

Relationship between energy allocation and gametogenesis in *Aulacomya atra* (Bivalvia: Mytilidae) in a sub-Antarctic environment

Relación entre reproducción y asignación de energía en *Aulacomya atra*
(Bivalvia: Mytilidae) en un ambiente sub-antártico

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Resumen.- Se analizó la gametogénesis y la variación temporal en la asignación de energía a diferentes órganos de *Aulacomya atra* del Canal Beagle (Tierra del Fuego). El periodo de desove de *A. atra* se extendió desde el final del invierno hasta la primavera, cuando el MGI (índice gónada-manto) resultó mínimo y el número de individuos desovados, máximo. En los machos maduros el incremento del MGI coincidió con un descenso en la densidad energética del manto-gónada (ED_{MG}), lo que está relacionado con un aumento de masa con menor energía. En hembras maduras el incremento de MGI se relaciona con un aumento de masa con valores más altos de energía, que determina el aumento de la ED_{MG} . Durante la maduración gonadal existen diferentes estrategias de asignación de energía en ambos sexos, aunque se alcanza similar contenido de energía en el manto-gónada; los machos mantienen gónadas más grandes, pero con menos energía por unidad de masa que las hembras. Además, en los individuos maduros desciende el valor del índice de la glándula digestiva (DGI), incrementa el MGI, y tiende a la depleción de la ED y del contenido energético, sugiriendo que este órgano actuaría como reservorio de energía.

Palabras clave: Energética, calorimetría, reproducción, bivalvo, Canal Beagle, *Aulacomya atra*

Abstract.- Gametogenesis and temporal variation in energy allocation to different organs were analyzed in *Aulacomya atra* from the Beagle Channel (Tierra del Fuego, Argentina). Our results show that *A. atra* presents an extended spawning period from late winter to spring when the lowest value of mantle-gonad index (MGI) and maximum number of spawned individuals were observed. The MGI in mature males increased as the energy density of the mantle-gonad (ED_{MG}) decreased, indicating an increase in mass of lower energy values. In contrast, the MGI in mature females increased as ED_{MG} increased, indicating an increase in mass of higher energy values. Consequently, males and females reached a similar energy content of the mantle-gonad (EC_{MG}) by means of different strategies: males have gonads of larger size but with lower energy by unit of mass than females. Besides, we observed a decrease in the digestive gland index (DGI) in concordance with an increase in the MGI of mature individuals, and a trend towards the depletion of ED and EC in the digestive gland during gonadal maturation. Our results evidence the role of the digestive gland as an energy reservoir and a different energy allocation strategy between sexes during gonadal maturation.

Key words: Energetics, calorimetry, reproduction, bivalve, Beagle Channel, *Aulacomya atra*

INTRODUCTION

In high-latitude marine environments, the strong seasonality observed in temperature and photoperiod influences food availability markedly (Clarke 1987). Consequently, accumulation of energy reserves in organisms can show temporal variations. The allocation of these reserves to different body components can provide relevant information on their functional roles and

relative importance for the survival and reproduction of organisms (Chaparro & Winter 1983, Lawrence & McClintock 1994, Lucas 1996, Ahn *et al.* 2003, Pérez *et al.* 2010).

Food availability and temperature are considered the main factors affecting growth and reproduction of bivalves (MacDonald & Thompson 1985a,b). Besides

influencing energy balance, these variables affect the way in which available resources are allocated for the maintenance of the cellular machinery, growth, and reproduction (Iglesias & Navarro 1991, Ahn *et al.* 2003, Barber & Blake 2006). The reproductive cycle requires high inputs of energy, which can be supplied directly by ingested food, by reserves previously stored in different tissues, or by a combination of both strategies (Jayabal & Kalyani 1986, Barber & Blake 2006, Vite-García & Saucedo 2008). The simultaneous study of the annual gametogenic cycle and the variation in energy content could give information about the specific pattern of energy storage and use (Morriconi *et al.* 2007).

Aulacomya atra (Molina 1782), commonly known as 'cholga' in Chile and Argentina and as 'Ribbed mussel' in South Africa, is a species distributed along the South American coasts; from southern Brazil to Tierra del Fuego on the Atlantic coast, and from the Beagle Channel to El Callao (Peru) on the Pacific coast (Castellanos 1967, Cancino & Becerra 1978, Navarro & Gutiérrez 1990¹, Zaiexo 2004). *A. atra* is also found in South Africa (Griffiths 1977, Barkai & Branch 1989). The Beagle Channel (Tierra del Fuego, Argentina) is the southernmost limit of the species distribution. The air temperature in the region is around 1.5°C in winter and 9°C in summer (Servicio Meteorológico Nacional 1986)² and the surface water temperature ranges between 4.4°C in August and 9.4°C in January (Balestrini *et al.* 1998). Therefore, the gametogenesis and energy allocation of *A. atra* in Tierra del Fuego could be influenced by physiological restrictions related to the low temperatures of this region.

Aulacomya atra occurs from the intertidal zone up to 25 m depths in most of the beds surveyed in the Atlantic and Pacific waters (Gamarra & Cornejo 2002, Zaiexo 2004). In Tierra del Fuego, this species inhabits the lower level of the intertidal zone, and thus, individuals are exposed to the air only exceptionally during the low tides and for short periods of time.

Few studies have analyzed the gametogenesis and energy content of *A. atra* in South America. In Argentina, Vinuesa & Tortorelli (1980) studied the reproductive cycle of this species, in populations from Puerto Deseado (47°44'S). In Chile, the gonadal cycles and the effect of temperature and food availability on gamete maturation

and spawning have been described for northern and southern populations (Jaramillo & Navarro 1995, Avendaño & Cantillán 2012).

