Invertebrate Reproduction and Development, xx:x (200x) x–x
Balaban, Philadelphia/Rehovot
0168-8170/0x/$05.00 © 200x Balaban

The reproductive cycle of *Eurhomalea exalbida* (Chemnitz, 1795) (Bivalvia: Veneridae) in Ushuaia Bay (54º 50' S), Beagle Channel (Argentina)

ELBA MORRICONI[1*], BETINA J. LOMOVASKY[1], JORGE CALVO [1] and THOMAS BREY[2]
[1]*Centro Austral de Investigaciones Cientificas (CADIC - CONICET). C.C. 92 (V9410BFD), Ushuaia, Tierra del Fuego, Argentina*
Tel. +54 (2901) 422310; Fax +54 (2901) 430644; email: jcem@arnet.com.arx
[2]*Alfred Wegener Institute for Polar and Marine Research (AWI). Columbusstrasse, 27515 Bremerhaven, Germany*

Received 9 November 2001; Accepted 5 May 2002

Summary

The reproductive cycle of *Eurhomalea exalbida* (Chemnitz, 1795) in Ushuaia Bay, Beagle Channel, was studied from October 1998 to December 1999. Clams were collected monthly by SCUBA diving at 3–5 m water depth. The degree of sexual maturity was determined histologically (n=338). The sex ratio was 1:1 (Chi-squared $p>0.05$). First sexual maturity occurred at 39 mm shell height in males and 40 mm in females (i.e., 4 years of age). Five distinct histological stages of sexual maturity could be established in males: (1) early active, (2) late active, (3) ripe, (4) partially spawned with recovery and (5) spent. Females had oocytes ranging from small and immature to large and fully developed in their ovaries throughout the year. Since we could not classify reproductive stages based on a qualitative histological scale of sexual maturity, quantitative measures using an image analyser were used. In males a high percentage of mature individuals were present between January and August. The percentage of early active and partially spawned individuals was highest in November 1998 (70%) and 1999 (60%). In females, the mean number of developed oocytes per unit of gonadal area in a cross section, the mean diameter (minor axis) of oocytes, and the percentage of gonadal area occupied by oocytes were lowest in November, indicating a spawning peak. These results indicate synchronous gonadal development and spawning in males and females. The more intensive spawning activity in November coincides with the higher biomass and production of phytoplankton in spring.

Key words: Reproductive cycle, gametogenesis, image analysis, clams, *Eurhomalea exalbida*

Introduction

The venerid *Eurhomalea exalbida* (Chemnitz, 1795) is a shallow sublittoral species widely represented in the extreme south of coastal South America, and is considered to be a potential economic resource in the Beagle Channel. Population dynamic study indicated an average abundance of 83 ind m^{-2} in the study site (Lomovasky et al., in press). The northern limits of its distribution are Chiloé (42° S) in the Pacific littoral zone (Soot-Ryen, 1959; Dell, 1964;

*Corresponding author.

Osorio et al., 1979) and the province of Buenos Aires (38° S) in the Atlantic littoral zone. The southern limit is the Beagle Channel (54°50′S) (Carcelles, 1944; 1950). The lowest temperatures within the species' geographic distribution are found in this southern zone with average monthly maximum and minimum temperatures of 8.7°C and 4.5°C, respectively (Hydrology Service of Austral Centre of Scientific Research). The importance of low temperatures as a factor that slows the rate of oogenesis and growth in bivalve molluscs has been widely demonstrated (Giese, 1959; Sastry, 1979; Pearse et al., 1991) and may be an important limitation on the capacity of the organisms to withstand commercial extraction. Consequently, studies of the biological characteristics of populations, such as growth and reproduction, are important for species that inhabit high latitudes. Only the population dynamics and morphology of some venerids in sub-Antarctic regions have been investigated thus far (Urban, 1994; Urban and Tesch, 1996).

Several species in this family (*E. exalbida, Venus (Ameghinomya) antiqua, Protothaca thaca*) are exploited commercially on the Pacific coast of Chile (Osorio et al., 1979), but investigations have only studied reproduction in populations of *Venus (A.) antiqua* in Ancud Bay (41°51′S, Lozada and Bustos, 1984) and Yaldad Bay (43°08′S, Stead et al., 1997). In coastal Argentina, reproduction in the clams *E. exalbida, Tawera gayi,* and *Venus (A.) antiqua* has been studied in Punta Loma, 42°47′S (Schuldt, 1975; Verdinelli and Schuldt, 1976). This last species is commercially exploited sporadically on the Argentinean coast (Ciocco et al., 1998).

