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Reproductive aspects of *Brachidontes rodriguezii* (Mollusca, Mytilidae) in the high intertidal: extreme environmental effects

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ABSTRACT

In this study we tried to reveal if under conditions of high-stress mussels presented some trade off about reproductive aspects like size/frequency of oocytes and the reproductive cycle of the mussel *Brachidontes rodriguezii*. This species dominates the rocky intertidal coast of Buenos Aires province, Argentine and is a prey item of several exploited species. Mussels were collected from December 2011 to May 2013 in the upper level of it distribution in the high intertidal coast. Gonad tissue samples were processed according to standard histological procedures. Four gonadal development stages were described for males and females all year round. According to the significant decrease in the frequency of mature oocytes, the spawning events were in February 2012/2013. This study also reports hermaphrodite and parasite mussels in very low frequency. We concluded that the frequency and duration of the post-spawning gonadal development stage were the most notable difference along the vertical distribution, suggesting that the high-stress condition of high intertidal impacts negatively on gonad restoration.

Key words: Mytilidae, stress conditions, gonad recovery.

Aspectos reproductivos de Brachidontes rodriguezii (Mollusca, Mytilidae) en el intermareal superior; efectos de condiciones ambientales extremas.

Contribution of authors:

R.P.: Sampling, processing of samples and writing of the manuscript.

G.J.: Sampling, processing of samples and writing of the manuscript.

B.N.: data analysis and writing of the manuscript.

T.M.E.: Sampling, processing of samples and writing of the manuscript.

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Conflict of interest:

En este estudio evaluamos si bajo condiciones de alto estrés los mejillines *Brachidontes rodriguezii* presentaban algún compromiso en aspectos reproductivos como el tamaño/frecuencia de los ovocitos y el ciclo reproductivo. Esta especie domina el intermareal rocoso de la provincia de Buenos Aires, Argentina y es presa de varias especies explotadas. Los mejillines se recolectaron desde diciembre de 2011 hasta mayo de 2013 en el nivel superior del intermareal costero. Se procesaron muestras de tejido gonadal según procedimientos histológicos estándar. Se describieron cuatro etapas de desarrollo gonadal para machos y hembras durante todo el año. De acuerdo con la disminución significativa en la frecuencia de ovocitos maduros, los eventos de desove fueron en febrero de 2012/2013. Este estudio también reporta mejillines hermafroditas y parásitos en muy baja frecuencia. Concluimos que la frecuencia y la duración de la etapa de desarrollo gonadal postdesove resultó ser la diferencia más notable a lo largo de la distribución vertical, sugiriendo que la condición de alto estrés del intermareal superior impacta negativamente en la restauración gonadal.

Palabras Clave: Mytilidae, condiciones estresantes, recuperación gonadal.

INTRODUCTION

The mussel *Brachidontes rodriguezii* (d'Orbigny, 1846) dominates intertidal rocky coasts and is distributed from Uruguay to North Patagonia in the South Atlantic Ocean (Penchaszadeh, 1973; Adami ***et al.***, 2013; Arribas ***et al.***, 2013); are considered ecosystem engineers and a key component of benthic intertidal communities (Borthagaray & Carranza, 2007; Arribas *et al.*, 2014). Inhabiting the intertidal coast environment means to face different conditions along the day, during the low tide, organisms in the high intertidal zone are exposed to aerial conditions for longer periods than organisms in the lower intertidal zone. These aerial conditions include high temperature and desiccation, along with a lower food supply (Connell, 1972); as long as mussels are exposed to the marine condition, food intake rises and the effect of high temperature decreases (Petes *et al.* 2008). Previous studies carried out in the same area revealed some reproductive features of *B. rodriguezii* in the low level of intertidal (Torroglosa & Giménez, 2019a) and given the differential exposure to aerial conditions reported for the study area (Torroglosa & Giménez, 2019b) and the probable effects of exposure to high temperature, the objective of this study were to describe reproductive features of males and females of *B. rodriguezii* in the high intertidal level on the coast of Buenos Aires Province, Argentina. We predicted that under conditions of high-stress mussels would exhibit some trade off in reproductive aspects like shifts in the reproductive cycle or the size of gametes leading to a successful reproductive strategy under extreme environmental conditions.