The objectives of this study were: a) to characterize gametogenesis in adult individuals of a population of *Aulacomya atra* located at the southernmost limit of its distribution; and b) to determine the allocation of energy to different organs, and its temporal variation in both males and females.

MATERIALS AND METHODS

STUDY SITE AND SAMPLING

Specimens of *Aulacomya atra* were collected every 3-4 months from October 2009 to October 2010 in the intertidal rocky shore of Ensenada Zaratiegui (Tierra del Fuego National Park) Beagle Channel, Argentina (54°51'S, 68°29'W; Fig. 1). To measure the seasonality of temperature throughout the year, one 1-wire Thermochron datalogger was deployed in the low edge of the mussel bed (mid-zone). This logger recorded air and water

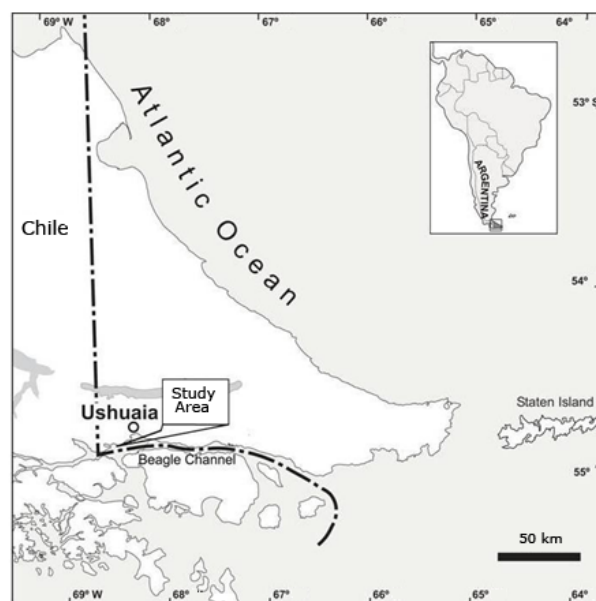


Figure 1. Sampling locality in Zaratiegui Embayment, Tierra del Fuego National Park, Tierra del Fuego, Argentina / Sitio de muestreo en Ensenada Zaratiegui, Parque Nacional Tierra del Fuego, Tierra del Fuego, Argentina

¹Navarro JM & A Gutiérrez. 1990. Sinopsis sobre el estado actual del cultivo de mitílidos en Chile. Cultivo de moluscos en Latinoamérica. Memorias de la Asociación Latinoamericana de Acuicultura, Segunda reunión de trabajo técnico, 189-210 pp.

²Servicio Meteorológico Nacional. 1986. Estadísticas meteorológicas (1971-1980). Estadística N° 36. Fuerza Aérea Argentina, Buenos Aires, 338 pp.

temperatures (depending on the tide level) every 1 h from October 2009 to October 2010. The monthly average day length was provided by the Servicio de Hidrografía Naval³. Individuals were transported to our laboratory and kept in sea water at 7°C for 24 h.

MANTLE-GONAD AND DIGESTIVE GLAND INDEXES

Shell length (maximum distance along the anterior posterior axis), width (maximum lateral axis) and height (dorsal/ventral axis) of each individual were measured to the nearest 0.01 mm using an electronic caliper, and total wet weight was recorded (0.01 g). The soft parts of the body were removed from the shells and weighed (± 0.00001 g). The mantle-gonad (MGI) and digestive gland (DGI) indexes were calculated for each individual as the ratio of organ wet mass (g) to total wet mass excluding the valves (g) multiplied by 100.

HISTOLOGICAL ANALYSES

A cross-sectional mantle-gonad portion was removed from each specimen, fixed in Bouin's solution over 24 h, washed with water and transferred to 70% alcohol. The portions were then dehydrated in an alcohol series, cleared in benzene, embedded in paraffin (Paraplast®), sectioned at 5 μ m and stained with Groat's hematoxylin and eosin (Humason 1962).

SEX DETERMINATION AND GAMETOGENIC STAGES

The slides were examined microscopically, the sex was identified and, when suitable, each individual was assigned to one of 6 gametogenic stages: immature (I), growing (G), mature (M), spawned with recovery (SR), spawned with recovery and atresia (SRA), and spawned (S) (Figs. 3 and 4). These stages were defined based on standard gonadal scales for *A. atra* and other mollusks (Vinueza & Tortorelli 1980, Jaramillo & Navarro 1995, Suárez *et al.* 2007, Oyarzún *et al.* 2011). Photomicrographs were taken with a digital camera attached to a Carl Zeiss® Axiostar microscope.

CALORIMETRIC DETERMINATION

Mantle-gonads and digestive glands were dried in an air circulating oven at 70°C to constant weight. After dry

mass was obtained, samples were ground and pellets were made with a press (Parr, model 2812®). The caloric contents of the pellets were measured in 30 individuals per season. The pellets (50-200 mg) were burned in a micro-bomb calorimeter (Parr, model 1425®), up to complete combustion (Lucas 1996). The energy density of the mantle-gonads (ED_{MG}) and digestive gland (ED_{DG}) were calculated as kJ/g ash-free dry mass (Boy *et al.* 2009, Pérez *et al.* 2010). The total energy content of the mantle-gonads (EC_{MG}) and digestive gland (EC_{DG}) were calculated as the product between ED_{MG} and ED_{DG} , and the total mass (g) of the mantle-gonads or digestive gland respectively. The values obtained were corrected for ash and acid content. The micro-bomb calorimeter was periodically calibrated with benzoic acid.

