

Reproductive biology of the limpet *Nacella (P.) deaurata* (Gmelin, 1791) in Bahía Lapataia (Beagle Channel)*

ELBA MORRICONI

Centro Austral de Investigaciones Científicas (CONICET), C.C. 92, (9410) Ushuaia, Tierra del Fuego, Argentina.
E-mail: rvcalvo@infovia.com.ar

SUMMARY: The reproductive cycle of a *Nacella (P.) deaurata* population that inhabits the lower intertidal zone in Lapataia Bay (54°52'S; 68°29'W) was studied. Monthly samples were collected, and specimens were measured and weighed, fixing the gonads in Bouin's fluid for histological analyses. The gonadosomatic index (GSI) was determined as a percentage of the ratio between gonadal and foot wet weight. Taking into account the presence and abundance of different cellular types, gonadal stages were established for males and females. The analyses of the variation of the gonadal stage percentages showed the first mature males and females in July. In 1989, mature females maintained a percentage higher than 30% until January. Spawning began in September and was massive in November. In 1990, the maximum percentage of mature females was found in September with all the specimens spawned in October. Nevertheless, the majority of the population was mature again in November. The male sexual cycle in 1989 showed the highest percentage of mature individuals in August, being high until October and slightly diminishing in November. The highest percentage of evacuated males was observed in November-December. In 1990, the cycle was similar to the one shown by the females, but without recovery in November. The GSI variability in males and females, and the adequate use of the GSI in determining the annual reproductive cycle were discussed. The biotic and environmental conditions that may act as a trigger for the spawning have been analyzed.

Key words: Archaeogastropods, Subantarctic limpets, reproductive cycle, spawning.

RESUMEN: BIOLOGÍA REPRODUCTIVA DE *NACELLA (P.) DEAURATA* (GMELIN, 1791) EN BAHÍA LAPATAIA (CANAL DEL BEAGLE). – Se estudió el ciclo reproductivo de *Nacella (P.) deaurata* en una población que habita el intermareal inferior en la costa sur de Bahía Lapataia (54°52'S; 68°29'O). Se realizaron muestreos mensuales, los ejemplares fueron medidos, pesándose las vísceras, pie y gónada, siendo esta última fijada en Bouin para su posterior procesamiento histológico. Se determinó el Índice Gonadosomático (IGS) como la expresión porcentual del peso gonadal sobre el peso húmedo del pie. Se establecieron histológicamente estadios gonadales tanto en hembras como en machos, de acuerdo con la presencia y abundancia de los diferentes tipos celulares. De acuerdo con el análisis de los porcentajes mensuales de los estadios gonadales, los individuos de ambos sexos se encontraron maduros a partir de julio. Las hembras maduras durante 1989 se presentaron en porcentajes superiores al 30% hasta enero. A partir de septiembre comenzó el desove, haciéndose masivo en la población en noviembre. En 1990 el pico máximo de maduración se alcanzó en septiembre. En octubre todos los individuos habían desovado, mientras que durante noviembre una gran proporción de la población se recuperó alcanzando nuevamente la madurez total. El ciclo sexual anual de los machos en 1989 mostró el mayor porcentaje de individuos maduros durante agosto, manteniéndose una alta proporción hasta octubre con un ligero descenso en noviembre. El máximo porcentaje de individuos evacuados se observó entre noviembre y diciembre. En 1990 el ciclo fue similar al que presentaron las hembras para el mismo año pero no se produjo recuperación de la maduración gonadal en noviembre. Se discute la variabilidad del IGS en ambos sexos y su validez para la determinación de los ciclos reproductivos anuales, analizándose las condiciones bióticas y ambientales que actuarían como desencadenantes del desove.

Palabras clave: Arqueogasterópodos, lapas subantárticas, ciclo reproductivo, desove.

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INTRODUCTION

Limpets are very common archaeogastropod molluscs that inhabit intertidal rocky shores. Many investigations have been made on their reproduction e.g., *Patella vulgata* (L., 1758) (see Orton *et al.*, 1956; Blackmore, 1969; Garwood, 1987), *Cellana radiata* (Born) (see Balaparameswara Rao, 1973), seven species of *Patella* (see Branch, 1974), *Cellana grata* (Gould, 1859) and *Patelloida pygmaea* (Dunker, 1860) (see Liu, 1994).

In cold waters the reproduction was studied in the Subantarctic limpet *Nacella maquarensis* (Finlay, 1927) (see Simpson, 1982) and in the Antarctic and Subantarctic limpet *Nacella concinna* (Strebels, 1908) (see Picken, 1980; Brêthes *et al.*, 1994). Nevertheless, there have not been histological studies on their reproductive cycles.

Nacella (Patinigera) deaurata (Gmelin, 1791) and *Nacella (Patinigera) magellanica* (Gmelin, 1791) are the two most conspicuous limpet species in the Beagle Channel due to their abundance and their relatively large sizes. *Nacella (P.) magellanica* inhabits the medium and superior intertidal zones while *Nacella (P.) deaurata* lives in the lower intertidal zone and the sublittoral zone (Morriconi and Calvo, 1993).

