Long-term survey on wild and cultured mussels (*Mytilus galloprovincialis* Lmk) reproductive cycles in the Ria de Vigo (NW Spain)

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Abstract

The gonadal development of cultured and wild mussels (*Mytilus galloprovincialis*) was studied in several localities of the Ria de Vigo (NW Spain) from December 1987 to June 1993. In the mussel populations examined, a stable reproductive pattern, with minor variations, was found. Gametogenesis takes place in late autumn and early winter. In late winter, gonad follicles are full of ripe gametes and some spawns may occur. However, the peak of the spawning season occurs in spring, coinciding with an increase in temperature in the area. After the first spawns, the gametogenesis begins again, quickly reaching new spawning conditions. Several spawns may occur until early summer. During late summer and autumn, there is a storage of reserves that will be used for the next gametogenesis.

Keywords: *Mytilus galloprovincialis*; Reproduction; Gametogenesis; Storage cells cycle

1. Introduction

Long-term studies of reproduction enable accurate predictions on population recruitment (Seed, 1976). Studies of reproductive cycles are more valuable when carried out over several years as the onset and duration of both gametogenesis and spawning in *Mytilus* can exhibit considerable temporal and spatial variation (Seed and Suchanek, 1992). This has been the subject of considerable research (Chipperfield, 1953; Lubet,

1959; Seed, 1976; Lowe et al., 1982; Newell et al., 1982; Brouseau, 1983; Dix and Ferguson, 1984).

Earlier studies of this area of Spain by Andreu (1954), Aguirre (1979) and Mariño et al. (1982) concluded that there are two spawning peaks during the year. The most important is in spring and the second in autumn. More recent studies indicate that there is only one spawning period with two spawning peaks from spring to summer (Ferrán et al., 1990; Ferrán, 1991; Villalba, 1995).

Despite their ecological importance, no studies on the reproductive patterns of the abundant natural mussel populations present in the intertidal shores of the Rias (Galicia, NW Spain) have been carried out.

The aim of the present work was to determine through quantitative stereology, the germinal and storage cells cycle from natural and cultured mussel populations over a prolonged period. Also, to determine the influence of depth on the reproduction of cultured mussels which has never been established.

2. Materials and methods

To determine the germinal and storage cells cycle in natural and cultured mussel from various environmental conditions in Ria de Vigo, two studies were planned.

The first was carried out from December 1987 to March 1990. The influence of depth and locality on the reproductive cycle were determined in mussel rafts from Liméns, an exposed area, and Meira, a protected area (Fig. 1). A total of 900 mussels, mean shell length 41.4 mm (SD = 6.3), were placed in 11 baskets and hung at 2-m depth where fresh water influence during the rainy season in the area is direct, and 5-m depth where this influence is presumably less. A total of 30 individuals were sampled monthly in the first year and bimonthly subsequently due to logistic limitations.

From January 1989 to December 1990, Domayo an inner and more protected area (Fig. 1) was studied together with the previous two (Liméns and Meira). In this experiment, 500 mussels, mean shell length of 45.3 mm (SD = 7.9), were placed in 13 baskets and

hung at 5-m depth, in all the localities (Liméns, Meira and Domayo). A total of 30 individuals were sampled bimonthly due to logistic limitations.

To determine the influence of the locality on the reproductive pattern of wild mussel populations established on natural substrates and on the raft's floats and anchor chains, a third study was carried out between October 1991 and May 1993. In the case of wild mussel populations established on natural substrates, two localities were chosen, Cabo Home, an exposed rocky shore, and San Adrian, a protected rocky shore (Fig. 1). Around 30 mussels of a mean shell length of 50.6 mm (SD = 0.59) were collected monthly from the lowest intertidal zone during low tide. Simultaneously, 30 mussels, mean shell length of 58.8 mm (SD = 0.94), were collected from the floats and anchor chains of culture rafts from Liméns and San Adrian. Salinity and temperature were recorded at each study site over the entire study period.

