Manuscript Details

Manuscript number	MERE_2020_788
Title	Embryonic shell shape as an early indicator of pollution in marine gastropods
Short title	Embryonic shell shape as an early biomarker of pollution of marine pollutants
Article type	Short communication

Abstract

Gastropods shell shape has been proposed as a good indicator of environmental changes while geometric morphometric (GM) is a powerful tool to detect such changes. Shell shape pattern in adults of Buccinanops deformis was proved to be correlated with imposex incidence and maritime traffic in populations of Patagonia. We explore through GM the shell shape variation of B. deformis intracapsular embryos in pre-hatching stages of development, in two populations with contrasting maritime traffic and imposex incidence. Embryonic shell shape from polluted and unpolluted areas were significantly different in apex, lateral, aperture and siphonal channel zones. The same shell shape pattern was observed previously in B. deformis adult specimens. Our results demonstrate that the shell shape is an early biomarker that could be used as a tool to detect the response to environmental changes during intracapsular embryonic development. The early exposition to contaminants could influence the concomitant fitness of adult gastropods.

Keywords	geometric morphometrics; maritime traffic; buccinanops; embryonic development; Patagonia; imposex, antifouling paints
Taxonomy	Biological Sciences, Earth Sciences

Corresponding Author	FEDERICO MÁRQUEZ
Order of Authors	Monica Primost, Andres Averbuj, GREGORIO BIGATTI, FEDERICO MÁRQUEZ

Suggested reviewers	Joana Vasconcelos, Italo Castro, Valeria Teso, Daniel Rittschof

Submission Files Included in this PDF

File Name [File Type]

cover letter.doc [Cover Letter]

Highlights.docx [Highlights]

Graphical abstract color RGB.tif [Graphical Abstract]

Primost et al..docx [Manuscript File]

declaration-of-competing-interests.docx [Conflict of Interest]

Credit Author Statement.docx [Author Statement]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

CONICET

IBIOMAR

Instituto de Biología de Organismos Marinos
Consejo Nacional de Investigaciones Científicas y Técnicas

Puerto Madryn, September 24, 2020

Professor F. Regoli
Editor MER

We are sending you our manuscript entitled "**Embryonic shell shape as an early indicator of pollution in marine gastropods**" for your editorial consideration to be published in the Marine Environmental Research (MER).

The main goal of this study is to study if the shell shape in pre-hatching *B. deformis* embryos is affected by environmental pollution. This is an original work that was not submitted elsewhere for publication. All scholars immediately involved have approved the manuscript.

We consider that the MER is appropriate to publish this article since it addresses broad visions and discusses a new methodological focus concerned with the studies of the embryonic shape variation as a biomarker due to marine pollution. Also, we are convinced that our study can be of crux importance for further studies since this approach yielded an enable rapid, continuous, fast, and low-cost monitoring protocols of the pollution's deleterious effects on marine coast. We hope you will consider this research of interest enough for publishing in the MER.

We look forward to hearing from you.