MATERIALS AND METHODS

Study area and sampling

This study was conducted in Villa Gesell (37° 16′S; 56° 53′W) (Fig. 1), a sandy beach with a dock where mussel beds cover the pier pilings, representing an excellent study site to evaluate the effects of tidal submergence on morphological and physiological parameters. Tides are semidiurnal and the tidal range is about 0.23–1.84 (SHN, 2012–2013). The water temperature was measured *in situ* during sampling, however, the tabulated values were considered more representative of the immediate thermal history of the studied organisms. Between 6-8 females were collected monthly between December 2011 and May 2013 from the upper limit of mussel beds. Shell length (SL) was recorded with a digital calliper (+0.1 mm). A transverse section of body mass was taken near the hinge region (Howard *et al.* 2004) and fixed in Bouin's solution for 13 h, post fixed and preserved in ethanol 70).

Histological procedure

Each fixed sample was embedded in methacrylate resin and sectioned at 6 mm. The sections were stained with haematoxylin and eosin. Four gonad development stages (GDS) – developing, mature, spawning and post-spawning – were assigned following Torroglosa and Giménez (2019a) to describe the reproductive cycle. To establish the reproductive season (spawning event) the size and frequency of vitellogenic oocytes were analysed monthly. Slides were examined with a light microscope (Zeiss Axiostar) at ×400. The area of all oocytes with conspicuous nucleoli within acini of five randomly selected areas was measured with the software AxioVision (2013) 4.8.2.

Fig. 1.
Map showing the geographic location of the sampling site of *B. rodriguezii* in intertidal areas of Villa Gesell (Argentina) and lateral view of *B. rodriguezii*. Scale: 5mm.

Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's honestly significant difference test Tukey post hoc analysis (Sokal & Rohlf, 1995) was used to evaluate differences in the variation of oocyte area while frequency of vitellogenic oocytes were analysed using t-Student test.

RESULTS

A total of 276 mussels were observed and assessed for their sex and gonad development stages; 112 were females, 157 were males and one was hermaphrodite (with male and female gametes within the same acini). Shell length of sampled mussels ranged from 9.60 to 23.20 mm. Males and females observed in this study were reproductively active throughout the study period; some parasite individuals (n=6) were founded and it was not possible to sex those individuals because gonads were full of sporocysts with cercariae of the family Bucephalidae.

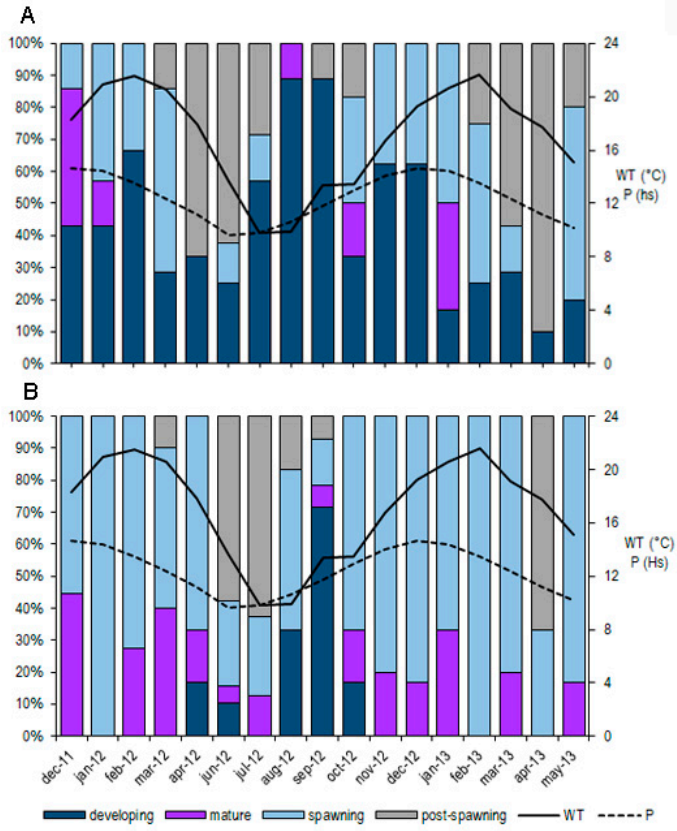

Fig. 2. Frequency distribution of gonad development stages of *B. rodriguezii*. (A) Females, (B) males. Secondary axis indicates water temperature (°C) and photoperiod (P).

