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The reproductive cycle of the Thecosomatous pteropod *Limacina retroversa* in the western South Atlantic

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Abstract. The reproductive cycle of *Limacina retroversa* in the Argentine Sea was studied by gonadal analysis. Samples were collected in 1978–1979. The relation between gonadal maturity and size is dependent on the season and the geographical area. Evidence of *in situ* reproduction was found at the beginning of spring and at the end of summer, indicating that the life cycle in the area consists of two generations per year. In accordance with the season of birth, both generations develop different strategies. Individuals born in spring mature early and reproduce before the end of summer. The offspring born in summer survive the cold season without reaching sexual maturity and reproduce in the following spring.

Introduction

Limacina retroversa (Fleming, 1823) is the most abundant holoplanktonic gastropod in the shelf waters (Argentine Sea) and slope waters off Argentina. It is carried from the West Wind Drift into the Argentine Sea where its northernmost distribution coincides with the Brazil-Malvinas (Falkland) front. Its geographical range extends westwards to approximately the 100 m isobath. Northward to about 40 °S, it spreads nearer the coast and may be found in high densities to the 50 m isobath (Dadon 1990).

The most extensive collection of quantitative zooplankton samples in the Argentine Sea was obtained during 1978–1979 cruises of the vessels "Walther Herwig" and "Shinkai Maru". Analysis of this collection (Dadon 1990) showed that the abundance of *Limacina retroversa* peaks on the continental shelf in autumn–winter. Then, its numbers gradually diminish in spring, and, finally, only scattered individuals are recorded in summer. This seasonal pattern clearly differs from the local pattern of primary and secondary production, characterized by a winter minimum and spring maximum (Carreto et al. 1981).

The population dynamics of *Limacina retroversa* off Argentina was postulated (Dadon 1990) to be predominantly regulated by passive migratory events. The importance of oceanic transport masks other factors. Reproductive activity by *L. retroversa* in the area has not been studied previously, and the question remained whether or not the population in the Argentine Sea, being at the northernmost boundary for the species, represented a sterile expatriate assemblage of individuals.

This paper focuses on an analysis of *in situ* reproductive activity by *Limacina retroversa* in the Argentine Sea. This is the first examination of the life cycle of this species in the southern hemisphere.

Materials and methods

After analyzing the "Walther Herwig" and "Shinkai Maru" collections, seven samples with high densities of *Limacina retroversa* for each of four seasons were selected (see Fig. 1). Samples were collected with 0.60 m diameter Bongo nets towed obliquely at three knots and were preserved in 5 to 7% buffered formalin. The mesh of the Bongo nets was 330 µm for the "Walther Herwig" tows and 505 µm for the "Shinkai Maru" tows.

Size measurements

Three different measures of shell size in *Limacina retroversa* have been used by different authors: the number of whorls (Chen and Bé 1964, Boltovskoy 1971), the maximal diameter (Lebour 1932, Hsiao 1939b, Redfield 1939, Conover and Lalli 1974) and shell height (Lalli and Wells 1978, Beckmann et al. 1987). When comparing the three measures, the first one is the crudest approximation to specimen size. The others (shell diameter and height), determined for 134 specimens, showed a correlation of 0.96979 ($P < 0.01$). In this case, and due to its wider range, shell height was chosen. In order to cover the complete series of early maturation stages, 83 µm intervals were used for shell heights ranging from 0.33 to 1.66 mm, while for individuals greater than 1.66 mm, 166 µm intervals were used. From every sample and for each size interval, 3 to 5 ind. were studied histologically. The specimens were dehydrated in increasing alcohol concentrations up to 96%. Butanol was used as a bleaching agent, and the material was embedded in paraffin at a temperature of 52 to 54 °C. The transversal serial sections were stained with Carazzi's

