

Comparative study of the female gametogenic cycle in three populations of *Buccinanops globulosus* (Caenogastropoda: Nassariidae) from Patagonia

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Abstract The populations of *Buccinanops globulosus* from three different sites of northern Patagonia were compared in the female reproductive cycle over a 2-year study period (September 2006–August 2008). These populations differed in demographic and life-history traits (sex ratios, size frequency distributions, growth rates and fecundity). Also, two of these populations show imposex-affected females (Bahía San Antonio, BSA and Bahía Nueva, BN), whereas the other (Playa Villarino, PV) is imposex-free and constitutes a commercial fishery ground for this species. The gametogenic activity of this species has not been studied earlier. Females showed the same gonadal stages in the three populations; however, there were differences in the female size at which each gonadal stage occurred. Monthly variation in gonadal stages and in oocyte size indicated that females of *B. globulosus* showed

a seasonal gametogenic pattern without the occurrence of a resting period. Evacuation of mature oocytes (up to 252.5 µm) occurred mainly from September to December (austral spring) in BSA and PV and from December to March (austral summer) in BN. Gonad maturation and the presence of females carrying egg capsules in the field coincided with changes in water surface temperature and a longer day length. Our results indicate that the occurrence of imposex in the populations studied apparently does not affect the gametogenic activity in females. This study provides further insights into the reproductive biology of *B. globulosus*. The identification of population variation in reproductive traits should lead to more effective management of the species.

Keywords Gonadal cycle · Imposex · Reproductive traits · Nassariidae · Patagonia

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Introduction

Determining reproductive processes in marine organisms is key to understand their reproductive output and population dynamics (Underwood and Keough 2001). The timing of reproduction represents a crucial life-history trait, which influences both the future prospect of offspring and the parental fitness (Varpe et al. 2007). Finely tuned breeding seasons may be important to provide juveniles with favorable environmental conditions.

The sexual activity of organisms living in fluctuating environments is usually a cyclic event, and gametogenic cycles are a fundamental part of this (Giese and Pearse 1974). Gametogenic cycles are intrinsic processes within an individual. However, they are influenced by factors that are both internal, such as accumulation of nutrients and

hormonal cycles, and external such as temperature and photoperiod. External factors may initiate and synchronize the timing of gametogenic events with environmental changes. Thus, the timing of reproduction may vary from species to species and among populations of the same species.

The nassariid whelk *Buccinanops globulosus* is an endemic macrofaunal species of coastal waters (up to 10 m depth) found from 34° to 48°S in the southwestern Atlantic Ocean (Pastorino 1993). Its habitat is restricted to sandy or muddy patches, where it spends most of the time buried (Scarabino 1977). Like most Neogastropoda species, *B. globulosus* is gonochoristic with internal fertilization. This species is considered as a bioindicator of TBT contamination in Patagonian waters because females may be imposex affected, i.e., characteristics of males superimposed onto females (Bigatti et al. 2009). As in other species of the genus, the egg capsules are attached to the callus and the adjacent region of the female shell (Penchaszadeh 1971, 1973; Averbuj and Penchaszadeh 2010; Avaca et al. 2012). At oviposition, egg capsules are filled with nurse eggs that provide nutritional support to a single developing embryo. Maternal body size is positively related to some proxies of reproductive success (number of nurse eggs, egg capsular area and total length at hatching) at least in one of the populations previously studied (Avaca et al. 2012). *B. globulosus* is a direct developer: Crawling juveniles emerge from the egg capsules and settle in the maternal environment, a behavior that ensures favorable conditions in the short term but limits dispersal (Penchaszadeh 1973). Even though this species constitute a fishery resource commercially exploited since 2000 in northern Patagonia (Narvarte 2006), the gametogenic cycle and other reproductive traits of this species have not been previously studied.

Our recent studies have shown that this nassariid species shows great variability among populations in several demographic and life-history traits (sex ratios, size frequency distributions, growth rates and fecundity; Narvarte 2006; Narvarte et al. 2008; Avaca et al. 2012, 2013a, b). This suggests that there may also be variation among populations in the species reproductive traits, such as gametogenic activity. Also, the occurrence of imposex may introduce variation between imposex-affected and imposex-free populations. While imposex is observed in *B. globulosus* females carrying egg capsules and thus reproduction does not appear to be compromised (Avaca et al. 2012), there is considerable interest in the knowledge of the gametogenic activity of imposex-affected females.

In the present study, we investigated the female gametogenic cycle in three populations of *B. globulosus* from Patagonia using histological techniques. We described female gonadal stages and seasonality of the gametogenic

activity to evaluate them in relation to population characteristics (origin and presence/absence of imposex) and to environmental conditions (day length and water temperature). We measured oocyte size as a descriptor of the gonadal activity and to compare this reproductive trait among populations. We also provided new information regarding gonadal status at mating and oviposition in order to illustrate other aspects of the reproductive biology of this species. The results will provide a more complete picture of the life history of this species and can be used for future comparisons among populations of *B. globulosus*. Also, they could help to identify potential links between reproduction and environmental variables.

Materials and methods

Sample collection

The present study was conducted in the northern Patagonia, Argentina, in Golfo San Matías (GSM; 40°50'S 65°10'W) and Golfo Nuevo (GN; 42°29'S 63°05'W). Specimens were collected monthly from September 2006 to August 2008 from three sites for which other life history features of *B. globulosus* were known (Narvarte et al. 2008; Avaca et al. 2013a, b). Two sampling sites were located in SMG (Bahía San Antonio [BSA]: 40°29'S, 63°01'W and Playa Villarino [PV]: 40°45'S, 64°40'W) and one in NG (Bahía Nueva [BN]: 42°46'S, 65°02'W; Fig. 1).

Specimens were attracted with biological baits (mainly discarded fish) and collected from the intertidal-low subtidal zones by SCUBA diving or handpicking, at high and low tide, respectively. This method showed to be efficient to catch individuals of all sizes at each sampling site (Narvarte et al. 2008). Individuals were kept in containers filled with sea water to maintain them alive. The presence of females carrying egg masses in the field was recorded at each sampling date. Also, non-systematic observations regarding mating activity (i.e., presence of pairs of individuals constituted by a male located above a female; Avaca et al. 2012) were performed.