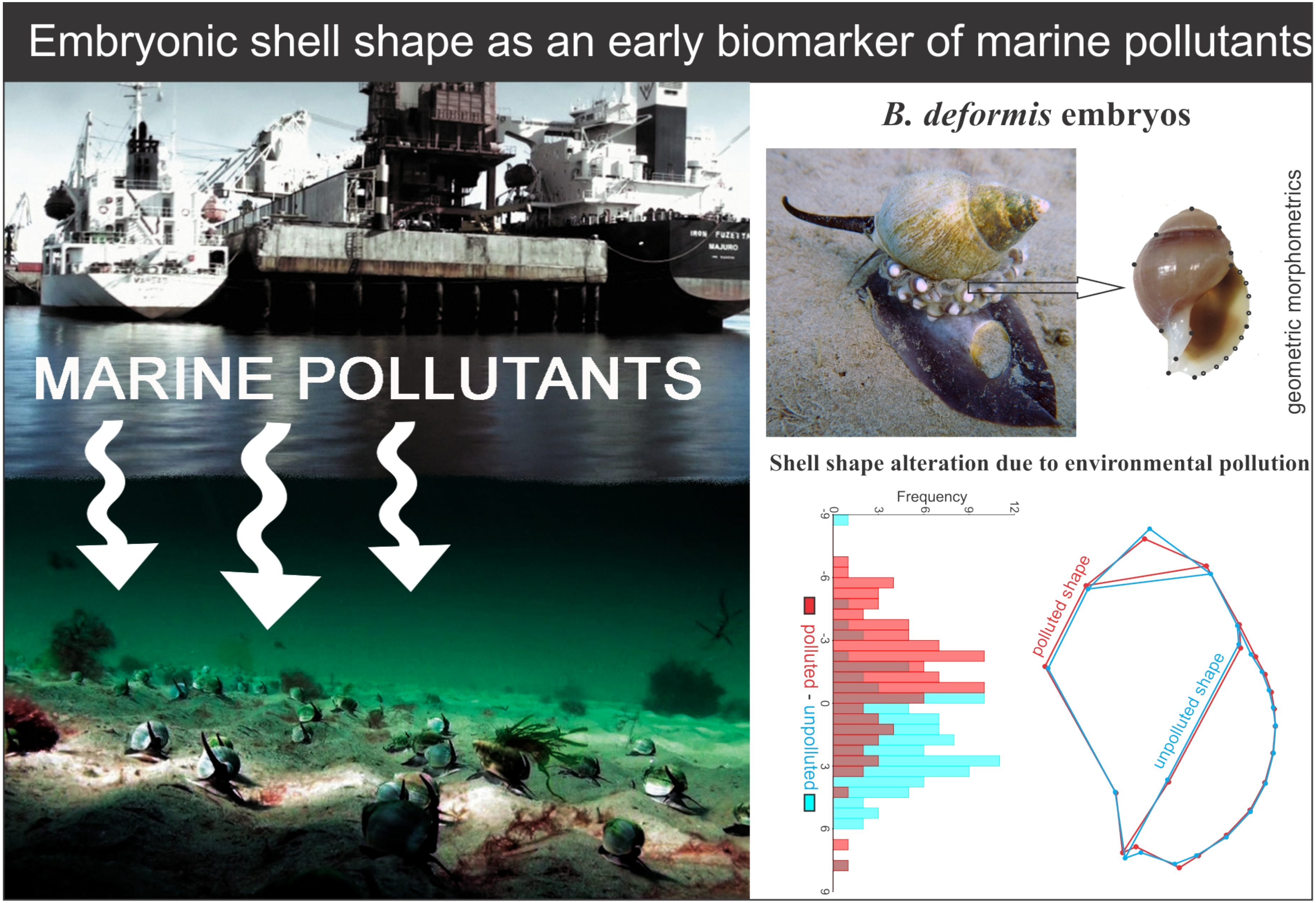
Yours sincerely,

Dr. Federico Márquez

IBIOMAR - CONICET
Blvd Brown 3600, Puerto Madryn (U9120ACF), Chubut- Argentina
Tel: (02965) 45-0401/ 45-1301/ 45-1375
Fax:(02965) 45-1543
fede@cenpat-conicet.gob.ar

Highlights

- **GM is a useful tool for detecting subtle changes in marine gastropods early development**
- **In polluted areas, shell shape pattern of intracapsular embryos is similar to adults**
- **Shell shape in pre-hatching *B. deformis* embryos is altered by environmental pollution**
- **Embryonic shell shape is an early biomarker of stress by environmental pollution**


Embryonic shell shape as an early biomarker of marine pollutants
MARINE POLLUTANTS
B. deformis embryos
geometric morphometrics
Shell shape alteration due to environmental pollution
Frequency
polluted - unpolluted
polluted shape
unpolluted shape

Embryonic shell shape as an early indicator of pollution in marine gastropods

M.A. Primost[a,b], A. Averbuj[b], G. Bigatti[b,c,d], F. Márquez[c,d*]

[a]Grupo de Investigación y Desarrollo Tecnológico en Acuicultura y Pesca (GIDTAP). Universidad Tecnológica Nacional, Facultad Regional Chubut. CONICET.
[b]LARBIM, Instituto de Biología de Organismos Marinos. IBIOMAR-CCT CENPAT-CONICET. Puerto Madryn, Argentina.
[c]Universidad Nacional de la Patagonia San Juan Bosco. Puerto Madryn, Argentina.
[d]Universidad Espíritu Santo, Ecuador.

*Corresponding Author: fede@cenpat-conicet.gob.ar

Abstract

Gastropods shell shape has been proposed as a good indicator of environmental changes while geometric morphometric (GM) is a powerful tool to detect such changes. Shell shape pattern in adults of *Buccinanops deformis* was proved to be correlated with imposex incidence and maritime traffic in populations of Patagonia. We explore through GM the shell shape variation of *B. deformis* intracapsular embryos in pre-hatching stages of development, in two populations with contrasting maritime traffic and imposex incidence. Embryonic shell shape from polluted and unpolluted areas were significantly different in apex, lateral, aperture and siphonal channel zones. The same shell shape pattern was observed previously in *B. deformis* adult specimens. Our results demonstrate that the shell shape is an early biomarker that could be used as a tool to detect the response to environmental changes during intracapsular embryonic development. The early exposition to contaminants could influence the concomitant fitness of adult gastropods.

Capsule summarizing main findings: The embryonic shell shape varies as a response to environmental pollution during intracapsular development.

Keywords: geometric morphometrics; maritime traffic; *buccinanops*; embryonic development; patagonia; imposex, antifouling paints

Introduction

Studies using shell shape as an indicator of changes in the environment are increasing (Harayashiki et al., 2020a). Geometric morphometrics (GM) turned out to be a useful tool for detecting subtle changes in response to environmental stressors (Conde-Padín et al., 2009; Conde-Padín et al., 2007; Sepúlveda and Ibáñez, 2012). It is known that persistent pollutants, industrial waste and drugs cause morphological changes and deleterious effects on marine organisms (His et al., 1999a; Matthiessen et al., 1995; Mensink et al., 1996; Zhu et al., 2011). In this sense, mollusks are among the most sensitive indicators in response to stressors such as tributyltin, heavy metals and polyaromatic hydrocarbons (Jobling et al., 2004; Oehlmann and Schulte-Oehlmann, 2003; Rittschof and McClellan-Green, 2005).

During the developmental process, many marine gastropod species protect their offspring by encapsulating early stages of development from environmental stressors such as salinity, desiccation, predation, pollution, etc. (Rawlings, 1994; 1999). However, little is known about the effectiveness of such encapsulation against pollutant molecules (Averbuj et al., 2017; Untersee, 2007).