Seasonal variations in MGI were analyzed using a one-way ANOVA, followed by Tukey HSD comparisons for an unbalanced design (Sokal and Rohlf 1995). Kendall Tau correlations were used to test for relationships between MGI and environmental variables such as photoperiod and water temperature (Zar 1984).

The sex ratio was analyzed with a Chi-square test, testing the hypothesis of a 1:1 male:female relation (Zar 1984). Sex could not be discriminated in the I stage, and the SRA and S stages were only found in females. Therefore, was performed a first analysis to test for significant differences in MGI and DGI between all gonadal stages without discriminating between sexes, by means of one-way ANOVAs. A two-way ANOVA was applied for each index (MGI and DGI) to test for significant differences between gonadal stages (G, M and SR) and sexes. Pairwise differences between gonadal stages were analyzed by unplanned Tukey HSD multiple comparisons for unequal N. When required, assumptions of normality (Kolmogorov-Smirnov test) and homogeneity of variances (Bartlett test) were previously verified (Sokal & Rohlf 1995). The same analyses were performed to study the variation in ED and EC both in mantle-gonads and digestive gland. ED_{DG} and EC_{DG} were transformed to ED_{DG}^2 and $\log EC_{DG}$ to meet the assumptions of the ANOVA model.

All statistical analyses were performed using the STATISTICA 6.0 Package (StatSoft Inc. 1998).

³Servicio de Hidrografía Naval de Argentina <<http://www.hidro.gov.ar/observatorio/sol/asp>>

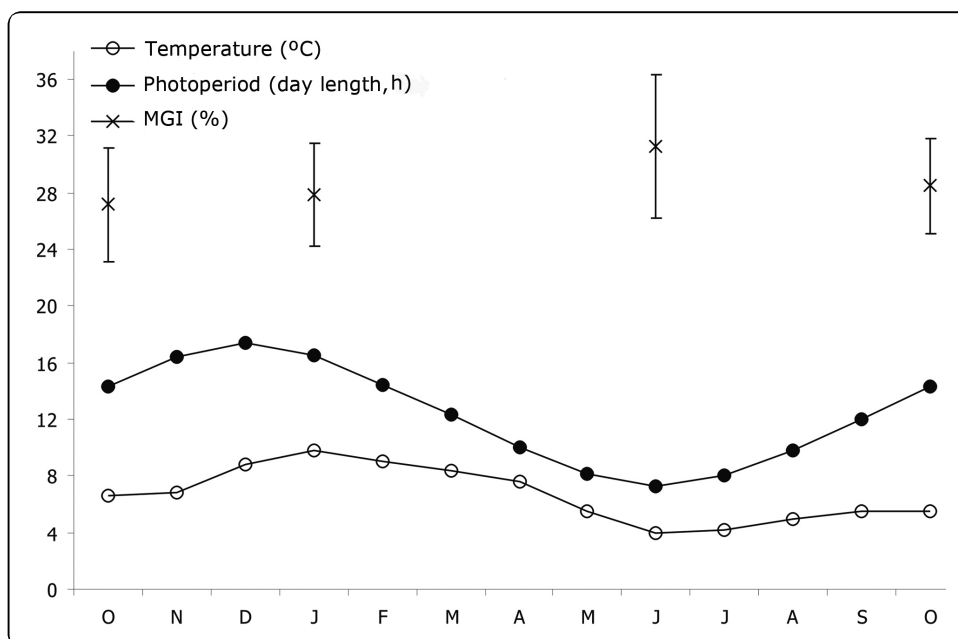


Figure 2. Mean mantle-gonad index (MGI \pm SD) for *Aulacomya atra* from the Beagle Channel (October 2009-October 2010). Monthly means of seawater temperature and day length in the Beagle Channel / Índice gónada-manto promedio (MGI \pm DE) de *Aulacomya atra* del Canal Beagle (octubre 2009-octubre 2010). Promedios mensuales de la temperatura del agua y horas diarias de luz en el Canal Beagle

RESULTS

SEX RATIO

On each sampling, 35 adult specimens, from 51 to 73 mm in shell length, were randomly collected from the low edge of the mussel bed. A total of 65 males, 53 females, 1 hermaphrodite, and 8 immature individuals were determined during the histological analysis of the samples. Immature individuals were not considered for the sex ratio analysis. The sex ratio of the Beagle Channel population of *A. atra* studied did not differ significantly from a 1:1 proportion (Chi-square test: $\chi^2 = 0.55$, g.l. = 1, $P = 0.46$).

TEMPERATURE, PHOTOPERIOD AND MANTLE-GONAD INDEX (MGI)

During the study period, the mean temperature recorded was 4.4°C in winter and 9.2°C in summer and day lengths varied between 7.3 h in June and 17.4 h in December (Fig. 2).

The MGI was significantly different between seasons (one-way ANOVA $F_{(3,122)} = 5.83$, $P < 0.001$, Fig. 2), being highest in June 2010 (Tukey HSD test for unequal N June 2010 vs. October 2009 and January 2010: $P < 0.01$, June 2010 vs. October 2010: $P < 0.05$). However, no correlation was observed between the MGI and water temperature or

day length (Kendall Tau correlations $\tau = -0.67$, g.l. = 3, $P = 0.17$ and $\tau = -0.55$, g.l. = 3, $P = 0.26$ for temperature and photoperiod, respectively; Fig. 2).