Laboratory techniques

In the laboratory, individuals were kept in containers filled with sea water and processed within the day of collection. Individuals were selected to cover the whole size range of each population (Narvarte et al. 2008; Avaca et al. 2013a). Thus, small individuals, suspected to be juveniles, were also included in the subsample for histological analysis. With some exceptions, each subsample contained at least ten individuals (Table 1). Based on a first examination, individuals a priori identified as females, were measured in

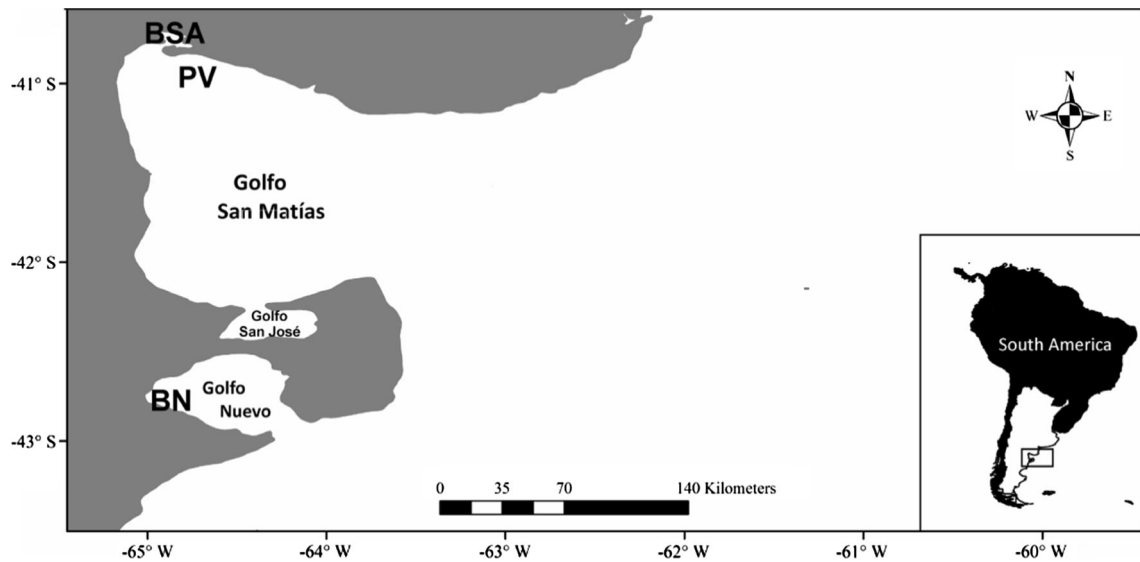


Fig. 1 Location of the sampling sites in Golfo San Matías and Golfo Nuevo in northern Patagonia. *PV* Playa Villarino, *BSA* Bahía San Antonio, *BN* Bahía Nueva

Table 1 Number of individuals histologically processed and number of oocytes measured (number of females used to measure the oocytes) for each population and collection date

Date	Bahía San Antonio		Playa Villarino		Bahía Nueva	
	Histology	Oocytes	Histology	Oocytes	Histology	Oocytes
Sep 06	12	263 (6)	10	186 (6)	15	289 (7)
Oct 06	11	159 (4)	9	215 (5)	24	284 (7)
Nov 06	11	103 (2)	6	180 (4)	25	219 (6)
Dec 06	12	105 (5)	9	236 (6)	19	268 (6)
Jan 07	14	311 (6)	9	119 (3)	14	248 (6)
Feb 07	16	256 (6)	15	251 (5)	20	267 (6)
Mar 07	8	204 (4)	16	318 (7)	13	250 (5)
Apr 07	14	266 (9)	10	140 (3)	7	250 (5)
Jun 07	8	160 (4)	10	100 (2)	NS	
Jul 07	NS		NS		12	285 (7)
Aug 07	13	253 (6)	19	272 (7)	14	251 (5)
Sep 07	14	279 (7)	14	287 (7)	12	217 (5)
Oct 07	17	180 (5)	14	266 (6)	14	252 (5)
Nov 07	16	251 (6)	14	225 (7)	8	196 (4)
Dec 07	15	224 (6)	5	77 (2)	10	248 (6)
Jan 08	16	237 (6)	15	281 (6)	11	231 (6)
Feb 08	16	300 (6)	14	250 (5)	13	292 (7)
Mar 08	10	207 (5)	17	121 (3)	13	150 (3)
Apr 08	20	253 (7)	NS		13	258 (6)
May 08	NS		17	395 (8)	NS	
Jun 08	11	228 (5)	NS		10	192 (5)
Aug 08	8	151 (5)	16	238 (6)	13	256 (5)
Sample size	262	4,390	239	4,157	277	4,903

NS: no sampling when weather conditions were bad or light hours were reduced

total shell length with Vernier calipers (± 0.01 mm). The shell was mechanically broken and the whole body of individuals removed to confirm the sex. Due to the occurrence of imposex in two of the populations studied,

BSA and BN (Bigatti et al. 2009; Avaca et al. 2013b), sex should be determined based on the presence of the albumen gland in females and by its absence and the presence of a penis in males. When present, the egg mass was carefully

detached from the shell and then classified according to the number of nurse eggs and the developmental stage of embryos into the following categories: Partly deposited, Early, Advanced and Hatched (Avaca et al. 2012).

Percentages of imposex-affected females were calculated as the number of females with penis and/or vas deferens with respect to all females sampled of each population. The sex of all individuals was later confirmed by histological examination of their gonads.

After removing the shell and according to individual size, the whole body, the entire gonad or only a section was fixed in Davidson's fluid for 24 h at 4 °C and subsequently stored in 70 % ethanol for histological analysis. Tissues were dehydrated using an ascending series of ethanol concentrations and then embedded in paraffin. Sections were cut at 5 µm with a microtome, stained with hematoxylin–eosin and observed under a light microscope. To describe the female gametogenic cycle, sections were classified according to gonadal developmental stage.

Monthly frequency distributions of oocyte diameter were estimated to quantitatively describe the gametogenic cycle of mature females. The oocytes still attached to the wall as well as those found free in the follicular lumen, both presenting a clear nucleus with nucleolus, were measured at their major and minor axes, and both measurements were used to calculate the mean oocyte diameter (MOD). The MOD was calculated for 20–50 oocytes per female. When possible, this process was repeated at least in five mature females for the monthly sample of each population (Table 1). To characterize oocyte diameter of immature females, two females in Proliferation and Growth I of each population were considered. All measurements were taken using a microscope and eye-piece reticle.

To test the intraspecific variation in egg size (measured as MOD), nested analysis of variance (ANOVA) was performed for females in Pre-evacuation, with females nested within population as a random effect. All assumptions were checked to be met before the analyses by visual inspection of residuals.