In Patagonia, the nassariid gastropod *Buccinanops deformis* (King, 1832) named as *Buccinanops globulosus* in previous works (Averbuj et al., 2017; Primost et al., 2015a; Primost et al., 2016, among others cited in this study), has been reported to present a highly sensitive response to environmental pollution (Bigatti et al., 2009; Giulianelli et al., 2020). *B. deformis* presents internal fertilization and the females carry the encapsulated offspring attached to their shells until the moment of hatching (Averbuj et al., 2014). This species lives on sandy bottoms and feeds mainly on carrion; the populations inhabiting Northern Patagonian gulfs showed reproductive and physiological alterations when exposed to anthropogenic pollutants. Gastropods exposed even to low levels of Tributyltin (TBT) are affected by imposex phenomenon (Gibbs and Bryan, 1986), defined as a superimposition of male sexual secondary characteristics. In particular, *B. deformis* populations registered 100% of imposexed females in harbor areas of Puerto Madryn (Primost, 2014). In this zone, moderate levels of polyaromatic hydrocarbons (PAHs) and heavy metals such as cadmium and lead were detected in sediments and organisms (Primost et al., 2018; Primost et al., 2017). Deleterious effects and morphological alteration could be observed in *B. deformis* offspring induced by moderate pollutant inputs (Averbuj et al., 2017; Márquez et al., 2017; Primost et al., 2015a; Primost et al., 2016). Moreover, gastropod inhabiting areas where maritime traffic and human activity are high showed shifts in the enzymes associated with detoxification

(Primost et al., 2015c), and in some morphological and reproductive aspects (Primost et al., 2015a). Although recent researches have shown that encapsulated embryos may respond to external stimuli (Solas et al., 2015) due to the presence of a semipermeable membrane in the egg capsule walls (Bigatti et al., 2014), it remains uncertain to what degree these morphological and physiological alterations observed in adults could be transferred to offspring during intracapsular development.

GM was used in *B. deformis* and *Odontocymbiola magellanica* (Gmelin, 1791) from Patagonian region, to study the shell shape variation related to the presence of pollutants (Márquez et al., 2011; Primost et al., 2016). In *B. deformis*, the shell shape in areas of high maritime traffic is globose with a shorter spire and a smaller relative size of the shell aperture. In contrast, the opposite shape (fusiform, elongated spired shell and bigger relative size of the aperture) is found in individuals from low maritime traffic areas (Primost et al., 2016). This pattern of shell shape variation was confirmed by comparing living populations of *B. deformis* with those inhabiting the same areas in pre-Hispanic times, where anthropic (including any maritime) activities were absent (Márquez et al., 2017).

Due to previous knowledge about *B. deformis* reproductive strategies, shape shell alterations and high sensitivity to marine pollution, the species is an optimal model of study. This work aimed to study if the shell shape in pre-hatching *B. deformis* embryos is affected by environmental pollution.

Materials and methods

In order to compare two sites with contrasting anthropogenic activities, the same sampling sites studied in Primost et al. (2016) were chosen: one site is located within the urban area of Puerto Madryn city (LPBH) where two important harbors and intense maritime activity are present; loading and unloading of raw materials derived from the aluminum industry, fishery products, and tourism are common activities in the area (APPM, 2017). The other site is Cerro Avanzado (CA) beach, 15 km away from Puerto Madryn city. CA is an area where maritime traffic is low (only a few small boats for recreational purposes), and human activities are scarce. Data from recent studies have reported that imposex incidence in *Buccinanops deformis* from CA beach is null, and TBT levels were not detectable in sediments or gastropods tissues (Del Brio et al., 2016; Primost, 2014). Both LPBH and CA are inside of Nuevo gulf and have the same oceanographic characteristics (Bökenhans, 2014).

B. deformis egg masses were hand-gathered by scuba diving during the spawning season in summer. The egg masses consist of a variable number of egg capsules attached to the female shell (Figure 1A) by a short stalk (Averbuj et al., 2014; Penchaszadeh, 1971). The spawning season ranges from September to March; by the end of this season, pre-hatching individuals are still observed inside the egg capsules. At the end of the intracapsular embryonic development, which last approximately four months, the embryos have a complete and colorful shell, and the egg capsules open easily even when a gentle pressure is applied.

Once the first hatched individuals in the field were observed (newly hatched individuals are visible at low tides), we proceeded to sample the egg masses attached to the females. In total, 20 females carrying egg capsules were carefully collected by scuba diving at each site (10 from LBPH and 10 from CA). Once in the laboratory, the embryos were excapsulated (artificially removed from the egg capsule). Egg capsules with multiple embryos were not used for this study (following Primost et al., 2015a).

For this study, a total of 190 embryos (92 from LPBH and 98 from CA) were numbered and photographed in apertural view, under a Carl Zeiss binocular magnifying glass equipped with AxioVision Rel.4.5 software (© Copyright Carl Zeiss Imaging Solutions). Before photographs were captured, the embryonic shell shape was digitalized using the same 2D-configuration of 12 landmarks, and 9 semilandmarks (Figure 1B), following Primost et al. (2016) and Márquez et al. (2017). Sliding semilandmarks were performed using TPSRelw software (Rohlf, 2004), employing the algorithm which minimizes the deformation between each specimen and the mean shape (Bookstein, 1997; Gunz and Mitteroecker, 2013; Gunz et al., 2005). After sliding the semilandmarks, all landmark configurations were superimposed by a Generalized Procrustes Analysis to remove translation, rotation and scale information (Rohlf and Slice, 1990; Slice et al., 1996). Centroid Size (CS) was used to scale the landmarks configurations to unit CS and was calculated as a proxy to size. Centroid size was calculated as the square root of the sum of the squared distances from the landmarks to the centroid, which they define (Zelditch et al., 2004).