GAMETOGENIC STAGES AND REPRODUCTIVE CYCLE

A total of 127 individuals were examined microscopically. The gonads of *A. atra* are located in the dorsal part of the visceral mass from which ventrally ramified tubules converge and open into the mantle cavity to release gametes into the sea water. Although this species is gonochoric, we found one hermaphrodite (1/127). Female mantle-gonads were brownish and granular, whereas male ones were light brown with a milky texture.

During summer, most individuals were in the growing stage (G) (Figs. 3a, 4a, 5a, and 5b), and only 10% of the whole sample was immature. Germinal cells were present in the tubules in all developmental stages, but the number of mature ova and spermatozoa was low. At the end of autumn, most of the individuals were mature (M) (tubules were almost full of ripe gametes) (Figs. 3b, 4b, 5a and 5b), except 25% of males, which were partially spawned with recovery (SR), in which the presence of a spermatocyte and spermatid band indicated an imminent gonadal recovery (Fig. 5b). During spring, almost all males and most females were partially spawned with recovery (SR)

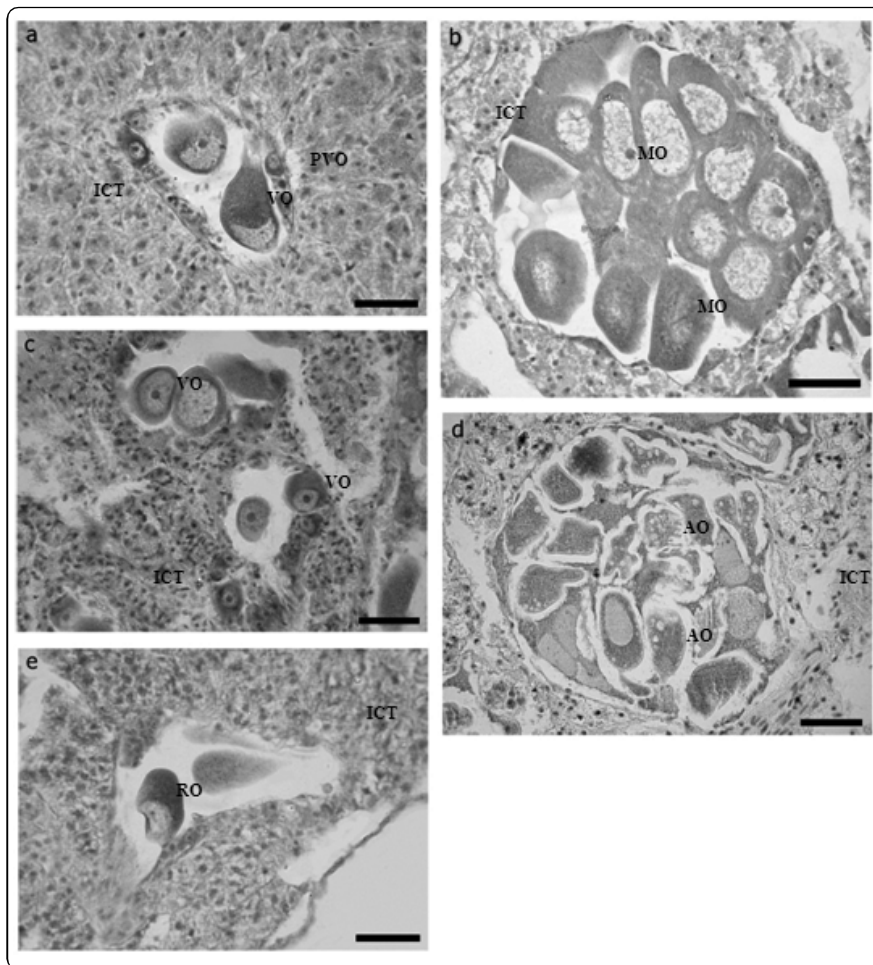


Figure 3. Histological sections of female gonads of *A. atra* from the Beagle Channel in different gametogenic stages: a) growing (G), b) mature (M), c) partially spawned with recovery (SR), d) partially spawned with recovery and atresia (SRA) and e) spawned (S). ICT: intertubular connective tissue, PVO: previtellogenic oocytes, VO: vitellogenic oocytes, MO: mature oocytes, AO: atresic oocytes, RO: relict ovas. Scale bar 50 μm / Secciones histológicas de gónadas femeninas de *A. atra* del Canal Beagle en diferentes estadios gametogénicos (G), b) maduro (M), c) parcialmente desovado con recuperación (SR), d) parcialmente desovado con recuperación y atresia (SRA) y e) desovado (S). ICT: tejido conectivo intertubular, PVO: oocitos pre vitelogénicos, VO: oocitos vitelogénicos, MO: oocitos maduros, AO: oocitos atrésicos, RO: ovas relictuales. Barra 50 μm