The objective of this study was to determine the sex ratio, the size at first maturity, and the reproductive cycle — in particular the spawning period and the period of maximum maturity — in a population of *E. exalbida* in the Beagle Channel.

Materials and Methods

Monthly samples were collected in Ushuaia Bay (54°50′S, Beagle Channel), in a subtidal flat with a water depth between 2 and 4 m at low tide. Sampling was conducted by SCUBA diving from October 1998 to December 1999. Mean surface temperature in the sampling zone was taken from SECEDOC (Hydrology Service of Austral Centre of Scientific Research, Argentina), ranging from 8.7°C in summer to 4.5°C in winter (Fig. 1). Salinity was measured sporadically with a Horiba U-10 meter, with values ranging from 30.9 to 32.9‰.

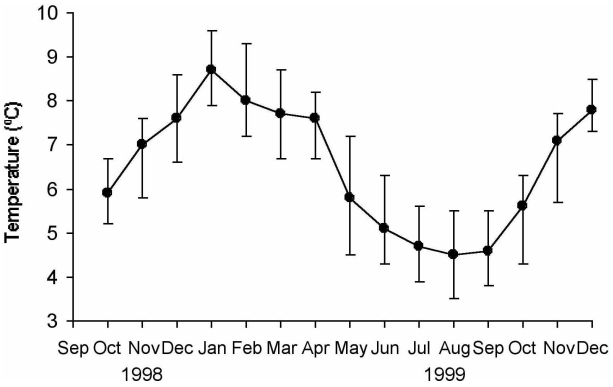

Fig. 1. Monthly mean with maximum and minimum seawater temperature in Ushuaia Bay, Beagle Channel, between October 1998 and November 1999.

Shell height (umbo to ventral axis, H), and length (anterior–posterior axis, L) were measured with electronic calipers to the nearest 0.01 mm and were recorded for each individual (n = 1196). A subsample was taken for histological analysis; the shells were removed and the soft parts (n = 138 females; 180 males; 20 undifferentiated) were fixed in Bouin's fluid and then washed with water and transferred to 70% alcohol. A cross-sectional block was dissected from each clam, dehydrated in an alcohol series, cleared in benzene, embedded in Paraplast, sectioned at 5 µm and stained with Groat's hematoxylin and eosin.

The stage of development of male gonads was determined by analysis of different levels of development of germinal cells in histological preparations.

A quantitative analysis of the histological preparations of female specimens was performed using images captured by a video camera (Panasonic KR222) and viewed using the Image-Pro Plus 3.0 software. Three microscopic fields (100×) per cross section were analysed for each specimen. In each field the non gonadal area was disregarded and the remaining area was expressed as the gonadal area in μm^2. The mean number of oocytes (divided into three size classes: <25 µm, 25–40 µm and >45 µm, minor axis) per 100 μm^2 gonadal area, the mean diameter (minor axis) of oocytes, and the percentage of gonadal area occupied by oocytes were determined.

Height at first maturity was based on 91 specimens between 24 and 48 mm H. Specimens were grouped into size classes (3 mm) and classified histologically as sexually active (with oocytes or sperm) and inactive (with scantly and small alveoli containing few sexual cells). The median of the cumulative frequency distribution of sexually active specimens was considered to represent the height at which 50% of animals are sexually mature.

The sex ratio was assessed for the total sample by examining gonadal smears and was tested against a 1:1 ratio with Chi-square tests.

Statistical Analysis

The monthly differences in mean oocyte diameter (minor axis) and the number of oocytes > 40 μm per gonadal area were analysed using an analysis of variance (ANOVA). The assumptions of normality and homogeneity of variances were tested and the appropriate transformations were applied when necessary. Unplanned comparisons were made (STATISTICA software) when significant differences were found.

Results

E. exalbida is gonorchoristic. The gonad is made up of interconnected gonadal alveoli and surrounds the digestive gland and the gut, infiltrating the muscular tissue of the foot. The alveoli contain different types of germinal cells and are separated by connective tissue. Individuals larger than 23 mm H could be sexed histologically and the largest specimen sampled measured 85.3 mm H. When fresh, female gonads are white and male gonads are beige to orange. The sex ratio did not differ significantly 1:1 (370 males; 379 females, χ^2 test, $p > 0.05$).