Frequency of gonadal development stages

In females, the four gonadal stages were observed throughout the whole period (Fig. 2A, Fig. 3A-D). The most frequent GDS observed was the developing stage. The proportion of females in the developing stage was high from July to September 2012 (more than 60%) and low (less than 30%) from January to May 2013. From December 2011 (when temperature started to increase), the spawning stages became more frequent (more than 50%) until April 2012, and then increased again between October 2012 and February 2013 following the rising temperature. The record of the mature stage was the lower during the period, and the post-spawning stage reached the highest values (more than 60 %) between April and June 2012 and then in March and April 2013. In males, the four gonadal GDS were observed also throughout the study period (Fig. 2B, Fig. 3E-H). The developing stage was recorded between April and October 2012 when temperature and photoperiod reached minimum values, on the other hand, the mature stage, was found almost all the months. increasing it frequency (more than 30 %) in December 2011, January 2012/2013, when temperature and photoperiod reached maximum values. The spawning stage was recorded during the whole period in high frequency (25-100%) while the post-spawning was bounded to the end of austral summer and winter.

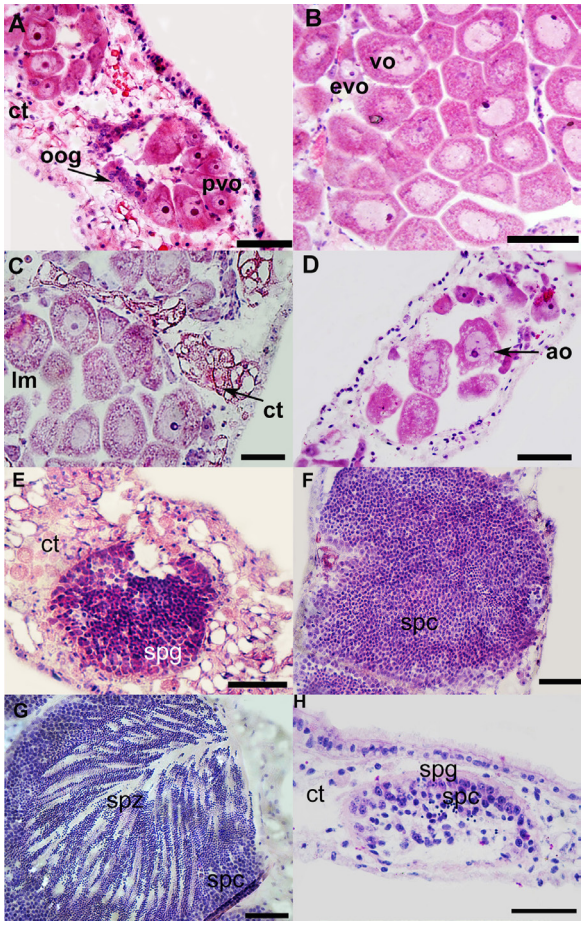

Fig. 3. Gonadal development stages of B. rodriguezii. (A) Developing female gonad. (B) mature or ripe female gonad. (C) Spawning female gonad with visible lumen inside acini as consequence of evacuation of gametes. (D) Post-spawning female gonad with atretic oocytes. (E) Developing male gonad. (F) Mature male gonad. (G) Spawning male gonad and (H) post-spawning male gonad. References: ao: atretic oocyte ct, connective tissue, evo: early vitellogenic oocyte, pvo: previtellogenic oocyte, spg: spermatogonia, spc: spermatocyte, spz: spermatozoa, vo: vitellogenic oocyte. Scales: A-D: 50 µm, E-H: 100 µm