Mussels were brought to the laboratory and, after shell removal, all tissues were fixed in Davidson's fixative (Shaw and Battle, 1957). An anterior transverse section was taken including mantle tissue. Histological sections with a thickness of 5 μ m were cut and stained with haematoxylin and eosin. The presence of gametes and storage cells were determined by quantitative stereology using a Weibel graticule following a method described by Lowe et al. (1982). Briefly, five random counts were performed on each histological slide. Results are given as a percentage of mantle volume occupied by the gamete development stages and cell types defined as follows: ripe gametes (RG), in the female: it is the stage in which oocytes were free within the follicles and some oocytes remained attached to the follicle wall; while in the male: it is the stage when follicles were filled by spermatozoa arranged in characteristic bands. Developing gametes (DG) in females: when rounded oocytes along with oocytes were found attached to the follicle wall; while in the male: when varying quantities of developing spermatogonic cells were present:

Vesicular cells of connective tissue (VCT)

Adipogranular cells (AC)

Empty follicles (EF).

As described by Ferrán (1991), the addition of volume percentage of ripe and developing gametes was used as a gonadal index ($DG + RG = GI$) and the addition of

volume percentage of vesicular cells and adipogranular cells was considered as a somatic index ($VCT + AC = SI$).

A Kruskal–Wallis test and Mann–Whitney test (Zar, 1984) were used for comparisons in gonad and storage cells cycles using gonad index of mussels studied. Differences in temperature and salinity from the sampled localities were assayed by means of a one-way ANOVA.

3. Results

3.1. Influence of locality and depth in the gonadal development of cultured mussels

Variations in percentage of the different cell types in the mantle volume of 2-m depth mussels from Liméns are shown in Fig. 2A. RG and DG were found throughout the study period with minimum values from late spring to early autumn, while maximum values were recorded from winter to spring. EF were also recorded throughout the study period attaining their higher values from mid winter to late spring, at this time, AC and VCT were less developed or absent. AC reached their maximum from mid summer to autumn while VCT raised from late spring to autumn. Graphic representation of GI and SI (Fig. 3A) clearly show their inverse and gradual fluctuation. GI reached its maximum value from winter to spring, just when SI reached its minimum values. The opposite was detected from summer to autumn. The variations on the percentage of the different cell types in the mantle volume of 5-m depth mussels were similar to those previously described (Fig. 2B) and also, the variations of GI and SI were similar (Fig. 3B). This similarity was corroborated by the Mann–Whitney test on GI which indicated that differences at different depths were not significant ($U = 152, p > 0.05$). A variation in the percentage of the different cell types in the mantle volume of 2-m and 5-m depth mussels from Liméns was detected in different years (Fig. 2).

The variations in the percentage of the different cell types in the mantle volume of 2-m and 5-m depth mussels from Meira are shown in Fig. 4A,B. DG and RG were detected throughout the study period. Their maximum values were found between winter and spring. This was more evident between December 1987 and June 1988, owing to the monthly sampling rate. The rest was sampled on a bimonthly basis. DG and RG were

scarce from late summer and autumn. EF were recorded in all sampled months with maximum values from late winter to spring and minimum values from middle summer and early winter. Between winter and late spring, AC were scarce or absent and their highest percentage was recorded during late summer and autumn. VCT were abundant from late spring to autumn and scarce from winter to early spring. GI and SI are shown in Fig. 5A,B. The fluctuation between depths was similar as it happened in Liméns. The Mann–Whitney test showed that the differences between depths were not significant ($U = 137, p > 0.05$).

No significant differences among GI cycles from both localities and depths ($H = 0.93, P > 0.05$, Kruskal–Wallis test) were found.

Similar to Liméns, some variation in percentage of the different cell types in the mantle volume of 2-m and 5-m depth mussels was detected in different years (Fig. 4).

Variations in the percentage of the different cell types in the mantle volume of Liméns, Meira and Domayo mussels are shown in Fig. 6. DG, RG and EF were detected throughout all the study period with maximum RG values in winter and spring. The greatest abundance of EF was also recorded during this period. AC and VCT were abundant from summer to autumn. GI and SI showed minor differences among the reproductive cycles of mussels cultured in the three localities (Fig. 7). Statistical analysis showed that these differences were not significant ($H = 1.55, p > 0.05$, Kruskal–Wallis test).

In spite of some variations being recorded from year to year, as a general pattern, GI increased during winter, reaching its maximum value in spring and decreasing gradually until autumn when minimum values were recorded.

