Invertebrate Reproduction and Development, 50:3 (2007) 155–162
Balaban, Philadelphia/Rehovot
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Reproductive biology of *Megabalanus azoricus* (Pilsbry), the Azorean Barnacle

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Received 5 April 2007; Accepted 14 September 2007

Summary

The reproductive biology of *Megabalanus azoricus* (Pilsbry), the commercially exploited barnacle in the Azores, was studied in an attempt to provide the scientific basis for the sustainable management of this heavily exploited regional marine resource. Both the Ospar Commission and WWF have expressed concern for this species, considering it at risk and in urgent need for scientific study. Barnacles were collected every month from shallow water (≈3 m depth) around São Miguel Island from October 2004 to September 2005. Individuals were measured and gonads processed for histology and analysed with a stereological method. *M. azoricus* has a hermaphroditic reproductive system with separate gonads and it was possible to describe the various stages of gametic maturation in both. Data on fertility were also obtained by egg counts and calculation of the Gonadosomatic index (GSI). Throughout the year, some specimens of *M. azoricus* can be found that rare mature, but two reproductive peaks were observed, one in January and a smaller one in July. A strong positive correlation between GSI and environmental factors such as photoperiod and water temperature was observed.

Key words: Azores, Cirripedia, *Megabalanus azoricus*, reproduction, fertility, gametogenesis, gonadal maturation

Introduction

The archipelago of the Azores (36–39ºN, 25–31°W) consists of nine volcanic islands forming three groups (western, central and eastern) located in the North Atlantic Ridge and has about 780 km of coastline (Pena and Cabral, 1997; Cruz, 2003). The Azores climate can be considered as subtropical and oceanic marine temperate, with mild temperatures, a small thermal amplitude, high precipitation and air humidity and persistent wind (Ferreira, 1980). From September to March the region is frequently crossed by the North Atlantic storm-track, whereas in late spring and summer the climate is influenced by the Azores anticyclone (Santos et al., 1995). The oceanographic conditions in the Azores are strongly influenced by the Gulf Stream jet, which in the central North Atlantic has a southern multi-branched current system with many unstable meanders and eddies at the Azores front (Pollard and Pu, 1985; Gould, 1985). The average winter sea surface temperature is typically 15–16°C and average summer temperature 22–24°C (Santos et al., 1995).

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São Miguel, located between 37º42′N, 25º08′W and 37º55′N, 25º52′W, is the largest and most highly populated island of the Azores archipelago (Pena and Cabral, 1997). Major hydrodynamism is observed in the winter during big storms. In the summer the island is frequently affected by offshore storm waves. The coast in São Miguel Island is very exposed (75%) or medium exposed (Macedo, 2002).

Barnacles have evolved particular features in biology and morphology, the most relevant of which is the development of sessile adults (Anderson 1986). Most species live on rocky shores and many are particularly common in exposed sites.

The complex life cycles of marine invertebrates have intrigued researchers for many years. Each phase of the life history presents different challenges and, consequently, a different set of adaptations are necessary for success (Wilbur, 1980). During the course of the evolution, two fitness-increasing strategies developed: the r- and the K-strategies, respectively (Southwood et al., 1974). Barnacles adopted the r-strategy, which is characterized by a high rate of propagation with high fecundity and larvae with short development time. This strategy occurs mainly with species specialized in colonizing new habitats with variable conditions, or with species with strongly fluctuating population sizes (Foster, 1987).

Most barnacles are hermaphroditic. Hermaphroditism in animals can assume different forms: simultaneous (or synchronous) hermaphroditism, when adult organisms have male and female sexual functions at the same time, or sequential hermaphroditism, when organisms mature as one sex and later change into the other sex, and therefore can only function as one sex at a given time (Ghiselin, 1969; Stubbings, 1975; Walker, 1992; Southward, 1998). However, barnacles adopt a sexual strategy different from other crustaceans. Being simultaneous hermaphrodites, with testis and ovary, termed digonic hermaphrodites, copulation can occur between sessile mating partners. Cross-fertilization is preferred to self-fertilization for reproduction (Bertness et al., 1991; Southward, 1998).