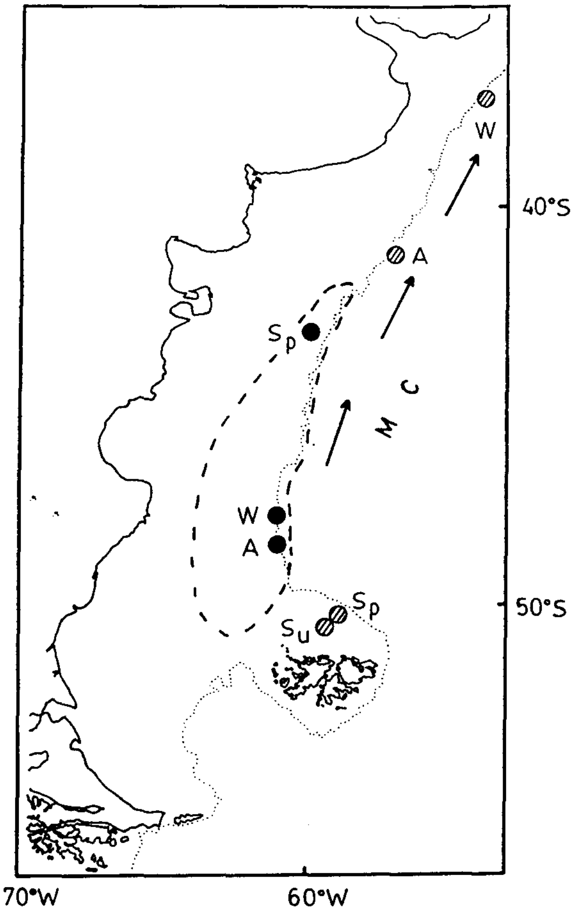

Fig. 1. Argentine Sea and oceanographic stations. Dotted line: 200 m isobath. MC: Malvinas (Falkland) Current. (●) Seasonal samples of the same aggregation. The mean position of the aggregation is indicated with a dashed line. The aggregation disintegrated in summer (Dadon 1990). (⊘) Seasonal samples where high densities of *Limacina retroversa* were detected. A: autumn 1978; W: winter 1978; Sp: spring 1978; Su: summer 1978–1979

hematoxyline and eosin. In order to minimize errors due to observation of tangential sections, diagnosis of gonadal stage was carried out only in sections with the retractor muscle situated in the center of the spiral.

Results

Relation between specimen size and gonadal maturity

Complete sequences from immature to hermaphroditic individuals were found at all of the stations. The maximally developed phase included at least 25% masculine elements; no specimens with less than 25% masculine elements (Stage f, Hsiao 1939a) were observed.

Through the examination of the genital system (i.e., seminal vesicle, seminal receptacle and accesory glands) and the density of germinal cells in the ootestis, the hermaphrodites with 50 to 25% masculine elements were separated into two physiological stages. In the first, germinal cells are densely arranged (Fig. 2) and spermatozoa can be seen in the gonoduct. The developed shell gland has columnar cells, with a high content of secretory material in the apical cytoplasmic portion (Fig. 3). The seminal vesicle contains spermatozoa (Fig. 4) which are missing in the seminal receptacle, indicating that copulation has not yet occurred.

The second physiological stage is characterized by a looser arrangement of the cells in the ootestis due to the absence of oocytes (Fig. 5). The shell gland is developed as in the former stage but it contains ova (Fig. 6), and there are spermatozoa in the seminal receptacle (Fig. 7). This indicates that copulation has already occurred and that individuals were near oviposition.

The size intervals corresponding to each maturation stage are illustrated in Fig. 8. Individuals smaller than 0.66 mm were always immature; in the 0.41 to 0.50 mm interval, it was possible to find individuals with nondeveloped gonads. In specimens larger than 0.66 mm, differences in the maturation stage among individuals of the same size from different stations were found. The greatest dispersion within one size class was observed in the 1.25 to 1.33 interval, which includes immature (in autumn) to spawned hermaphrodite individuals (in summer).

Discussion

Factors related to gonadal maturation

The relationship between shell size and gonadal maturity is influenced by several factors. First, the relationship varies with season. Reproductive maturity occurs at a smaller size during summer than during spring. This is clearly evident when comparing the minimal size of the oldest stage. This difference is not due to the omission of maturation stages as suggested by Hsiao (1939b), for a complete developmental series could be found in all the analyzed samples. Intermediate stages were never missing. Instead, a shortening of the different stages, or a "telescoping" effect (Spoel 1967), was observed.

A second factor is geographic. In contrast to shelf waters, slope waters turn over quickly due to the Malvinas (Falkland) Current (Fig. 1). Earlier sexual maturity appears to occur in the slope area. The gonads of specimens of comparable size captured over the slope were more developed than those from specimens collected over the shelf. This is especially evident in winter (Fig. 8), when hermaphrodites with up to 50% male elements were recorded over the slope while shelf specimens of the same size had not yet reached that stage. These differences between slope and shelf populations are related to different hydrographic dynamics, reflecting distinct previous histories of the specimens in the two areas (Dadon 1990).

Environmental factors might initiate or stop the maturation process. Thus, even though reproductive maturity depends on age, the age cannot be deduced from the size or the reproductive stage of the individual.