Male gonadal stages

1. Early active (Fig. 2A)

Gonads at this stage contain abundant, round alveoli, with very little interalveolar space. In the alveoli a row of spermatogonia and a wide band of spermatocytes and spermatids form a germinal layer. The spermatozoa become arranged with tails pointing toward the center of the lumen.

2. Late active (Fig. 2B)

At this stage the alveoli are larger. Although the band of spermatocytes and spermatids remains conspicuous, it is narrower than in the previous stage. The quantity of spermatozoa increases and in a few alveoli they are found disorganised.

3. Ripe (Fig. 2C)

The alveoli are very large and touching each other, leaving no interalveolar space. The band of spermatocytes and spermatids is very reduced, and the lumen is wide and full of spermatozoa. In some alveoli the spermatozoa are disorganised, and ready to be expelled to the tubules.

4. Partially spawned with recovery (Fig. 2D)

Partially empty alveoli with a decrease in the number of spermatozoa filling the lumen are observed. The presence of a spermatocyte and spermatid band indicates an imminent gonadal recovery. This stage is difficult to distinguish from the early active stage, the greatest difference lying in the somewhat disorganised state of the spermatocyte and spermatid band and the irregular shape of the alveoli due to their partial contraction.

5. Spent (Fig. 2E)

This stage is characterised by small and irregular alveoli, with few and disorganised spermatocytes and spermatozoa, and abundant interalveolar connective tissue. Few individuals were found at this stage.

6. Inactive (Fig. 2F)

This stage was only observed in juveniles. The gonads have small and few alveoli, with much interalveolar tissue. The alveoli contain spermatogonia and a few spermatozoa.

The male reproductive cycle was determined by an analysis of the monthly percentage of gonadal development stages.

Male reproductive cycle

An analysis of the monthly percentages of the stages of development (Fig. 3) shows that a high percentage of individuals (60–100%) were mature for a large part of the year (January–August), with a low percentage of partially spawned with recovery individuals. In November, 1998, a low percentage of early active individuals (25%) and a high proportion of partially spawned with recovery individuals (58.33%) were observed. In November, 1999, 58.33% of the specimens were observed in the partially spawned with recovery stage and none in the early active stage, which may indicate that their recovery was slower than in the previous year. At the end of spring (December), spawning ceases, increasing the numbers at the late active stage.

Female reproductive cycle

Histological observations of female gonads revealed different types of germinal cells. Oogonia (5–7.5 μm), small oocytes adhering to the alveolar walls