According to Buhl-Mortensen and Høeg (2006), barnacles do not spawn freely, the eggs being fertilized inside the brood chamber and forming ovigerous lamellae in the mantle cavity. Each barnacle produces two lamellar structures and the incubation period can vary from weeks to months (Gerhart et al., 1990). During this period the embryos reach the first nauplius stage (Lee et al., 1999; O'Riordan and Murphy, 2000). Egg retention until hatching could be an important feature in the evolutionary success of barnacles, reflecting a more efficient energy allocation (Buhl-Mortensen and Høeg, 2006). Gregarious behaviour may also be an important adaptive feature, since barnacles save energy which would otherwise be used for locomotion and defence and invest it in growth and reproduction (Wu et al., 1977).

Megabalanus azoricus is an insular Atlantic species inhabiting shallow subtidal bedrock of St. Helena Island, the Azores, Madeira and the Canaries Archipelagos (Wirtz et al., 2006). This species is the largest and the third most common shallow-water barnacle of the Azores. *M. azoricus* has long been collected for food in the Azores islands and, according to Santos et al. (1995), this species may be in danger of over-exploitation. A high impact of direct exploitation of resources on rocky shore communities is usually reflected in declining populations (Thompson et al., 2002). Therefore, the huge increase (115%) in the exploitation levels of this barnacle in recent years and its absence in places where large healthy populations used to be present has led to a conservation alert. In consequence, this species is currently being proposed for urgent action under the OSPAR Convention (1998–2006) and is also considered to be threatened by the WWF (2004).

To our knowledge, the reproductive biology of *M. azoricus* has not been studied to date. Thus, the objective of this study was to gain an understanding of the main aspects of the reproductive cycle of this barnacle to provide the baseline knowledge necessary for the management and conservation of this resource.

Materials and Methods

The study was carried out on São Miguel Island, Azores. Between 16 and 25 specimens were sampled in the subtidal zone (0–3 m) during each of four periods: October 2004 (autumn); January 2005 (winter); April 2005 (spring) and July 2005 (summer).

Measurements of shell as operculum maximum diameter, basal maximum diameter, carina maximum height, rostrum maximum height, width and length of right and left tergum and scutum, were taken to the nearest 0.05 mm using digital callipers. In addition, total fresh weight (soft part plus attached opercular plates), gonad weight and edible mass (whole soft body parts determined as fresh weight minus operculum plates weight) of each individual were determined to the nearest 0.1 mg.

To study gonad maturation, both ovaries and testes were excised from 10 specimens during each sampling period. They were fixed in 10% formalin and embedded in paraffin. Serial sections, 7-µm thick, were stained with Mayer's haemalum and eosin (Martoja and Martoja-Pierson, 1970). The relative volumetric density of each cell type was estimated using the M168 Weibel Multipurpose Test System (Weibel, 1979), under the light microscope.

Histological classification was determined according to the degree of development of the ovary and testis and also by the presence/absence of different stages of oocytes and male germ cells.

Four stages of spermatogenesis were identified, based on the classification of Griffond et al. (1991) and Curdia et al. (2005): (1) spermatogonia, medium-sized cells rectangular in shape when viewed by light microscopy, with a large nucleus in relation to the quantity of cytoplasm, always located near the acinus epithelium; (2) spermatocytes, smaller than spermatogonia with basophilic cytoplasm; (3) spermatids, smaller than spermatocytes, spheroidal in shape and slightly more basophilic than spermatocytes; (4) spermatozoa — with strong basophilic heads and eosinophilic tails. Spermatocytes I and II could not be distinguished from each other.

Following Hill and Bowen (1976) and Curdia et al. (2005), three stages of development were distinguished during oogenesis: (1) previtellogenic oocytes (PV), small, rounded and with strong basophilic cytoplasm; (2) vitellogenic oocytes (V), larger, irregular in shape, sometimes with multiple visible nucleoli, cytoplasm with slight granulations and lightly basophilic; (3) maturing oocytes (M), larger than vitellogenic oocytes, round in shape with eosinophilic and granular cytoplasm.