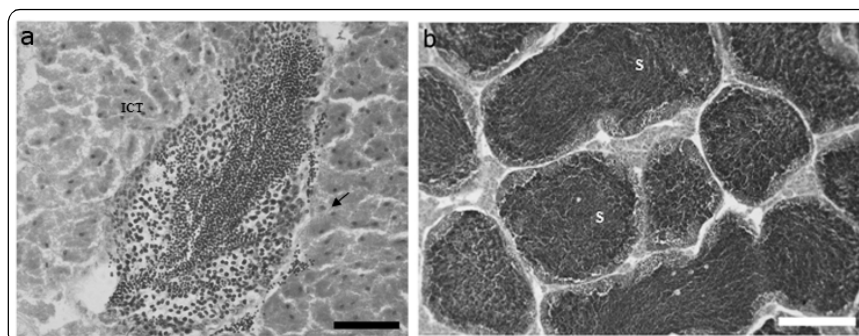


Figure 4. Histological sections of male gonads of *A. atra* from the Beagle Channel in different gametogenic stages: a) growing (G) and b) mature (M). ICT: intertubular connective tissue, Arrow: developing germinal layer, S: spermatozoa. Scale bar 50 μm / Secciones histológicas de gónadas masculinas de *A. atra* del Canal Beagle en diferentes estadios gametogénicos: a) crecimiento (G) y b) maduro (M). ICT: tejido conectivo intertubular, Flecha: capa germinal en desarrollo, S: espermatozoides. Barra 50 μm

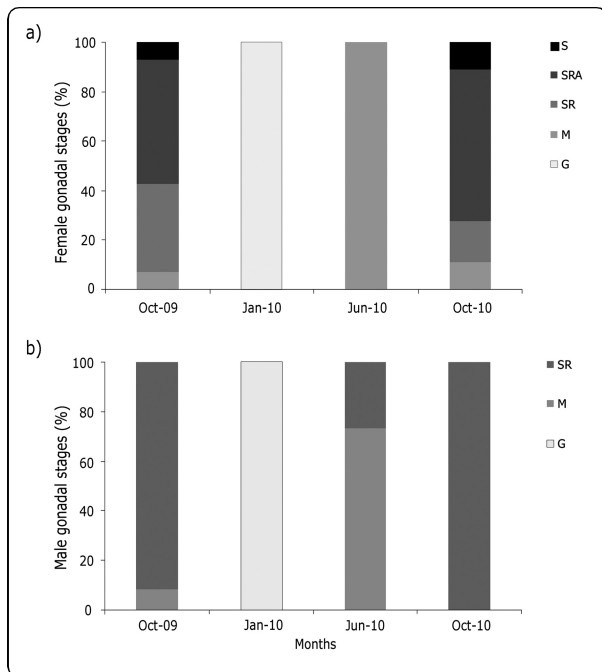


Figure 5. Seasonal changes in the percentage of females (a) and males (b) of *A. atra* in the different gametogenic stages (G growing, M mature, SR spawned and recovery, SRA spawned and recovery with atresia, and S spawned) between October 2009 and October 2010 / Cambios estacionales en el porcentaje de hembras (a) y machos (b) de *A. atra* en diferentes estadios de maduración gonadal (G en maduración, M maduro, SR desovado con recuperación, SRA desovado con recuperación y atresia) entre octubre 2009 y octubre 2010

(Figs. 3c, 5a and 5b), with an increased percentage of females with signs of follicular atresia (SRA) (Figs. 3d and 5a). Atresia and degeneration of gametes during spawning, followed by recovery, were recorded. In spermatozoa, lysis is not as evident as in oocytes, since the former have virtually no cytoplasm. The remaining spring females (~10%) were either mature (M), or spawned (S) (Figs. 3e and 5a).

MANTLE-GONAD INDEX (MGI), DIGESTIVE GLAND INDEX (DGI) AND GONADAL STAGES

When analyzing overall MGI and DGI (not discriminating between sexes), we found that values varied significantly among gonadal stages (ANOVA $F_{(5,117)} = 3.62$, $P < 0.01$ and $F_{(5,117)} = 5.16$, $P < 0.001$, for MGI and DGI, respectively). Individuals in the M stage had a significantly higher MGI than those in the SRA stage (Fig. 6a), and a significantly lower DGI than those in the G, SR and SRA stages (Fig. 6b). When only the G, M and SR stages were compared, significant differences in MGI were observed between stages and sexes (ANOVA $F_{(2,88)} = 6.09$, $P < 0.01$, $F_{(1,88)} =$

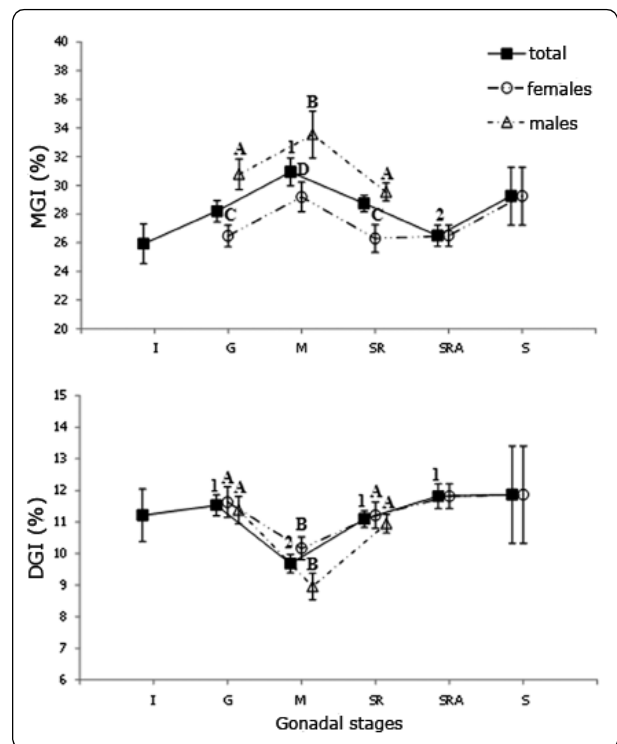


Figure 6. Mean mantle-gonad index (a, MGI) and digestive gland index (b, DGI) of *A. atra* for each gonadal stage (I immature, G growing, M mature, SR spawned and recovery, SRA spawned and recovery with atresia, S spawned). Error bars show standard error of mean MGI and DGI values. Significant differences in indexes are indicated by different numbers for comparisons between all gonadal stages without discriminating between sexes and different letters for comparisons between gonadal stages and sexes / Índice gónada-manto promedio (a, MGI) e índice de la glándula digestiva (b, DGI) de *A. atra* para cada estadio de maduración gonadal (I inmaduro, G en maduración, M maduro, SR desovado con recuperación, SRA desovado con recuperación y atresia, S desovado). Las barras de error indican el error estándar respecto de los valores de MGI y DGI promedio. Las diferencias significativas son indicadas con números distintos para comparaciones entre todos los estadios sin discriminar sexos y con letras distintas para las comparaciones entre estadios y sexos

