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Feeding behavior of the mussel *Mytilus galloprovincialis* (L.) in a Mediterranean estuary: A field study

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ABSTRACT

The feeding behavior of the mussel *Mytilus galloprovincialis* was investigated in the field on top of a mussel raft in Alfacs Bay, NW Mediterranean Sea. The experiments were performed in November 2006 and February, April and July 2007 using a flow-through filter feeding device. Total particulate matter (TPM), particulate organic matter (POM) and particulate inorganic matter (PIM) were calculated for the bay water, as well as the feces and pseudofeces of the mussels. These were used with the biodeposition method to estimate several feeding-physiological parameters, such as the clearance rate (CR), rejection rate through pseudofeces production (RP), organic ingestion rate (OIR), absorption rate (AR) and absorption efficiency (AE). The results showed that the characteristics of available suspended matter for mussels in terms of particle concentration (TPM: mg/l) and organic content (*f* defined as POM/TPM) ranged 1.03–2.30 mg/l, and 0.48 to 0.73 respectively throughout the study period. This indicates a rather stable feeding environment of low concentrations of high organic content particles despite the wide range of temperatures recorded (from 10 to 26 °C). However, a characteristic of such a variation pattern of particle suspension (TPM and *f*) was that short-term variations (in the course of days) covered the whole range of annual variation. Accordingly, physiological parameters characterizing both food acquisition and absorption in mussels were found to respond to short-term variations in food regime. Pseudofeces production in mussels was low (less than 5% in most cases) and they tended to reduce their clearance rate instead of increasing their pseudofeces production in response to rising particle concentration. The absorption efficiency was positively related to the organic content of the seston particles. There was also a positive correlation between clearance rate and absorption efficiency. The reduction of clearance, ingestion and absorption rates obtained in July highlights a negative influence of high water temperatures upon feeding and digestive processes of mussels.

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1. Introduction

Mussels are filter feeding bivalves, ideal for aquaculture due to their wide distribution and adaptability. Mussels are harvested for human consumption worldwide (Figueiras et al., 2002; Mortensen et al., 2006) with production of the species *Mytilus galloprovincialis* and *Mytilus edulis* above 300,000 t per year (FAO, 2009), mostly cultured in Spain, the leading mussel producer in Europe (Pérez Camacho et al., 1991; Keldany, 2002). Mussels (*M. galloprovincialis*) on the Spanish Mediterranean coast are mainly cultured in the two Ebro Delta bays, Fangar and Alfacs, with an annual production of 3000 t per year (Ramón et al., 2005a). They are cultured in suspension on a total of 166 fixed rafts divided between the bays of Alfacs (90 farms) and Fangar (76 farms). One raft is composed by rectangular wooden frames measuring 200 m × 15 m, from which 2 to 3 m long mussel ropes are suspended. Mussel growth follows a seasonal pattern in Fangar Bay, with higher rates from March to May and the lowest rates in winter. The growth and mortality of mussels cultured in the two bays are affected greatly by the high seawater temperatures reached during July and August, which lead to a cessation in growth and high mortalities of adults and juveniles (Ramón et al., 2005a).

Areas that have bivalve cultures are characterized by high primary production that can sustain the grazing pressure of the bivalves. Facing the Mediterranean Sea, Thau Lagoon (SE France) has high primary production (Gasc, 1997), with particulate organic matter (POM) values ranging from 0.1 to 1.7 mg l^{-1} (Gangnery et al., 2004). The study site, Alfacs Bay (NE Spain), has a chlorophyll *a* level that is one order of magnitude above the surrounding Mediterranean Sea (Delgado, 1987). POM values in the study site range from 1 to 3.4 mg l^{-1} (Ramón et al., 2005b) higher than in the Galician Rías (NW Spain), the largest mussel

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producer in Europe (Pérez Camacho et al., 1991; Keldany, 2002). In addition to the POM, food quality expressed as POM/TPM can have a significant influence on the growth of bivalves due to its positive effect on the food absorption efficiency (Bayne et al., 1987; Navarro et al., 1991; Urrutia et al., 1996). There is a high percentage of organic content in the water of both Ría de Arousa and Alfacs Bay, and values can reach up to 50–60% (Babarro et al., 2003; Ramón et al., 2005b).

