rolling refers to the rotation along the longitudinal axis (dorsal–ventral direction). It is important to check that these effects do not occur when the picture is taken because they cannot be mathematically standardised and they can influence the dataset. To capture shell shape, we defined a configuration of 11 landmarks and 11 semi-landmarks (Fig. 2). All specimens were digitised by one observer (PMA) using a TPS dig2 software (Rohlf 2004*a*). Semi-landmarks were digitised on outline curves of the shell, for which the exact location on the curve cannot be identified and, hence, is statistically estimated. We used the sliding landmark algorithm for this purpose, which minimises the bending energy, a measure of local shape difference, between each individual and the sample average (Gunz *et al.* 2005; Gunz and Mitteroecker 2013). Effect of rotation, translation and scale were eliminated by Procrustes analysis (Rohlf and Slice 1990) by using TPS Relw software (Rohlf 2004*b*). The Procrustes coordinates of the aligned individuals were then used as shape variables to perform the multivariate statistical analyses by MorphoJ software (Klingenberg 2011).

Imposex

Sex in females was determined on the basis of the presence or absence of sexual accessory glands (albumen, capsule and pedal glands). Males lack these glands and present a conspicuous penis. In a subsample of 160 females, we determined the imposex incidence (percentage of females with imposex) and the mean female penis length (FPL), which was measured with a 0.1-mm precision digital calliper.

Data analysis

Female penis length was compared between sites by Kruskal–Wallis test with $P < 0.05$ significance and Dunn contrasts (Infostat statistical software, Di Rienzo *et al.* 2012). The centroid size was used as a proxy for shell size and was calculated for each specimen as the square root of the sum of the squared deviations of landmarks from the centroid (Bookstein 1991; Zelditch *et al.* 2004).

To evaluate the independence between the shape (Procrustes coordinates of the aligned individuals) and size (centroid size) variables (allometric effects), we conducted a multivariate regression (Bookstein 1991; Klingenberg 2011).

To assess whether sites were significantly different, as well as to visualise the distance between the average shapes of each site, we calculated a hierarchical cluster analysis with statistical inference and single linkage (Florek *et al.* 1951) based on the Mahalanobis distances (Valdano and Di Rienzo 2007). Also, to recover an axis of maximum discrimination among sites, a canonical variate analysis (CVA) was performed. Finally, a multivariate regression between average Procrustes coordinates of the aligned individuals from each site (as an independent variable) and imposex incidence (as a dependent variable) was performed by MorphoJ software (Klingenberg 2011).

Results

Imposex

Females from areas of high maritime traffic (LPBH and PC) showed the highest values of imposex incidence and FPL (Fig. 1). Females from CA and AM showed the lowest values. Significant differences in FPL among sites were detected ($K = 117.197$, $P < 0.0001$, $n = 160$). A maximum value for FPL was 6.31 mm in the LPBH site. No penis development was observed in AM beach and only a *vas deferens* development in one female was observed in CA beach, where FPL could not be calculated.

Morphometric data

In total, 313 females of the gastropod *Buccinanops globulosus* were analysed (LPBH = 50, PC = 53, PE = 59, PP = 49, CA = 52 and AM = 50). The multivariate regression of shape