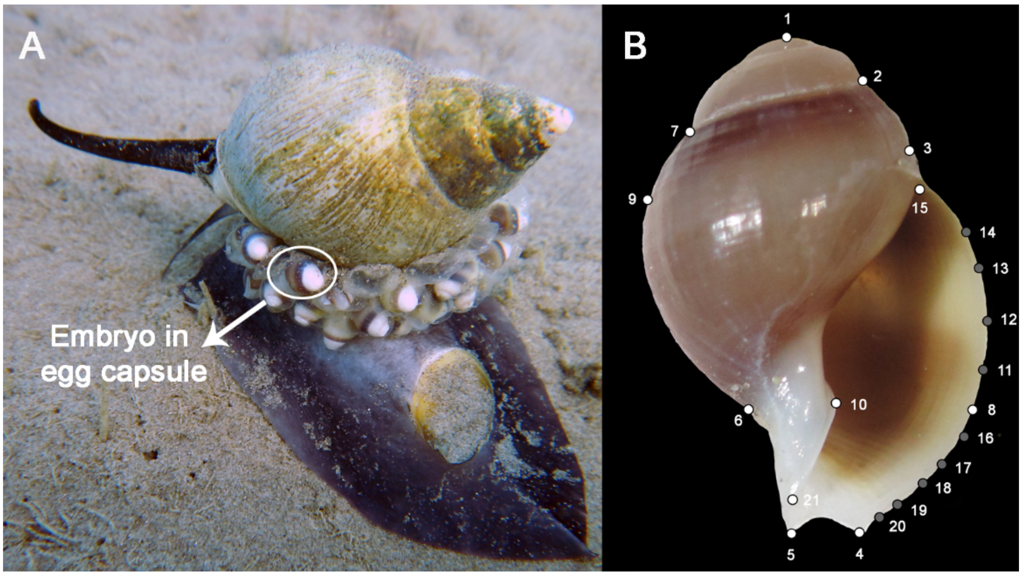


Figure 1. *Buccinanops deformis* female carrying its encapsulated embryos. Egg capsules are attached to the female shell (A). Landmarks (white dots) and semilandmarks (grey dots) in *B. deformis* embryos (B).

The relationship between shape and size (allometry) was tested using a multivariate regression (pooled within-site) between dependent variables (aligned individuals) and CS as an independent variable. To know and test for the maximum differences of the embryos shell shapes between sites (LPBH and CA), a discriminant analysis (DA) was performed. We estimated the readability of discrimination using a leave-one-out cross-validation procedure. Finally, to test for differences in the shell shape means between the two sites, we calculated a Hotelling T-square with a permutation test (1000 permutation runs). All GM statistical analyses were made in MorphoJ v.1.06d (Klingenberg, 2011).

Results

Embryos shell allometry was statistically significant ($p = 0.003$). The percent shape variation explained by size increment was 2.4%; thus, we performed all subsequent analyses with allometric corrections using the residual regression scores as new shell shape variables.

The discriminant analysis (DA) showed that the range of shell shapes was different between sites. As there are only two groups, there is a single axis of shape differences and values are indicated with histogram bars proportional to their frequency (Figure 2). The mean shell shapes were statistically significant ($p < 0.0001$, T-square= 189.333) between LPBH and CA individuals. The mean shell shape from LPBH was more globular than the CA site. The embryos from the polluted site presented shells with lateral expansion, retracted apex and aperture with a little extension of the siphonal channel. The embryos from the unpolluted site were represented by the opposite (slender) shape change (Figure 2). The cross-validated classification analysis showed that 71.1% and 73.46% of the LPBH and CA individuals, respectively, were correctly classified.