Environmental variables

To assess the relationship between the pattern of gonadal activity and temperature, the water surface temperature (°C) was recorded in situ with a multiparameter probe (YSI 556 MPS) between July 2007 and May 2008 at the time of sample collection. Also, sea surface temperature (SST) regimes based on estimations available in public access databases (single 4 × 4 km grid cells closest to the point of interest; AVHRR Pathfinder 5, www.poet.jpl.nasa.gov NOAA-NASA) were analyzed for the 1997–2007 period at a selected geographic point (GSM: 41°25'S, 64°26'W; GN: 42°32'S, 64°52'W). The photoperiod cycle, also considered

as an environmental variable that may influence the reproductive activity of *B. globulosus*, was obtained from the Servicio de Hidrografía Naval de Argentina, available online for different lighthouses of Argentina (<http://www.hidro.gov.ar>; SMG: San Matías lighthouse; NG: Morro Nuevo lighthouse).

Results

Body size and sex determination

A total of 778 individuals from the three populations were histologically analyzed. The size range of individuals was 10.6–31.7 mm for BSA (mean ± SD = 19.1 ± 4.3; $n = 262$), 13.5–67.2 mm for PV (40.0 ± 15.2; $n = 239$) and 12.5–45.0 mm for BN (29.5 ± 15.2; $n = 277$). The sex of individuals identified a priori as females was confirmed by histological observation of the gonad in 82.8 % of the samples on average. In BSA, 83.1 % were females, 8.6 % lacked gonadal tissue (undifferentiated) and 8.2 % were males ($n = 202$, 21 and 20, respectively). In PV, 80.2 % were females and the remaining was undifferentiated ($n = 187$ and 46, respectively). In BN, 83.5 % were females, 10 % were undifferentiated and 6 % were males ($n = 229$, 17 and 28, respectively). Of the females analyzed, 22.5 and 90 % showed imposex in BSA and BN, respectively (Fig. 2). No female from PV was imposex affected. Some individuals from the three populations (BSA $n = 19/262$, PV $n = 6/239$, BN $n = 3/277$) showed numerous sporocysts of an unidentified trematode in the digestive gland but not in the gonads; these individuals were not used for the study of the gametogenic cycle.

Gonadal structure and gonadal stages

The ovary was situated in the first whorl of the shell and could be easily distinguished from the digestive gland because of its different coloration. However, it could not be accurately separated from it, so it was not possible to calculate a gonadosomatic index. The color of the ovary varied from whitish to beige, being most of the times yellowish, whereas the digestive gland was brownish. Sometimes, it was possible to directly visualize the “eggs” by transparency in the ovary.

Based on histological observations, gonad sections were classified into six stages: Undifferentiated, Proliferation and Growth I, Growth II, Pre-evacuation, Evacuation and Post-evacuation. Undifferentiated individuals were characterized by the lack of differentiated gonadal tissue. In these individuals, the digestive gland was at the first whorl. When the gonad was developed, many follicles were present. During Proliferation and Growth I (Fig. 3a), few

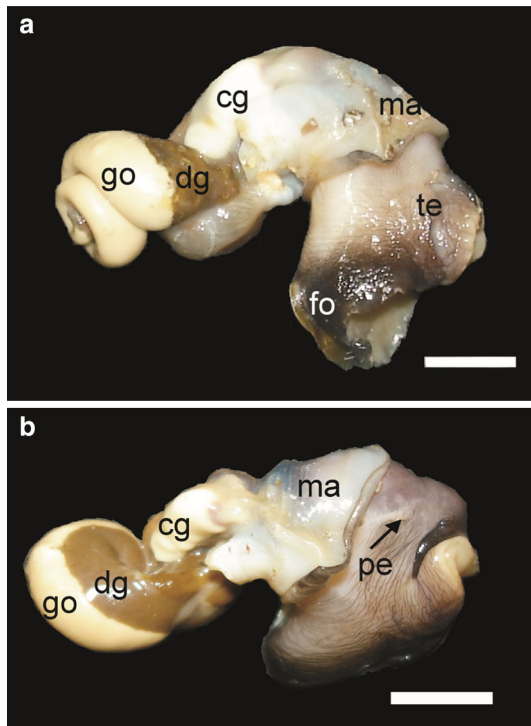


Fig. 2 *Buccinanops globulosus*. **a** Normal female from Playa Villarino, **b** imposex-affected female from Bahía Nueva. *cg* capsule gland, *dg* digestive gland, *fo* foot, *go* gonad, *te* tentacle, *ma* mantle, *pe* penis. Scale bars 1 cm

separate follicles embedded in connective tissue were observed. The gonadal tissue was composed of oogonia and primary oocytes (MOD mean \pm SD = $35.6 \pm -5.7 \mu\text{m}$, $n = 137$), but no vitellogenic or degenerating oocytes were present. At this stage, the follicular lumen was scarce and oogonia/oocytes showed a well-developed nucleus and nucleolus. Other cell types, such as the companion cells, were found surrounding the oocytes. As the oocytes grew in size (MOD mean \pm SD = $64.1 \pm 21.7 \mu\text{m}$, $n = 4,985$), eosinophilic granules presumably of vitellus appeared in the cytoplasm and the number of other types of cells decreased (Growth II; Fig. 3b). At this stage, oocytes were oblong, and major and minor axes were distinguished. The follicles became more closely packed, and the connective tissue between them was reduced. At Pre-evacuation (Fig. 3c), the follicles were full of large vitellogenic oocytes (MOD mean \pm SD = $124.7 \pm 27.7 \mu\text{m}$; $n = 4,468$) that were attached to the follicular wall and occupied the entire lumen. The connective tissue disappeared and follicles became highly compressed. The cytoplasm of oocytes was uniformly filled with eosinophilic granules, making it difficult to identify nuclei and nucleoli. During Evacuation (Fig. 3d, e), there were free oocytes in the lumen (maximum MOD = $252.5 \mu\text{m}$) and large oocytes were still attached to the wall. The lumen of some follicles became emptier, and some pre-vitellogenic

oocytes were observed near the follicle wall. Thus, females with gonads in Evacuation had two cohorts of oocytes, smaller oocytes in the germinal layer (range $20\text{--}80 \mu\text{m}$) and larger ones that were being released (range $120\text{--}252 \mu\text{m}$). At the Post-evacuation stage (Fig. 3f), the follicles lost their typical shape and varied between large empty follicles to empty small follicles dispersed in a network of connective tissue. The gonadal tissue was composed of a thin layer of oogonia and oocytes (MOD mean \pm SD = $42.0 \pm 26.2 \mu\text{m}$; $n = 2,599$) that restarted the cycle. Also there were reabsorbed oocytes and other gametogenic remains.