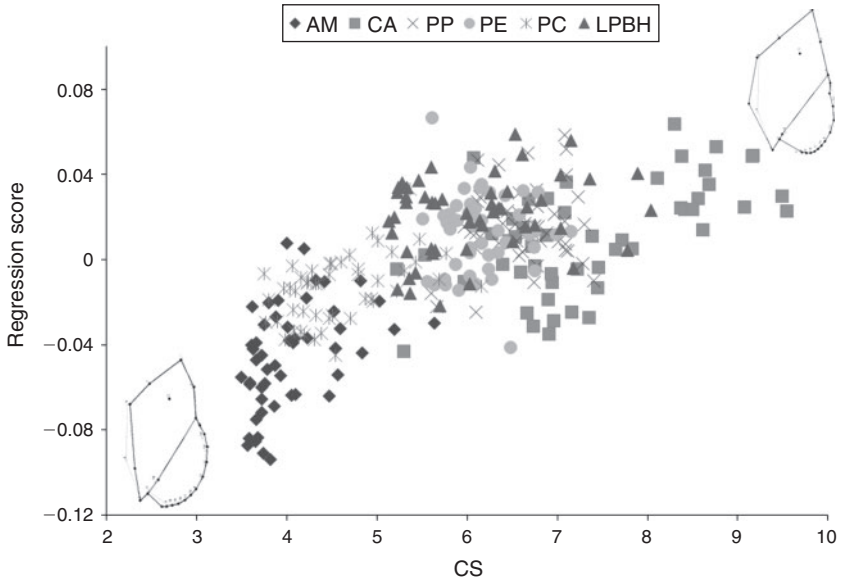

Fig. 3. Shape scores as a function of centroid size, illustrating static allometric growth in females of *Buccinanops globulosus*. Shapes at the opposite extremes of the range of allometric variation are presented by using wire-frame graphs, showing the average shell shape; the change from the grey circles to the black dots indicates the predicted landmark shift corresponding to an increase (right) and decrease (left) of centroid size by a scale factor of 5.

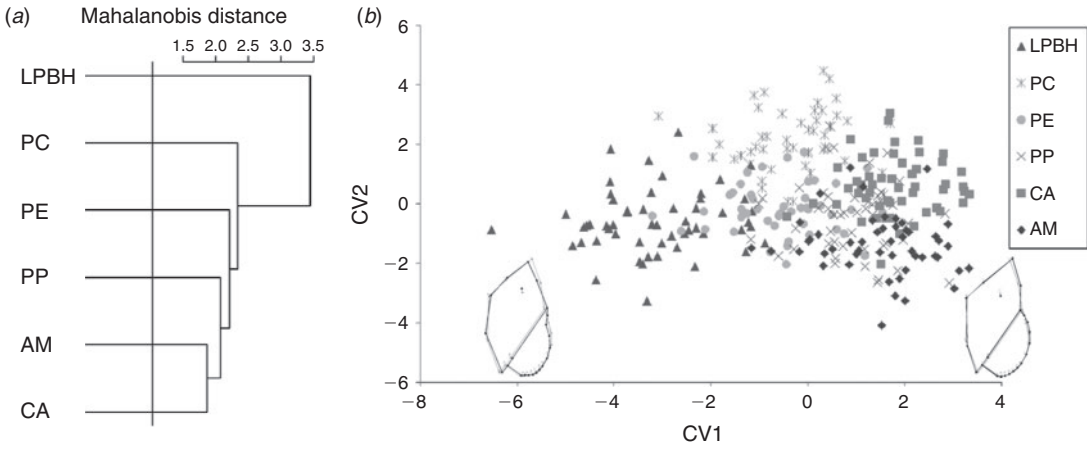

Fig. 4. Shell-shape comparisons among sites. (*a*) Single-linkage cluster analysis of adult females from different sites, showing the relationships among them. The cut-off criterion ($P > 0.05$) obtained with the MDGC test (Multivariate Dirienzo Guzman Casanoves, proposed by Valdano and Di Rienzo 2007) is indicated with a vertical line. Six statistically different groups of shell shapes were identified by this method. (*b*) Canonical analysis of the maximum overall shell-shape variation along the first two canonical axes. Wire-frame diagrams show shape changes from mean shape (black dot) to the positive (scale factor of 4) and negative extremes (scale factor of −6) on the canonical variate (CV1) axis.

on size was highly statistically significant (permutation test with 10 000 random permutations, $P < 0.0001$), and accounted for 13.08% of the total amount of shape variation. The relationship between shell shape and size of adult females was allometric. The main shell-form variation related to bigger individuals was associated with the shape of the callus zone, which was wider (more globose) in bigger individuals, which also presented a shorter relative size of the shell aperture (Fig. 3). For subsequent analyses, we used the regression residuals as the new allometric-free shell-shape variables. The dendrogram calculated on shape Mahalanobis distance matrix showed that each site had a characteristic shell shape, with significant statistical differences among the sites (cut criteria are represented by a vertical line at $P = 0.05$, Fig. 4*a*). The groups presenting minor distances were the AM and CA beaches (with low maritime traffic), whereas the harbour (LPBH) site (the site with high maritime traffic) was the most divergent regarding the shell shape (Fig. 4*a*).