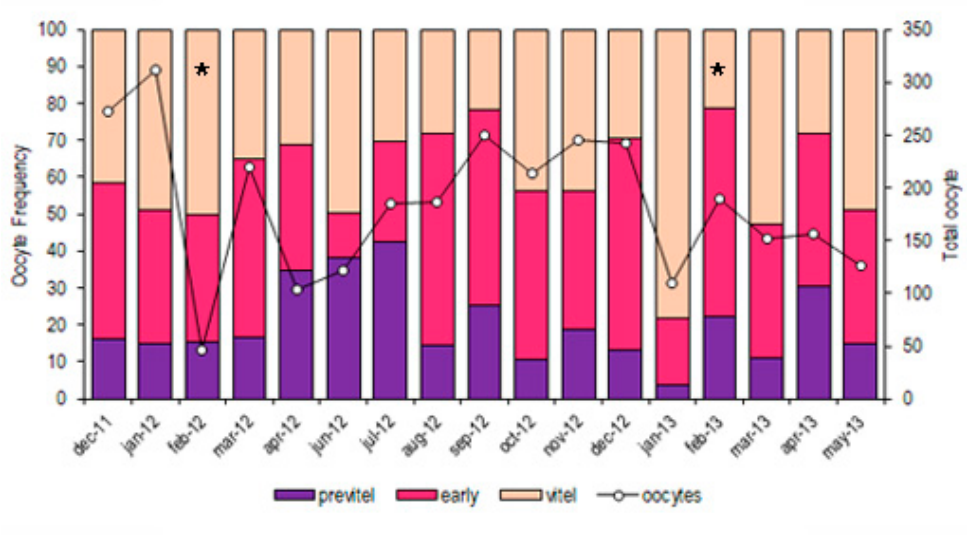

Fig. 4. Relative frequency of different oocyte stages: previtellogenic oocytes (previtel, early vitellogenic oocyte (early) and vitellogenic oocytes (vitel) between December 2011 and May 2013. Asterisks indicates significant decrease of vitellogenic oocytes frequency and white dots the total number of oocytes.

Oocyte size and frequency

The histological examination of females allowed us to determine the size of oocytes in the upper level of the intertidal (Fig. 4). The mean area of previtellogenic oocytes was 128.35 mμ^2 and did not showed significant differences in the area during the study period ($F_{(16, 615)}$=1.03; p-value=0.42). The early vitellogenic oocytes mean area was 457.96 mμ^2 and showed significant differences along months ($F_{(16, 1314)}$=2.22, p-value=0.0047) reaching maximum values in July 2012 (528.33 mμ^2) and May 2013 (555.45 mμ^2) when temperature decrease. Finally, vitellogenic oocytes mean area was 1165.38 mμ^2 showed significant variation in size between months ($F_{(16, 1202)=}$10.03; p-value<0.0001). The monthly mean area of vitellogenic oocytes varied between 800 and 1,300 μm2 monthly, reaching the higher sizes according to Tukey's test by winter and early spring between August and October 2012 (when the mean size reached the highest value 1354.70 mμ^2).

The main spawning events were associated to the significant decreased in the frequency of vitellogenic oocytes, according to the results in Figure 3 we found two spawning events throughout the study period, February 2012 (*t*=2.87, df=11, p-value=0.02) and February 2013 (*t*=2.98, df=11, p-value=0.01). However, by early autumn (April 2012) and winter (July to September) the frequency of oocytes decreased, suggesting little peaks of evacuation besides the main events in austral summer.

DISCUSSION

The results of this study showed different reproductive aspects of male and female of *Brachidontes rodriguezii* in the high intertidal. The stress conditions increase in the higher intertidal zone as consequence of tides, organisms spend less time feeding and the longer exposure to aerial conditions leads to desiccation (Petes *et al.* 2008; Helmuth *et al.* 2011). Previous studies carried out in the same area revealed some reproductive features of *B. rodriguezii* in the low intertidal (Torroglosa & Giménez, 2019a) and a differential growth probably due to tides (Torroglosa & Giménez, 2019b).

