



Reproductive aspects and energy allocation compromise to aerial exposure in the intertidal mussel *Brachidontes rodriguezii*. A case study at two tidal levels

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ABSTRACT

Histological and energetic parameters were evaluated seasonally between bivalves from two tidal levels. Results suggest different reproductive patterns between individuals from two contrasting intertidal zones, having also different spawning timing. Females of the lower level spawned in Spring and Autumn while females of the upper level did in Summer with a lower number of oocytes, possibly due to differential food availability between the two intertidal zones. The lipids content in the oocytes did not vary between seasons but varied in the digestive gland, being higher in Autumn – Winter, and suggesting that the digestive gland is a lipid reservoir. The energy density of females was similar throughout the seasons and levels, while in males it varied seasonally. Additionally, energy condition index is proposed as a proxy of stressful conditions, as air exposure time (therefore, to the food availability), mussels from the lower level reached a greater energy condition index than those from the upper level.

1. Introduction

The mussel *Brachidontes rodriguezii* (d'Orbigny, 1842) is the most abundant species in rocky intertidal shores of the north Argentinean coast and has a wide geographic distribution from the Buenos Aires Province (36° 32'S) to Patagonia (42° 45'S) (Penchaszadeh, 1973; Adami et al., 2004). This mussel is also found on artificial substrates (Scelzo et al., 1996) like pillar of fishing piers where vertical arrangement contains individuals subjected to different environmental conditions. Its wide distribution, the condition of ecosystem engineer (Borthagaray and Carranza, 2007) and recent studies considering *B. rodriguezii* a sentinel species (e.g. Laitano and Fernández-Giménez, 2016; Oliva et al., 2015, 2017; Quintas et al., 2017; Buzzi and Marcovecchio, 2018; Arrighetti et al., 2019) support the ecological relevance of the species. However, the effect of environmental stress on energy allocation patterns of this species remains unknown.

The intertidal habitat is a stressful environment in which species are exposed to highly variable oceanic and atmospheric environmental conditions (Helmuth et al., 2002). The organisms are living close to

their physiological tolerance limits (e.g., Tsuchiya, 1983), facing exposure to air during tidal regimes (Helmuth, 1999; Helmuth et al., 2002), in addition to the environmental changes resulting from climate change (Somero, 2002). Even slight increases in aerial temperature could have serious sublethal and lethal consequences for these organisms (Petes et al., 2007, 2008).

Organisms continually adjust physiological processes and energy allocation to fulfill growth, reproduction, and homeostasis (Gadgil and Bossert, 1970; Reznick, 1992). In bivalves, as energy storage is regulated mainly by food availability and temperature (MacDonald and Thompson, 1985a, 1985b; Joaquim et al., 2011), energy reserves in bivalve reproduction are correlated with environmental conditions (Holland, 1978). In organisms' strategies to allocate energy under stress conditions, reproductive processes may be compromised in an attempt to allocate more energy to maintain homeostasis (Stearns, 1992; Wingfield and Sapolsky, 2003). In the intertidal, environmental stress increases along a vertical gradient, because organisms in the higher level are exposed to air conditions for a longer period than those at low tide (Connell, 1972; Menge and Sutherland, 1987). Also, in the upper

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intertidal limit exhibit physiological consequences of prolonged air exposure as reduced growth (Menge et al., 2002; Somero, 2002; Torroglosa and Giménez, 2019a), protein damage (Halpin et al., 2004) and higher oxidative stress (Andrade et al., 2018).

Bivalve storage tissues undergo seasonal variations in biochemical composition and cellular structure in relation to the reproductive cycle and environmental conditions. The influence of these reserves and the timing of use vary among species, or populations of the same species (Bayne, 1976). Generally, reserves are accumulated prior to gametogenesis as glycogen, lipid and protein substrates, and when developing gametes these can be derived directly from food or, when this source is scarce, from reserves stored in the tissues (Mathieu and Lubet, 1993; De la Parra et al., 2005). The digestive gland is involved principally in the storage of lipids and, to a lesser extent, glycogen (Le Pennec et al., 2001). Lipids and glycogen constitute energy reserves particularly for gametogenesis (Anjos et al., 2017; Matias et al., 2016). Particularly, lipids play an important role in the reproductive process, being the main reserve in oocytes and larvae (Gallager and Mann, 1986; Le Pennec et al., 1990; Labarta et al., 1999; Matias et al., 2011; Joaquim et al., 2016).

The investigation of the reproductive and energy allocation pattern of organisms in dynamic habitats contributes to better understand the effects of stressors, such as aerial exposure, that implicate physiological challenges to organisms and consequently for e.g. intertidal populations. In this context, this study aimed to analyze and compare the energy allocation pattern and reproductive aspects of *B. rodriguezii* from two intertidal zones with contrasting environmental conditions.