Few studies on the biology of these limpet species have been carried out although they are abundant along the Patagonian coast. Guzmán (1978) determined the spatial pattern and density of *N. magellanica* in the Magellan Straits and Guzmán and Ríos (1987) established the age and growth of this species. Morriconi and Calvo (1993) determined the environmental influence on shell allometric growth in the *Nacella (P.) deaurata* Beagle Channel population.

The aim of this study was to establish the annual reproductive cycle and the frequency and duration of the spawning cycle of *Nacella (P.) deaurata* based on the histological description of gonadal tissues. Moreover, the biological and environmental parameters that could trigger the spawning were analyzed.

MATERIAL AND METHODS

Sampling was carried out from March 1989 to November 1990 in Bahía Lapataia (54°52' S; 68°29' W) in the Beagle Channel (Fig. 1). The sampling area is protected against the dominant south-westerly winds.

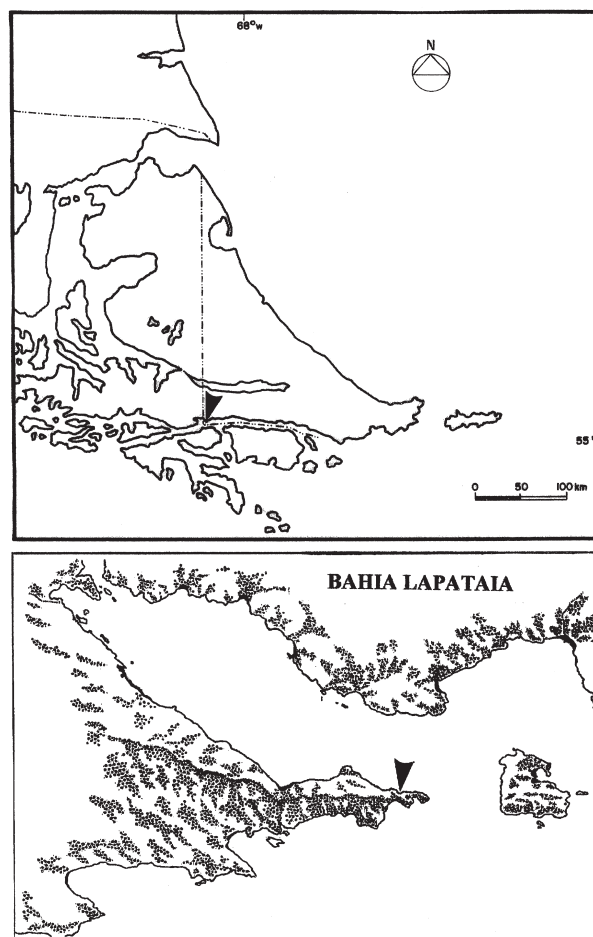


FIG. 1. – Sampling area (arrows) in Bahía Lapataia (54° 52' S; 68° 29' W).

Monthly samples of around 30-40 individuals were collected by diving. The shell of each specimen was measured along its greatest length using vernier callipers to the nearest 0.5 mm. The individuals were removed from their shells and the soma dissected out. The soft parts were fixed in Bouin's fixative for at least 48 hours, washed in tap water and kept in 70 % ethyl alcohol. Afterwards the foot, the digestive mass and the gonad were separated and the wet weight of each part was obtained. The gonad was sectioned in three parts: anterior, central and posterior. Following dehydration and clearing, the gonads were embedded in Paraplast, sectioned (5 μ m) and counterstained with hematoxylin-eosin. A total of 536 individuals, ranging from 15 to 65 mm in shell length, were described histologically.

The Gonosomatic Index (GSI) was expressed as percentage (100 * wet gonad weight/wet foot weight). The differences in the values of GSI between males and females were tested using the Mann-Whitney U -Test (Sokal and Rohlf, 1995).

