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The reproductive cycle of white clam *Spisula solida* (L.) (Mollusca: Bivalvia): Implications for aquaculture and wild stock management

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ABSTRACT

The dynamics of the white clam's (*Spisula solida*) reproductive cycle along with its nutrient storage and exploitation strategy in the Algarve coast (Portugal) was studied throughout the year 2003. The timing of gametogenic development and spawning of *S. solida* were analysed through histological preparation using qualitative and quantitative criteria. Condition index and biochemical composition were determined in order to provide information on energy storage and utilization. Seawater temperature is a primary environmental factor determining reproductive development and spawning of *S. solida*; reproductive activity occurred during low temperatures. The spawning period began in late winter as a consequent response to the increase in seawater temperature and extended through spring. During this period, the condition index and the gonadal index decreased. In June, most of the population was spent and big resting oocytes appear dispersed in the gonad. In summer, the specimens were found to be in the resting phase and condition index increased to its maximum value as a consequence of reserves storage. Gametogenic activity was initiated coincident with decreasing temperature in September, but a sudden increase of this environmental parameter in October disturbed the gametogenic process and a second spawning occurred. In this period, the synchronism between males and females of the population was lost. The striking consumption of glycogen reserves developed during the previous August and consequent biosynthesis of lipids during gamete formation occurred. In the following two months, reproductive synchronism was restored, and storage of reserves and gametogenesis took place concurrently. In December the entire population was in the ripe stage of gonadal development. Moreover than a consequence of gametogenesis during autumn/winter, lipid behaviour reflected the energy accumulation process and its conversion to somatic development in spring/summer. The reproductive strategy adopted by *S. solida* makes possible broodstock manipulation in terms of conditioning in aquaculture. The information obtained in this study is important for assessing sustainable management of wild stocks as well as for estimating its potential for aquaculture production.

1. Introduction

The white clam *Spisula solida* (Linnaeus 1758) has been reported from off the south of Iceland, and the Norwegian Sea, to the Atlantic coast of the Iberian Peninsula, Morocco and Madeira (Tebble, 1966). This species is of commercial importance in Ireland (Fahy et al., 2003), Spain (Peña et al., 2005) and Portugal (Gaspar and Monteiro, 1999) where it is the target of a dredge artisanal fishery, and *S. solida* may have commercial fishery potential in other countries including France and Morocco (Gaspar and Monteiro, 1999). However, in the past decade the synergistic action of the intensive harvest of *S. solida*, coupled with the rapid growth rate and short lifespan of the species has resulted in large inter-annual fluctuations in stock abundance and periodic recruitment failure (Joaquim et al., 2008). In order to try to reverse this negative trend, it is of utmost importance to improve the management of the fishery and to develop restocking programs supported by aquaculture advances to rebuild the reproductive viability of depauperate populations.

Knowledge of the reproductive cycle of *S. solida* is fundamental for developing management strategies (e.g. protect spawning stock and/or larval settlement) (Shaw, 1965; Manzi et al., 1985; Sbrenna and Campioni, 1994) and is crucial for establishing successful hatchery-based production (Gribben et al., 2004; Peharda et al., 2006). The reproductive cycle of *S. solida* was investigated by Gaspar and Monteiro (1999) for a population off Vilamoura (southern Portugal). In their study, the seasonal gonadal development was followed using qualitative histological methods. However, the subjectivity inherent to these methods has led us to think that the use of a quantitative histological method (oocyte diameter) would be important to confirm patterns observed from qualitative analyses. Barber and Blake (1981) suggest that qualitative staging is necessary to describe the reproductive events

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pertaining to gamete development but that detailed quantitative information eliminates the subjectivity and semantic problems associated with qualitative description and thereby enhance our ability to extract ecologically meaningful information.

Several authors have reported the relationship of the reproductive cycle with energy storage and utilization cycles and with local environmental conditions in a wide variety of bivalves (e.g. Costa Muniz et al., 1986; Fernandez Castro and Vido de Mattio, 1987; Massapina et al., 1999; Camacho et al., 2003). In general, reserves accumulate prior to gametogenesis in the form of glycogen, lipid and protein substrates, and subsequently are utilized in the production of gametes when metabolic demand is high (Mathieu and Lubet, 1993). However, that relationship has not been studied in *S. solida*. The main objectives of this study were to determine from reproductive staging of histological preparations (using qualitative and quantitative criteria), from condition index analyses, and from biochemical composition assays, the reproductive cycle and the pattern of energy storage and utilization in a population of *S. solida* from the Algarve (southern Portugal).

2. Materials and methods

2.1. Sample collection

Forty-five adult specimens (30 to 33 mm shell length) were collected monthly during 2003 by local dredgers from a site off Fuzeta (Algarve, southern Portugal; Fig. 1) at water depths ranging from 4 to 8 m. Mean monthly surface seawater temperature (SST) data were provided by Tunipex. SST was measured daily with an YSI multiparameter probe.