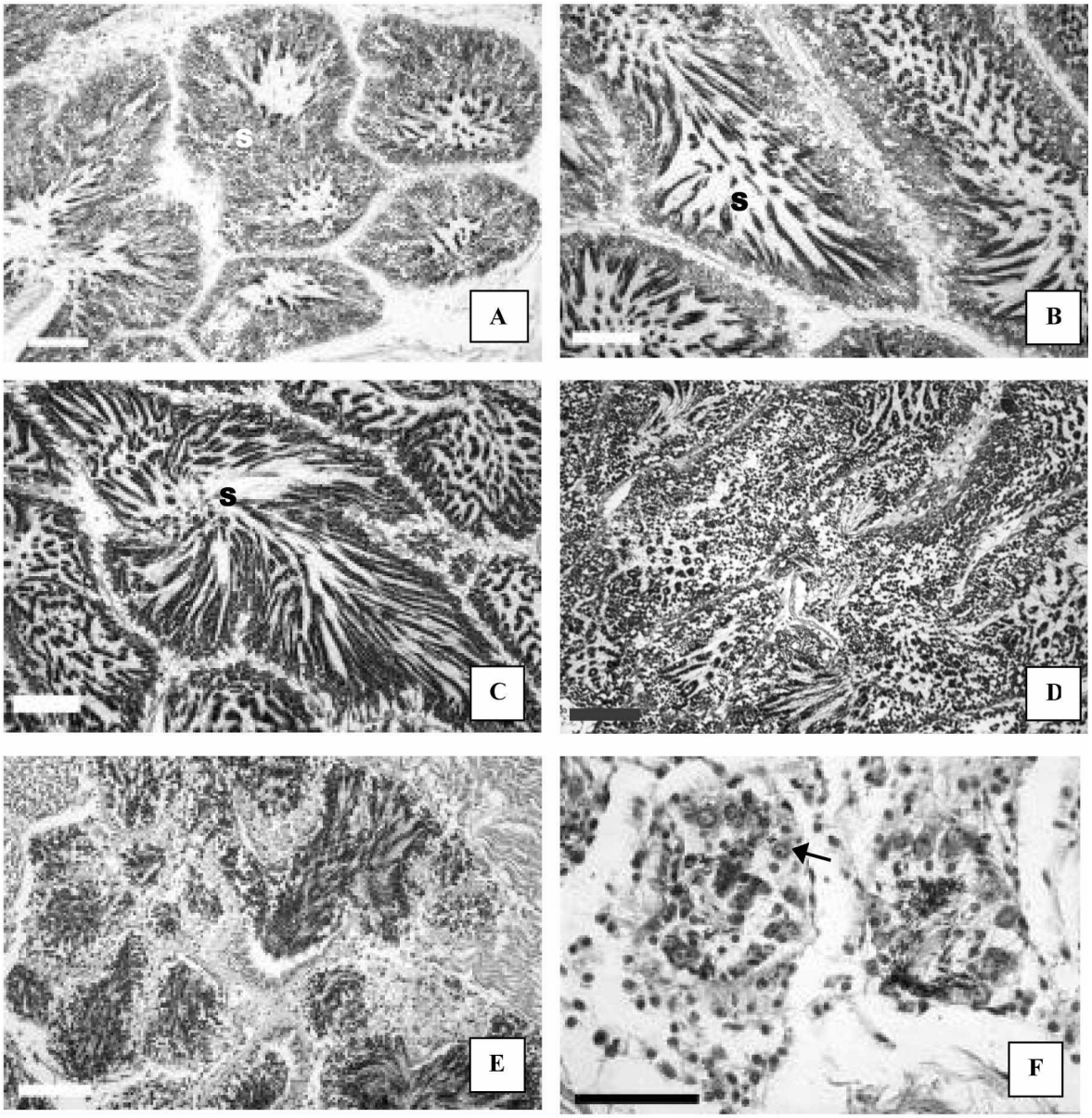

Fig. 2. Photomicrographs of gonadal stages of males of *E. exalbida.* A. early active: (Sc) spermatocytes; B. late active: (Sz) spermatozoa; C. ripe; D. partially spawned; E. spent; F. inactive: (arrow) spermatogonia. Scale bar = 100 µm (A–E) and 50 µm (F).

(< 25 µm), pedunculate oocytes (25–40 µm), and spherical free oocytes (> 40 µm) were distinguished, the latter being considered full grown or near maturity (Fig. 4A). Mature oocytes were observed using fresh smears. They measured between 82 and 103 µm and were surrounded by a jelly coat. Histological processing produces a contraction of oocyte diameter of approximately 40%.

The monthly mean number of mature oocytes (diameter >40 µm; Fig. 5) showed little variation over the period February–October. In November, 1998, the number of mature oocytes per gonadal area was lower than in the other months of the year, indicating spawning had occurred. These values for November, 1998, were significantly different from the values observed in January, 1999 (one-way ANOVA, $F = 2.105$; $p < 0.05$; and subsequent modified Tukey test: November vs. January, $p < 0.01$). Histological images of the gonads in November (Fig. 4B) showed significant numbers of mature oocytes in the interior of the alveoli, indicating that spawning was only partial. Following the spawning peak in November there was

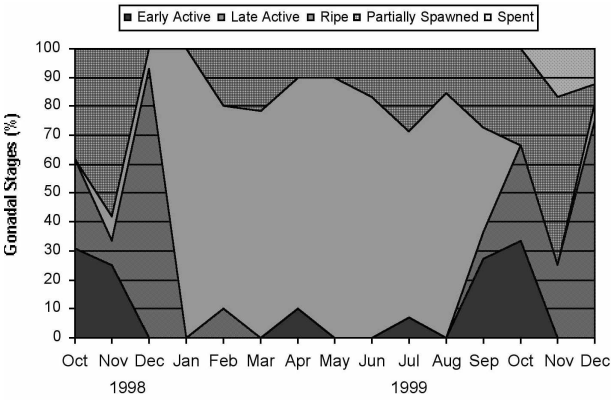

Fig. 3. Percentage of males of *E. exalbida* in each gonadal stage, between October, 1998, and December, 1999 (n = 184).