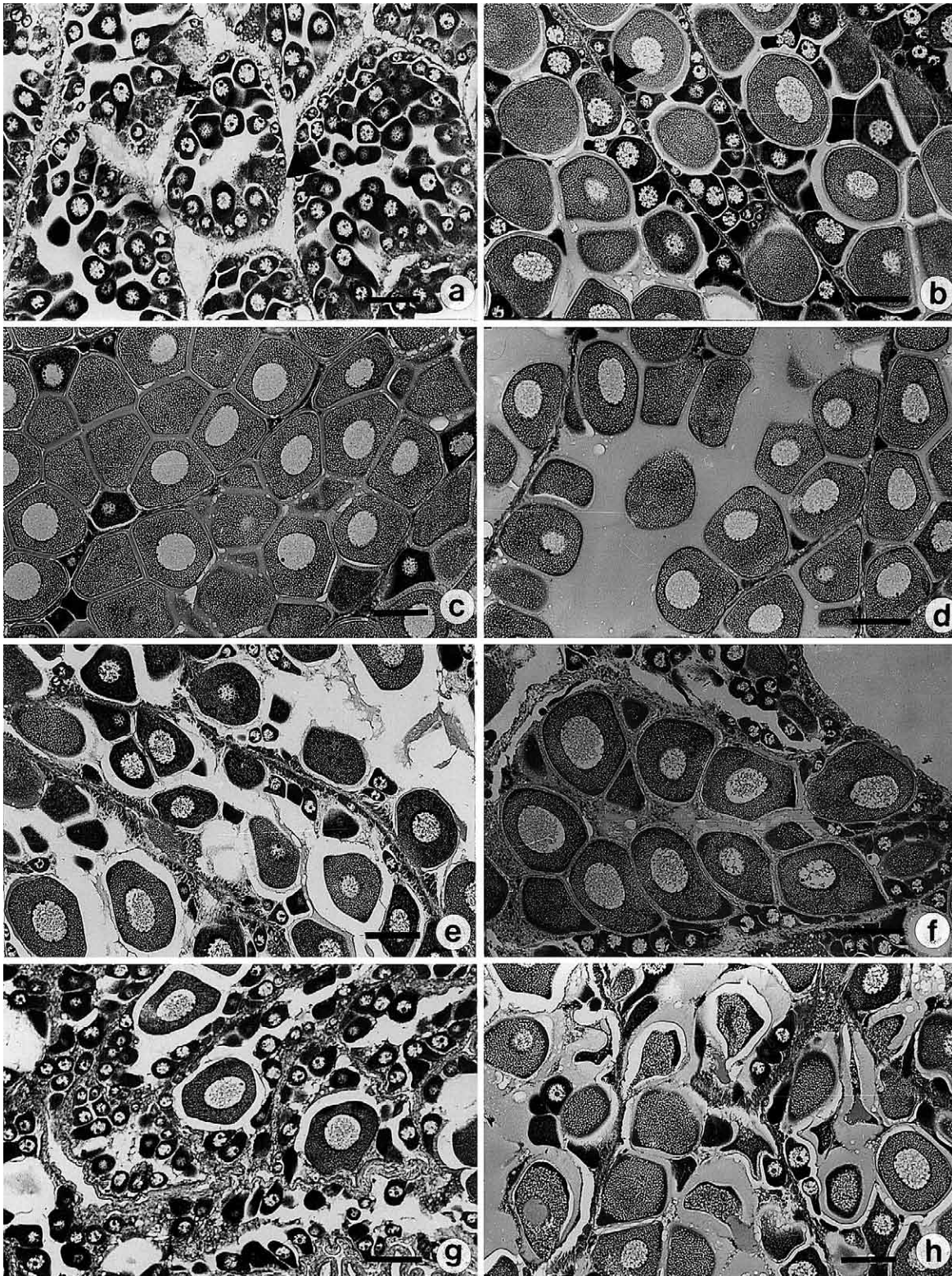


FIG. 2. – *Nacella (P.) deaurata*. Gonadal female stages. Scale bar = 100 μ m; a) Early developing stage. Nests of oogonia (arrows). Numerous basophilic oocytes; b) Developing stage: oocytes with deposition of eosinophilic granules in the basal region of cytoplasm (arrow), acidophilic oocytes; c) Ripe stage: acidophilic oocytes fill the tubular lumen, small number of basophilic oocytes against the trabeculae; d) Ripe stage: homogeneous eosinophilic matrix in the tubular lumen, between the free oocytes; e) Partially spawned with recovery stage: acidophilic oocytes free in the tubular lumen, basophilic and intermediate oocytes; f) Partially spawned with no recovery stage: small basophilic oocytes close to the tubular wall, mature oocytes in the tubular lumen with a basophilic cytoplasmic zone, oocytary remains in the lumina; g) Totally spawned stage: very scarce mature oocytes are free in the tubules, basophilic oocytes close to the tubular wall; h) Oocytes in different degree of atresia.

RESULTS

Nacella (P.) deaurata is a dioecious species. It has an impaired gonad located at the ventral part of the animal, underneath the visceral mass. The gonad occupies a small portion of the posterior region in the immature specimens. The gonad grows in anterior and lateral directions, over the outer margins of the visceral mass, during maturation. In males, the gonad is yellow in maturing and ripe specimens, while it is brown-reddish in spawned individuals. The female gonad is brown during maturation and becomes darker in totally spawned specimens.

In both sexes, the gonad is formed by tubules that extend from the ventral towards the dorsal region forming branches. The tubules support a germinal epithelium and are separated by connective tissue trabeculae.

Developmental stages of the female

Early developing stage

The trabeculae are enlarged. The tubules have a wide lumen occasionally containing cytoplasmic remains and phagocytes.

Oogonial clusters are attached to the tubule wall (Fig. 2a). They are more numerous in the ventral zone. These cells have a rounded central nucleus (5 μm in diameter) and a conspicuous nucleolus. The basophilic oocytes (13-50 μm in diameter, Fig. 2a) usually show a pyriform shape and have an oval nucleus with several peripheric nucleoli. Oocytes (40-80 μm in diameter) with numerous small chromophobic vacuoles in their cytoplasm are present at the end of this stage.

Developing stage

The trabeculae are thin. In the tubules oogoniae and basophilic oocytes are less numerous than in the early developing stage. Eosinophilic granules appear in the basal region of the cytoplasm near to the trabeculae (Fig. 2b) in the basophilic oocytes with chromophobic vacuoles. These intermediate oocytes (90 μm in diameter) show an eosinophilic layer, the chorion, which is not continuous in the region attached to the trabeculae. There are also few acidophilic oocytes with the cytoplasm full of eosinophilic granules (Fig. 2b). These oocytes (100 -130 μm in diameter) have a conspicuous chorion.