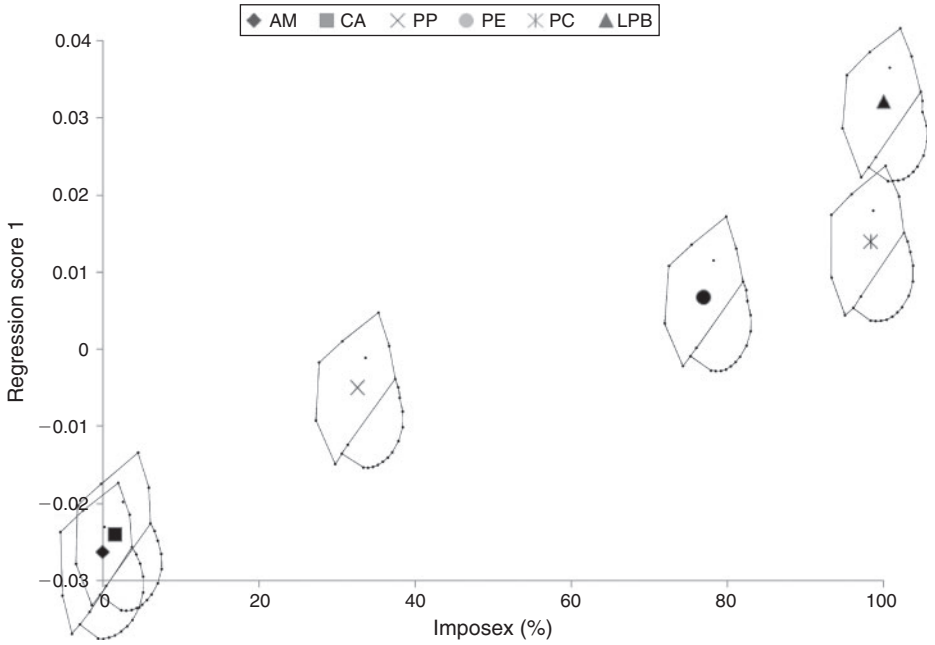

Fig. 5. Scatter plot of shape (scores along the vector of regression coefficients) *v*. imposex incidence. Wire-frame diagrams show the average shell shape at each site.

The 90.23% of variation in shell shape was explained in canonical Axes 1–3. The most conspicuous trait separating shapes along the CV1 (51% of the variance) was the globular shape in the negative values *v*. fusiform shape in the positive values (Fig. 4*b*). Globular individuals from the harbour have a shorter spire and siphonal canal, and a smaller relative size of the shell aperture. The individuals from low maritime traffic were represented by the opposite shape change with elongated-spired shell and a bigger relative size of the shell aperture.

A significant relationship between the average shell shape from each site and imposex incidence was detected by multivariate regression analysis (permutation test with 10 000 random permutations, $P < 0.001$) and accounted for 61.45% of the total amount of shape variation. Fig. 5 suggests that the effect of imposex on shape is the same as that represented by CV1, namely that individuals presenting the highest imposex incidence tend to be more globose with a shorter relative size of the shell aperture, than are individuals with a low or null imposex incidence.

Discussion

The gastropod *B. globulosus* is a good model to evaluate the effect of the environmental contamination on the shell shape, because variations in pollution in a small area reflect different shell shapes. In the harbour area of Nuevo Gulf (LPBH), the presence of TBT in sediments (up to 174.81 ng Sn g^{-1} DW) and in the body of the gastropod *O. magellanica* (up to 150 ng Sn g^{-1} DW in gonads) were recently detected in the same sites as we sampled in Nuevo Gulf, Patagonia (Bigatti *et al.* 2009; del Brío 2011). In addition, pollutants such as PAHs (Commendatore and Esteves 2007; Massara Paletto *et al.* 2008; Torres *et al.* 2013) were measured in sediments and, recently, Primost (2014) found heavy metals in the tissue of *B. globulosus* from the LPBH area. The GM analysis showed a high association between the incidence of imposex and gastropod shell shape. Heavy metals and TBT compounds have been associated with differences in growth rate in bivalves and limpets (Waldock and Thain 1983; Ruiz *et al.* 1994; Almeida *et al.* 1998; Tablado and López Gappa 2001; Coelho *et al.* 2006; Sokołowski *et al.* 2008; Smolarz and Bradtke 2011), and environmental gradients represent a source of variation that can exert its influence on shell shape (Caravajal-Rodríguez *et al.* 2005; Conde-Padín *et al.* 2007; Cuña *et al.* 2011). In the present work, we detected significant differences in shell shape related to different imposex incidences among areas separated by 20 km, so latitudinal gradients are not influencing our results. Individuals with a higher incidence of imposex presented rounded shells and gastropods with a lower incidence of imposex presented elongated shells. A similar pattern of shell-shape variation was reported by Márquez *et al.* (2011) in the same zone, who detected rounded *v*. elongated shells in the gastropod *Odontocymbiola magellanica* from Nuevo Gulf depending on the imposex incidence and TBT pollution in the tissues of individuals (del Brío 2011).