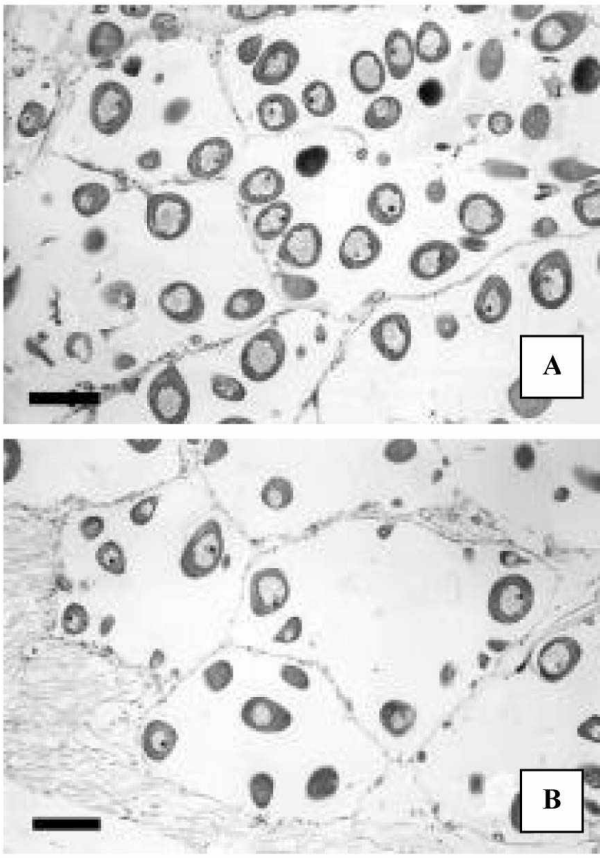

Fig. 4. Photomicrographs of females of *E. exalbida*. A. mature gonad: free oocytes in the lumen; B. partially spawned gonad: immature oocytes attached to the alveolar wall and few free oocytes in the lumen. Scale bar = 100 µm.

a marked decrease in the number of oocytes with small diameters (<25 and 25–40 µm). Mature oocytes (> 40 µm) increase in quantity until January when the maximum number of mature oocytes was observed

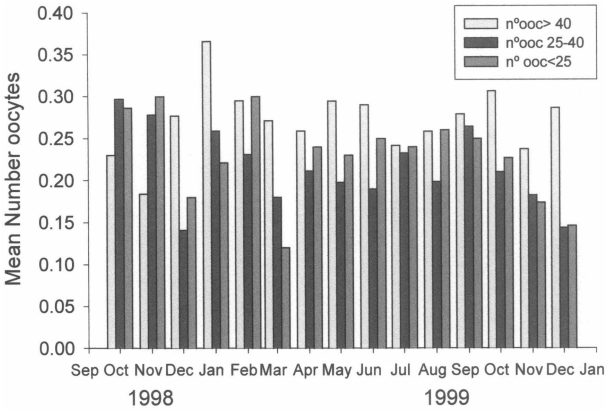

Fig. 5. Mean number of oocytes per 100 µm^2 of gonadal area of *E. exalbida* from October, 1998, to December, 1999 (n = 117).

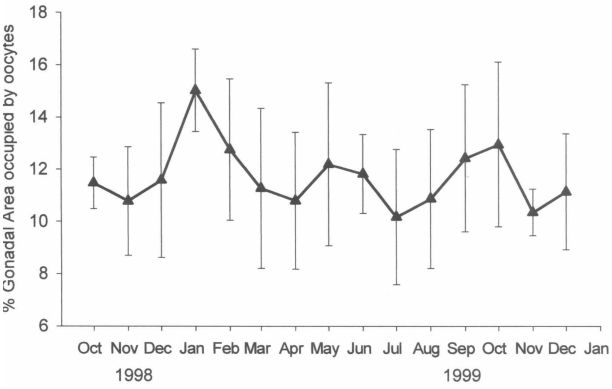

Fig. 6. Percentage of gonadal area occupied by oocytes ±SD of *E. exalbida* from October, 1998, to December, 1999 (n = 117).

