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Variability of imposex development and reproductive alterations in the Patagonian gastropod *Buccinanops globulosus* inhabiting a polluted harbour area

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Abstract: The aim of this work was to study the reproductive output and imposex parameters of the caenogastropod *Buccinanops globulosus* inhabiting a harbour area, with high maritime traffic and pollution. The results showed higher imposex incidence for non-gravid females than for gravid females (carrying egg capsules). Among imposex-affected females, mean penis size was significantly larger in non-gravid (3.4 mm) than gravid (1.5 mm) females. In *B. globulosus* usually a single juvenile hatches per egg capsule, but in this study capsules with multiple embryos were found. Overall, these results suggest a relationship between imposex development and alterations on the reproductive output of *B. globulosus* females living in adverse environmental conditions (polluted area).

Key words: Maritime traffic, Offspring, Pollution, Reproduction, TBT.

Resumen: El objetivo de este trabajo fue estudiar parámetros reproductivos y desarrollo de imposex en el caenogasterópodo *Buccinanops globulosus* que habita en una zona portuaria de alto tráfico marítimo y con presencia de contaminantes. Los resultados mostraron una mayor incidencia de imposex en hembras sin ovicápsulas que en hembras con ovicápsulas. Entre las hembras con imposex, el desarrollo del pene fue significativamente mayor en aquellas sin ovicápsulas (3.4 mm) que en las hembras con ovicápsulas (1,5 mm). En *B. globulosus* comúnmente un solo juvenil eclosiona por cada cápsula, sin embargo, en este estudio se registraron capsulas con múltiples embriones. Todos estos resultados sugieren una relación entre el desarrollo de imposex y alteraciones en los parámetros reproductivos de las hembras de *B. globulosus* que habitan en sitios con condiciones ambientales adversas (área contaminada).

Palabras clave: Tráfico marítimo, Descendencia, Contaminación, Reproducción, TBT.

INTRODUCTION

Harbour activities, in particular maritime traffic, imply the release of toxic substances such as tributyltin (TBT) (Fent, 2003). This toxic compound can be accumulated, persisting in the sediments for up to 2.5 years (de Mora *et al.*, 1995). In female caenogastropods, the neoformation of a penis or *vas deferens* in individuals exposed to TBT is known as imposex (Gibbs & Bryan, 1996). Because imposex is irreversible (Stroben *et al.*, 1992) researchers worldwide have used this phenomenon as a marine pollution biomarker associated to maritime traffic (Axiak *et al.*, 2003, Ketata *et al.*, 2008). In areas highly polluted with TBT, sterile females and popula-

tion declines have been observed (Oehlmann *et al.*, 2007). In Argentinean harbour areas, several gastropod species have been reported to be affected by imposex, including *Buccinanops globulosus*, and its congeners *B. monilifer* and *B. cochlidium* (Penchaszadeh *et al.*, 2001, Bigatti *et al.*, 2009, Averbuj & Penchaszadeh, 2010a). Pollutants such as TBT, heavy metals and polycyclic aromatic hydrocarbons (PAHS) were detected in Nuevo Gulf at north Patagonia, Argentina (Gil *et al.*, 2006, Massara Paletto *et al.*, 2008, Bigatti *et al.*, 2009). The highest concentrations of TBT were reported in the harbour area of Nuevo Gulf, with up to 174.81 ng (Sn).g⁻¹ in sediments and 345.27 ng (Sn).g⁻¹ in body tissues of the gastropod *Odontocymbiola magellanica* (del Brio, 2011).

Buccinanops globulosus presented high values of imposex parameters in moderately polluted areas with TBT concentrations of 0.4 ng (Sn).g⁻¹ dry sediment; thus, it was classified as a good indicator due to its high sensitivity to low concentrations of TBT (Bigatti *et al.*, 2009).

Buccinanops globulosus lives mostly buried in sandy or muddy bottoms (Scarabino, 1977). This species is edible and is part of an expanding artisanal fishery (Narvarte, 2006, Averbuj *et al.*, 2014). Adult females spawn once a year a variable number of egg capsules that are attached to their own shells (Penchaszadeh, 1971). Generally one *B. globulosus* crawling juvenile, measuring between 3-7 mm, hatches from each egg capsule (Penchaszadeh, 1971, Narvarte, 2006, Averbuj *et al.*, 2014).

The aim of this work was to study the reproductive output and imposex parameters of *B. globulosus* inhabiting a polluted area.