Ripe stage

The trabeculae are very thin. The lumen of the distended tubules is full of mature acidophilic oocytes (120-150 μm in diameter) (Fig. 2c). Scarce basophilic oocytes are attached to the tubule walls. The presence of a homogenous eosinophilic matrix in the tubular lumen, between the free oocytes, is usual (Fig. 2d). Mature oocytes are less densely packed in the dorsal zone of the gonad, probably due to a gradual expulsion of them.

Partially spawned with recovery stage

This stage is similar to the developing stage. The main morphological difference is that more acidophilic oocytes are found free in the tubular lumen, which is wider than in developing stage (Fig. 2e). The presence of basophilic and intermediate oocytes indicates that a quick recovery of gonadal maturation is happening.

Partially spawned with no recovery stage

This stage is characterized by the presence of oogonia and small basophilic oocytes attached to the tubular wall. Mature oocytes are still frequent in the tubular lumen, with many of them becoming elongated and showing a basophilic zone. Oocytary remains are usually observed in the lumina (Fig. 2f).

Totally spawned stage

The trabeculae are thick. The number of basophilic oocytes is variable. The remaining mature oocytes are very scarce, with only two or three of them in each tubule. There is usually necrotic oocytary material in the lumina (Fig. 2g).

Oocytary atresia

In all gonadal stages, except for the early developing one, a variable number of different vitellogenic oocyte types showing lysis is found. The atretic oocytes have an irregular shape and a hypertrophied chorion. The cytoplasm changes the stainability, becoming very basophilic and increasing the vacuolisation. The nucleus stains stronger than in healthy oocytes and shrinks (Fig. 2h).