The shell-shape variation in *B. globulosus* was previously studied by GM in two sites of the San Matías (SM) Gulf, and it was compared with the same site we studied in Nuevo Gulf (PC beach in the present work) by Avaca *et al.* (2013). Those authors stated that individuals from Bahía San Antonio (in SM) and PC present similarities in shell shape, but differ from individuals from Playa Villarino, another site in the SM Gulf, arguing that shell shape differences would be a product of different environmental conditions (different wave exposition as a principal cause, although not measured in that work). However, analysing the results of Avaca *et al.* (2013) in the context of our paper, their results seem to be similar to those found in our work. Both sites

inside bays (Bahía San Antonio and PC) are affected by organotin-pollution compounds (Bigatti *et al.* 2009), individuals analysed by those authors presented rounded shells, as in the present work, whereas in Playa Villarino (free imposex area; Avaca *et al.* 2015), individuals present elongated shells. Therefore, we consider that the patterns of shell-shape variation found by Avaca *et al.* (2013) coincide with our findings, and may be related to exposure to pollutants from maritime-traffic areas.

In agreement with Márquez *et al.* (2011), our results corroborated that this GM method could be used for monitoring natural resources without a need to use destructive or extractive techniques. Besides, the association between the shell shape and imposex incidence could be applied to determine qualitative TBT pollution. Therefore, the shell shape could be used in environmental monitoring programs to analyse the TBT coastal pollution through collection of shells on the intertidal.

This first descriptive cause–effect study of natural patterns of shell-shape variation in this gastropod will provide the information to conduct controlled laboratory experiments or animal transplants in the field sites, directed to test the hypothesis that TBT influences shell shape. Linking our results and those of experimental manipulative methods would improve our knowledge in this sense. Our results may be applied to evaluate the consequences of an exposure to pollutants such as TBT on the shell shape of many gastropods species.

Acknowledgements

This study was partially funded by PIP 11220120100067CO, PICT 1232, PICT 078 and PADI Foundation. All research work including sampling and laboratory researches comply with Argentine current laws. We especially thank two anonymous reviewers for their valuable suggestions, which enriched the discussion in the manuscript.

References

Almeida, M. J., Moura, G., Pinheiro, T., Machado, J., and Coimbra, J. (1998). Modifications in *Crassostrea gigas* shell composition exposed to high concentrations of lead. *Aquatic Toxicology* **40**, 323–334. doi:10.1016/S0166-445X(97)00062-3

Aizieu, C. (1986). TBT detrimental effects on oyster culture in France – evolution since antifouling paint regulation. In 'Proceedings of the Oceans '86 Symposium', 23–25 September 1986, Washington, DC, USA. pp. 1130–1134. (IEEE: New York.) doi:10.1109/OCEANS.1986.1160376

Alzieu, C. (2000). Impact of tributyltin on marine invertebrates. *Ecotoxicology* **9**, 71–76. doi:10.1023/A:1008968229409

Alzieu, C., Sanjuan, J., Deltreil, J. P., and Borel, M. (1986). Tin contamination in Arcachon Bay: effects on oyster shell anomalies. *Marine Pollution Bulletin* **17**, 494–498. doi:10.1016/0025-326X(86)90636-3

APPM (2013). Resumen Movimiento De Mercaderias Año 2013. (Administración Portuaria de Puerto Madryn.). Available at http://www.appm.com.ar/sites/default/files/estadistica_2013_final.pdf [Verified 5 October 2015].

Avaca, M. S., Narvarte, M., Martín, P., and Van der Molen, S. (2013). Shell shape variation in the Nassariid *Buccinanops globulosus* in northern Patagonia. *Helgoland Marine Research* **67**, 567–577. doi:10.1007/S10152-013-0344-5

Avaca, M. S., Martín, P., van der Molen, S., and Narvarte, M. (2015). Comparative study of the female gametogenic cycle in three populations of *Buccinanops globulosus* (Caenogastropoda: Nassariidae) from Patagonia. *Helgoland Marine Research* **69**, 87, 99. doi:10.1007/S10152-014-0418-Z