In order to identify gonadal maturation state, scores for volumetric density were summed for each specimen and converted to percentages.

To obtain the median diameter of PV, V and M, maximum and minimum diameter of 40 oocytes per ovary in 30 female gonads were measured using an eyepiece micrometer. Only oocytes simultaneously showing cytoplasm, nucleus and at least one nucleolus were measured.

The gonadosomatic index (GSI) was calculated for the ovary for all samples. The small size of testes invalidated their precise excision and measurement and therefore prevented the GSI calculation for male gonads. The GSI was calculated according to Maddock and Burton (1998) and Favaro et al. (2003) as gonad weight (g) divided by body eviscerated weight (g) × 100.

Brooding activity, defined as the percentage of animals carrying egg masses (Cruz and Hawkins, 1998), was determined in 25 specimens sampled each month from October 2004 to September 2005.

The potential spawning was estimated from the average number of eggs in the mantle cavity. To measure fecundity we used lamellae removed from ten individuals with scutum maximum length between 18.2–22.6 mm. Lamellae were removed from the mantle cavity and the total number of eggs was estimated from total egg volume, after counting an egg sub-sample corresponding to 20% of the gonad.

Data were analysed with ©Statistica V.5.1. (StaSoft, Inc.) package software. Relative volumetric density of each stage in oogenesis and spermatogenesis was compared using a one-way ANOVA or a Kruskal–Wallis test whenever data did not comply with ANOVA assumptions.

Results

No significant differences were found in the relationship between the gametogenic maturation stage and the morphometric parameters. The edible mass and the gonad weight presented positive significant correlations ($r^2 > 0.50$) with the morphometric parameters, except with the maximum diameter of operculum. Consequently, tergum length was chosen as the reference measure.

The ovaries of *M. azoricus* are composed of multiple spherical lobules consisting of branched ovarian tubules interspersed in a connective tissue stroma. Histological observations revealed three types of female germinal cells (Fig. 1; Fig. 2A): (1) previtellogenic or immature oocytes, with maximum and minimum diameter of 27.57±1.11 µm and 21.22±0.84 µm, respectively, and a mean volume of $7.2\times10^4 \pm 7.3\times10^3$ µm^3; (2) vitellogenic oocytes, with a maximum and minimum diameter of 56.78±1.71 µm and of 43.78±1.54 µm, respectively and a mean volume of $6.1\times10^5 \pm 5.8\times10^4$ µm^3; cell nucleus with a mean diameter and volume of 18.03±0.37 µm and $2.6\times10^4 \pm 1.5\times10^3$ µm^3, respectively, and occupying 4.3% of the cell; (3) mature oocytes, with maximum and minimum diameter of 118.24±2.91 µm and 93.78±2.19 µm, respectively, and a mean volume of $5.3\times10^6 \pm 3.5\times10^5$ µm^3. The nucleolus was 25.65±0.47 µm in diameter and $7.3\times10^4 \pm 3.9\times10^3$ µm^3 in volume, and occupied 1.4% of the cell.

In each ovarian follicle of *M. azoricus* all the maturation stages of oocytes can be found throughout the sampling period. Differences in the diameter of the oocytes at different stages were tested with one-way ANOVA (F= 75.73, P <0.0001). Oocytes grow to reach a maximum diameter of 200 µm (Fig. 3). Previtellogenic oocytes showed a maximum diameter under 50 µm; vitellogenic oocytes ranged from 50 to 90 µm, with the majority being 60–70 µm in maximum diameter. Most maturing oocytes had a diameter of 120–130 µm (Fig. 3).