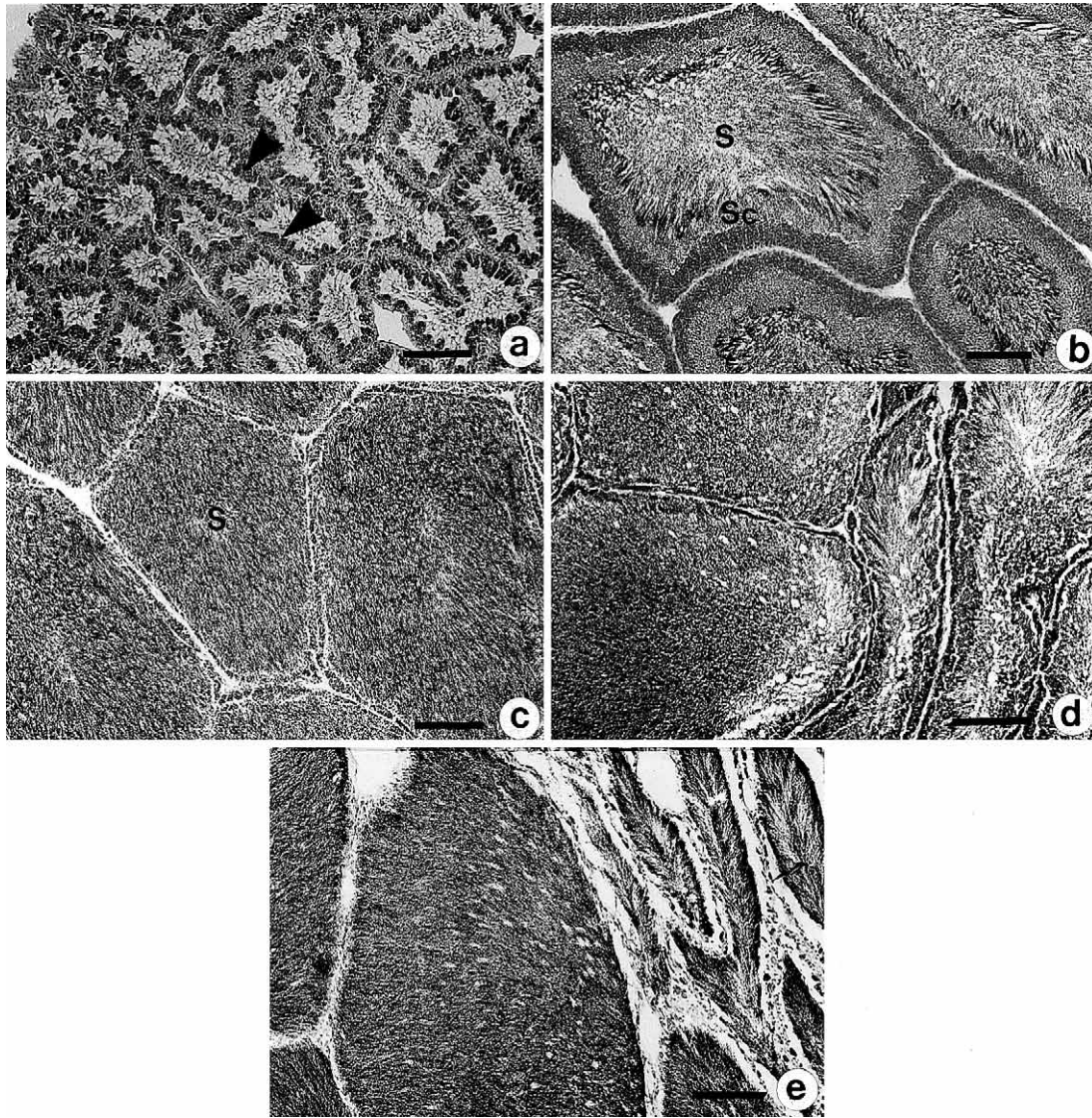


FIG. 3. – *Nacella (P.) deaurata*. Gonadal male stages. Scale bar = 100 μm ; a) Early developing stage: thick connective trabeculae with spermatogonia close to them (arrows); b) Developing stage: tubules with a wide layer of germinal series (Sc: spermatocytes; S: spermatozoa); c) Ripe stage: high number of spermatozoa fills the enlarged tubules; d) Ripe stage: at left tubules with densely packed spermatozoa, at right evacuated tubules with an irregular band of spermatocytes and loosely spermatozoa in the lumen; e) Ripe stage: at left tubules with densely packed spermatozoa, at right evacuated tubules with low number of spermatozoa.

Developmental stages of the male

Early developing stage

The trabeculae are thick. One or two layers of spermatogonia are attached to them. The tubule lumen is wide and contains a net of cells that fill it (Fig. 3a).

Developing stage

Layers of spermatogonia, spermatocytes and spermatids are found from the tubule wall towards the

lumen. The spermatozoa fill the tubular lumen (Fig. 3b).

Ripe stage

The trabeculae are very thin. The tubules are enlarged, with only a band of spermatogonia attached to the tubular wall and free spermatozoa filling the lumen (Fig. 3c). The spermatozoa decrease in number in the dorsal zone of the gonad, probably due to a gradual expulsion of them. In addition, some individuals show one part of the gonad partially spawned while the rest of the gonad remains with a ripe aspect (Figs 3d and 3e).

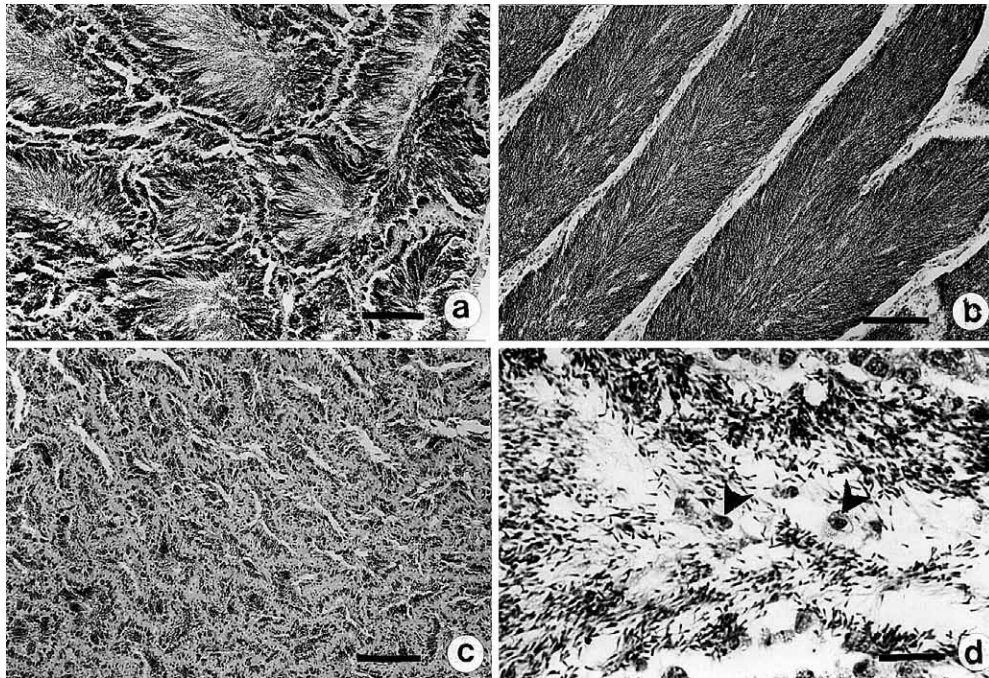


FIG. 4. – *Nacella (P.) deaurata*. Gonadal male stages. a), b), c); scale bar = 100 μ m; d) scale bar = 20 μ m; a) Partially spawned with recovery stage: tubules with an irregular band of germinal line, loose spermatozoa in the tubular lumen.; b) Partially spawned with no recovery stage: abundant spermatozoa in the tubules; c) Totally spawned: shrunk tubules with scarce spermatozoa; d) Totally spawned: phagocytes among the spermatozoa.

Partially spawned with recovery stage

The tubules are smaller than in the developing stage. The cells of germinal line are abundant and form an irregular band. There is a noticeable decrease in the number of spermatozoa. Phagocytes are frequently observed in between them (Fig. 4a).