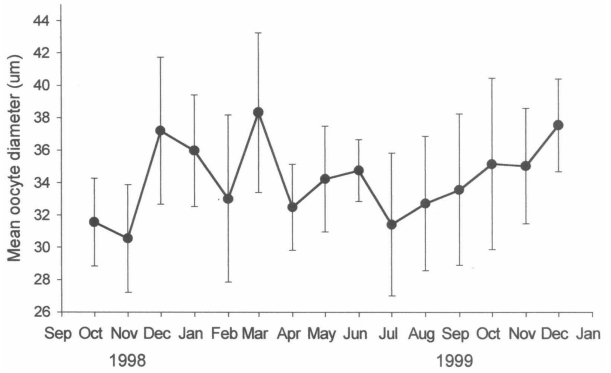

Fig. 7. Mean oocyte diameter (minor axis) ± SD of *E. exalbida* from October, 1998, to December, 1999 (n = 117).

(Fig. 5). In April and July there was also a decrease in the number of mature oocytes, although it was less marked than that in November. The percentage of the gonadal area occupied by oocytes (Fig. 6) was smallest in November (1998 and 1999), April and July. The

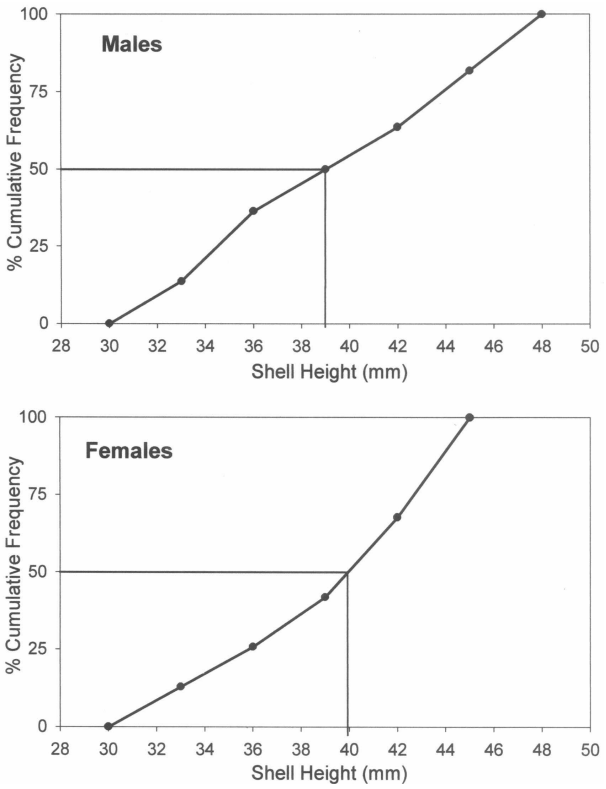

Fig. 8. Size at first maturity of *E. exalbida* in the Beagle Channel. (n = 41 males, N = 50 females).

mean diameter (minor axis) of oocytes (Fig. 7) was also smallest in November, 1998, and July, 1999, while the largest mean diameters were observed in December, 1998, and March, 1999 (one-way ANOVA, $F = 3.358$; $p < 0.001$; and subsequent modified Tukey test: November vs. December $p<0.05$; July vs. December: $p<0.05$; July vs. March: $p<0.01$).

Height at first maturity

In males, the mean height at first maturity was approximately 39 mm H and in females it was about 40 mm H (Fig. 8). These heights correspond to individuals of 4 years of age (Lomovasky et al., in press). Relationships between height and length was H = 0.9529, L = 1.8436 ($R^2 = 0.983$). The smallest males and females with active gonads measured were 32 and 30 mm H, respectively.

Discussion

Variable proportions of oocytes in different stages of maturity were observed in the gonads of female individuals of *E. exalbida* over time, yet this variation was not sufficiently marked to establish qualitative gonadal stages in the reproductive cycle. However, the quantification of diverse gonadal parameters (the percentage of gonadal area occupied by oocytes, the number of oocytes per gonadal area and the mean oocyte diameter) by analysis of microscopic images, allowed the female reproductive cycle to be understood and permitted the periods of maturity and spawning to be identified.

In both sexes a spawning peak was detected in November (1998 and 1999; Figs. 3, 5, 6 and 7). These results partially coincide with the findings of Schuldt (1975), who found that spawning in *E. exalbida* occurs from August to November in a study site located in the province of Chubut (42°47′S).