MATERIALS AND METHODS

Females of *B. globulosus* were collected during February 2012 when egg capsules with embryos in late stages of development are most abundant (Averbuj *et al.*, 2014). The "gravid females" occur mainly from October to March, when the water temperature ranges between 11°C and 18°C (Averbuj *et al.*, 2014). This work was performed at Luis Piedra Buena Harbour (LPBH, 42°45'45" S and 64°1'51" W), a high maritime traffic area with ~730 vessels per year (APPM, 2013). In this area sporadic catches of *B. globulosus* are carried out for human consumption.

Buccinanops globulosus individuals were captured by SCUBA diving, then placed in glass aquaria and sexed by presence (female) or absence (male) of the accessory glands. The gastropods were counted and classified into three categories: female, male and gravid female (carrying egg capsules). This species was chosen considering its sensitivity to TBT and the fact that the egg capsules are attached to the female's own shell (Penchaszadeh, 1971, Averbuj & Penchaszadeh, 2010b), allowing for a straightforward estimation of reproductive output of each gravid female. Total shell length (TSL), female penis length (FPL) and male penis length (MPL) were measured. The imposex percentage (I%), was estimated and the relative penis length index (RPLI) was calculated according to (Gibbs & Bryan, 1994). Reproductive output was estimated for gravid females (Fig. 1) when embryos were at hatching stage (Averbuj *et al.*, 2014). The fol-

Table 1: Imposex and reproductive output parameters (mean ± SD, where corresponds) in *B. globulosus*. Abbreviations: FPL= female penis length, MPL= male penis length, RPLI= relative penis length index.

	Imposex %	FPL (mm)	RPLI	MPL (mm)
Without EC	100	3.40±0.95	25.03	13.25 ±5.6
With EC	89	1.50±1.05	11.06	

lowing parameters were considered for reproductive output: a) number of egg capsules per female (# EC), b) number of embryos per egg capsule, c) total number of embryos per female and d) mean embryo size per female. Egg capsules with lysed eggs/embryos or undeveloped embryos were not considered but special attention was taken for cases of embryo malformation. To estimate the mean embryo size (d), 10 hatchling embryos were randomly removed from each of 15 gravid females and measured to determine embryo shell length (ESL) under a stereomicroscope. The Mann-Whitney test was used to test for differences in the mean female shell length and mean female penis length (FPL), between gravid and non-gravid imposex females. In all cases the STATISTICA 7® software was used.

RESULTS

A total of 67 individuals of *Buccinanops globulosus* were studied. The minimum TSL of gravid females was 37.78 mm and the total shell length (TSL) was not significantly different (U=243.5; p=0.713; n=43) between gravid (41.88±2.40 mm) and non-gravid (41.58±2.56 mm) females. Imposex parameters (I%, FPL and RPLI) were higher in females without egg capsules than in gravid females (Table 1). Furthermore, all females without imposex incidence were gravid. Penis length was significantly larger in non-gravid than gravid females (U=44.0, p<0.0001, n=41) (Table 1). None of the studied females were sterilized or exhibited an occluded gonopore.

On average 47.89±2.7 egg capsules occurred per female, with 56.73±4.3 total embryos per females and 1.19±0.07 embryos per egg capsule. A high proportion (74%) of gravid females carrying egg capsules with multiple embryos (ME: >1 embryo/capsule) was recorded. On average, 17.3% of the egg capsules contained multiple embryos. The mean embryo shell length (ESL) was