Averbuj, A., and Penchaszadeh, P. E. (2010). On the reproductive biology and impact of imposex in a population of *Buccinanops monilifer* from Mar del Plata, Argentina. *Journal of the Marine Biological Association of the United Kingdom* **90**, 729–734. doi:10.1017/S0025315409990956

Averbuj, A., Rocha, M. N., and Zabala, S. (2014). Embryonic development and reproductive seasonality of *Buccinanops globulosus* (Nassariidae) (Kiener, 1834) in Patagonia, Argentina. *Invertebrate Reproduction & Development* **58**, 138–147. doi:10.1080/07924259.2013.875949

Bigatti, G., and Carranza, A. (2007). Phenotypic variability associated with the occurrence of imposex in *Odontocymbiola magellanica* from Golfo Nuevo, Patagonia. *Journal of the Marine Biological Association of the United Kingdom* **87**, 755–759. doi:10.1017/S0025315407055762

Bigatti, G., Primost, M. A., Cledón, M., Averbuj, A., Theobald, N., Gerwinski, W., Arntz, W., Morriconi, E., and Penchaszadeh, P. E. (2009). Biomonitoring of TBT contamination and imposex incidence along 4700 km of Argentinean shoreline (SW Atlantic: from 38S to 54S). *Marine Pollution Bulletin* **58**, 695–701. doi:10.1016/J.MARPOLBUL.2009.01.001

Bookstein, F. L. (1982). Foundations of morphometrics. *Annual Review of Ecology and Systematics* **13**, 451–470. doi:10.1146/ANNUREV.ES.13.110182.002315

Bookstein, F. L. (1991). 'Morphometric Tools for Landmark Data. Geometry and Biology.' (Cambridge University Press: New York.)

Caravajal-Rodríguez, A., Conde-Padín, P., and Rolán-Alvarez, E. (2005). Decomposing shell form into size and shape by geometric morphometric methods in two sympatric ecotypes of *Littorina saxatilis. The Journal of Molluscan Studies* **71**, 313–318. doi:10.1093/MOLLUS/EYI037

Chagot, D., Alzieu, C., Sanjuan, J., and Grizel, H. (1990). Sublethal and histopathological effects of trace levelsb of tributyltin fluoride on adult oysters *Crassostrea gigas. Aquatic Living Resources* **3**, 121–130. doi:10.1051/ALR:1990012

Chesman, B. S., and Langston, W. J. (2006). Intersex in the clam *Scrobicularia plana*: a sign of endocrine disruption in estuaries? *Biology Letters* **2**, 420–422. doi:10.1098/RSBL.2006.0482

Coelho, M. R., Langston, W. J., and Bebianno, M. J. (2006). Effect of TBT on *Ruditapes decussatus* juveniles. *Chemosphere* **63**, 1499–1505. doi:10.1016/J.CHEMOSPHERE.2005.09.016

Commendatore, M. G., and Esteves, J. L. (2007). An assessment of oil pollution in the coastal zone of Patagonia, Argentina. *Environmental Management* **40**, 814–821. doi:10.1007/S00267-005-0221-3

Conde-Padín, P., Grahame, J. W., and Rolán-Alvarez, E. (2007). Detecting shape differences in species of the *Littorina saxatilis* complex by morphometric analysis. *The Journal of Molluscan Studies* **73**, 147–154. doi:10.1093/MOLLUS/EYM009

Cuña, V., Saura, M., Quesada, H., and Rolán-Alvarez, E. (2011). Extensive micro-geographical shell polymorphism in a planktotrophic marine intertidal snail. *Marine Ecology Progress Series* **427**, 133–143. doi:10.3354/MEPS09033

del Brío, F. (2011). Determinacion de TBT, factores de acumulación e imposex, en el volutido *Odontocymbiola magellanica* en la Bahia Nueva, Chubut. B.Sc.(Licenciatua) Thesis, Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn Argentina.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., and Robledo, C. W. (2012) InfoStat versión (2012). Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Available at http://www.infostat.com.ar [Verified 22 October 2015].

Dyrynda, E. A. (1992). Incidence of abnormal shell thickening in the pacific oyster *Crassostrea gigas* in Poole Harbour (UK), subsequent to the 1987 TBT restrictions. *Marine Pollution Bulletin* **24**, 156–163. doi:10.1016/0025-326X(92)90244-Z

Florek, K., Lukaszewicz, J., Perkal, J., and Zuvrzycki, S. (1951). Sur la liaison et la division des points d'un ensemble fini. *Colloquium Mathematicae* **2**, 282–285.