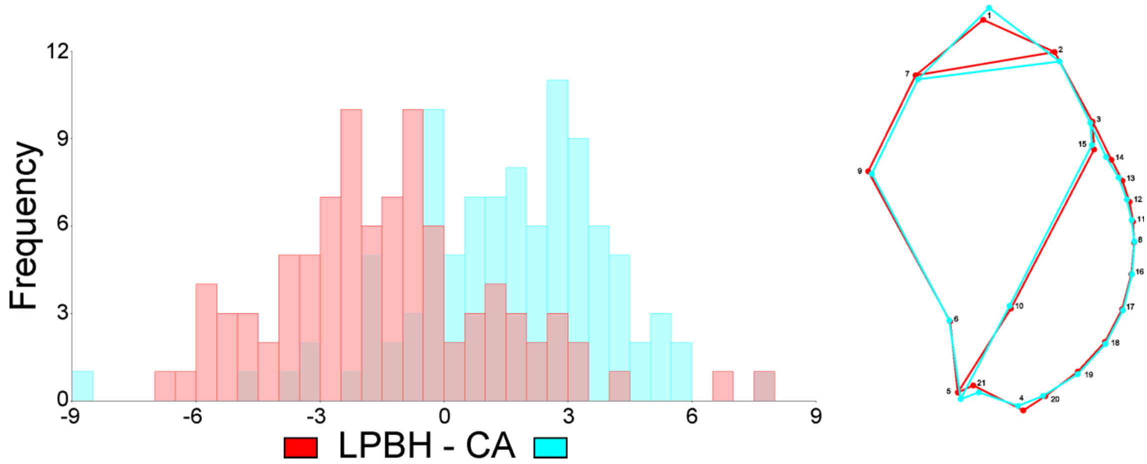


Figure 2. Discriminant analysis of *Buccinanops deformis* embryos shell shape differences between polluted (LPBH) and unpolluted (CA) sites. Frequencies of the discriminant scores predicted by a jackknife (leave-one-out) cross-validation are shown using histogram bars. Unpolluted (red vector) and polluted (light blue vector) mean shapes are visualized using wireframe drawings magnified 2 times.

Discussion

Environmental pollution is associated with chronic effects on organisms, including shell shape alterations in marine gastropods. In particular, butyltin compounds (tributyltin-TBT- and derivatives) have been extensively studied due to the chronic effects they pose on non-target organisms such as marine gastropods. Physiological disorder, imposex development, oxidative stress and morphological and reproductive alterations have been attributed to TBT bioaccumulation in Patagonian marine gastropods (Bigatti and Carranza, 2007; Bigatti et al., 2009; Del Brio et al., 2016; Primost et al., 2015a; Primost et al., 2016; Primost et al., 2015b; Primost et al., 2015c). From 2005 onwards, the use of GM techniques in marine gastropods has increased to detect shell shape changes (Harayashiki et al., 2020a). Moreover, GM applied to ecotoxicology studies has allowed a new and complementary analysis that became a potent tool for estimating the impact of anthropic stress (Harayashiki et al., 2020b; Márquez et al., 2011; Núñez et al., 2012; Piñeira et al., 2008; Savriama et al., 2015).

In this work, through GM techniques we were able to detect that the pattern of shell shape changes in *Buccinanops deformis* embryos, a pattern which is similar to the one displayed by the adults from the same populations when they are exposed to a variety of environmental pollutants typically associated to maritime traffic. The variation in the adults shape was related to the intensity of marine traffic and concomitant pollution as well as to the levels of imposex and stress indicators in the same harbor area (Márquez et al., 2017; Primost et al., 2016). In agreement with the hypothesis of Márquez et al. (2017), we propose that the globular shape presented in embryos from the polluted area could reduce body contact with the sediment where the hatched juveniles will live in. Therefore, the globular shell shape reported in previous works in marine gastropods exposed to maritime pollutants appears as a sustained response to pollution in such cases (Márquez et al., 2017; Primost et al., 2016).

Encapsulated development partially isolates the embryos from the surrounding environment (Chaparro et al., 1999). Despite being encapsulated, deleterious effects in embryos produced by exposure to environmental pollutants were reported (Beiras and Bellas, 2008; Bellas, 2008; Wu et al., 2014). It is known that small organic molecules can pass through the egg-capsule wall of marine gastropods (Bigatti et al., 2014; Leroy et al., 2012). Thus, it is likely that the egg capsules of *B. deformis* exchange substances with the surrounding medium. In this sense, a LD_{50} % TBT experiment determined that excapsulated embryos mortality of *B. deformis* was significantly higher than that of encapsulated ones (Averbuj et al., 2017).

In bivalve mollusks, marine pollutants such as TBT and its derivatives can be transferred from females to larvae during oogenesis (Inoue et al., 2006). Variable levels of pollutants such as PAH, PCB and TBTs have been recorded in the gonads of marine gastropod females and egg capsules from polluted areas, as well as in early stages of other invertebrate species (Bellas, 2007; Cima et al., 1996; Goldberg et al., 2004; His et al., 1999b; Stroben et al., 1992). Taking into account these results, we proposed that shell shape variations in gastropods embryos from harbor areas could be caused both due to maternal transference and environmental exposure of egg capsules during the development phase.

Particular attention should be given to populations living in high maritime traffic areas where a variety of persistent pollutants are concentrated in environmental matrices such as water and sediment, and bioaccumulated through the food webs compromising consumers health. Given the results achieved, and taking into account the background on mollusks bioaccumulation capacity, we recommend performing more studies of pollutants transference in trophic webs and maternal transfer, especially for shellfish resources as *B. deformis*.

Our results showed that the adults and embryos from the same site presented the same shell shape variation, indicating that the environmental conditions influence the shape of *B. deformis* adults since early stages of development. Consequently, we suggest that the globular shell shape in *B. defromis* could be used as an inexpensive biomarker to control and prevent the commercialization and consumption of gastropods from polluted sites.

Acknowledgements

We thank Facundo Irigoyen for their help during field activities. This is publication #XXXXX of the Laboratorio de Reproducción y Biología Integrativa de Invertebrados Marinos (LARBIM). Authors are members of CONICET.

Author contributions

Authors' individual contributions M.A. Primost: Investigation, Methodology, Resources, Writing - Original Draft. A. Averbuj: Methodology, Resources, Writing - Review & Editing. G. Bigatti: Resources, Writing - Review & Editing, Funding acquisition. F. Márquez: Investigation, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Review & Editing. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Funding:

This contribution has been partially funded by the ANPCyT PICT-2018-3197 (FM) and PICT 2018-04386 (AA).