The six gonadal stages were recognized in the individuals from the three populations. However, the size at which each gonadal stage occurred was different among populations (Fig. 4). The shell length of individuals with undifferentiated gonads ranged from 10.6 to 18.4 mm (mean \pm SD = 12.8 ± 1.9), 13.5–28.6 mm (20.0 ± 4.6) and 12.9–22.8 mm (17.7 ± 3.3) for BSA, PV and BN, respectively. The minimum sizes of mature females (at Growth II, Pre-evacuation, Evacuation and Post-evacuation) were 15.6, 38.7 and 19.76 mm for BSA, PV and BN, respectively. Once females reached maturity, their gonads presented different stages of development independently of their body size. Imposex-affected females, both from BSA and BN, had all the gonadal stages found in the non-affected females. Also imposex-affected females did not show any evident difference in their development in comparison with the non-affected females.

The gonadal stages observed in females found in copulation were Pre-evacuation (62.5 %), Evacuation (12.5 %) and Post-evacuation (25.0 %). These females were sampled between September and October 2007 and ranged in size from 19.7 to 23.7 mm ($n = 4$) and 51.6–56.6 mm ($n = 4$) for BSA and PV, respectively. Also, the presence of spermatozoa was recorded in the *bursa copulatrix* of two females from BSA that were not found in pairs, but whose whole body was histologically processed. These females were sampled in August 2008. Their total shell lengths were 18.7 and 23.2 mm, and the gonads of these females were in Growth II and Pre-evacuation, respectively.

The gonadal stages of females carrying egg capsules varied according to the stage of the egg mass they were carrying. These females were 18.2–25.2 mm ($n = 10$), 50.1–57.6 mm ($n = 9$) and 21.2–36.8 mm ($n = 15$) for BSA, PV and BN, respectively. Eighty percent of the females carrying egg masses from BN showed imposex, whereas none of the females carrying egg masses from BSA were imposex affected. All the females with egg masses partially deposited ($n = 4$) and females with egg masses in Early stage of development ($n = 16$, 61.5 %) showed gonads mostly in Evacuation, whereas females

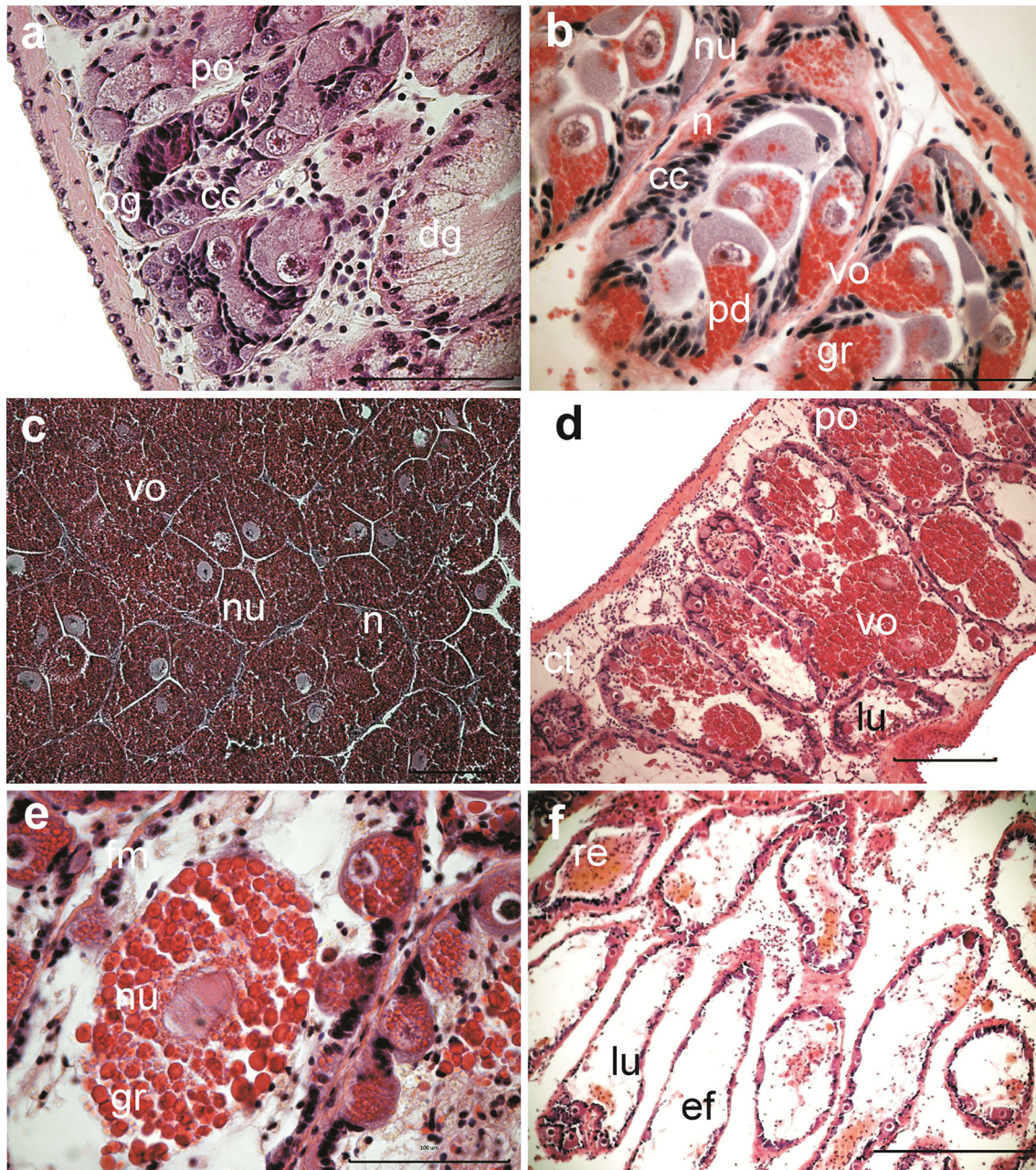


Fig. 3 *Buccinanops globulosus*. Female gonad at different developmental stages. **a** Proliferation and Growth I: follicles with oogonia and pre-vitellogenic oocytes surrounded by companion cells. **b** Growth II: oocytes attached to the follicular membrane still in contact with companion cells and the cytoplasm charged by vitellogenic granules. **c** Pre-evacuation: cytoplasm of oocytes fully occupied by vitellum and reduction in the lumen space. **d** Evacuation: general aspect of the ovary with the presence of free large vitellogenic oocytes in the lumen of the follicles. **e** Detail of a follicle at the