Gibbs, P. E., and Bryan, G. W. (1986). Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom* **66**, 767–777. doi:10.1017/S0025315400048414

Gibbs, P. E., and Bryan, G. W. (1987). TBT paints and the demise of the dog-whelk, *Nucella lapillus* (Gastropoda). In 'Proceedings of the Oceans '87 Symposium', 28 September–1 October 1987. pp. 1482–1487. (IEEE: New York.) doi:10.1109/OCEANS.1987.1160635

Gil, M. N., Torres, A., Harvey, M., and Esteves, J. L. (2006). Metales pesados en organismos marinos de la zona costera de la Patagonia argentina continental. *Revista de Biología Marina y Oceanografía* **41**, 167–176.

Gunz, P., and Mitteroecker, P. (2013). Semilandmarks: a method for quantifying curves and surfaces. *Hystrix* **24**, 103–109. doi:10.4404/HYSTRIX-24.1-6292

Gunz, P., Mitteroecker, P., and Bookstein, F. L. (2005). Semilandmarks in three dimensions. In 'Modern Morphometrics in Physical Anthropology'. (Ed. D. E. Slice.) pp. 73–98. (Kluwer Academic/Plenum: New York.)

Klingenberg, C. P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* **11**, 353–357. doi:10.1111/J.1755-0998.2010.02924.X

Levinton, J. S. (2001). 'Marine Biology: Function, Biodiversity, Ecology', 2nd edn. (Oxford University Press: New York.)

Márquez, F., González, R. J., and Bigatti, G. (2011). Combined methods to detect pollution effects on shell shape and structure in neogastropods. *Ecological Indicators* **11**, 248–254. doi:10.1016/J.ECOLIND.2010.05.001

Márquez, F., Nieto Vilela, R. A., Lozada, M., and Bigatti, G. (2015). Morphological and behavioral differences in the gastropod *Trophon geversianus* associated to distinct environmental conditions, as revealed by a multidisciplinary approach. *Journal of Sea Research* **95**, 239–247. doi:10.1016/J.SEARES.2014.05.002

Massara Paletto, V., Commendatore, M. G., and Esteves, J. L. (2008). Hydrocarbon levels in sediments and bivalve mollusks from Bahía Nueva (Patagonia, Argentina): an assessment of probable origin and bioaccumulation factors. *Marine Pollution Bulletin* **56**, 2100–2105. doi:10.1016/J.MARPOLBUL.2008.08.026

Narvarte, M. A. (2006). Biology and fishery of the whelk *Buccinanops globulosum* (Kiener, 1834) in northern coastal waters of the San Matías Gulf (Patagonia, Argentina). *Fisheries Research* **77**, 131–137. doi:10.1016/J.FISHRES.2005.11.006

Oehlmann, J., Stroben, E., Schulte-Oehlmann, U., and Bauer, B. (1998). Imposex development in response to TBT pollution in *Hinia incrassata* (Ström, 1768) (Prosobranchia, Stenoglossa). *Aquatic Toxicology* **43**, 239–260. doi:10.1016/S0166-445X(98)00058-7

Pastorino, G. (1993). The taxonomic status of *Buccinanops* d'Orbigny, 1841 (Gastropoda: Nassariidae). *The Veliger* **36**, 160–165.

Penchaszadeh, P. E., Averbuj, A., and Cledón, M. (2001). Imposex in gastropods from Argentina (south-western Atlantic). *Marine Pollution Bulletin* **42**, 790–791. doi:10.1016/S0025-326X(01)00098-4

Piñeira, J., Quesada, H., Rolán-Álvarez, E., and Caballero, A. (2008). Genetic impact of the Prestige oil spill in wild populations of a poor dispersal marine snail from intertidal rocky shores. *Marine Pollution Bulletin* **56**, 270–281. doi:10.1016/J.MARPOLBUL.2007.10.020

Pontones, J. (2014). Análisis de los patrones espaciales y temporales de la energía y altura de las olas en el Golfo Nuevo y su efecto sobre algunas especies marinas. B.Sc.(Licenciatua) Thesis, Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn, Argentina.

Primost, M. A. (2014). Ecotoxicología y alteraciones morfo-funcionales en gasterópodos marinos expuestos a contaminación por organometales y metales. Ph.D. Thesis, Universidad Nacional de La Plata, La Plata Argentina.