2. Materials and methods

2.1. Sample collection

Patches of *B. rodriguezii* were collected with a paint scraper seasonally, in December 11, 2012 (Spring), March 10, 2013 (Summer), June 3, 2013 (Autumn) and September 10, 2013 (Winter), at two tidal levels: lower (0.5 m) and higher (1.1 m) at the fishing pier of Villa Gesell (37° 16' 49.5" S; 56° 58' 55.4" W). The tidal regime is mixed mainly semidiurnal with tidal range of 0.23–1.84 (SHN, 2012–2013). The individuals were transported in water from the study site to the laboratory.

In the laboratory, only adult individuals (shell length greater than 12 mm; Torroglosa and Giménez (2018)) were selected; the valves were removed and there were assigned sex from the mantle coloration (brown and white mantles for females and males respectively). The sex assignment was subsequently confirmed by histology.

2.2. Environmental conditions

The aerial exposure time (T, Fig. 1) of each level was estimated considering harmonic analysis of tidal and using the tide tables (SHN, 2012–2013) taking into account the daily rising and falling tides of the periods included for each month:

$$T_{(L)} = \sum [\arccos(2 * (0.5 \text{ m} - \text{HLT})/A) * \Delta t/180] \quad (1)$$

$$T_{(U)} = \sum [\arccos(2 * (1.1 \text{ m} - \text{HLT})/A) * \Delta t/180] \quad (2)$$

Where HLT: height low tide (m); A: amplitude (m) (height variation between high and low tide); Δt : time between high and low tidal heights (h).

In the lower level were considered low tides those below 0.5 m, in case the tides surpassed this value in the whole day, and it was considered 24 h submersion.

In the upper level were considered high tides those above 1.1 m, in case the tides did not reach this value in the whole day, it was considered 24 h aerial exposure. The monthly photoperiod (hours of daylight per 24 h) and monthly mean sea surface temperature (SST)

(Spring: 20 °C; Summer: 20.5°C; Autumn: 10.9 °C; Winter: 11.8 °C) was provided by the Servicio de Hidrografía Naval from the permanent station. Pinamar (37°07' S; 56°51' W), nearest the sampling area (<http://www.hidro.gov.ar>). The air temperature was calculated from (<http://siga2.inta.gov.ar/en/datoshistoricos/>) considering the average high and low temperatures of all days of the month prior sampling (Fig. 1). Although water temperature was measured *in situ* when sampling monthly mean values were considered representative of the immediate thermal history of the studied organisms.

2.3. Histological analysis

The soft parts of nine-ten individuals of each sex from each season and level were fixed in 10% formalin solution and dehydrated in ethanol graduated series, embedded in resin (Leica HistoResin®) and sectioned at 5 μm with an electronic microtome (Leica RM 2155®) for histological analysis.

The histological sections for each female and male from both levels were stained with hematoxylin-eosin (H/E). Slides were examined with a light microscope (Zeiss Axiostar) at $\times 400$. Gonads of both sexes were categorized into four stages (developing, ripe, spawning and post-spawning) following Torroglosa and Giménez (2019b) (Fig. 2). When more than one developmental stage occurred simultaneously within a single individual, the criteria for assigning the stage was based upon the condition of the majority of the section.

To avoid overestimation during counting and measurement of oocytes were only considered the oocytes with nucleus and nucleoli and counted from five randomly selected sections and oocytes areas were measured using the software AxioVision (2013) 4.8.2.

2.3.1. Lipids analysis of female gonad and digestive gland

Sudan black B (SBB) histochemical techniques were used to identify lipids (Bayliss High, 1990) on gonads and digestive gland of each individual, following the methodology standardized by Rodríguez-Jaramillo (2004) further applied by Gómez-Robles et al. (2005) and Gómez-Robles and Saucedo (2009). Overall, were quantified in females' gonad: Lipid content of oocytes (LC_O) and Lipid Index of oocytes (LI_O); and in digestive gland sections: Lipid content of digestive gland (LC_{DG}) and Lipid Index of digestive gland (LI_{DG}). For this purpose, from each individual, 10–12 vitellogenic oocytes and 7 digestive tubules were randomly selected, observed under a light microscope (Zeiss®), and photographed with a Canon® digital camera.

The images were processed with Image-Pro Plus 6.0 software and were measured the areas occupied by: the oocyte (O), its nucleus (N) and total lipid inclusions within the cytoplasm area of oocytes (LC_O , μm^2) whereas in digestive gland, there were measured the areas occupied by: digestive tubules (DT), lumen of digestive tubules (LDT) and total lipid inclusions within the cytoplasm area of digestive tubules (LC_{DG} , μm^2).

LI_O were calculated as:

$$\text{LI}_O = \text{LC}_O / (O - N)$$

The cytoplasm area of oocytes was calculated as the difference between O and N.

Finally, LI_{DG} were calculated as:

$$\text{LI}_{DG} = \text{LC}_{DG} / (\text{DT} - \text{LDT})$$

The cytoplasm area of digestive tubules was calculated as the difference between DT and LDT.