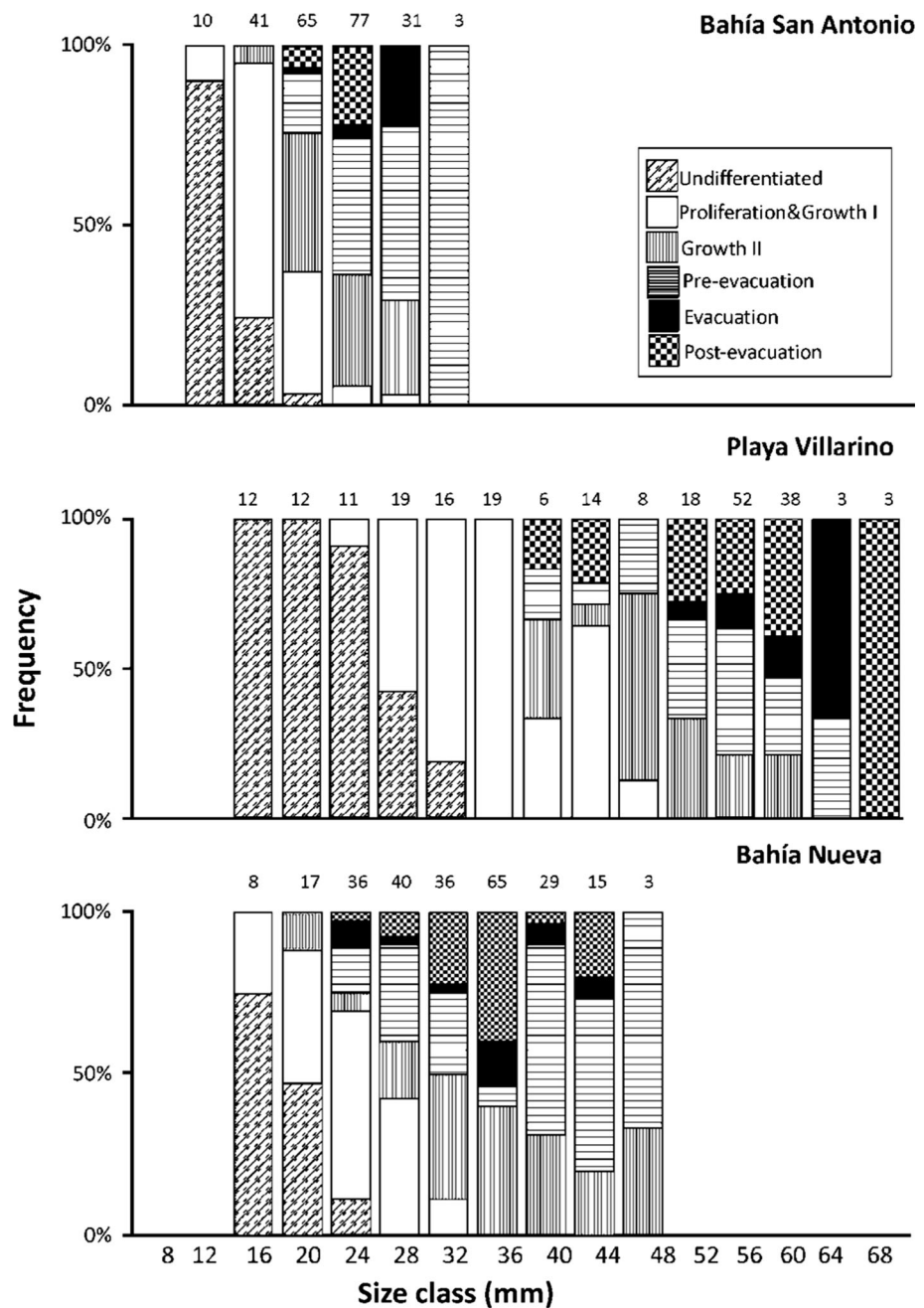
Evacuation stage; the presence of small oocytes in the follicular wall indicates the restarting of the growth process. **f** Post-evacuation: empty follicles lost their typical shape and showed only some gametogenic remains. *cc* companion cells, *dg* digestive gland, *ef* empty follicle, *fn* follicular membrane, *gr* vitellogenic granules, *re* gametogenic remains, *lu* lumen, *n* nucleus, *nu* nucleolus, *og* oogonia, *po* pre-vitellogenic oocyte, *vo* vitellogenic oocyte. Scale bars = 100 μ m (**a**, **b**, **f**); 200 μ m (**c**, **e**); 250 μ m (**d**)

with egg masses in the Advanced ($n = 7$, 100 %) and Hatched stages ($n = 7$, 85.7 %) showed gonads mainly in Post-evacuation. Also, one female with a Hatched egg mass showed the gonad in Growth II.

Gametogenic cycle

Histological examination of gonad sections indicated similar gametogenic activity patterns for most females from

Fig. 4 Gonad maturity stages by body size class and population studied. Numbers above the columns the number of whelks analyzed in each size class