Rechimont, M. E. (2011). Patrón de distribución y abundancia de invertebrados bentónicos en costas del Golfo Nuevo. B.Sc.(Licenciatua) Thesis, Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn Argentina.

Rohlf, F. J. (2004*a*). Software TPSDig Version 1.40. Department of Ecology and Evolution, State University of New York, New York.

Rohlf, F. J. (2004*b*). Software TPSRelw, Relative Warps Analysis, 1.35. Department of Ecology and Evolution, State University of New York, New York.

Rohlf, F. J., and Marcus, L. F. (1993). A revolution morphometrics. *Trends in Ecology & Evolution* **8**, 129–132. doi:10.1016/0169-5347(93)90024-J

Rohlf, F. J., and Slice, D. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. *Systematic Biology* **39**, 40–59.

Ruiz, J. M., Bryan, G. W., and Gibbs, P. E. (1994). Chronic toxicity of water tributyltin (TBT) and copper to spat of the bivalve *Scrobicularia plana*: ecological implications. *Marine Ecology Progress Series* **113**, 105–117. doi:10.3354/MEPS113105

Scammell, M. S., Batley, G. E., and Brockbank, C. I. (1991). A field study of the impact on oysters of tributyltin introduction and removal in a pristine lake. *Archives of Environmental Contamination and Toxicology* **20**, 276–281. doi:10.1007/BF01055916

Scarabino, V. (1977). Moluscos del Golfo San Matías, provincia de Río Negro, República Argentina. Inventario y claves para su identificación. Vol. IV 25 (31–32), Comunicaciones de la Sociedad malacológica del Uruguay, Montevideo, Uruguay.

Schulte-Oehlmann, U., Oehlmann, J., Bauer, B., Fioroni, P., and Leffler, U.-S. (1998). Toxico-kinetic and -dynamic aspects of TBT-induced imposex in *Hydrobia ulvae* compared with intersex in *Littorina littorea* (Gastropoda, Prosobranchia). *Hydrobiologia* **378**, 215–225. doi:10.1023/A:1003270528688

Smolarz, K., and Bradtke, K. (2011). Bioindicative potential of shell abnormalities occurring in the clam *Macoma balthica* (L.) from the Baltic Sea. *Marine Pollution Bulletin* **62**, 1421–1426. doi:10.1016/J.MARPOLBUL.2011.04.031

Sokołowski, A., Pawlikowski, K., Wołowicz, M., Garcia, P., and Namieśnik, J. (2008). Shell deformations in the Baltic clam *Macoma balthica* from southern Baltic Sea (the Gulf of Gdansk): hypotheses on environmental effects. *AMBIO: A Journal of Human Environment* **37**, 93–100. doi:10.1579/0044-7447(2008)37[93:SDITBC]2.0.CO;2

Tablado, A., and López Gappa, J. (2001). Morphometric diversity of the pulmonate limpet *Siphonaria lessoni* in different coastal environments. *Scientia Marina* **65**(1), 33–41.

Torres, P. J., Commendatore, M. G., Primost, M. A., and Bigatti, G. (2013). Hidrocarburos poliaromáticos (PAHs) en *Buccinanops globulosus* afectados por imposex. In 'Proceedings of the I Congreso Argentino de Malacología (CAM)', September 2013, La Plata Argentina. p. 99. (Sociedad Argentina de Malacología: La Plata, Argentina.)

Valdano, S. G., and Di Rienzo, J. A. (2007). Discovering meaningful groups in hierarchical cluster analysis. An extension to the multivariate case of a multiple comparison method based on cluster analysis. *InterStat*, 1–28.

Waldock, M. J., and Thain, J. E. (1983). Shell thickening in *Crassostrea gigas*: organotin antifouling or sediment induced? *Marine Pollution Bulletin* **14**, 411–415. doi:10.1016/0025-326X(83)90445-9

Waldock, M. J., Thain, J. E., and Waite, M. E. (1996). An assessment of the value of shell thickening in *Crassostrea gigas* as an indicator of exposure to Tributyltin. In 'Organotin: Environmental Fate and Effects'. (Eds M. A. Champ and P. F. Seligman.). pp. 219–238. (Chapman and Hall: London.)

Zelditch, M. L., Swiderski, D. L., Sheets, H. D., and Fink, W. L. (2004). 'Geometric Morphometrics for Biologists: a Primer.' (Elsevier Academic Press: New York.)