In the same population of *E. exalbida* analysed in this study, Lomovasky et al. (2001) determined the energy content (KJ/g AFDW) and Relative Condition Index (RCI; see Lomovasky et al., 2001) in three groups of organs: foot-visceral mass (gonad, digestive gland and gastrointestinal tract), adductor muscles, and gills-mantle-siphons. The energy content and RCI of gills-mantle-siphons and adductor muscles did not vary over time, indicating that they do not function as reserve organs. The energy content and RCI of the gonad-digestive gland-gastrointestinal tract decreased significantly in November, coinciding with the peak of the spawning period indicated by the current study. The greatest values of energy content and RCI for the gonad-digestive gland-gastrointestinal tract and the highest degree of ripeness as determined in the present study (Figs. 3 and 5) were observed in January.

In the Ushuaia Bay population of *E. exalbida*, the gonads recovered quickly following each gametic emission, hence a large percentage of individuals were mature throughout a large part of the year (Figs. 3 and 5). In mature female gonads the largest oocytes never totally occupied the alveoli (Fig. 4A), suggesting a gradual expulsion of oocytes. Coinciding with these observations, a small percentage of males (15–20%) partially evacuates spermatozoa throughout the year (Fig. 3). No evidence of a period of sexual quiescence was found in the study population. These results are in agreement with findings in others clam species that inhabit high latitudes. An extended spawning period was described in *Laternula elliptica* from Antarctic waters (Urban and Mercuri, 1998), and in the Baltic Sea in *Astarte borealis*, *A. elliptica* and *Cyprina islandica* (von Oertzen, 1972).

Temperature and food supply are considered the most important environmental factors in the regulation of gamete maturation and spawning in bivalves (Brey and Hain, 1992; MacDonald and Thompson, 1985;

Pearse et al., 1991). The spawning peak observed during November in the studied population of *E. exalbida* coincides with increasing sea temperature (Fig. 1) and a high concentration of phytoplankton (Hernando and San Román, 1999) enhancing the prospects for larval survival. In fact, in this species larval settlement is higher between November and February (Lomovasky et al., in press). During the rest of the year (March to October), however, partially spawned specimens are found (Fig. 3), and this is consistent with the small pulses of larval settlement occurring at these times (Lomovasky et al., in press).

The low and scattered reproductive effort observed in *E. exalbida* coincides well with the low P/B ratio, low mortality rate and a long life span (up to 70 years, Lomovasky et al., in press), all pointing towards a life history strategy characterised by low rates and slow turnover.

Acknowledgements

We thank Mr. D. Aureliano and Mr. A. Ferlito for their assistance in sampling and Mr. D. Aureliano for histological processing. Mr. C. Schroeder from the Hydrology Service (Austral Centre of Scientific Research, Argentina) is thanked for temperature data. We wish to thank Dr. John Pearce and an anonymous reviewer whose contributions improved the first version of this paper.

This work was supported by grants from Fundación Antorchas (Project 13817/4-2000), Bilateral Cooperation in Science and Technology German–Argentinean (BMBF and SETCIP; ARG 001/99 MAR - AL/A99-UXIII/12) and Consejo de Investigaciones de la Universidad Nacional de la Patagonia San Juan Bosco (CAFHCS Nº 238-97).

References

Brey, T. and Hain, S., Growth, reproduction and production of *Lissarca notorcadensis* (Bivalvia: Philobryidae) in the Weddell Sea, Antarctica, Mar. Ecol. Prog. Ser., 82 (1992) 219–226.

Carcelles, A., Catálogo de los moluscos marinos de Puerto Quequén, Rev. Museo La Plata, Secc.Zool., 3 (1944) 233–309.

Carcelles, A., Catálogo de los moluscos marinos de la Patagonia, Rev. Museo Arg. Cs. Nat. Bno. Riv., Buenos Aires, 2 (1950) 41–100.

Ciocco, N.F., Lasta, M.L. and Bremec, C., Pesquerías de bivalvos: mejilón, vieiras (tehuelche y patagónica) y otras especies. In: El mar argentino y sus recursos pesqueros. Tomo 2, Los moluscos de interés pesquero. Cultivos y estrategias reproductivas de bivalvos y equinoideos, E. Boschi (ed.), INIDEP, 1998, pp. 143–166.