2.4. Calorimetric analysis

On other individuals, twenty from each level (ten females and ten males) and sampling season it was recorded the total wet weight (± 0.0001 g) and the shell length (L, maximum distance along the anterior – posterior axis, ± 0.01 mm) with an electronic caliper. Valves

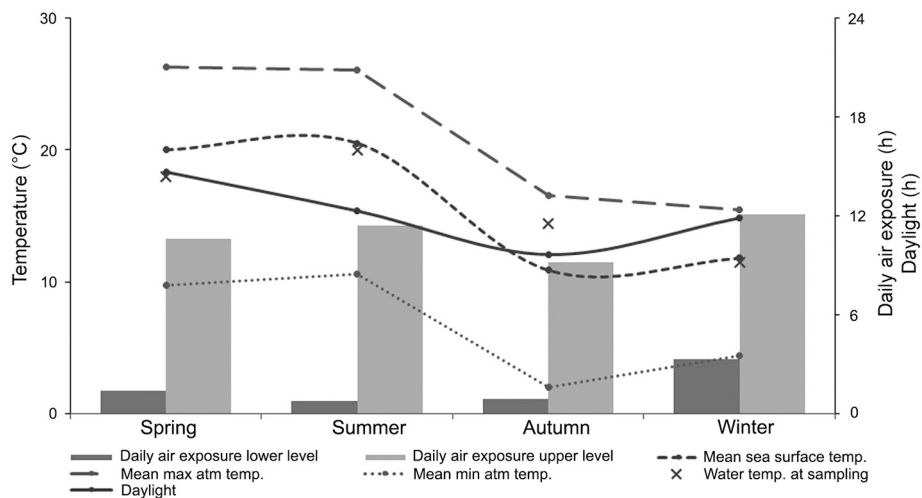


Fig. 1. Average water temperature, atmospheric temperature (maximum and minimum) and photoperiod for the period October 2012–September 2013 and measured on each sampling (x), daily aerial exposure (gray bars) for the lower level and upper level.

were removed and the soft parts of each whole individual were weighed (flesh wet weight, ± 0.0001 g) and dried in an air-circulating oven at 60 °C until constant flesh dry weight (DW). Samples were stored at -18 °C until calorimetric analyses were performed.

The total soft parts dried were ground and one pellet per individual was made with a press (Parr model 2812). The pellets were weighed and burned in a micro-bomb calorimeter (Parr model 1425) to complete combustion (Lucas, 1996). When individual pellets did not reach the minimum weight required by the micro-bomb calorimeter (0.02 g, dry mass), "pools" of individuals of equal dry weight were made. The values obtained were corrected for ash and acid content and expressed as kJ/g ash-free dry mass (energy density ED, kJ/g AFDM) (Boy et al., 2009; Cossi et al., 2015). Benzoic acid calibrations were done periodically.

An energy condition index (EI, J/mm³) was calculated per individual as:

$$EI = (ED * DW) / L^3$$

2.5. Statistical analyses

Variation in total oocytes and Lipid Index of oocytes (LI_o) were

tested using two-way ANOVA (level and season as factors). Lipid content (LC_{DG}) and Lipid Index of digestive gland (LI_{DG}), energy density (ED) and energy condition index (EI) were analyzed using multi-factorial ANOVA (sex, season and level as factors). The assumptions of normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene’s test) were tested, and transformations were applied when necessary. Significant differences were compared using multiple pairwise Tukey test (Sokal and Rohlf, 1995) and significant interactions were analyzed by simple effects analysis.

When the assumptions required by parametric tests were not reached, non-parametric tests were performed, followed by multiple pairwise comparisons. Variations in oocytes area, Lipid Content of oocytes (LC_o) were analyzed using a non-parametric test (Kruskal–Wallis). Differences between sexes and levels were tested using paired T test (Zar, 1984; Sokal and Rohlf, 1995) or Mann–Whitney non-parametric test whenever necessary.

All statistical analyses were performed using the software Statistica 7.1., p = 0.05 was taken as significance threshold.