3.2. Influence of locality in the gonadal development of wild mussel populations

Variations in the percentage of the different cell types in the mantle volume of mussels from Liméns and San Adrian floats and anchor chains are shown in Fig. 8. In Liméns (Fig. 8A), DG and RG were detected throughout the study period, with maximums from winter to late spring. AC were the less abundant cell type except in autumn. VCT were

found throughout all the study with maximums in late summer and autumn. Similar results were found in San Adrian (Fig. 8B). In this locality, RG reached their maximum in June 1992, but a year later, their value in the same month was low. However, in general, these values followed a seasonal pattern. EF increased in spring with low values after that season. The percentage of AC present was always low with a minimum in spring, their maximums were attained in summer and autumn. VCT values raised in summer and autumn. Fluctuations in GI and SI values were similar in both localities and differences were not significant ($H = 1.32$, $p > 0.05$, Kruskal–Wallis test) (Fig. 9A,B).

Variations in the percentage of the different cell types in the mantle volume of mussels from Cabo Home and San Adrian are shown in Fig. 10. In Cabo Home, RG were abundant between winter and early summer reaching a maximum percentage in spring. AC reached their maximum values between summer and autumn. A similar pattern was recorded for VCT (Fig. 10A). Similar results were found in San Adrian (Fig. 10B), a maximum percentage of RG was found during winter and spring while a maximum percentage of EF was found in late spring and maximum values of AC and VCT in summer and autumn. Differences between GI in both localities were not significant ($H = 1.40$, $p > 0.05$, Kruskal–Wallis test) (Fig. 11A,B).

Some variation in the percentage of the different cell types in the mantle volume of wild mussels was detected in different years, however, the general pattern is maintained.

Comparisons between GI of wild mussel populations settled in natural and artificial substrates showed some differences, although they were not statistically significant ($H = 0.94$, $p > 0.05$, Kruskal–Wallis test).

3.3. Influence of temperature and salinity on gonadal development of wild and cultured mussel populations

The variations in temperature and salinity recorded during the study period are shown in Fig. 12. The increases in RG and EF values were associated with the water temperature rise that occurs during spring and early summer. When the water temperature decreased there was an increase of AC and VCT percentage. Temperatures ranged from 10 to 12°C in winter and from 18 to 20°C in summer. Salinity values ranged from 31 to 36

ppt. Differences of temperature and salinity from different localities studied were not significant (ANOVA, $F = 0.917$, $p > 0.05$ for temperature and ANOVA, $F = 2.674$, $p > 0.05$ for salinity).

3.4. General gonadal and somatic indices patterns

A general pattern was found throughout the study period in all the localities studied with some variation from year to year (Figs. 2–11). During the summer and autumn, an increase in storage cells was detected, in order to accumulate energy that will be used in gametogenesis. Then, from spring to early summer, a major spawn takes place. However, minor random spawns were detected during the rest of the year. Year to year variations on GI were found, although the differences among localities and substrates were not significant. The reproductive cycle of male and female were similar. The proportion of males and females was around 50% and only three hermaphrodites were found in a total of 3800 sampled mussel.

4. Discussion

As it has been experimentally confirmed, there are exogenous and endogenous factors that regulate the reproductive cycle in bivalve molluscs like mussels, oysters and pectinids (Seed, 1976; Devauchelle and Mingant, 1991; Robinson, 1992; Seed and Suchanek, 1992; Couturier, 1994). In the first instance, the most important factors are temperature, salinity, photoperiod and food; in the second, nutrient reserves, hormonal cycles and the genotype. Differences between reproductive cycles from mussels located in different environmental conditions could be explained by these factors. A short reproductive period (Lande, 1973) is common in cold waters. Several spawns in a wide reproductive period may occur in mussel populations in the south of the Northern Hemisphere (Seed, 1976). Our results could be included in the last situation and are in accordance with those previously described (Ferrán, 1991; Villalba, 1995). Year to year variations have also been described previously. Seed (1976) showed interannual variations on the reproductive cycle in five mussel populations from England coast sampled over a 5-year period, although as in our case, a general reproductive pattern could be established.

Our results showed minor variations at a local level. Seed (1976) described that the gonadal development was faster in mussels from the low intertidal zone, than in those from the upper zone and related these results to food availability. In our study, wild populations from natural substrates were located in the low intertidal profile and we can assume that food availability was relatively constant. No differences were detected with the gonadal development of wild mussel populations settled on artificial substrates (permanently submerged floats and chains of the rafts). Previous studies in the area associated local variations in gonadal cycle with environmental conditions, without using any statistical analysis (Ferrán, 1991; Villalba, 1995). The differences found in the gonadal development of mussels were not statistically significant.