20.35, $P < 0.01$ and $F_{(2,88)} = 0.17$, $P = 0.84$, for stage, sex and interaction, respectively). The MGI was higher in males than in females for all stages, and individuals in the M stage had a significantly higher MGI than those in the G and SR stages for both sexes (Fig. 6a).

The DGI showed significant differences between gonadal stages (ANOVA $F_{(2,88)} = 11.2$, $P < 0.001$), but not between sexes ($F_{(1,88)} = 2.55$, $P = 0.11$). The interaction between both factors was not significant for the DGI ($F_{(2,88)} = 0.82$, $P = 0.45$). Animals in the M stage had a

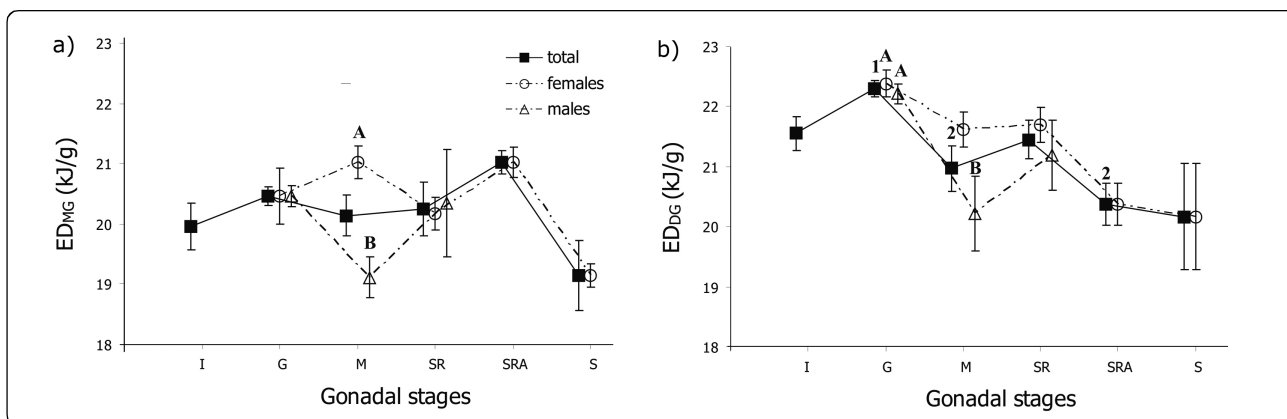


Figure 7. Mean energy density of the mantle-gonads (a, ED_{MG}) and digestive gland (b, ED_{DG}) of *A. atra* for each gonadal stage (I immature, G growing, M mature, SR spawned and recovery, SRA spawned and recovery with atresia, S spawned). Error bars show standard error of mean ED values. Significant differences in indexes are indicated by different numbers for comparisons between all gonadal stages without discriminating between sexes and different letters for comparisons between gonadal stages and sexes / Densidad energética promedio del manto-gónada (a, ED_{MG}) y de la glándula digestiva (b, ED_{DG}) de *A. atra* para cada estadio de maduración gonadal (I inmaduro, G en maduración, M maduro, SR desovado con recuperación, SRA desovado con recuperación y atresia, S desovado). Las barras de error indican el error estándar respecto de los valores de ED promedio. Las diferencias significativas son indicadas con números distintos para comparaciones entre todos los estadios sin discriminar sexos y con letras distintas para las comparaciones entre estadios y sexos

significantly lower DGI than those in the other two stages (Fig. 6b).

ENERGY DENSITY OF MANTLE-GONADS (ED_{MG}) AND DIGESTIVE GLAND (ED_{DG})

ED_{MG} did not vary significantly among gonadal stages when analyzed without discriminating between sexes (ANOVA $F_{(5,48)} = 1.52$, $P = 0.200$; Fig. 7a). However, an interaction between sexes and stages was observed when the ED_{MG} of the G, M, and SR stages was compared (ANOVA $F_{(2,31)} = 3.66$, $P < 0.05$). The ED_{MG} was lower in mature males than in mature females (Fig. 7a).

Moreover, significant differences among gonadal stages were found for ED_{DG} (ANOVA $F_{(5,48)} = 4.91$, $P < 0.001$, Fig. 7b). Individuals in the G stage had higher ED than those in the M and SRA stages (Fig. 7b). Analysis of the ED_{DG} of the G, M, and SR stages showed significant differences between stages and sexes (ANOVA $F_{(2,31)} = 6.33$, $P < 0.01$, $F_{(1,31)} = 4.42$, $P < 0.05$ and $F_{(2,31)} = 1.20$, $P = 0.3164$, for stage, sex and interaction, respectively). The ED_{DG} values of mature males were lower than those of individuals in the growing stage (Fig. 7b).