2.2. Laboratory analysis

In the laboratory, clams were placed in 0.45 µm-filtered seawater at 20 °C for 24 h to purge their stomachs before histological, condition index and biochemical analyses. Following the 24 h purging period, each clam was dissected and wet meat weight was determined.

2.2.1. Histology

Ten individuals of each sex from each monthly sample were examined histologically to determine the gametogenic stages of both sexes. The visceral mass was separated from siphons and gills and fixed in San Felice solution for 24 h, then transferred to 70% ethyl alcohol (ETOH) for storage. For microscopic examination, tissues from these samples were dehydrated with serial dilutions of alcohol and embedded in paraffin. Thick sections (6–8 µm) were cut on a microtome and stained with haematoxylin and eosin. The histologically prepared slides were examined using a microscope at 40× magnification and each specimen was assigned to a stage which represented the gonad condition. Clam reproductive maturity was categorized into six stages using the scale proposed by Gaspar and Monteiro (1998). When more than one developmental stage occurred simultaneously within a single individual, the staging criteria decision was based upon the condition of the majority of the section. For each one of those stages a numerical ranking was assigned as follows (Gosling, 2003): Inactive (0); Early active (3); Late active (4); Ripe (5); Partially spawned (2); Spent (1). A mean gonad index (GI) was then calculated using the method proposed by Gosling (2003):

$$GI = \frac{\sum n^{o} \text{ individuals from each development stage} \times \text{stage numerical ranking}}{n^{o} \text{ total individuals in each sampling month}}$$

The GI ranged from 0 (all individuals in the sample are resting) to 5 (all individuals are ripe).

The images of each slide were recorded with a Nikon DSFi 1 camera and subsequently analysed using the freely available image analysis software Image J 1.38s. In order to obtain quantitative data the diameter of all oocyte with visible nuclei from five randomly selected female images were was measured.

2.2.2. Condition index

Dry meat and shell weights of 20 clams from each sample were determined after oven drying at 80 °C for 24 h. Meat samples were then ashed at 450 °C in a muffle furnace, ash weight determined, and organic matter weight calculated as the ash free dry meat weight (AFDW). The condition index (CI) was calculated according to Walne and Mann (1975):

$$CI = \frac{\text{dry meat weight (mg)} - \textit{ash} \text{ (mg)}}{\text{dry shell weight (mg)}} \times 100$$

2.2.3. Biochemical composition

The meat of five clams of each monthly sample was frozen and stored at −20 °C for biochemical analyses. For each specimen, protein was determined using the modified Lowry method (Shakir et al., 1994), glycogen content was determined from dried (80 °C for 24 h) homogenate using the anthrone reagent (Viles and Silverman, 1949) and total lipids were extracted from fresh homogenised material in chloroform/methanol (Folch et al., 1957) and estimated spectrophometrically after charring with concentrated sulphuric acid (Marsh and Weinstein, 1966). Duplicate determinations were performed in all cases and values are expressed as a percentage of AFDW. Caloric

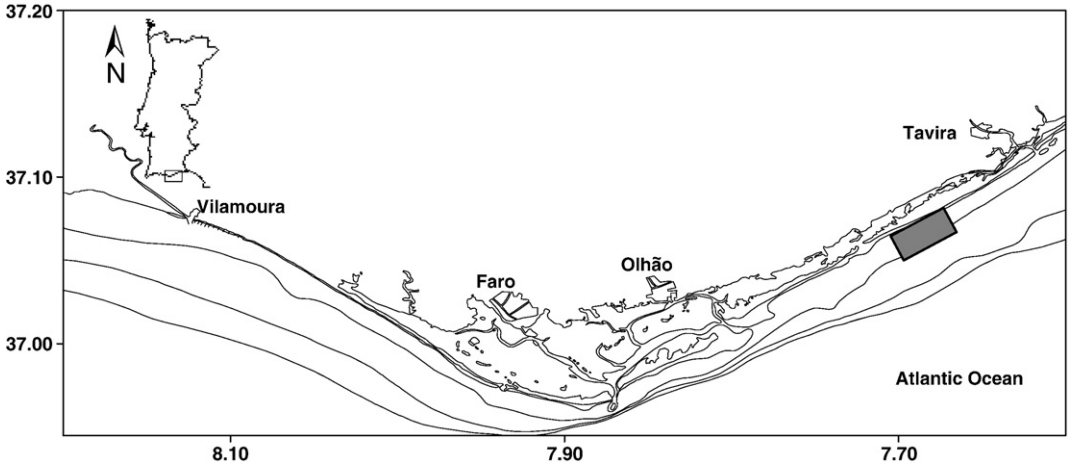

Fig. 1. Location on the Algarve coast where *Spisula solida* were collected.