References

APPM, 2017. Administración Portuaria de Puerto Madryn. Available at http://www.appm.com.ar/estadisticas

Averbuj, A., Primost, M.A., Giulianelli, S., Bigatti, G., 2017. Acute toxicity of tributyltin to encapsulated embryos of a marine gastropod. Molluscan Research 38, 29-33, https://doi.org/10.1080/13235818.2017.1357671

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

Beiras, R., Bellas, J., 2008. Inhibition of embryo development of the *Mytilus galloprovincialis* marine mussel by organic pollutants; assessment of risk for its extensive culture in the Galician Rias. Aquaculture 277, 208-212, https://doi.org/10.1016/j.aquaculture.2008.03.002

Bellas, J., 2007. Toxicity of the booster biocide Sea-Nine to the early developmental stages of the sea urchin *Paracentrotus lividus* Aquatic Toxicology 83, 52-61, https://10.1016/j.aquatox.2007.03.011

Bellas, J., 2008. Prediction and assessment of mixture toxicity of compounds in antifouling paints using the sea-urchin embryo-larval bioassay. Aquatic Toxicology 88, 308-315, http://10.1016/j.aquatox.2008.05.011

Bigatti, G., 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, https://doi.org/10.1017/S0025315407055762

Bigatti, G., Giraud-Billoud, M., Vega, I.A., Penchaszadeh, P.E., Castro-Vazquez, A., 2014. Embryonic development in the Patagonian red snail *Odontocymbiola magellanica* (Neogastropoda: Volutidae): Morphology and biochemistry. Zoologischer Anzeiger - A Journal of Comparative Zoology 253, 372-381, http://dx.doi.org/10.1016/j.jcz.2014.03.001

Bigatti, G., Primost, M.A., Cledón, M., Averbuj, A., Theobald, N., Gerwinski, W., Arntz, W., Morriconi, E., Penchaszadeh, P.E., 2009. Biomonitoring of TBT contamination and imposex incidence along 4700 km of Argentinean shoreline (Bigatti et al., 2009). Marine Pollution Bulletin 58, 695-701, https://doi.org/10.1016/j.marpolbul.2009.01.001

Bökenhans, V., 2014. Estimación de la edad y crecimiento de dos poblaciones de *Buccinanops globulosus* del Golfo Nuevo, Facultad de Ciencias Naturales. Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn, p. 53.

Bookstein, F.L., 1997. Shape and the information in medical images: A decade of the morphometric synthesis. Computer Vision and Image Understanding 66, 97-118, https://doi.org/10.1006/cviu.1997.0607

Chaparro, O.R., Oyarzun, R.F., Vergara, A.M., Thompson, R.J., 1999. Energy investment in nurse eggs and egg capsules in *Crepidula dilatata* Lamarck (Gastropoda, Calyptraeidae) and its influence on the hatching size of the juvenile. Journal of Experimental Marine Biology and Ecology 232, 261-274, http://dx.doi.org/10.1016/S0022-0981(98)00115-4

Cima, F., Ballarin, L., Bressa, G., Martinucci, G., Burighel, P., 1996. Toxicity of organotin compounds on embryos of a marine invertebrate (*Styela plicata*; Tunicata). Ecotoxicology and Environmental Safety 35, 174-182 https://doi.org/10.1006/eesa.1996.0097

Conde-Padín, P., Caballero, A., Rolán-Álvarez, E., 2009. Relative role of genetic determination and plastic response during ontogeny for shell-shape traits subjeted to diversifying selection. Evolution 63, 1356-1363, https://doi.org/10.1111/j.1558-5646.2009.00636.x

Conde-Padín, P., Grahame, J.W., Rolán-Alvarez, E., 2007. Detecting shape differences in species of the *Littorina saxatilis* complex by morphometric analysis. Journal of Molluscan Studies 73, 147-154, https://doi.org/10.1093/mollus/eym009

Del Brio, F., Commendatore, M.G., Castro, I.B., Gomes Costa, P., Fillmann, G., G., B., 2016. Distribution and bioaccumulation of Butyltins in the edible gastropod *Odontocymbiola magellanica*. Marine Biology Research 12, 608-620, https://doi.org/10.1080/17451000.2016.1169296

Gibbs, P.E., 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, https://doi.org/10.1017/S0025315400048414

Giulianelli, S., Primost, M.A., Lanari, C., Bigatti, G., 2020. RXR expression in marine gastropods with different sensitivity to imposex development. Scientific Reports 10, https://doi.org/10.1038/s41598-020-66402-1

Goldberg, R.N., Averbuj, A., Cledón, M., Luzzatto, D., Nudelman, N.S., 2004. Search for triorganotins along the Mar del Plata (Argentina) marine coast: finding of tributyltin in egg capsules of a snail *Adelomelon brasiliana* (Lamarck, 1822) population showing imposex effects. Applied Organometallic Chemistry 18, 117-123, https://doi.org/10.1002/aoc.590

Gunz, P., Mitteroecker, P., 2013. Semilandmarks: a method for quantifying curves and surfaces. Hystrix 24, 103-109, https://doi.org/10.4404/hystrix-24.1-6292