Maturing oocytes occupied more than 60% of the gonadal volume throughout the sampling period, growing from winter (October) to spring (April), when

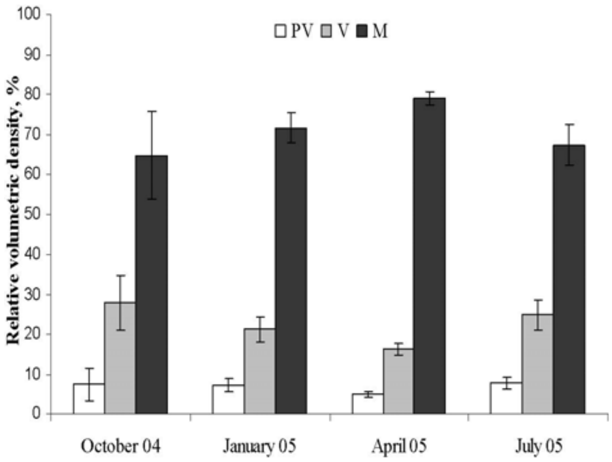

Fig. 1. Relative volumetric density (mean ±SE) of the oogenic stages in *Megabalanus azoricus*, from October 2004 to July 2005: pre-vitellogenic (PV), vitellogenic (V) and maturing oocytes (M).

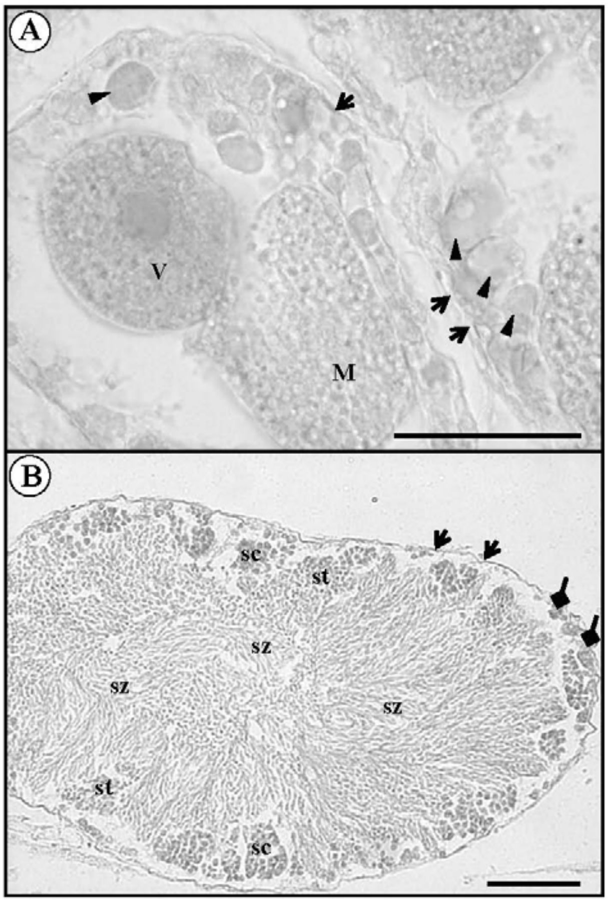

Fig. 2. Histological section of *Megabalanus azoricus* gonads. A: Ovary; arrows, germinal layer; arrowheads, previtellogenic oocytes; V, vitellogenic oocytes; M, maturing oocytes. Scale bar = 50 µm. B: Testicle; lozenge arrowheads, spermatogonia; sc, spermatocytes; st, spermatides; sz, spermatozoa. Scale bar = 50 µm.

the highest percentage is reached, and decreasing after the summer (Fig. 1). Ovaries in April showed the lowest values of pre-vitellogenic and vitellogenic oocytes (Fig. 1). In October, the highest diversity of relative volumetric density of the different oogenic stages was observed.

The testes consisted of numerous lobed seminiferous tubules lying around the alimentary canal and included cells at different stages of spermatogenesis. Based on staining affinity and the relative position in the tubule, four types of cells were distinguished: spermatogonia, spermatocytes, spermatids and spermatozoa.

Throughout the year at least 40% of the testis volume was occupied by spermatozoa. The relative volumetric density of spermatozoa increased from winter (January) to summer (July), when it occupied almost 60% of the testis volume (Fig. 4; 2B).

The highest GSI value was observed in spring (April) and the lowest in winter (January) (Fig. 5). The GSI values were significantly different between January and July ($F = 3.48$, $p < 0.05$).