Life cycle of *Limacina retroversa* in the Argentine Sea

The Argentine Sea shows a typical temperate-cold hydrographic cycle with seasonal variations in primary and

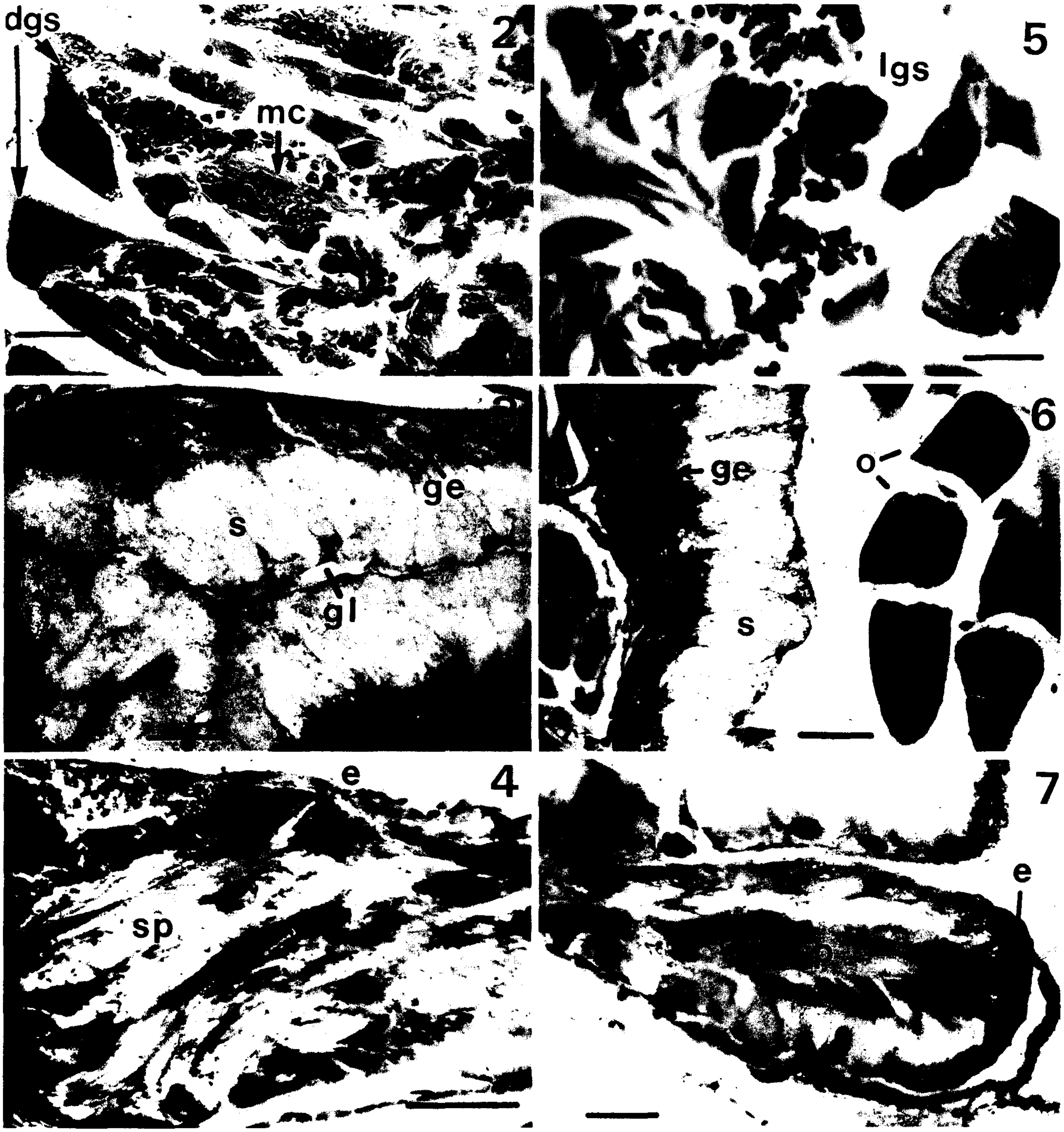

Fig. 2 to 7. *Limacina retroversa.* Genital system. Fig. 2. Ootestis. Fig. 3. Shell gland. Fig. 4. Seminal vesicle. Fig. 5. Ootestis. Fig. 6. Seminal receptacle. Fig. 7. Shell gland. dgs: dense gonadal stroma; e: epithelium; ge: glandular epithelium; gl: gland lumen; lgs: lax gonadal stroma; mc: masculine germ cells; o: ova; s: secretion; sp: spermatozoa. Bar = 40 µm

secondary production. These variations are not synchronous over the whole area; in fact, biological production peaks first in the northern area and in slope waters, and then it progresses southward and from the slope to the coast (Carreto et al. 1981). These authors reported that the lowest values of zooplanktonic biomass are found in winter. Springtime warming of the waters is accompanied by phytoplankton blooms, which are followed by an increment in the global zooplanktonic production (Carreto et al. 1981). Spring zooplankton maxima were observed for both shelf and oceanic species belonging to different groups (Chaetognatha:Ramírez and Viñas 1983; Euphausiacea, Cladocera and Copepoda: Ramírez 1981).