The conclusion of Andreu (1954), Aguirre (1979) and Mariño et al. (1982) regarding a second spawning in autumn inferred from settlement studies is explained by the settlement sequence in the area recently described by Cáceres-Martinez et al. (1993, 1994) and Cáceres-Martinez and Figueras (1997), which has two components. The first is characterised by the settlement of competent larvae following the spawning peak from early spring to early summer, the second by the settlement of local dispersed post-larvae from mid summer to autumn.

The low incidence of hermaphrodites suggest that *Mytilus galloprovincialis* has a stable gonochorism (Brousseau, 1983).

As a general conclusion, gametogenesis takes place in the Ria de Vigo (NW Spain) during late autumn and early winter. In late winter, gonad follicles are full of ripe gametes and some spawns may occur. However, massive spawns occur in spring coinciding with an increase in temperature and chlorophyll-a concentration in the area (Andreu, 1976; Iglesias et al., 1984) providing favorable conditions for larval growth (Aguirre, 1979). After the first spawns, fast gametogenesis may occur, restoring the spawning condition, and several random spawns may take place until early summer. In autumn, a second increase in chlorophyll-a concentration in the area takes place (Iglesias et al., 1984). This increase in food availability for mussels in the area may favour the accumulation of reserves during this period.

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Fig. 1. Map showing the study area (Ria de Vigo), indicating the locations of mussel raft-culture areas with rectangles. Dots indicate sampled mussel beds.

Fig. 2. Variations of the volume percentage of the mantle components from mussels submerged at 2-m (A) and 5-m (B) depth in the exposed culture area of Liméns, Ria de Vigo, Spain, during the study period.

Fig. 3. Variations of the GI ($\pm$ SD) and SI ($\pm$ SD) from mussels submerged at 2-m (A) and 5-m (B) depth in the exposed culture area of Liméns, Ria de Vigo, Spain, during the study period.

Fig. 4. Variations of the volume percentage of mantle components from mussels submerged at 2-m (A) and 5-m (B) depth in the central and protected culture area of Meira, Ria de Vigo, Spain, during the study period

Fig. 5. Variations of the GI ($\pm$ SD) and SI ($\pm$ SD) from mussels submerged at 2-m (A) and 5-m (B) depth in the central and protected culture area of Meira, Ria de Vigo, Spain, during the study period.

Fig. 6. Variations of the volume percentage of mantle components from mussels cultured in localities of Liméns an exposed culture area (A), Meira a central and protected culture area (B) and Domayo an inner and protected culture area (C), Ria de Vigo, Spain, during the study period.

Fig. 7. Variations of the GI ($\pm$ SD) and SI ($\pm$ SD) from mussels cultured in localities of Liméns, an exposed culture area (A), Meira, a central and protected culture area (B) and Domayo, an inner and protected culture area (C), Ria de Vigo, Spain, during the study period.

Fig. 8. Variations of the volume percentage of mantle components from mussels attached on floats and chains of culture rafts form Liméns, an exposed culture area (A) and San Adrián, an inner and protected culture area (B), Ria de Vigo, Spain, during the study period.

Fig. 9. Variations of the GI ($\pm$ SD) and SI ($\pm$ SD) from mussels cultured in localities of Liméns, an exposed culture area (A) and San Adrián, an inner and protected culture area (B), Ria de Vigo, Spain, during the study period.

Fig. 10. Variations of the volume percentage of mantle components of mussels from Cabo Home, an exposed rocky shore (A) and San Adrián, a protected rocky shore (B), Ria de Vigo, Spain, during the study period

Fig. 11. Variations of the GI ($\pm$ SD) and SI ($\pm$ SD) of mussels from Cabo Home, an exposed rocky shore (A) and San Adrián, a protected rocky shore (B), Ria de Vigo, Spain, during the study period.

Fig. 12. Variations in temperature and salinity recorded during the study period. L: Liméns; RSA: protected rocky shore San Adrian; CH: exposed rocky shore Cabo Home; SA: mussel raft San Adrian.