Individuals with egg lamellae were recorded throughout the year except in November, March and August (Fig. 6). Production peaks occurred in January and June. There was a significant positive relationship between body weight and the number of eggs produced ($R^2 = 0.93$). The mean fecundity of *M. azoricus* was found to be 9.8×10^4 eggs/individual, ranging from 5.9×10^4 in smaller individuals (18.2 mm tergum length), to 2.5×10^5 in the largest (22.6mm TL).

Discussion

The standard morphometric parameters analyzed for *M. azoricus* did not present a linear relationship with maturation states, but displayed good positive relations with an r^2 superior to 0.50, between edible mass and gonad weight. Consequently, these measurements can be used in non-destructive studies (e.g., photography) in the future, similar to those carried out by Schmidt-Nielsen (1984) and Klingenberg and Monteiro (2005).

Morphologically, like most balanomorph barnacles, *M. azoricus* is hermaphroditic with simultaneous development of both testis and ovary. The structure of reproductive organs was shown to be similar to other balanomorph species, such as *Chthamalus dalli* and *Chthamalus malanyensis* (Korn and Kolotukhina 1984; Yan et al. 2006). Our results indicate that *M. azoricus* is capable of producing sperm, mature oocytes and eggs simultaneously.

Stereological analysis of the female gonads revealed asynchrony in oogenesis, since each gonad can show the three developmental stages (PV, V and M) at the same

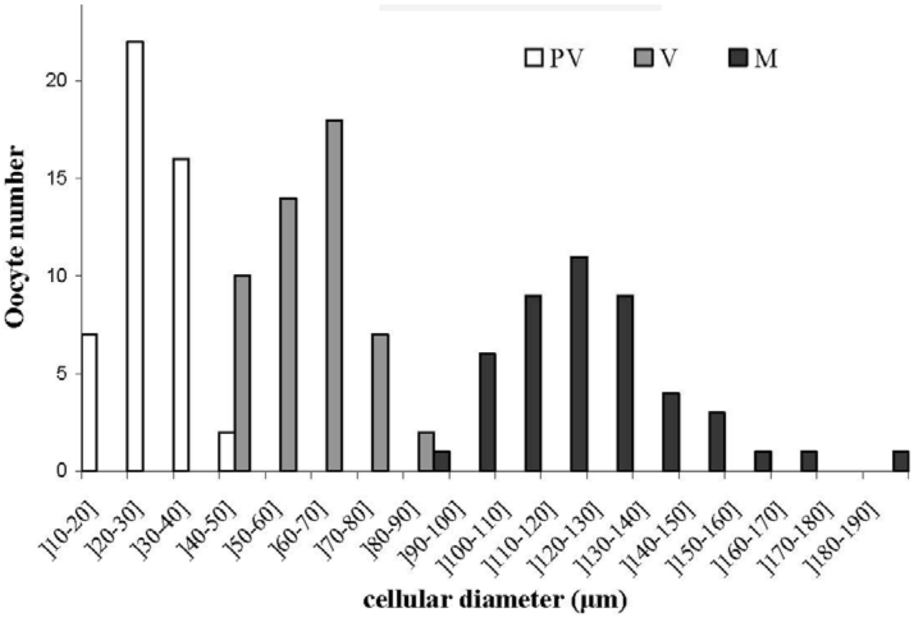

Fig. 3. Cellular diameter of three different development stages in ovary of *Megabalanus azoricus*: pre-vitellogenic (PV), vitellogenic (V) and maturing ocytes (M).