ENERGY CONTENT OF MANTLE-GONADS (EC_{MG}) AND DIGESTIVE GLAND (EC_{DG})

When analyzing overall EC_{MG} (not discriminating between sexes), we found that values varied significantly among gonadal stages (ANOVA $F_{(5,48)} = 10.08$, $P < 0.001$).

Individuals in the G and M stages had higher energy content than those in the I and SR stages (Fig. 8a). When only the G, M and SR stages were compared, significant differences in EC_{MG} were observed between stages (ANOVA $F_{(2,31)} = 17.39$, $P < 0.001$, $F_{(1,31)} = 0.5583$, $P > 0.05$ and $F_{(2,31)} = 3.25$, $P = 0.052$, for stages, sex and interaction, respectively). Animals in the SR stage had lower EC_{MG} than those in the G and M stages for both sexes (Fig. 8a).

The energy content of the digestive gland ($\log EC_{DG}$) differed among gonadal stages (ANOVA $F_{(5,48)} = 3.23$, $P < 0.05$), although post-hoc comparisons were unable to show significant differences (Fig. 8a). Furthermore, when the $\log EC_{DG}$ of G, M and SR was analyzed, no interaction was observed between gonadal stage and sex (ANOVA $F_{(2,31)} = 2.36$, $P = 0.111$) and significant differences were observed between stages ($F_{(2,31)} = 6.72$, $P < 0.01$) but not between sexes ($F_{(1,31)} = 0.63$, $P = 0.434$). Males in the G stage had higher $\log EC_{DG}$ than mature males and than males in the SR stage (Fig. 8b).

DISCUSSION

Our results show that the *Aulacomya atra* population living in the intertidal zone of the Beagle Channel has an extended spawning period from late winter to spring, when the lowest value of MGI and the maximum number of spawned (SR, SRA and S) individuals were observed (Figs. 2 and 5). MGI increased until winter, reaching its maximum in June (the coldest and darkest month) (Fig. 2). Variation patterns of MGI (Fig. 6a) were similar in both

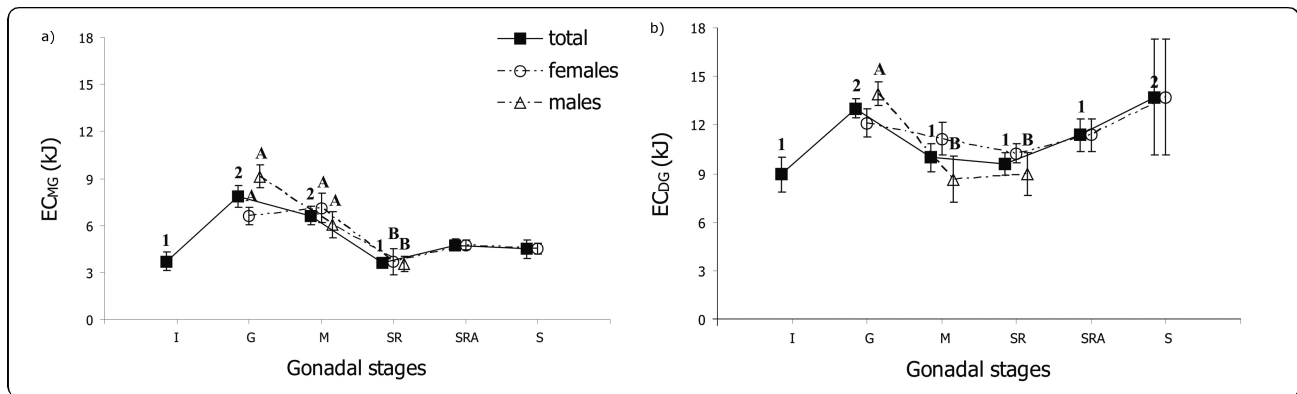


Figure 8. Mean energy content of the mantle-gonad (a, EC_{MG}) and digestive gland (b, EC_{DG}) of *A. atra* for each gonadal stage (I immature, G growing, M mature, SR spawned and recovery, SRA spawned and recovery with atresia, and S spawned). Error bars show standard error of mean EC values. Significant differences in indexes are indicated by different numbers for comparisons between all gonadal stages without discriminating between sexes and different letters for comparisons between gonadal stages and sexes / Contenido energético promedio del manto-gónada (a, EC_{MG}) y de la glándula digestiva (b, EC_{DG}) de *A. atra* para cada estadio de maduración gonadal (I inmaduro, G en maduración, M maduro, SR desovado con recuperación, SRA desovado con recuperación y atresia, S desovado). Las barras de error indican la desviación estándar respecto de los valores de EC promedio

sexes, as found for a Chilean population of *A. atra* (Jaramillo & Navarro 1995), *Anodonta gabillotta pseudodopsis* (Sereflisan *et al.* 2009) and *Mytilus chilensis* (Oyarzún *et al.* 2011).

Environmental conditions, especially temperature, photoperiod, and food availability, influence gamete maturation and spawning of marine invertebrates (Griffiths 1977, Lawrence & Soame 2004, Santos *et al.* 2011). Even though no correlation was observed between MGI and temperature or photoperiod in the time period studied, a trend was observed. The highest MGI (mature stage) values occurred in early June, when day length was shortest and water temperature was lowest. Subsequently, MGI decreased in coincidence with the increase in day length and water temperature. In October, when day length was 13-14 h, most of the individuals were found in gonadal recovery and spawned stages, coincident with the increase in primary production (Almandoz *et al.* 2011).