Partially spawned with no recovery stage

There are one or two layers of spermatogonia against the tubular wall. Spermatozoa are abundant in the lumen of the tubules (Fig. 4b).

Totally spawned stage

The connective trabeculae are very thick. The tubules are shrunk. A variable number of spermatogonia is found against the tubular wall (Fig. 4c). Few spermatozoa and some phagocytes are found among them (Fig. 4d).

Seasonal variation of the gonadal cycle

Females (Fig. 5)

The gametogenesis started in February and con-

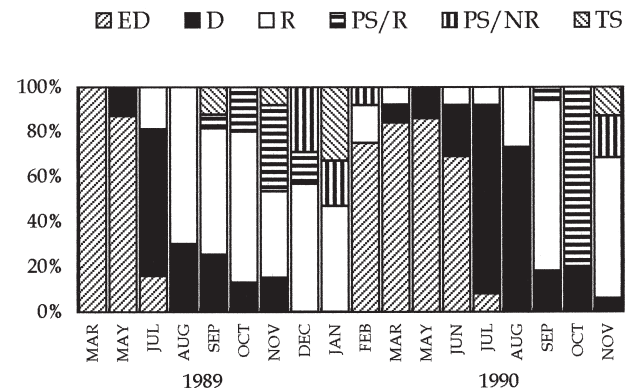


FIG. 5. – Gonadal female stages (% of specimens) of *Nacella (P.) deaurata*. ED: Early developing stage. D: Developing stage. R: Ripe stage. PS/R: Post spawned with recovery stage. PS/NR: Post spawned with no recovery stage. TS: Totally spawned stage.

tinued until June with most of the individuals in the early developing stage (more than 70%). The maximum percentage of individuals in the developing stage was found in July. Ripe individuals were observed in winter, spring and the beginning of summer in 1989-1990. Partially spawned with recovery stages were found in October-December while in December and January partially spawned with no recovery and totally spawned stages were observed, indicating the end of the reproductive cycle.

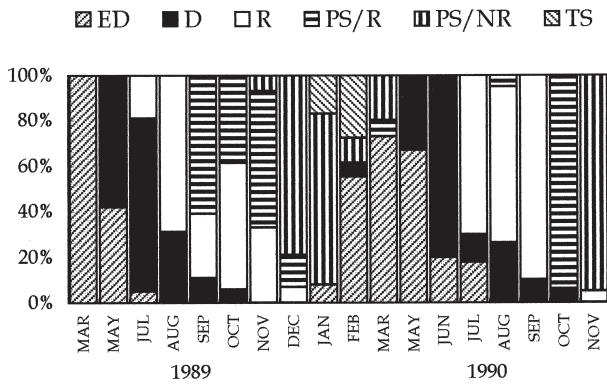


FIG. 6. – Gonadal male stages (% of specimens) of *Nacella (P.) deaurata*. ED: Early developing stage. D: Developing stage. R: Ripe stage. PS/R: Post spawned with recovery stage. PS/NR: Post spawned with no recovery stage. TS: Totally spawned stage.

In 1990, the maturation cycle was similar to the one observed in 1989 but a major spawning with recovery occurred in October. The population showed a quick recovery with over 60% of the population in ripe stage in November.

Males (Fig. 6)

Male limpets showed a similar reproductive cycle to females. The highest percentage of ripe individuals was observed in August 1989, however this percentage remained still high until November. During these months, most of the limpets were in partially spawned with recovery stage. The population was found in partially spawned with no recovery and totally spawned stages during December and January.

The gonadal cycle during 1990 followed a similar pattern to that noted in 1989 up to June. High percentages of ripe individuals were found from July to September. Partially spawning individuals with recovery were found in October, while there were about 95% of the individuals in partially spawned with no recovery stage in November.

Gonosomatic Index

Variations in the Gonosomatic Index for males and females of *N. (P.) deaurata* are shown in Fig. 7. The highest GSI values were found in August 1989 and September 1990 for males. The highest female GSI were found in August and September for 1989 and September for 1990. The maximum GSI were higher in 1990 than those found in 1989 for both sexes ($p < 0.001$). The GSI mean values of each gonadal stage were higher in males than in females

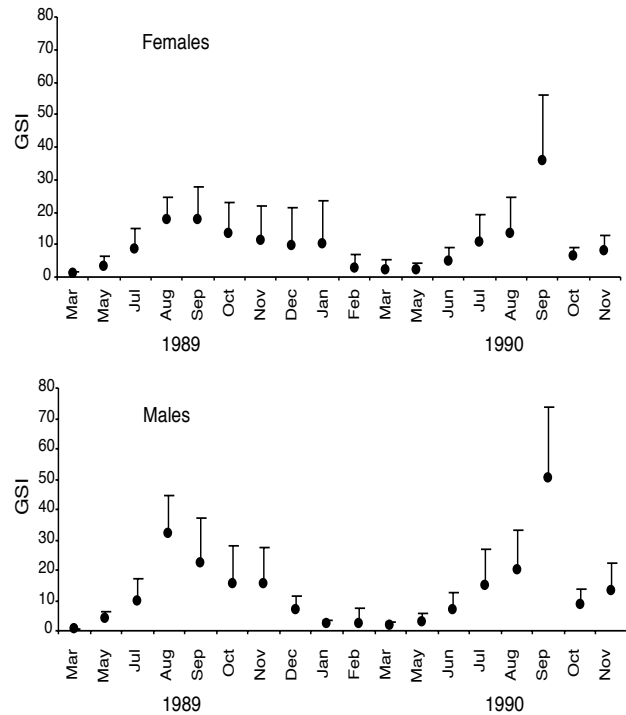


FIG. 7. – Mean Gonosomatic index (GSI) of *Nacella (P.) deaurata*. Bar = Standard Deviation.