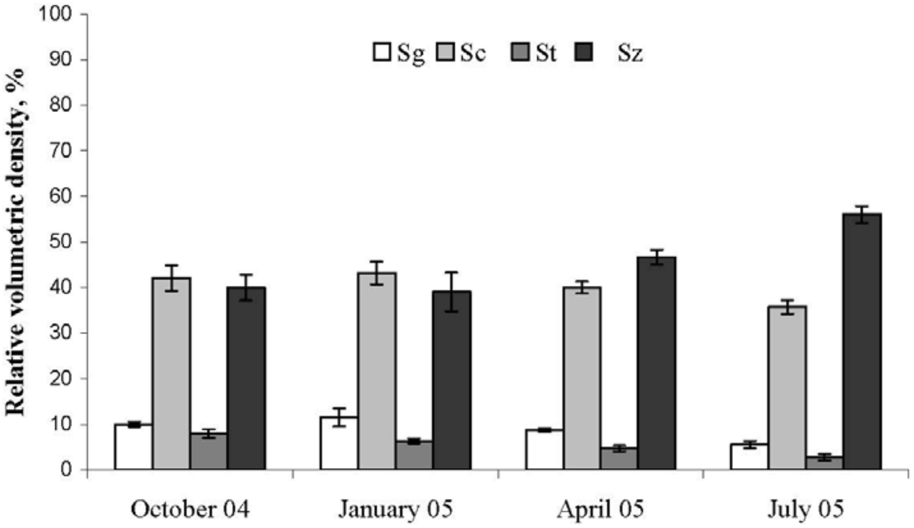

Fig. 4. Relative volumetric density (mean ± SE) of the spermatogenic stages in *Megabalanus azoricus*, from October 2004 to July 2005: spermatogonia (Sg), spermatocytes (Sc), spermatids (St) and spermatozoa (Sz).

time. An identical situation was observed by Cancino et al. (1998) for *Austromegabalanus psittacus*. Spawning in *M. azoricus* was determined to be partial in that individuals can present both ovigerous lamellae and an abundance of maturing oocytes in the ovary.

There is a relationship between latitude and the duration and type of spawning (Blaxter and Hunter, 1982). In high latitudes, with a short summer period, barnacles have total spawning during a short period (Blaxter and Hunter, 1982). Towards the equator, in tropical and subtropical latitudes, the reproductive period is longer and involves partial spawning.

More than 65% of the female gonad is occupied with mature oocytes throughout the year, which largely results from the large volume occupied by individual mature oocytes, which are 73 times larger than pre-vitellogenic cells and 9 times larger than vitellogenic cells. Moreover, the time and energy invested in producing mature cells is greater than for the previtellogenic or vitellogenic stage.

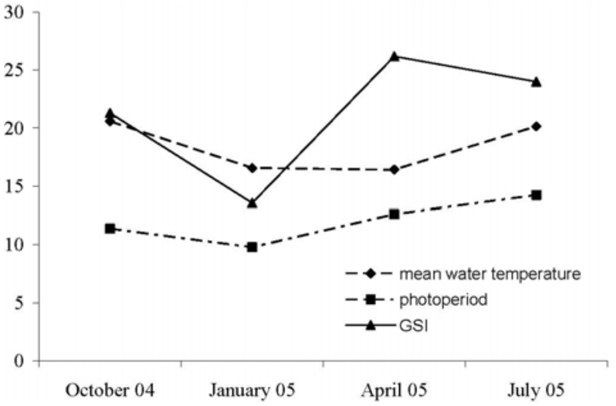

Fig. 5. Water temperature, photoperiod and GSI (average sea surface temperatures) for the period of November 2001 to May 2003 in the geographical area of São Miguel (37E–38EN; 26E–25EW). Data obtained from PODAAC–ESIP (http://podaac-esip.jpl.nasa.gov).

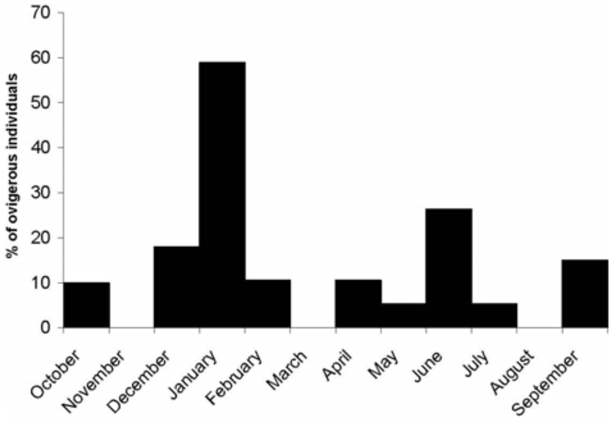

Fig. 6. Ovigerous *M. azoricous* during the year at São Miguel Island.