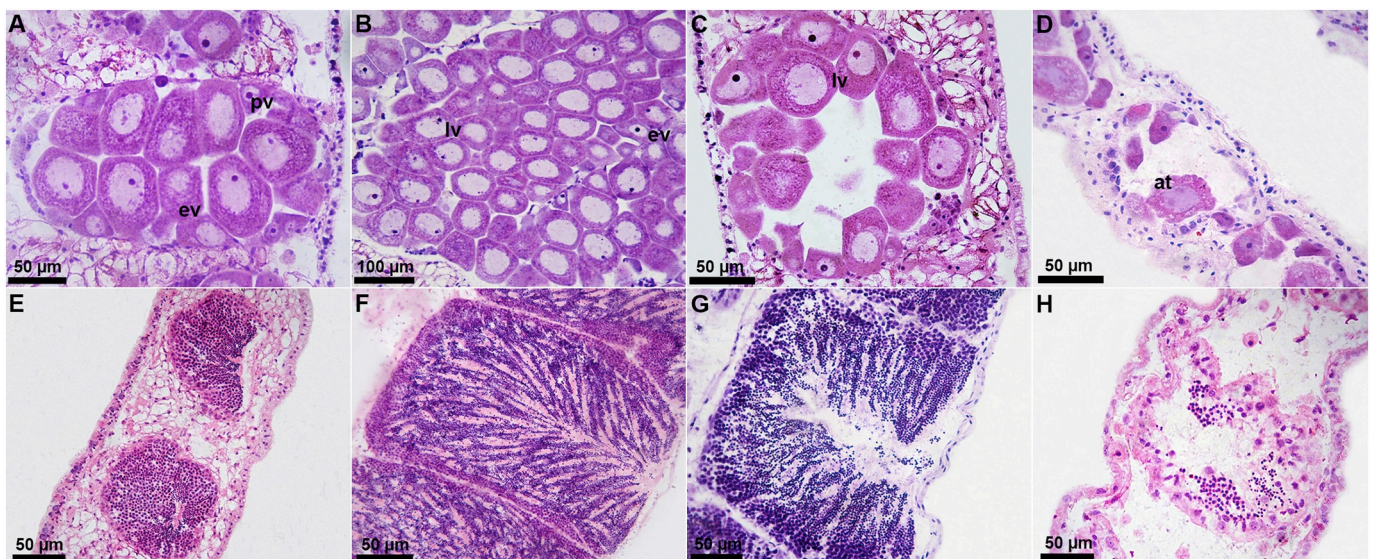


Fig. 2. Histological sections of the gonad of *B. rodriguezii* showing gonadal stages: Females A–D A. Developing. B. Ripe. C. Spawning. D. Post-spawning. ev: early vitellogenic, pv: previtellogenic, lv: late vitellogenic, at: atretic oocyte. Males E–H E. Developing, F. Ripe, G. Spawning, H. Post-spawning.

3. Results

3.1. Emersion time

The lower level of the fishery pier remained exposed to air in Spring 43.3 h/month (1.4 h/day), Summer 24.0 h/month (0.8 h/day), Autumn 27.1 h/month (0.9 h/day) and Winter 100.3 h/month (3.3 h/day) (Fig. 1). In the upper level, the air exposure time was in Spring 319.4 h/month (10.6 h/day), Summer 342.4 h/month (11.4 h/day), Autumn 341.1 h/month (9.2 h/day) and Winter 362.5 h/month (12.1 h/day) (Fig. 1).

3.2. Individuals size and weight

Size range of chosen individuals from the lower level was 12.1–24.2 mm and in the upper level was 12.3–20.0 mm, whereas the dry weights were: 0.018–0.131 g and 0.013–0.069 g respectively.

3.3. Seasonality of reproduction

3.3.1. Reproductive stages

In the lower level, both sexes showed gonads in the developing stage in higher proportion in Summer (30%). Spawning stage was observed in greater proportion in Spring (55.6% females, 60% males) and Autumn (30% females, 44.4% males). The ripe stage was observed in all seasons (33.3–60% females, 30–50% males) (Fig. 3A).

In females from upper level (Fig. 2B), developing stage was observed in greater proportion in Summer (50%), and Winter (60%). The spawning stage was the predominant in Summer (50% females, 50% males) (Fig. 3B). Post-spawning stage was observed mainly in females from upper level (Fig. 3B).

Most males from both levels were in ripe and spawning stages (Fig. 3B).

3.3.2. Total number of oocytes and oocyte area

Total number of oocytes were significantly varied in levels and seasons (two-way ANOVA $F_{1,71} = 18.12$, $p = 0.0001$, $F_{3,71} = 3.95$, $p = 0.011$, $F_{1,71} = 1.11$, $p = 0.3520$ for level, season and interaction, respectively). In Summer, mean oocyte number in the lower level (75.3 ± 22.4) was higher than in the upper level (31.7 ± 15.9). The lowest mean number of total oocytes occurred at the upper level in Summer (31.7 ± 15.9), whereas the highest value occurred at the lower level in Winter (88.4 ± 23.1) (Tukey test $p < 0.05$; Table 1).

The area of previtellogenic oocytes was significantly different between levels (Kruskal–Wallis $H_{1,652} = 4.0$, $p = 0.045$) (Table 1) at

Winter was higher in the lower level than in the upper (Mann–Whitney $U = 5932.5$, $p < 0.05$). Significant differences were observed in the late vitellogenic oocytes area between levels (Kruskal–Wallis $H_{1,2449} = 6.05$, $p = 0.014$) and seasons (Kruskal–Wallis $H_{3,2449} = 67.8$, $p = 0.000$). At Spring was higher in the upper level than in the lower one (Mann–Whitney $U = 20664.0$, $p < 0.05$), while the opposite was found in Winter (Mann–Whitney $U = 62418.5$, $p < 0.05$).