Similar results were found by Tortorelli (1987) in a nearby locality. The population studied by Tortorelli reached gonadal maturity in winter and then had an extended period of spawning (from the end of winter until summer), which was associated with the decrease in sea water temperature. Influence of seasonality on spawning of *A. atra* was also found by other authors in populations from the South Pacific Ocean. In Yaldad Bay (43°7'S; 73°43'W), Jaramillo & Navarro (1995) observed periods of greater spawning intensity in April, August-November and February, and reported that food availability was the most important factor for successful development of gametogenesis and spawning. In Caleta Punta Arenas

(21°38'S; 70°09'W), Avendaño & Cantillán (2012) found continuous spawning throughout the year, with 2 important peaks during August-September and October-November, associated with a decrease in water temperature. In a population of Puerto Deseado, in the South Atlantic Ocean (47°45'S; 65°55'W), Vinuesa and Tortorelli (1980) reported 2 spawning periods (January and April) but found no relationship with sea water temperature. The spawning period of the southernmost population of *A. atra* studied here seems not to differ from the general pattern described for other populations of this species, and seems to be associated with the increase in water temperature after winter and probably also with primary productivity.

Oocyte degradation and atresia have been observed in other mytilids, namely *Mytilus edulis* (Pipe 1987) and *Mytilus galloprovincialis* (Suárez *et al.* 2007). In *M. galloprovincialis*, Suárez *et al.* found that atresia and degradation of gametes are more intense during winter and infrequent in spring, when the main spawning occurs, and that non-favorable environmental conditions could explain the occurrence of this phenomenon (Suárez *et al.* 2007). Besides, Paulet *et al.* (1988) found that temperature may act as a cue in *Pecten maximus* and that spawning takes place only if water temperature reaches 15.5-16°C, and that otherwise, pronounced atresia occurs in mature gonads. Although the regulating mechanism of oocyte atresia is not well known, some factors such as low temperature, nutritional deficit (Galap *et al.* 2004) and environmental pollution (Steele & Mulcahy 1999) could be involved. We found atresia in *A. atra* during spring, coinciding with the

spawning period, and only in females, although it likely occurs in males as well. Degradation and atresia in male gametes are difficult to detect because spermatozoa have a very small amount of cytoplasm (Suárez *et al.* 2007).

We found evidences that the high energy demands of gonadal maturation are supplied at least by the intertubular connective tissue of gonads and by the digestive gland. The histology and cellular organization of reproductive tissues in *A. atra* showed that the intertubular connective tissue and gonadal tubules present an inverse growth cycle (Figs. 3 and 4), as previously reported for other populations of this species (Vinuesa & Tortorelli 1980, Jaramillo & Navarro 1995), and for other bivalves in general (Gosling 2003, Oyarzún *et al.* 2011). Gonadal tubules showed their maximum development during sexual maturity when intertubular tissue was minimal. Several authors have suggested that intertubular tissue provides the energy needed during gametogenesis in bivalves. *Mytilus edulis* females (and maybe also males), for example, accumulate reserves in the mantle off the gonadal cells (Gabbott & Peek 1991). In the present study, we observed a decrease in DGI in concordance with the increase in MGI in mature individuals (Fig. 6) and a trend towards the depletion of ED and EC of the digestive gland during gonadal maturation, *i.e.* from growing individuals to mature ones (Figs. 7b and 8b), although it was significant only for males. These findings suggest the translocation of energy from the digestive gland to the gonads during gonadal maturation in *A. atra*.

In the population under study, energy allocation differed between sexes. Mature females allocated more energy per unit organ to mantle-gonads than mature males (Fig. 7a) since sperm cells require less energy than oocytes to complete maturation. After maturity, the ED_{MG} of males and females converged to similar values, and decreased abruptly at spawning (Fig. 7a). We also found that males and females reached similar EC_{MG} (Fig. 8a) values by means of different strategies: males had gonads of larger size (Fig. 6a) but with lower energy by unit of mass (energy density) than females (Fig. 7a) (sperm cells have less reserve substances than oocytes) (Pérez *et al.* 2010). With the beginning of the spawning period, a sharp decrease in MGI occurred given by the decrease in the mass of mantle-gonads in relation to the whole mass of the individual. Summarizing, the increase in MGI in mature males occurred together with a decrease in ED_{MG} (indicating an increase in mass of lower energy values), whereas the increase in MGI in mature females occurred

together with an increase in mass of higher energy values, such as vitellum (Pérez *et al.* 2010), which increased ED_{MG}.

Several authors have studied patterns of storage and ultimate utilization of energy reserves in mussels by measuring proximal composition, which is an indirect method (Gabbott 1975, Zandee *et al.* 1980, Hawkins *et al.* 1985). In contrast, the present study is the first to study energy allocation patterns in ribbed mussels through direct calorimetric determinations. Our results evidenced the role of the connective tissue of gonads and the digestive gland as energy reservoirs for gonadal maturation in *A. atra* of the Beagle Channel, and a different energy allocation strategy between sexes during gonadal maturation. Given that our results refer to individuals of *A. atra* living in the intertidal area of the southern limit of the species distribution, and considering the results on other bivalve species, which have shown differences in relative energy invested in reproduction among intertidal individuals as well as differences in reproductive cycles and gonad indexes between intertidal and subtidal populations (Alfaro *et al.* 2003, Petes *et al.* 2008), studies implementing calorimetric determinations in subtidal individuals from the same locality are needed. Moreover, studies of energy allocation to gametogenesis are needed on other populations of *A. atra* along its wide distribution. This will help us understand the plasticity in the life-history of the species.

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