Gunz, P., Mitteroecker, P., Bookstein, F.L., 2005. Semilandmarks in three dimensions, in: Slice, D.E. (Ed.), Modern morphometrics in physical anthropology. Klewer Academic/Plenum, New York, pp. 73-98

Harayashiki, C.A.Y., Márquez, F., Cariou, E., Castro, Í.B., 2020a. Mollusk shell alterations resulting from coastal contamination and other environmental factors. Environmental Pollution 265, 114881, https://doi.org/10.1016/j.envpol.2020.114881

Harayashiki, C.A.Y., Martins, C.P., Márquez, F., Bigatti, G., Castro, Í.B., 2020b. Historical shell form variation in *Lottia subrugosa* from southeast Brazilian coast: Possible responses to anthropogenic pressures. Marine Pollution Bulletin 155, 111180, https://doi.org/10.1016/j.marpolbul.2020.111180

His, E., Beiras, R., Seaman, M.N.L., 1999a. The assessment of marine pollution - bioassays with bivalve embryos and larvae, in: Southward, A.J., Tyler, P.A., Young, C.M. (Eds.), Advances in Marine Biology. Academic Press, pp. 1-178

His, E., Heyvang, I., Geffard, O., Montaudouin, X.D., 1999b. A comparison between oyster (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*) larval bioassays for toxicological studies. Water Research 33, 1706-1718, https://doi.org/10.1016/S0043-1354(98)00381-9

Inoue, S., Oshima, Y., Usuki, H., Hamaguchi, M., Hanamura, Y., Kai, N., Shimasaki, Y., Honjo, T., 2006. Effects of tributyltin maternal and/or waterborne exposure on the embryonic development of the Manila clam, *Ruditapes philippinarum*. Chemosphere 63, 881-888, https://doi.org/10.1016/j.chemosphere.2005.07.047

Jobling, S., Casey, D., Rodgers-Gray, T., Oehlmann, J., Schulte-Oehlmann, U., Pawlowski, S., Baunbeck, T., Turner, A.P., Tyler, C.R., 2004. Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. Aquatic Toxicology 66, 207-222, https://doi.org/10.1016/j.aquatox.2004.01.002

Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11, 353-357, https://doi.org/10.1111/j.1755-0998.2010.02924.x

Leroy, F., Comtet, T., Brante, A., Leroux, C., Riera, P., 2012. Can encapsulated embryos of *Crepidula fornicata* (L.) use extracapsular dissolved organic matter? An experimental study with a 13C-enriched amino acid. Journal of Molluscan Studies 78, 100-104, https://doi.org/10.1093/mollus/eyr044

Márquez, F., González, R.J., Bigatti, G., 2011. Combined methods to detect pollution effects on shell shape and structure in Neogastropods. Ecological Indicators 11, 248-254, https://doi.org/10.1016/j.ecolind.2010.05.001

Márquez, F., Primost, M.A., Bigatti, G., 2017. Shell shape as a biomarker of marine pollution historic increase. Marine Pollution Bulletin 114, 816-820, http://dx.doi.org/10.1016/j.marpolbul.2016.11.018

Matthiessen, P., Waldock, R., Thain, J.E., Waite, M.E., Scropehowe, S., 1995. Changes in periwinkle (*Littorina littorea*) populations following the ban on TBT-based antifoulings on small boats in the United Kingdom. Ecotoxicology and Environmental Safety 30, 180-194, http://dx.doi.org/10.1006/eesa.1995.1023

Mensink, B.P., Everaarts, J.M., Kralt, H., ten Hallers-Tjabbes, C.C., Boon, J.P., 1996. Tributyltin exposure in early life stages induces the development of male sexual characteristics in the common whelk, *Buccinum undatum*. Marine Environmental Research 42, 151-154, http://dx.doi.org/10.1016/0141-1136(95)00041-0

Núñez, J.D., Laitano, M.V., Cledón, M., 2012. An intertidal limpet species as a bioindicator: Pollution effects reflected by shell characteristics. Ecological Indicators 14, 178-183, https://doi.org/10.1016/j.ecolind.2011.07.015

Oehlmann, J., Schulte-Oehlmann, U., 2003. Mollusc as bioindicators, in: Markert, B.A., Breure, A.M., Zechmeister, H.G. (Eds.), Trace Metals and other Contaminants in the Environment. Elsevier, Netherlands, pp. 577-635.

Pechenik, J.A., Hsieh, J., Owara, S., Wong, P., Marshall, D., Untersee, S., Li, W., 2001. Factors selecting for avoidance of drilled shells by the hermit crab *Pagurus longicarpus*. Journal of Experimental Marine Biology and Ecology 262, 75-89, http://dx.doi.org/10.1016/S0022-0981(01)00284-2

Penchaszadeh, P.E., 1971. Aspectos de la embriogenesis de algunos gasterópodos del genero *Buccinanops* d´Orbigny, 1841 (Gastropoda, Prosobranchiata, Buccinidae). Physis 30, 475-482.

Piñeira, J., Quesada, H., Rolán-Álvarez, E., 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, http://dx.doi.org/10.1016/j.marpolbul.2007.10.020

Primost, M.A., 2014. Ecotoxicología y alteraciones morfo-funcionales en gasterópodos marinos expuestos a contaminación por organometales y metales, Facultad de Ciencias Naturales y Museo. Universidad Nacional de La Plata, La Plata, p. 165.