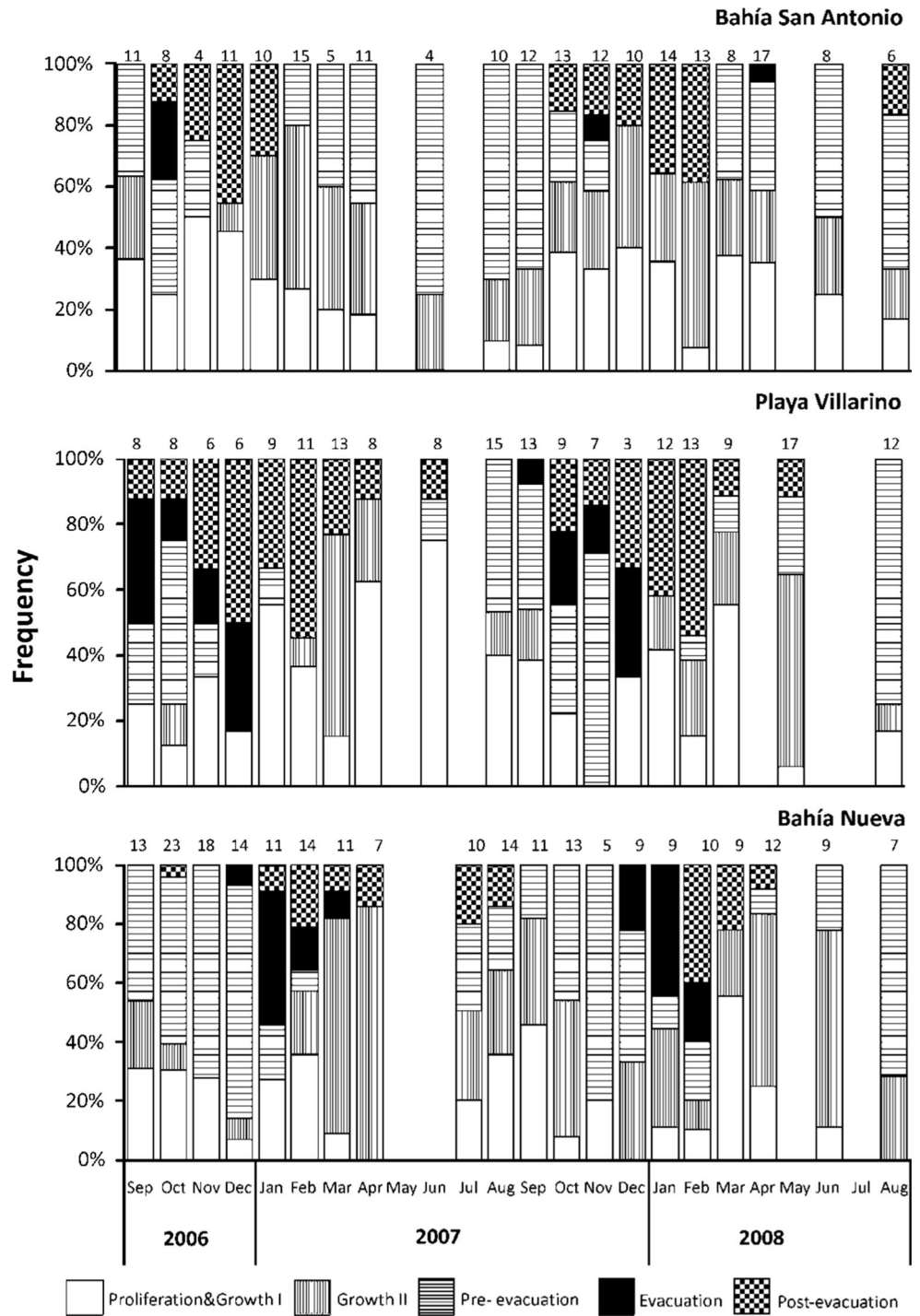


the three populations studied with differences related to seasonality (Fig. 5). Both mature and immature gonads (in Proliferation and Growth I) were present all year round at each population. Different stages of development occurred simultaneously within mature females of each population. Evacuation of oocytes occurred mainly from September to December (spring) in BSA and PV and from December to March (summer) in BN. In females from PV, Post-evacuation was found during almost all the study period (except August–September 2007 and August 2008), with an evident presence of reabsorbed oocytes and other gametogenic remains. Mature females developed oocytes (Growth II and

Pre-evacuation) during all the study period but mainly after evacuation (i.e., autumn and winter). In females from BSA, this growth process may produce another peak of evacuation in early autumn (e.g., April 2008).

The MOD for each population showed an annual variation in coincidence with the stages of gonads (Fig. 6). The maximum MOD in most females from BSA and PV was found from August to September–November, with large vitellogenic oocytes ready to be evacuated (late winter and spring). Then, the MOD decreased reaching minimum values between December and February (summer). By this time, oocyte size dispersion was low and a new oocyte

Fig. 5 Seasonal variation in gonad maturity stages found in females of *B. globulosus*. Numbers above the columns indicate the number of mature females observed in each date



cohort (median $\approx 40 \mu\text{m}$) started to develop thereafter. Some females from BSA showed a rapid increase in MOD and toward late March some oocytes were ready to be released (180–200 μm). At that moment, another evacuation peak may occur in coincidence with the occasional presence of females carrying egg capsules in the field, for example, in April 2008. In contrast, females from PV

showed a slight increase in MOD, suggesting that most of them were in the same stage, recovering from Evacuation. In both populations, as a result of oocyte maturation, MOD continued increasing until August to November, when larger sizes were reached and the cycle restarted (180–200 μm , maximum MOD = 232.5 and 253.7 μm in February 2008 for BSA and September 2006 for PV,

Fig. 6 Box plots showing monthly variation in the mean oocyte diameter (MOD) in the populations studied. Samples sizes are presented in Table 1

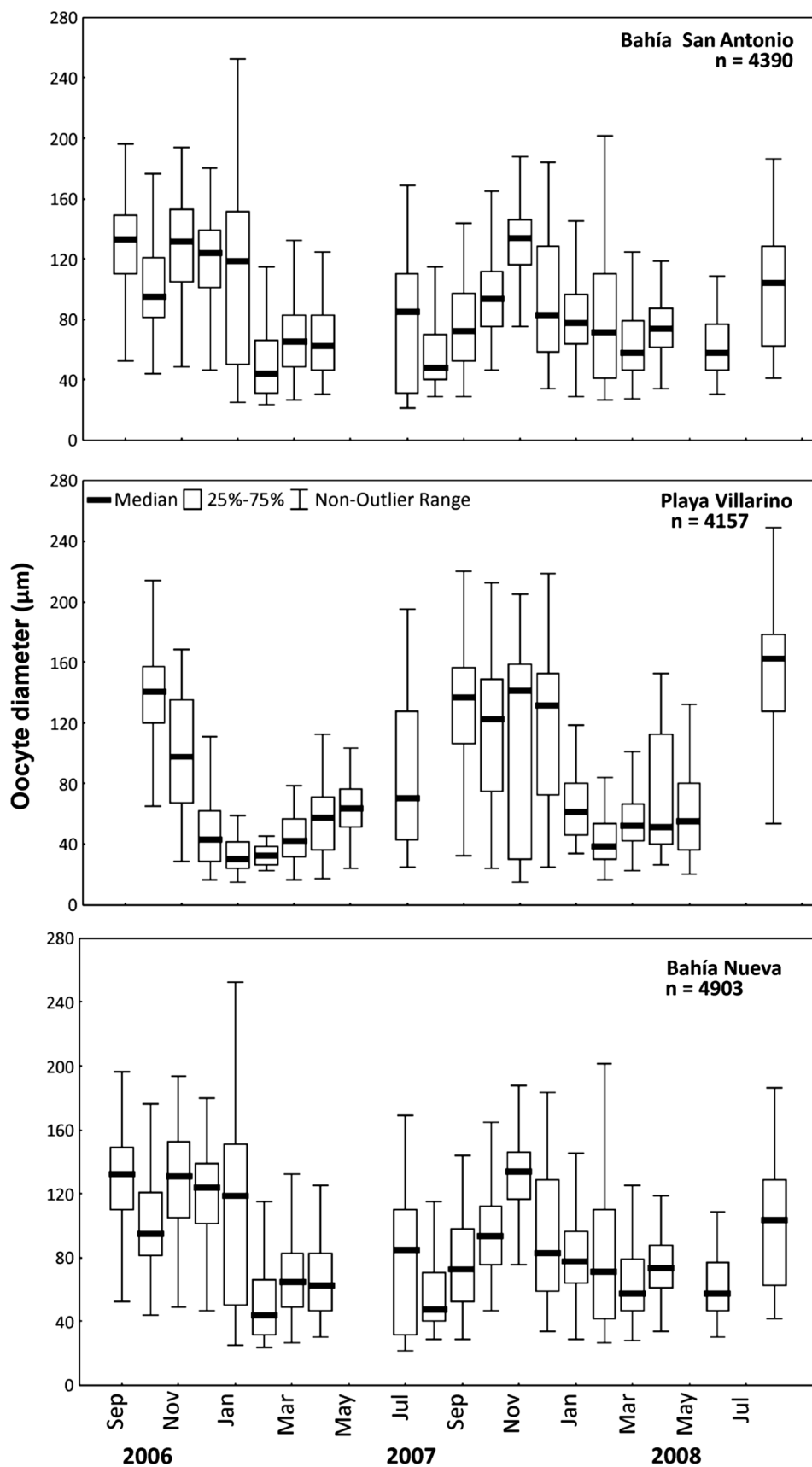


Table 2 Nested analysis of variance (ANOVA) of oocyte size at Pre-evacuation stage in females from Bahía San Antonio, Playa Villarino and Bahía Nueva