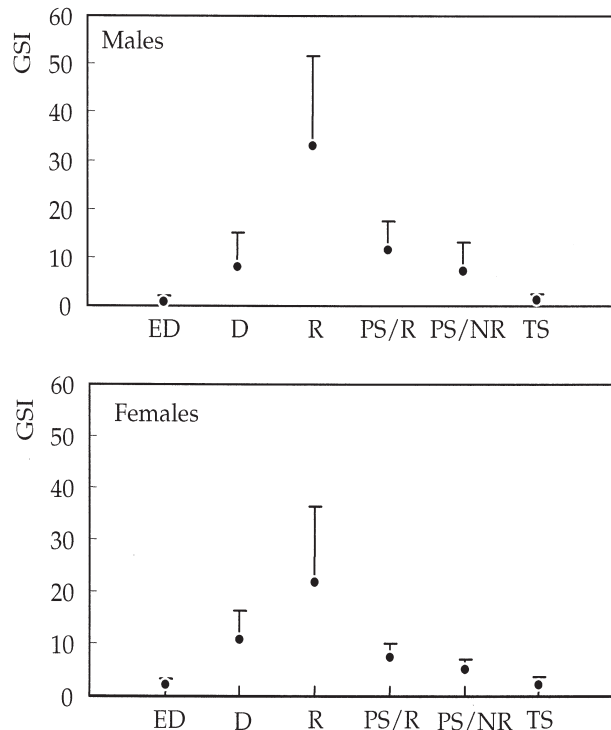


FIG. 8. – Mean Gonosomatic index (GSI) of *Nacella (P.) deaurata* for each gonadal stage. ED: Early developing stage. D: Developing stage. R: Ripe stage. PS/R: Post spawned with recovery stage. PS/NR: Post spawned with no recovery stage. TS: Totally spawned stage. Bar = Standard Deviation.

but the differences between sexes (Fig 8) were not significant except for the ripe stage ($p < 0.001$).

DISCUSSION

Several different methodologies have been used to study reproductive cycles in molluscs. The monthly variation of the Gonosomatic Index has frequently been used in species with gonads easy to separate from the rest of the soma, since it is a good indicator of gonadal changes throughout the year (Grant and Tyler, 1983). Several authors (Garwood, 1987; Catalan and Yamamoto, 1993; Liu, 1994; Brêthes *et al.*, 1994) have described gonadal cycles in limpets using this method. Nevertheless, it should be used only when the maturation cycles are synchronous. When asynchronous reproductive cycles are studied, the use of histological observations is necessary in order to obtain a better understanding of the reproductive process. In this study the GSI has been used complementary to the histological analysis.

Nacella (P.) deaurata monthly GSI values (Fig. 7) showed large dispersion probably due to the existence of different gonadal stages in each month (asynchronous cycle). The GSI values of each gonadal stage showed large dispersion in specimens of the same size, reaching a maximum in the ripe stage (Fig 8), due to the increasing variability of the gonadal weight during the maturation process. Previous studies on *Nacella (P.) magellanica* have shown similar dispersion in the GSI values of ripe individuals (Morriconi, unpubl.).

Dispersion in GSI values of ripe *N. (P.) deaurata* individuals can be related to three different histological characteristics: 1) the mature gametes were more loosely arranged dorsally than ventrally in the gonads of ripe individuals of both sexes indicating gradual gamete liberation. 2) Some ripe males showed a portion of the gonad evacuated while the rest of the gonad remained mature (Fig. 3d, 3e). 3) Parts of the tubules of some ripe females showed an eosinophilic matrix filling the lumen that therefore contained a smaller number of mature oocytes (Fig. 2d).

The reproductive effort of a population is usually measured as the difference in GSI between the ripe and spawned individuals (Parry, 1982; Fletcher, 1984). In *N. (P.) deaurata*, that value was 21 % in males and 14% in females (Fig. 8). A higher reproductive effort in males than females was also

observed in different species of South African *Patella* (see Branch, 1974) and in *Nacella macquarensis* (see Simpson, 1982) while in *Cellana nigrolineata* (Reeves) (Catalan and Yamamoto, 1993), *Cellana grata* and *Patelloidea pigmaea* (see Liu, 1994) the difference between sexes was not significant.

There were some differences in the reproductive cycle between the two studied years (Fig. 5; Fig 6) in *N. (P.) deaurata*. The spawning was gradual in 1989 starting in July for both sexes, and continuing until December (males) and January (females). In 1990, a massive spawning occurred for both sexes in October. About 70% of females were ripe again in November but due to the low gonadal weight they had, the mean GSI value was lower than the one found in September (Fig 7). The males did not show any recovery this year. These variations could indicate plasticity in the reproductive response to environmental factors.