Primost, M.A., Averbuj, A., Bigatti, G., 2015a. Variability of imposex development and reproductive alterations in the Patagonian gastropod *Buccinanops globulosus* inhabiting a polluted harbour area. Revista del Museo Argentino de Ciencias Naturales n.s. 17, 167-171.

Primost, M.A., Bigatti, G., Márquez, F., 2016. Shell shape as indicator of pollution in marine gastropods affected by imposex. Marine and Freshwater Research 67, 1948-1954, http://dx.doi.org/10.1071/MF15233

Primost, M.A., Commendatore, M., Torres, P.J., Bigatti, G., 2018. PAHs contamination in edible gastropods from north Patagonian harbor areas. Marine Pollution Bulletin 135, 828-831, https://doi.org/10.1016/j.marpolbul.2018.08.021

Primost, M.A., Gil, M.N., Bigatti, G., 2017. High bioaccumulation of cadmium and other metals in Patagonian edible gastropods. Marine Biology Research 13, 774-781, https://doi.org/10.1080/17451000.2017.1296163

Primost, M.A., Giulianelli, S., Gil, M.N., Bigatti, G., 2015b. Acute toxicity in two edible marine gastropods with different sensitivity to Tributyltin. Pan-American Journal of Aquatic Sciences 10, 172-178, https://doi.org/10.1080/13235818.2017.1357671

Primost, M.A., Sabatini, S.E., Di Salvatore, P., Ríos De Molina, M.C., Bigatti, G., 2015c. Oxidative stress indicators in populations of the gastropod *Buccinanops globulosus* affected by imposex. Journal of the Marine Biological Association of the United Kingdom 97, 35-42, http://dx.doi.org/10.1017/S0025315415002155

Rawlings, T.A., 1994. Encapsulation of eggs by marine gastropods: effects of variation in capsules from on the vulnerability of embryos to predation. Evolution 48, 1301-1313, https://doi.org/10.1111/j.1558-5646.1994.tb05314.x

Rawlings, T.A., 1999. Adaptations to physical stresses in the intertidal zone: The egg capsules of neogastropod molluscs. American Zoologist 39, 230-243, https://doi.org/10.1093/icb/39.2.230

Rittschof, D., McClellan-Green, P., 2005. Molluscs as multidisciplinary models in environment toxicology. Marine Pollution Bulletin 50, 369-373, https://doi.org/10.1016/j.marpolbul.2005.02.008

Rohlf, F.J., Slice, D., 1990. Extensions of the procrustes method for the optimal superimposition of landmarks. Systematic Biology 39, 40-59, https://doi.org/10.2307/2992207

Savriama, Y., Stige, L.C., Gerber, S., T., P., Alibert, P., David, B., 2015. Impact of sewage pollution on two species of sea urchins in the Mediterranean Sea (Savriama et al., 2015): Radial asymmetry as a bioindicator of stress Ecological Indicators 54, 39-47, https://doi.org/10.1016/j.ecolind.2015.02.004

Sepúlveda, R.D., Ibáñez, C.M., 2012. Clinal variation in the shell morphology of intertidal snail *Acanthina monodon* in the Southeastern Pacific Ocean. Marine Biology Research 8, 363-372, https://doi.org/10.1080/17451000.2011.634813

Slice, D.E., Bookstein, F.L., Marcus, L.E., Rohlf, F.J., 1996. Appendix I: a glossary for geometric morphometrics, in: Marcus, L., Corti, M., Loy, A., Naylor, G., Slice, D. (Eds.), Advances in Morphometrics. Plenum Press, New York, pp. 531-551.

Solas, M.R., Hughes, R.N., Márquez, F., Brante, A., 2015. Early plastic responses in the shell morphology of *Acanthina monodon* (Mollusca, Gastropoda) under predation risk and water turbulence. Marine Ecology Progress Series 527, 133-142, https://doi.org/10.3354/meps11221

Stroben, E., Oehlmann, J., Fioroni, P., 1992. The morphological expression of imposex in *Hinia reticulata* (Gastropoda: Buccinidae): a potential indicator of tributultin. Marine Biology 13, 625-636, https://doi.org/10.1007/BF00349706

Untersee, S., 2007. Effects of Encapsulated Development and Larval Dispersal on Susceptibility to Phenol and Copper for Marine Gastropods *(Untersee, 2007)*. ProQuest, Tufts University, Massachusetts, USA, p. 114.

Wu, L., Chen, Z., Zhu, J., Hu, L., HUANG, X., Shi, H., 2014. Developmental toxicity of organotin compounds in animals. Frontiers in Marine Science 1, 1-4, https://doi.org/10.3389/fmars.2014.00039

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

Zhu, X., Zhou, J., Cai, Z., 2011. TiO2 nanoparticles in the marine environment: Impact on the toxicity of tributyltin to abalone (*Haliotis diversicolor supertexta*) embryos. Environmental Science & Technology 45, 3753-3758, https://doi.org/10.1021/es103779h

Declaration of interests

▯ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author contributions

Authors' individual contributions M.A. Primost: Investigation, Methodology, Resources, Writing - Original Draft. A. Averbuj: Methodology, Resources, Writing - Review & Editing. G. Bigatti: Resources, Writing - Review & Editing, Funding acquisition. F. Márquez: Investigation, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Review & Editing. All authors reviewed the manuscript.