At the lower level the previtellogenic oocyte area (Table 1) was significantly lower in Autumn than in Winter (Kruskal–Wallis $H_{3,503} = 9.9$, $p = 0.019$). In Spring at the lower level, the late vitellogenic oocytes area was lower than in other seasons, and Summer showed differences with Autumn and Winter (Kruskal–Wallis $H_{3,1607} = 63.2$, $p = 0.000$).

In the upper level, differences were observed in late vitellogenic oocytes (Kruskal–Wallis $H_{3,842} = 49.7$, $p = 0.000$) with Spring, Summer and Autumn differing from Winter (Table 1).

3.4. Lipid Content (LC) and Lipid index (LI)

3.4.1. Lipid Content (LC_O) and Lipid index (LI_O) of oocytes

No significant differences were found in the LC_O between levels or seasons (Kruskal–Wallis $H_{1,551} = 3.3$, $p = 0.071$; $H_{3,551} = 1.2$, $p = 0.875$ respectively; Fig. 4A). LI_O differed significantly only between seasons, being higher in Winter than in other seasons (ANOVA $F_{3,543} = 6.3$, $p = 0.000$; Tukey test $p < 0.05$; Fig. 4B).

3.4.2. Lipid content (LC_{DG}) and Lipid index (LI_{DG}) of digestive gland

No significant differences were found in the LC_{DG} between sex (ANOVA $F_{1,137} = 1.5$, $p = 0.229$) but were detected among levels and seasons (ANOVA $F_{1,137} = 13.9$, $p = 0.001$; $F_{3,137} = 22.8$, $p = 0.000$; $F_{3,137} = 3.2$, $p = 0.026$ for levels, seasons and interaction, respectively). The highest LC_{DG} was observed during Winter at the lower level (Tukey test $p < 0.05$; Fig. 4C).

The LI_{DG} (Fig. 4D) differed only between seasons (ANOVA $F_{3,142} = 41.8$, $p = 0.000$), with the lower values in Spring and Summer ($LI_{DG} = 0.08$) than in Autumn and Winter ($LI_{DG} = 0.13$) (Tukey test $p < 0.05$).

3.5. Energy density

Significant differences were observed in energy density (ED) (Fig. 5A) between seasons (ANOVA $F_{3,139} = 4.6$, $p = 0.004$). In Spring and Summer were found lower ED values than in Winter (Tukey test $p < 0.05$). A significant interaction between level and sex was found (ANOVA $F_{1,139} = 8.4$, $p = 0.004$). In the upper level during Spring and

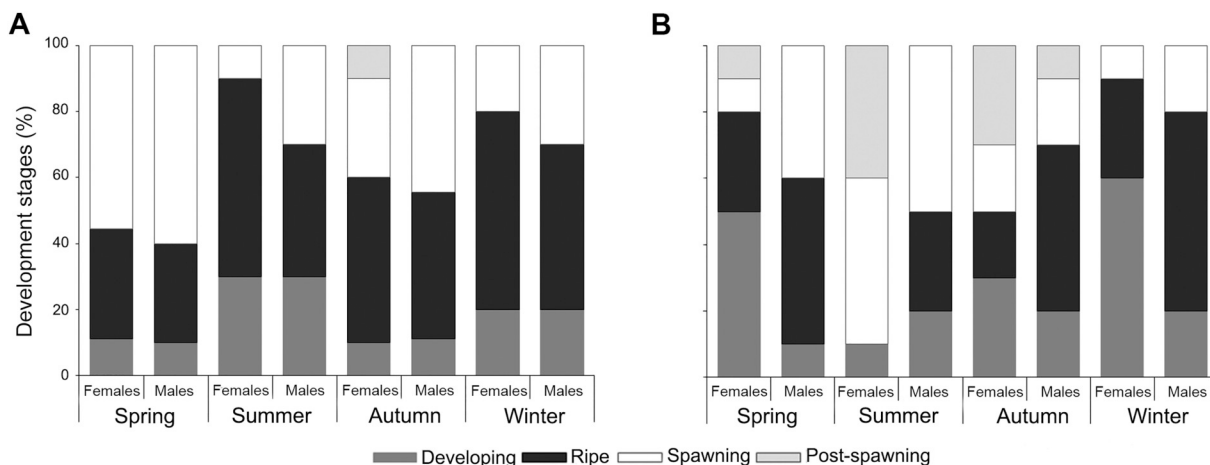


Fig. 3. Seasonal variation in the gonad developmental stages of *B. rodriguezii* at two levels of air exposure (Lower, Upper): A. females and males of lower level, B. females and males of upper level.

Table 1
Seasonal variation of oocytes of *B. rodriguezii* at the two tidal levels (Lower, Upper).