Spermatogenesis in *M. azoricus* was shown to be similar to that in other barnacle species (e.g., *Balanus amphitrite*, *Semibalanus balanoides*, *Austromegabalanus psittacus*), with spermatozoa occupying at least 50% of the testis throughout the year.

Male and female gonads are mature throughout the year, suggesting that *M. azoricus* is characterized by simultaneous hermaphroditism with cross-fertilization, where each barnacle can play both male and female roles. The number of female gametes is limited by the high levels of energy invested in each (Clutton-Brock and Vicent, 1991).

Although the results of our study seem to indicate two reproductive peaks for *M. azoricus*, ovigerous lamellae are present throughout the year, excluding November, March and August. In the January peak more than half of the population presented ovigerous lamellae. The photophase is lowest in this month and water temperatures are also low. The strong hydrodynamic activity in this period might favour larval dispersion, water oxygenation and increased particulate material in the water column. Consequently, barnacles may be able to divert energy from respiration and feeding and expend it on reproduction. This reproductive peak may also result from the retention of larvae during winter, giving a longer period for embryonic development at the lower temperatures. Barnacles like *M. azoricus*, living at high tide levels, may well be very sensitive to day-length changes, perhaps because the contrast between day and night is greater, whereas at lower shore levels illumination is reduced by overlying turbid water. Spawning may also be triggered by sea roughness (Orton et al., 1956; Bowman and Lewis, 1986). In Azores, sea roughness is higher in winter, arguing in favour of an extended spawning period during this season. This can be extremely important to *M. azoricus* since this species lives in the intertidal and shallow subtidal and is therefore highly exposed to sea roughness.

The June reproductive peak coincides with a long photoperiod, warmer water temperature and follows the local spring phytoplankton bloom (e.g., Couto, 2004). In fact, Crisp (1986) reported that the signal for embryo hatching in *Balanus balanoides* and *Tetraclita pacifica* is provided by the parental incubation of the egg masses, in response to the uptake of food from the plankton. Moreover, it is known that temperature, food availability and photoperiod play an important role in the reproductive cycle of barnacles (Southward and Crisp, 1956; O'Riordan and Murphy, 2000).

As expected, the GSI showed significant differences during the year but the application of the Tukey test was inconclusive, a continuous rather than a discrete GSI pattern resulting from the pattern of reproductive activity in *M. azoricus*. It seems that while a part of the population is investing energy in spawning, another part is using resources in the production of mature gametes.

Thus, we can conclude that in the Azores *M. azoricus* presents a reproductive pattern marked by an uninterrupted capacity to produce offspring throughout the year, albeit with the retention of a potential to respond to favorable environmental conditions, as shown by the high percentage of individuals with egg lamellae in January.

As described by Olivares et al. (1991) for *Paralabraxhumeralis valenciennes,* the GSI of *M. azoricus* shows a strong relationship with abiotic factors (photoperiod and water temperature). These factors can affect the GSI either independently or synergistically. Indeed, these abiotic factors strongly influence the ecological niche (Clare 1987; Planque and Fromentin, 1996; Saether 1997) where this species occurs.

The main goals of this study have been fulfilled. However, more studies are needed to provide the fundamental knowledge on recruitment, growth and population dynamics required for the establishment of conservation guidelines. A histological study involving a larger sample with a larger size range in selected months would be advisable to give strength to the present results and to find the first maturation size. Nevertheless, from the results presented, it would be advisable to apply precautionary protection measures in June when high reproductive activity coincides with high exploitation levels.

Acknowledgments

We thank João Brum, Dr. Rui Costa, Dr. André Amaral and Paulo Melo for technical assistance and to Dr. Carmo Barreto for helpful comments on the manuscript. This research was funded by research project POCI/MAR/58185/2004.

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