	<i>df</i>	Mean squares	<i>F</i>	<i>P</i>
Population	2	5,407.2	0.16	0.85
Population (female)	15	34,333.7	121.30	<0.0001
Error	882	282.9		

Females per population 6; replicates 50

Bold value indicates statistically significant ($P < 0.0001$)

respectively). Females from BN showed the maximum MOD values between September 2006 and January 2007 and between October 2007 and February 2008 (spring and summer). The maximum MOD value was 252.5 μm in January 2007. The minimum values of MOD were found after evacuation (median values = 43.7 and 57.5 μm in February 2007 and March 2008, respectively). As MOD values increased, the frequency size distribution became more dispersed with different cohorts of oocytes.

Although the population had no significant effect on the size of oocytes at Pre-evacuation, a large component of variance related to females was found within populations (70.6 of the total variance; Table 2).

Environmental variables and timing of reproduction

The minimum temperatures recorded in situ were 7.1 °C in BSA (August 2007), 7.7 °C in PV (July 2007) and 8.6 °C in BN (August 2007). The maximum values were recorded in January (summer) and were higher in BSA (27 °C) and BN (22.1 °C) than in PV (21.6 °C). The maximum increase in temperature was recorded between September and October in BSA (13–22 °C), between November and December in PV (13–18 °C) and between December and January in BN (18–21.3 °C). The day length was minimum in June (9 h; GSM: 9 h: 17', GN: 9 h: 04') and maximum in December (15 h; GSM: 15 h: 02', GN: 15 h: 17') at the three sites studied (Fig. 7).

The maximum reproductive activity, considered as Evacuation stage presented in the gonad and/or occurrence of females carrying egg capsules in the field, in the three populations and both years coincided with an increase in temperatures from winter to spring jointly with a long day (Fig. 7). The deposition of egg capsules started earlier in populations from GSM (BSA and PV, September 2006 and October 2007) than in the population from NG (BN, November 2007 and December 2006). The presence of females carrying egg capsules along the reproductive season was continuous in BSA and BN populations, but not in the PV population. From May to August, both day length and sea temperature decreased and, when sampling was carried out, no females carrying egg capsules were observed.

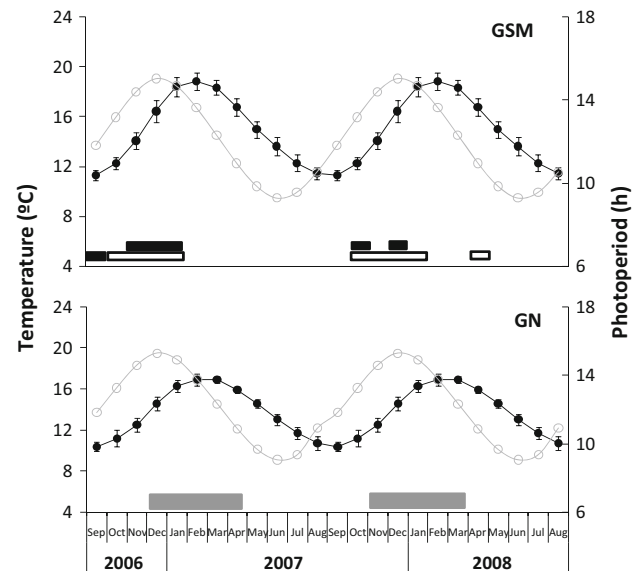


Fig. 7 Mean values and standard deviation of sea surface temperature (period 1997–2007, full circles), photoperiod (open circles) and oviposition in the populations of *B. globulosus* studied (shaded bars, black Playa Villarino, white Bahía San Antonio, and gray Bahía Nueva). Data from NOAA-NASA (www.poet.jpl.nasa.gov) and Servicio de Hidrografía Naval (www.hidro.gov.ar). GSM Golfo San Matías, GN Golfo Nuevo

Regarding the presence of pairs of individuals, observations performed at BN showed mating activity throughout the year (mating pairs were recorded in: November 2006, December 2006, February–April, July and October–November 2007 and January 2008). Fewer data were obtained at BSA (September and November–December 2007) and PV (August and October 2007), but in these cases, the observation effort was also lower.

Discussion

The populations of *B. globulosus* studied showed no differences in their gonadal stages, oocyte size and the annual pattern of gametogenic activity despite their differences in demographic and life-history traits. The results of this work confirm that female gametogenic activity occurs with no apparent differences between imposex-affected populations (BSA and BN) and an imposex-free population (PV) of the species. Gametogenic activity was related to an increase in water temperature and in day length, and only slight differences between northern populations and the southern one were detected. We also found that the gonad development was directly related to the characteristic body size of each population, i.e., immature stages at larger sizes in PV than in the other two populations. This finding is especially important for a sustainable management of this

Table 3 Environmental cues related with oviposition in Neogastropoda species inhabiting the southwestern Atlantic Ocean

Environmental cues	Species	Family	Location	Habitat	Source
Temperature rising	<i>Zidona dufresnei</i>	Volutidae	38°20'S; 57°37'W	Subtidal	Giménez and Penchaszadeh (2002)
	<i>Adelomelon brasiliana</i>	Volutidae	38°20'S; 57°37'W	Subtidal	Cledón et al. (2005)
	<i>Adelomelon beckii</i>	Volutidae	38°20'S; 57°37'W	Subtidal	Arrighetti and Penchaszadeh (2010)
Extended day length	<i>Odontocymbiola magellanica</i>	Volutidae	42°43'S; 65°01'W	Subtidal	Bigatti et al. (2008)
	<i>Adelomelon ancilla</i>	Volutidae	42°48' S; 64°54'W	Subtidal	Penchaszadeh et al. (2009)
Extended day length + temperature rising	<i>Buccinanops globulosus</i>	Nassariidae	40°29'S; 63°01'W 40°45'S; 64°40'W 42°46'S; 65°02'W	Intertidal-low subtidal	This study
Extended day length + low temperature	<i>Trophon geversianus</i>	Muricidae	42°46'S; 64°59'W	Intertidal	Cumplido et al. (2010)
	<i>Buccinanops cochlidium</i>	Nassariidae	42°25'S; 64°31'W	Subtidal	Averbuj et al. (2010)

fishery, taking into account that there are differences in size at maturity among populations (unpublished data).