Level	Season	Oocyte (n) + SD	Oocyte area (μm^2) + SD		
			Previtellogenic	Early vitellogenic	Late vitellogenic
Lower (n = 9)	Spring	68.6 ± 22.9	256.8 ^{ab} ± 100.0	962.8 ± 310.5	2115.9 ^{ca} ± 499.5
Upper (n = 10)		57.0 ± 27.5	243.5 ± 97.8	939.8 ± 296.7	2389.9 ^{abca} ± 651.2
Lower (n = 10)	Summer	75.3 ± 22.4*	277.0 ^{bc} ± 96.1	939.3 ± 292.6	2395.8 ^a ± 531.6
Upper (n = 10)		31.7 ± 15.9*	262.6 ± 108.7	912.8 ± 340.1	2398.5 ^{ab} ± 544.6
Lower (n = 10)	Autumn	67.7 ± 30.5	243.5 ^a ± 112.2	953.5 ± 303.6	2278.1 ^b ± 456.9
Upper (n = 10)		44.9 ± 34.8	243.3 ± 106.6	969.6 ± 340.1	2237.5 ^b ± 431.3
Lower (n = 9)	Winter	88.4 ± 23.1	280.9 ^{ca} ± 98.9	914.9 ± 321.8	2247.8 ^{ba} ± 459.9
Upper (n = 10)		70.7 ± 33.0	241.8* ± 109.6	974.8 ± 338.6	2068.7 ^{ca} ± 360.3

Number of oocytes (n ± SD) and Oocyte area (μm^2), Statistical differences between levels are denoted with asterisk. Statistical differences between seasons of each level for previtellogenic, early vitellogenic and late vitellogenic oocytes are indicated with different letters (a, b, c, d; Kruskal–Wallis multiple comparisons test).

Summer, males' ED was lower than females' ED (Tukey test $p < 0.05$). Finally, the interaction between season, sex and level was significant (ANOVA $F_{3,139} = 4.1, p = 0.008$). In Summer, males of the upper level displayed lower ED than: males of Autumn, females of Spring and Winter and also males of the lower level in Winter (Tukey test $p < 0.05$). Also, in males of the lower and females of the upper level during Winter were found greater values than males at lower level in Autumn (Tukey test $p < 0.05$).

3.6. Energy condition index

The energy condition index (EI) varied between level and also the interaction between the season and level factors (ANOVA level: $F_1,$

$_{121} = 31.3, p = 0.000$, season × level: $F_{3, 121} = 11.11, p = 0.000$). In Winter, there were found greatest EI values in the lower level (Tukey test $p < 0.05$; Fig. 5B).

4. Discussion

The present results suggest different energy allocation patterns between sexes in *B. rodriguezii*. Female energy density remained relatively constant throughout the year, with higher values in the individuals living under more stressful conditions (e.g. higher exposure to air, temperature variation, desiccation, reduced food availability). Whilst males showed a marked seasonal depletion of the energy density, during summer and autumn in the upper and lower levels respectively.