Dell, R.K., Amphineura, Scaphopoda and Bivalvia. In: Antarctic and Subantarctic Mollusca, Discovery Rep., 33 (1964) 93–250.

Giese, A.C., Comparative physiology: annual reproductive cycles of marine invertebrates. Ann. Rev. Physiol., 21 (1959) 547–576.

Hernando, M. and San Román, N., Preliminary data on chronic effects of ultraviolet radiation on the growth of some phytoplankton species of the Beagle Channel, Argentina. Sci. Mar., 63 (1999) 81–88.

Lomovasky, B.J., Morriconi, E. and Calvo, J., Energetics variation of the striped clam *Eurhomalea exalbida* (Chemnitz, 1795) in Ushuaia Bay, Beagle Channel (54°50′S). J. Shellfish Res., 20 (2001) 1089–1094.

Lomovasky, B.J., Brey, T. Morriconi, E. and Calvo, J. Growth and production of the venerid bivalve *Eurhomalea exalbida* in the Beagle Channel, Tierra del Fuego. J. Sea Res., in press.

Lozada, E. and Bustos, H., Madurez sexual y fecundidad de *Venus antiqua antiqua* King & Broderip 1835 en la Bahía de Ancud (Mollusca: Bivalvia: Veneridae). Rev. Biol. Mar. Valparaiso, 20 (1984) 91–112.

MacDonald, B.A. and Thompson, R.J., Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus.* II. Reproductive output and total production. Mar. Ecol. Prog. Ser., 25 (1985) 295–303.

Osorio, C., Cifuentes, J.A. and Fischer, S.M., Moluscos marinos de importancia económica en Chile. Biol. Pesq. Chile, 11 (1979) 3–47.

Pearse, J.S., McClintock, J.B. and Bosch, I., Reproduction of Antarctic benthic marine invertebrates: tempos, modes, and timing. Amer. Zool., 31 (1991) 65–80.

Sastry, A., Pelecypoda (excluding Ostreidae). In: Reproduction of Marine Invertebrates. Vol. 5, Molluscs: Pelecypods and Lesser Classes, A.C. Giese and J.S. Pearse (eds.), Academic Press, New York, 1979, pp. 113–292.

Schuldt, M., Consideraciones sobre la reproducción de los pelecípodos chiónidos en la Cholguera de punta Loma, Golfo Nuevo, Chubut. Physis, 88 (1975) 137–146.

Shafee, M.S., Reproduction of *Perna picta* (Mollusca: Bivalvia) from the Atlantic coast of Morocco. Mar. Ecol. Prog. Ser., 53 (1989) 235–245.

Soot-Ryen, T., Pelecypoda, Acta Univ. Lund., 2(6) (1959) 1–86.

Stead, R.A., Clasing, E., Navarro, J.M. and Asencio, G., Reproductive cycle and cohort formation of *Venus antiqua* (Bivalvia: Veneridae) in the intertidal zone of southern Chile. Rev. Chilena His. Nat., 70 (1997) 181–190.

Urban, J.H., Adaptations of six infaunal bivalves species of Chile: Coexistence resulting from differences in morphology, burrowing depth and substrate preference. Arch. Fish. Mar. Res., 42 (1994) 183–193.

Urban, J.H. and Tesch, C., Aspects of the population dyna-

mics of six bivalve species from Southern Chile. Results of the 'Victor Hensen cruise to the Magellan Strait and the Beagle Channel in October/November 1994. Arch. Fish. Mar. Res., 44 (1996) 243–256.

Urban, J.H. and Mercuri, G., Population dynamics of the bivalve *Laternula elliptica* from Potter Cove, King George Island, South Shetland Islands. Antarctic Sci., 10 (1998) 153–160.

Verdinelli, M.A. and Schuldt, M., Consideraciones preliminares sobre aspectos de la dinámica poblacional y reproducción de la almeja rayada (*Ameghinomya antiqua* King- Chionidae) en Punta Loma, Golfo Nuevo-Chubut. Rev. Museo La Plata, Secc.Zool., 119 (1976) 183–202.

von Oertzen, J.A., Cycles and rates of reproduction of six Baltic Sea bivalves of different zoogeographical region. Mar. Biol., 14 (1972) 143–149.