The gonads of both sexes recovered quickly after the spawning and had no resting phase. The gametic proliferation (early developing stage) started immediately (Fig 5 and 6). The same occurred in *N. (P.) magellanica* (Morriconi, unpubl.) and *Patelloidea alticostata* (Angas) (Fletcher, 1987) while a protracted resting phase was observed in *P. vulgata* (see Orton *et al.*, 1956). Several environmental factors have been considered as spawning stimulus. The spawning season in *N. (P.) deaurata* was coincident with a rise in water temperature (Fig. 9) as has been reported in *P. longicosta* (Lamarck) and *P. oculus* (Branch 1974) and in *N. concinna* (see Picken, 1980; Brêthes *et al.*, 1994). Branch (1974) stated that spring and summer spawner species such as *P. longicosta* and *P. oculus* are restricted to warm waters while winter spawner species such as *P. cochlear* (Born), *P. granatina* (L.), *P. granularis*

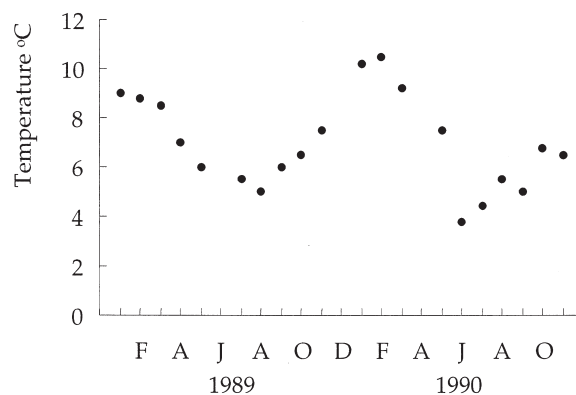


FIG. 9. – Seasonal variation in sea temperature at the sampling site.

(L.), *P. argenvillei* (Krauss) and *P. barbara* are restricted to cold waters. Fritchman (1962) found a similar tendency. Nevertheless, *N. (P.) deaurata* and *N. (P.) magellanica* which inhabit the Beagle Channel Subantarctic waters (with temperatures from 4°C to 10.5°C, Fig. 9) spawn in spring-summer. Moreover, *N. macquariensis*, another Subantarctic limpet, and *N. concinna*, an Antarctic and Subantarctic limpet, are summer spawners (Simpson, 1982; Picken, 1980; Brêthes *et al.*, 1994).

Orton *et al.* (1956), Ballantine (1961), Bowman and Lewis (1977) and Thompson (1980) suggested that spawning could be triggered by storms, and Grange (1976) correlated spawning to rough waters. Nevertheless, these environmental factors are not likely spawning triggers in the *N. (P.) deaurata* populations studied in this paper because they are living in a protected zone.

A relation between spawning and increase of chlorophyll *a* has been proposed by Himmelman (1981) and Jørgensen (1981). Regarding gastropod species with planktotrophic larvae, Underwood (1974) suggested that spawning occurs when the phytoplankton concentration is higher. Several authors have claimed that egg size is useful for predicting larval life style because species with small eggs typically develop into planktotrophic larvae (Havenhand, 1995; Jaeckle, 1995; Bhaud and Duchêne, 1996). Many *Patella* species have high fecundity values and the diameter of the oocytes does not exceed 180 µm (Branch, 1974) suggesting the production of planktonic larvae. *N. concinna* has small ripe oocytes (about 170 µm; Morriconi, pers. obs.) and produces free larvae, probably planktonic ones (Picken, 1980). In this species the spawning was correlated with the phytoplankton bloom (Picken, 1980; Brêthes *et al.*, 1994). The spawning of *N. (P.) deaurata* and *N. (P.) magellanica* (Morriconi, unpubl.) of the Beagle Channel populations happened simultaneously with the spring increase in primary production (Hernando, pers. comm.). High egg numbers (Morriconi, unpubl.) as well as small eggs (150 µm) would indicate that these species have planktotrophic larvae and therefore, the increase in productivity could have been one of the spawning triggers in both studied species. Nevertheless, the studies performed in four species of Archaeogastropoda (Hadfield *et al.*, 1997) and in *N. macquariensis* (see Simpson, 1982) suggest that their veligers are non-feeding, consequently the spawning and the phytoplankton bloom could have no causal correlation.

Although Thorson (1950) concluded that in polar invertebrates non-pelagic development is the rule, more recent evidence reveals that this mode of development is common in crustaceans and prosobranch gastropods but not for echinoderms or bivalves (Pearse *et al.* 1991; Clarke, 1992; Hain and Arnaud, 1992). Nevertheless, the Antarctic and Subantarctic prosobranch gastropods such as *N. concinna* (see Picken, 1980), *N. macquariensis* (see Simpson, 1982), *N. (P.) deaurata* and *N. (P.) magellanica* (Morriconi, unpubl.) seem to be exceptions of the Thorson rule because they may have pelagic development.

More comparative studies are necessary in order to establish the reproductive strategies of Antarctic and Subantarctic molluscs.

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