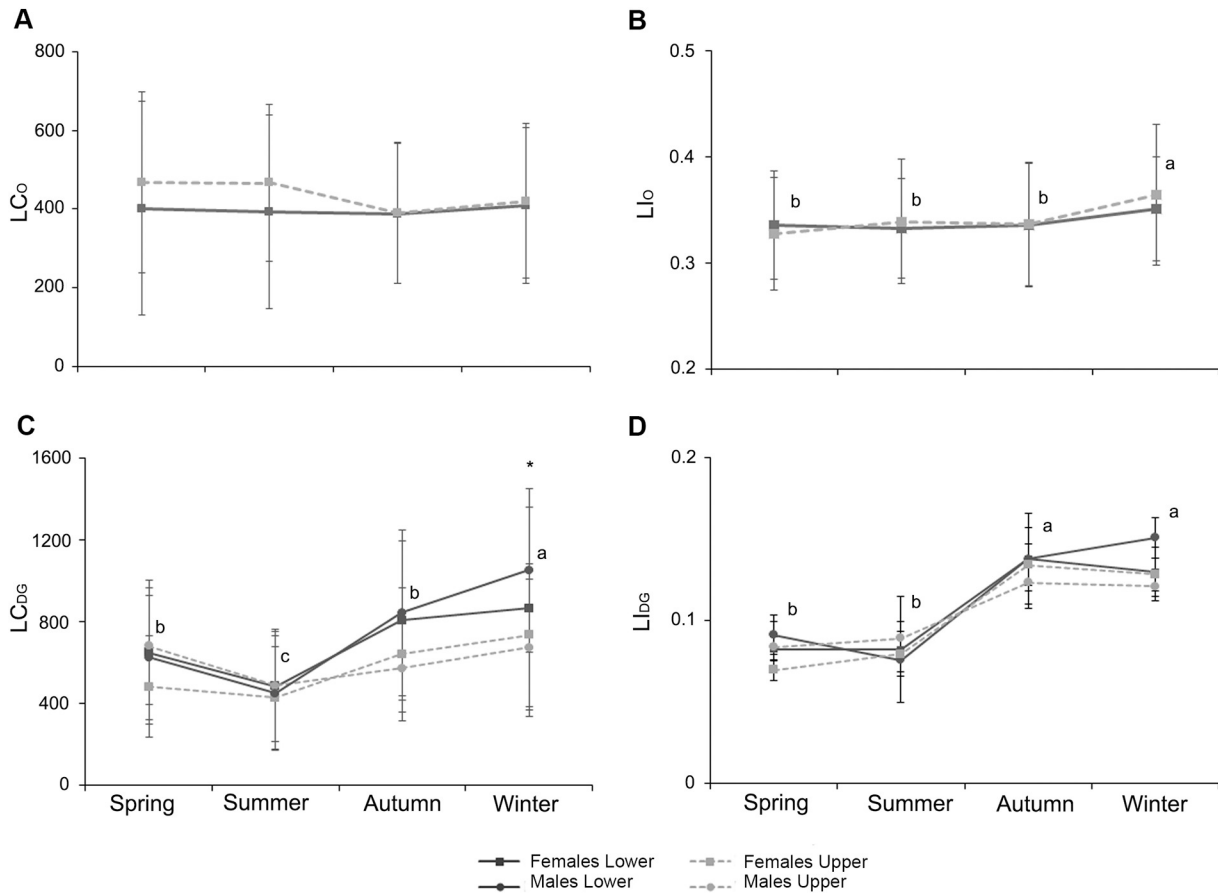


Fig. 4. Seasonal variation of: A. Lipid content oocyte (LC_O ; mean ± SD), B. Lipid index oocyte (LI_O ; mean ± SD), C. Lipid content digestive gland (LC_{DG} ; mean ± SD), D. Lipid index digestive gland (LI_{DG} ; mean ± SD) in *B. rodriguezii* at two levels of air exposure (Lower, Upper). Statistical differences between seasons are indicated with different letters (a, b, c, d; Tukey test, $p < 0.05$); by statistical differences between levels are denoted with an asterisk (Tukey test, $p < 0.05$).

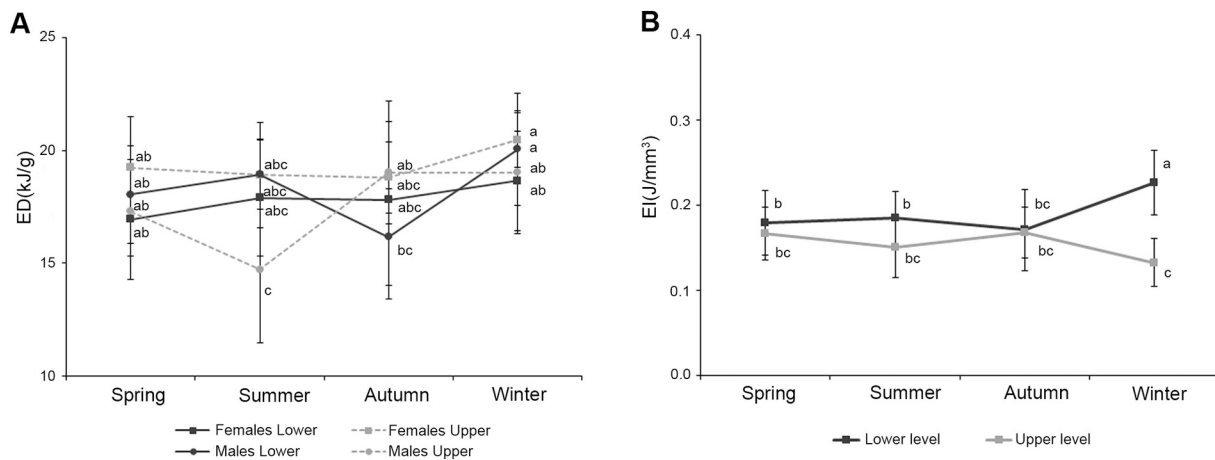


Fig. 5. Seasonal variation of: A. Energy density (ED kJ/g; mean \pm SD). B. Energy condition index (EI J/mm³; mean \pm SD) in *B. rodriguezii* at two levels of air exposure (Lower, Upper). Statistical differences are indicated with different letters (a, b, c, d; Tukey test $p < 0.05$).

Furthermore, mussels from the upper level reached in general a poorer EI than those from the lower level, in accordance with Connor and Robles (2015) who also found evidence of energetically costly environmental stress, such as attenuated growth rates in more exposed mussels. And this poor condition, i.e. low IE, is even stronger during winter when the longest air exposure time (and food restriction) is attained. Some studies already found evidences of decreased energy allocated to growth and reproduction in intertidal mussels under environmental stress (e.g. Petes et al., 2007; Connor and Robles, 2015), which would be allocated to physiological defenses, hence survival (Hofmann and Somero, 1995; Somero, 2002).