The histological examination showed males and females active all year round, potentially capable of spawning year-round. However, the frequency of the gonadal development stages changed through time. During summer, even by ending spring, when temperature rise, there was in increase in the frequency of males and females with spawning gonads. This observation suggests a relation between temperature and gonad development. As Fearman & Moltschaniwskyj (2010) mention, developing rates of gametes depend on temperature, being fast at low temperature and slow when temperature increases. This would probably due to the energetic demands of gametogenesis and food supply; the low food intake, as result of the reduce time that mussel in high intertidal remain submerge, may impact negatively in the storage tissue development affecting energy availability. Our results showed developing gonads all year round, with higher frequency in females than male, low frequency of mature gonads in both sex and from February-Mach to September-October the presence of gonads in post-spawning stage. *B. rodriguezii* in the low level of intertidal showed a similar pattern of GDV frequency, the proportion of mature/ripe gonads is higher and the post-spawning stage is less frequent (Torroglosa & Gimenez, 2019a); the post-spawning stage reached 40 % in the low level while in this study we observed 60% or even 90% of mussel in this condition. This result suggests a lower recovery capacity in the upper

level, due to more stressful conditions and probably more investment in protection processes, as was described for the intertidal mytilid *M. californianus* (Petes *et al.* 2008).

In terms of the size of oocytes, the mean area of previtellogenic oocytes did not differ during the study period, mean size was around 130 $m\mu^2$ and previous results in Villa Gesell showed similar values with mean areas ranging between 30–400 $m\mu^2$, reaching in October 2012 the highest mean value of the entire period (260.92 $m\mu^2$) (Torroglosa & Giménez, 2019a). The same for the early vitellogenic oocytes showed a significant increased in size reaching maximum values in austral winter (~550 $m\mu^2$), while in the lower level, early vitellogenic oocytes reached the highest mean value of the entire period (691.16 $m\mu^2$) by November 2012 in austral spring (Torroglosa & Giménez, 2019a). Finally, for the vitellogenic oocytes, in this study record an increase in oocyte size reaching the maximum mean value 1354.70 $m\mu^2$ by October 2012 while in the lower level vitellogenic oocytes area reached the highest mean value (1623.63 $m\mu^2$) by December 2011 (Torroglosa & Giménez, 2019a). The oocytes sizes reported in the lower level were a little bigger than our results for the high level. Previous studies also showed that *B. rodriguezii* has a lower growth index in high intertidal (Torroglosa & Giménez, 2019b). A similar situation was observed in populations of *Mytilus edulis* under stressful conditions: lower somatic growth and smaller oocytes (Bayne *et al.* 1982).

Based on the vitellogenic oocyte frequency decrease, the reproductive season for *B. rodriguezii* females in the upper level of high intertidal in Villa Gesell showed major peaks of evacuation in February. Our previous results in the lower level of intertidal showed a more extended reproduction season from February to May (Torroglosa & Giménez, 2019), however in both levels he evacuation of gametes match in time. This apparent spawning synchronization may be due to *B. rodriguezii* is an external fertilization species, and therefore, if there are more gametes in the water at the same time, the chances of fertilization increases.

In this study one hermaphrodite individual was found, and the arraignment of reproductive tissue observed had already been described for *B. rodriguezii.* According to literature, in Villa Gesell, there were registered the presence of hermaphrodite individuals with two modalities of hermaphrodite gonad: male and female gametes within the same acini and male and female acini (Torroglosa & Giménez, 2019). The low frequency of hermaphrodite individuals suggests a possible shift of sexual strategy from dioecious to a more plastic sexual strategy that may be highly dependent on habitat characteristics, even more in the hard environmental conditions of high intertidal. Our study also showed the prevalence of sporocysts of the family Bucephalidae in a very low (2%); according to literature the presence of this kind of parasite in frequent in mature mussels during the whole year and often with low incidence (Ojeda *et al*, 2021).

The results of this study partially supported the hypothesis proposed, we found some trade-offs in reproductive aspects of *B. rodriguezii* in the higher limit of the intertidal under stress conditions.

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