The abundance pattern of *Limacina retroversa* is clearly different. This species peaks in autumn–winter, occupying practically its whole range in the area and occurring in swarms of up to 67 000 ind. per 1000 m^3 (Dadon 1990). Histological analysis in the present study revealed that although the hermaphrodite stage was detected in

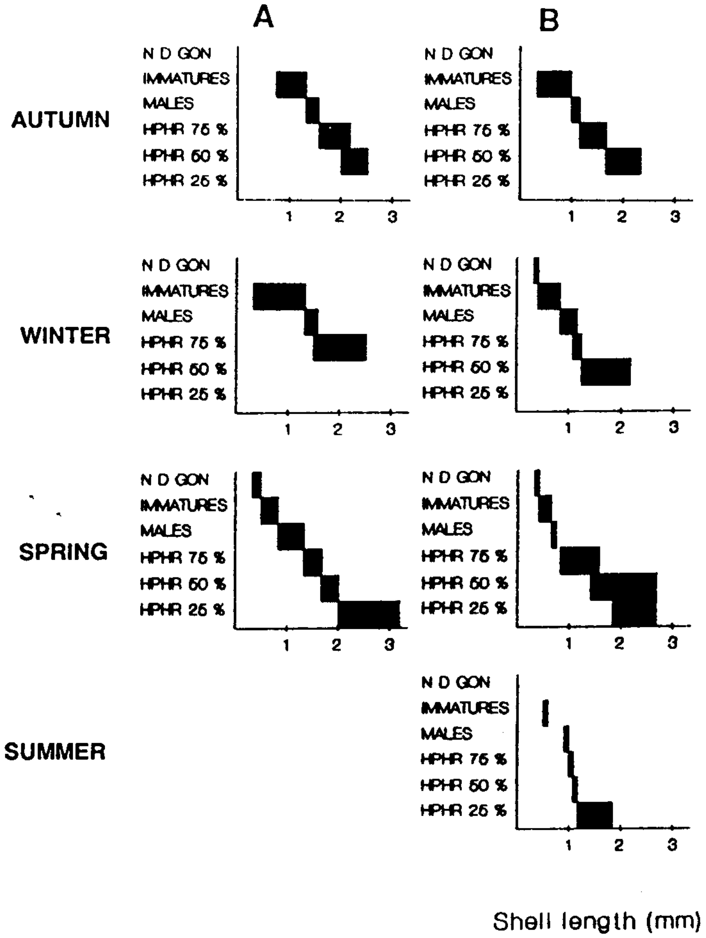

Fig. 8. *Limacina retroversa*. Relationship between maturation stages and size at each oceanographic station. A: seasonal samples of the same aggregation. The aggregation disintegrated in summer (Dadon 1990). B: Stations where high densities of *L. retroversa* were detected. N D GON: nondeveloped gonads; HPHR 75%: hermaphrodites with more than 75% masculine elements; HPHR 50%: hermaphrodites with 75 to 50% masculine elements; HPHR 25% hermaphrodites with 50 to 25% masculine elements

autumn–winter (Fig. 8), there was no evidence of reproduction during the cold period. Reproduction takes place in spring, simultaneously with most other species in the area. The numbers lost from the local population in the winter, are, however, not fully replaced by the spring spawning (Dadon 1990). Towards the end of the summer, the swarm in shelf waters disintegrates and only a few individuals remain on the shelf. Probably the individuals die after spawning, as observed in other species of the genus (*L. inflata*, *L. bulimoides*, *L. trochiformis*: Wells 1976; *L. helicina*: Kobayashi 1974).

The detection of spawning specimens in summer in the proximity of the Malvinas Islands (Fig. 1) indicates that the seasonal cycle of the species may be somewhat more complicated. These individuals have a considerably smaller size (minimum: 1.25 mm) than the individuals spawning in spring (minimum: 1.99 mm), which implies that there is a second generation that originates in the area.

It can be concluded that *Limacina retroversa* exhibits a two generation annual life cycle in the Argentine Sea. Generations born in different seasons show strong differences in developmental strategies. Individuals which are born in spring mature early and, taking advantage of favorable environmental conditions, complete their reproductive cycle and breed before the end of the warm summer season. The offspring produced in summer can apparently survive the cold season in shelf waters without reaching sexual maturity and reproduce in the following spring.

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