Different reproductive patterns were found here on *B. rodriguezii*'s females from the two tidal levels studied. According to Torroglosa and Giménez (2019b), once adulthood is achieved individuals are at least potentially capable of spawning year-round. However, females at long air exposure (having more energy density, but less energy condition index) showed only one marked spawning episode in Summer at highest sea water temperature, while those of the lower level presented two more pronounced spawning events, in Spring and Autumn. A similar pattern was found in *Mytilus californianus*, whose individuals from the upper intertidal zone presented only one spawning peak (an also at Summer) (Petes et al., 2007, 2008). Differences in the spawning pattern found between levels might be explained by the differential environmental conditions, i.e. thermal stress and less food intake at higher levels, and according to Petes et al. (2007) less energy allocated to growth and reproduction, i.e. one spawning peak.

Differences in the reproductive patterns were also observed in oocytes number, but not in oocytes area. Mussels in optimal micro-environments reach sexual maturity faster and reallocate a greater fraction of assimilated energy to gamete production (Thompson, 1984). The oocytes number was fairly constant in the lower level, and in the upper one oocytes are less numerous and also showed a marked decrease during the spawning peak (Summer). This could be due to the increased cost to cope with higher physiological stress in females in the upper level in decrement of the allocation of energy to reproduction, reaching only to produce a small amount of oocytes during a limited period in the year.

Although high frequencies of atretic oocytes may occur at several points in the marine bivalves' reproductive cycle (Beninger, 2017), relict and atretic oocytes was observed mainly in post-spawning females of the upper level. Some authors have reported a characteristic "phenomenon of oocyte atresia" as a reallocation of energy to slowed down basal metabolisms through gamete's resorption (Borzone et al., 2003; Suárez et al., 2005; Azpeitia et al., 2016; Rouabhi et al., 2019). Further, it has been suggested that atresia occurs after thermal stress (Suárez-Alonso et al., 2007; Azpeitia et al., 2016) or environmental

contamination (Steele and Mulcahy, 1999; Ortiz-Zarragoitia and Cajaraville, 2006; Rouabhi et al., 2019). Accordingly, the smaller proportion of ripe females in the upper level, would suggest the lack of enough energy to mature gametes quickly after spawning, i.e. an energetic trade-off between reproduction and stress resistance (Petes et al., 2008).

Spawning males were observed in all seasons, in a similar pattern to females of both levels. It should be noted that post-spawning males were only observed in Autumn in the upper level. The interaction between food availability and water temperature is associated to timing of reproduction and reproductive success (Pérez-Camacho et al., 2003). The annual bimodal cycle of the primary production in the Argentinean Sea, typical of temperate-cold water ecosystems, shows a maximum concentration of phytoplankton in spring, and a secondary maximum in the first months of autumn (Campagna et al., 2006). Accordingly, higher concentrations of chlorophyll were reported during spring and autumn (Carreto et al., 1995). Spawning in the lower level, when the highest concentrations of phytoplankton occur, would be an adaptive strategy of *B. rodriguezii* improving larval survival.

Although glycogen is widely known as a long-term energy reserve in many aquatic invertebrates (Lucas, 1996), several authors also suggested the importance of lipid stored in oocytes during gametogenesis, as energy sources for embryonic and early larval development in bivalve species (Dorange and Le Pennec, 1989; Le Pennec et al., 1990; Massapina et al., 1999; Cannuel and Beninger, 2005; Joaquim et al., 2016). Part of the lipids, like triglyceride reserves, are used to synthesize vitellogenin-like proteins that promote the formation of the yolk (Arcos et al., 2009). The lipid index has been used as a reliable criterion of gonad condition (Gómez-Robles et al., 2005; Gómez-Robles and Saucedo, 2009). The similar lipid content and lipid index of oocytes found between levels suggest the same pattern of lipid storage females, regardless of environmental conditions such as air exposure. And the highest lipid values observed in winter would be driven mainly by autumn's secondary peak of phytoplankton in the Argentinean Sea.

The highest lipid content in the digestive gland observed in the lower level, and its variation at both levels reveals that lipids are stored in the digestive gland at a higher rate when productivity increases, as reported for *Pecten maximus* (Le Pennec et al., 2001). Consequently, our results suggest that the digestive gland of *B. rodriguezii* plays a crucial role, in the transference of energy to gonads during gametogenesis, as already described for other bivalve species (Pérez et al., 2013; Angel-Dapa et al., 2010; Robinson et al., 1981; Pazos et al., 1996; Barber and Blake, 2006; Gómez-Robles and Saucedo, 2009), and this transference would be not only of glycogen but also lipids.

Intertidal species, mussels in particular, are frequently exposed to environmental changes related to tidal regimes that include a multitude

of stressors (Andrade et al., 2018). Sessile organisms are likely to face the highest probability of exposure to physiological stress due to their immobility (Menge and Sutherland, 1987) which they avoid or tolerate by developing adaptive strategies (Andrade et al., 2018, 2019). The findings of the present study suggest that females and males of *B. rodriguezii* exhibit differential energetic and reproductive strategies in response to stressful conditions, with females showing different spawning patterns between the two tidal levels, and different seasonal patterns of energy density between sexes. Also the energy condition index arise a sensitive proxy of stressful conditions, as aerial exposure.

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