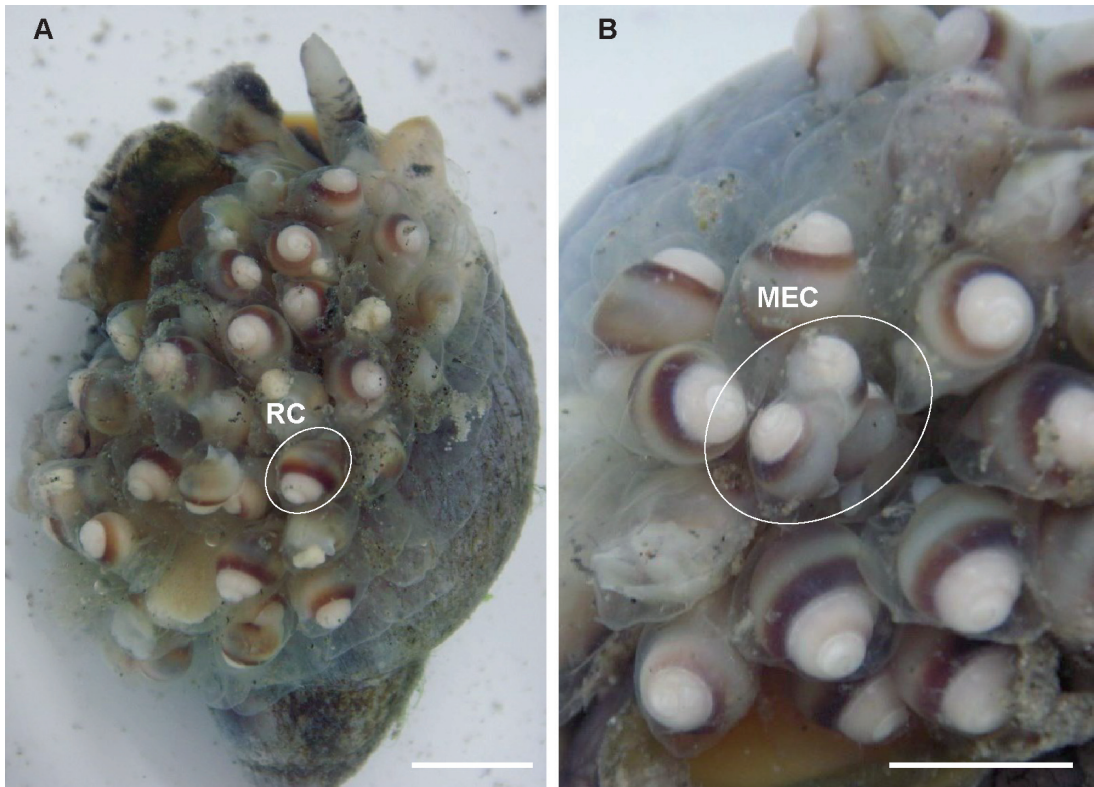


Fig. 1. Gravid females of *B. globulosus* with conspicuous egg capsules in late stages of development. (A) General aspect of the capsule mass, regular capsules with 1 embryo; (B) Capsules with multiple embryos. Abbreviations: MEC, multiple embryos capsule, RC, regular capsule. Scale bars = 10mm.

5.04±0.06 mm. No malformed embryos were observed during this study.

DISCUSSION

The reproductive output, defined as the mean number of embryos (and their sizes) spawned per female, has been shown to be susceptible to variability in different environmental conditions (Ramirez Llodra, 2002). The effects of pollutants on gastropod reproductive output has been associated with histological changes in gonads and aborted egg capsules in females (Gibbs *et al.*, 1991, Axiak *et al.*, 2003, Oetken *et al.*, 2004). Although generally in *B. globulosus* only one embryo is developed per egg capsule (Penchaszadeh, 1971), a population recently studied in Golfo Nuevo showed 0.22% of egg capsules with multiples embryos (Averbuj *et al.*, 2014). Our results showed that gravid females presented on average a large proportion of egg capsules with multiple embryos (17.3%). Differences in imposex parameters between gravid and non-gravid females are

remarkable. Imposex-affected gravid females showed significantly smaller penis than non-gravid ones, suggesting that the later may be more severely affected by imposex, since exposition to the endocrine disrupter tributyltin (TBT) induces a concentration -and time- dependent imposex development in gastropod females (Bettin *et al.*, 1996). Similarly, in *B. monilifer* from Mar del Plata (northern Argentina), females that succeed in spawning showed significantly lower imposex incidence (imposex % and penis length) than the rest of the female population (Averbuj & Penchaszadeh, 2010a). It is important to note that the larger penis recorded in non-gravid females were not related to female sterilization by gonopore occlusion, although it clearly evidence major imposex affection. This suggests either major exposure or individual sensibility to TBT (and probably other pollutants that might affect reproductive output) in *B. globulosus*.

Higher penis development infemales with reduced reproductive capacity may be suggesting an alteration of TBT on the reproductive physi-

ology, in particular affecting gonadic processes (Ramirez Llodra, 2002, Oehlmann & Schulte-Oehlmann, 2003). The studied population of *B. globulosus* could be negatively affected, limiting the number of females capable of reproduction and, complementarily altering its reproductive output under unfavorable environmental conditions as reported for other gastropods species (Marshall *et al.*, 2008, Chatzinikolaou & Richardson, 2010, Collin & Spangler, 2012). This pattern, evident in the genus *Buccinanops* due to its particular breeding behavior, may be occurring in other gastropod species inhabiting similar polluted environments.

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