The gametogenic cycle was characterized by the growth of oocytes during autumn and winter months and evacuation of gametes during late spring–summer months. By the end of the summer, individuals had evacuated and deposited egg capsules, and during autumn, without the occurrence of a resting period, the gametogenic activity had restarted. The mean oocyte size was a good descriptor of this temporal pattern. Many studies carried out at temperate regions have shown that different cues may stimulate reproductive activity. Among these cues, temperature and photoperiod have been the factors more pointed out to affect induction of oviposition in Neogastropoda species inhabiting the coast of the Argentine sea (Table 3).

Variation in the timing of the reproductive season is common within the geographic range of a species; for example, *Buccinum undatum* in the Gulf of St. Lawrence (Canada) shows a late spring summer reproduction in contrast with the winter breeding period reported in England and Sweden (Martel et al. 1986a, b; Kideys et al. 1993; Valentinsson 2002). In this study, the females from the northern populations started their reproductive activity earlier than those of the southern population. Also, females from bays (Bahía San Antonio and Bahía Nueva) showed a longer reproductive season than females from Playa Villarino. Regarding differences in seasonality, some threshold temperature may trigger reproduction and that value is reached slightly later at the southern population. For instance, during spring and early summer, a given temperature is reached approximately 1 month later in Golfo Nuevo than in Golfo San Matías (this study). Orton (1920) suggested that each species has a critical breeding temperature, which should be reached before reproductive activities proceed (Orton's rule, Thorson 1946). However,

Orton's rule does not strictly apply in many cases, and temperature is now considered an environmental cue rather than a driving force (Clarke 1993). This may applied for *B. globulosus* at the populations studied. The photoperiod cycle is a feature of latitude, and since in this study differences in the photoperiod cycle between sites were negligible, this feature seems not to contribute to the variation observed.

Although the pattern of size–age at maturity has not been described yet (unpublished data), it is clear that size at maturity varied largely according to the female origin. Females from Playa Villarino showed mature gonads at a size about two times larger (38.7 mm) than females from the other populations (19.8 and 15.6 mm and, for BN and BSA, respectively), whereas females carrying egg capsules were also larger in PV than in the other two populations (Narvarte et al. 2008; Avaca et al. 2012; this study). This finding is especially important taking into account that *B. globulosus* constitutes a fishery resource (e.g., Gendron 1992; Roggers-Bennett et al. 2004; Shelmerdine et al. 2007; Eversole et al. 2008).

Despite the occurrence of imposex, no differences at light microscopic level were detected in the gonadal stages between females from affected populations (Bahía San Antonio and Bahía Nueva) and the non-affected population (Playa Villarino). The percentages of imposex-affected females at each population, 21 % BSA and 90 % BN, were concurrent with previous reports for the species and populations (Bigatti et al. 2009). Imposex-affected females had gonads in all the stages described, even Evacuation, thus indicating that all events related to gametogenic activity take place normally. At low intensity, imposex is not expected to affect the reproductive capacity of females. Imposex-affected females of many species (e.g., *Bolinus brandaris* Ramón and Amor 2001; *Buccinum undatum*

Mensink et al. 2002; *Rapana venosa* Mann et al. 2006) present a normal histological structure of the ovary. Also, similarities in the ultrastructure of oogenesis between not severely imposex-affected and normal females have been reported, for example, in *Hexaplex trunculus* (Axiak et al. 2003) and *Babylonia areolata* (Muenpo et al. 2011). Imposex has been found to alter the reproductive function by blocking the pallial part of the oviduct; when this occurs, ova cannot be laid and females are effectively sterilized (e.g., *Nucella lapillus*; Gibbs and Bryan 1986; Gibbs et al. 1987), or copulation is prevented due to the opening of the *bursa copulatrix* (e.g., *Ocenebra erinacea* Gibbs et al. 1990; Oehlmann et al. 1992; *Hexaplex trunculus* Axiak et al. 1995). Also, both modes of sterilization may occur in the same species (e.g., *Ocenebrina aciculata* Oehlmann et al. 1996). For *B. globulosus*, the occurrence of imposex-affected females carrying normal egg masses has been reported in one of these populations (Bahía Nueva) indicating that copulation and depositing of egg capsules also occurs normally (Avaca et al. 2012; this study).

Our results indicate that all females of *B. globulosus* found in pairs were mature, and most of them were in Pre-evacuation thus suggesting that copulation occurs mainly before the evacuation of oocytes from the gonad. Mature females were present throughout the year, and at least for the Bahía Nueva population, this was in concordance with the presence of mating pairs in the field almost all year. The presence of spermatozoa in the *bursa copulatrix* of females with oocytes in maturing process suggest that this species may store sperm as described in other neogastropods (e.g., *B. undatum* Martel et al. 1986a; *Neptunea antiqua* Power and Keegan 2001; *Odontocymbiola magellanica* Bigatti et al. 2008). Further studies of the histological structure of the *bursa copulatrix* are needed to confirm this and to establish whether sperm is stored for short or long periods. We observed that gonads of all females carrying partially deposited egg masses or egg masses in early stage of development were in Evacuation, thus indicating that once gametogenesis is completed, oviposition can start soon after. The maximum sizes observed in oocytes at Evacuation (252.5 µm, this study) coincided with the mean size of nurse eggs present in the egg capsules of this species (240.8 µm; Avaca et al. 2012), thus suggesting that nurse eggs may be unfertilized oocytes or fertilized oocytes without karyogamy (Fretter and Graham 1994). Embryo development within the egg capsule was accompanied by different gonadal stages, from Post-evacuation to Growth II at the moment of hatching, indicating that soon after egg laying, the gonads begin a new cycle of gametogenesis. This information contributes to fill some gaps regarding the reproductive biology of this species.

This study provides further insights into the reproductive biology of *B. globulosus* and constitutes one of the first

reports on the gametogenic cycle in species of the genus *Buccinanops* along with the report of Averbuj et al. (2010) on *B. cochlidium*. Our findings highlight that to attain a more complete understanding of population variability in reproductive processes and ultimately in population demography, reproduction should be examined across the spectrum of demographic and environmental conditions available to conspecifics. The population dynamics of *B. globulosus*, as many other gastropod species, is influenced by human activities such as fishery and marine pollution. Also, the identification of population variation in reproductive traits should lead to more effective management of the species.

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