Different approaches have been used to study the feeding behavior of mussels. Foster-Smith (1975) calculated filtration rates from the particle removal rates from a fixed volume of suspension. For a better approach to natural conditions, other authors estimated clearance rates by monitoring the removal of suspended particles as water passed through mussels; therefore, the filtration rates could be calculated as the clearing of water particles from the environmental water (Riisgård, 1977; Bayne and Widdows, 1978; Widdows et al., 1979). Similarly, Yahel et al. (2005) based his filtration rates study on the simultaneous pair wise collection of the water inhaled and exhaled by some filter feeding invertebrates in situ by means of scuba diving. Nevertheless, this technique cannot be used in some environments due to, i.e. weather conditions. Therefore, there was need to design new devices which simulated real conditions in order to better understand the feeding behavior in the field. Smith and Wikfors (1998) developed an automated rearing chamber system for studies of shellfish feeding behavior, and Babarro et al. (2000) used a portable box raft experimental chamber to conduct experiments in situ on top of mussel rafts. Different portable filter feeding devices have been designed in order to better understand the feeding physiological parameters of mussels (Filgueira et al., 2006; Grizzle et al., 2006). Nowadays, there are different methods for measuring filtration rates in suspension feeding bivalves, each of which should be used according to the particular experimental conditions and taking into account their advantages and disadvantages (reviewed by Riisgård, 2001).

Ecological and commercial information on the mussel *M. galloprovincialis*, one of the most important bivalve species in the Mediterranean Sea, is still needed in this area. Many studies have been conducted with *M. edulis*; however, the results may not be totally applicable to *M. galloprovincialis*. Studies have been performed with these species on the Atlantic side of their geographical distribution, but the Atlantic environmental conditions, especially the temperature regime, are quite different to the Mediterranean conditions. In this sense, there is a lack of physiological data that would help to predict the potential for mussel cultivation in the Mediterranean (Sarà and Pusceddu, 2008).

The aim of the present study is to determine the main physiological parameters related to feeding behavior of the mussel *M. galloprovincialis* in a Mediterranean estuary where bivalve aquaculture takes place. For a more realistic approach the experiments were carried out in the field at four different periods of the year. These results will be useful to improve shellfish aquaculture management in Alfacs Bay.

2. Materials and methods

2.1. Experimental design and animals

The filter feeding experiments were performed from November 2006 to July 2007 on top of a mussel raft in Alfacs Bay, NW Mediterranean Sea. Four filter feeding experiments, lasting 2 h each, were carried out per sampling period (i.e., November 2006, and February, April and July 2007), corresponding to 2 consecutive days in 2 consecutive weeks in each period, except for July, in which only 3 experiments were carried out. Each experiment was conducted with a different group of mussels of similar sizes; the mussel sizes in each sampling period corresponded to the growth cycle in the area.

Mussels, *M. galloprovincialis*, were collected from a mussel aquaculture farming site in Alfacs Bay (Ebro Delta) the day before each experiment. Twenty mussels per experiment were collected, and epiphytes and other encrusting organisms were removed from the shells. A little plastic hook and loop fastener was glued to one of the two shells of each individual. When the glue dried, mussels were hung back on the raft where the experiments were performed the following day. Acclimation was not necessary as the mussels were always submerged in the bay water. Fake mussels were made by collecting four of the twenty fresh mussels, taking out the flesh and gluing the shells back together to act as controls.

2.2. Flow-through devices

Two portable filter feeding flow-through devices were designed to simulate in vivo conditions of mussel feeding (Fig. 1). One portable filter feeding device consisted in a common PVC tank (length 560 mm, width 300 mm, height 150 mm) that received bay water from an underwater pump hung from the mussel raft poles at 1 m depth. Laterally, the tank was provided with an extra flow exit tube. Aeration was added to mix the bay water in the common tank. Ten rubber tubes emerged from the lower part of the tank and each tube was connected to an individual PVC aquarium. The individual aquaria measured $45 \times 180 \times 60$ mm (length × width × height). Each aquarium contained a single mussel except for two of the aquaria, which contained one fake mussel each and were used as controls. The mussels were positioned near the flow exit tube of the aquaria and attached to the bottom with a piece of plastic hook and loop fastener. The flow of bay water from the common tank to each aquarium was regulated through a manual valve and maintained at a constant rate of $12\ l\,h^{-1}$. This flux was determined by previous laboratory experiments and $12\ l\,h^{-1}$ showed a homogeneous distribution of particles between aquaria and no water recirculation occurred in any of the aquaria.

2.3. Characteristics of the bay water

Bay water (1 l) from the tanks with fake mussels (acting as controls) was collected every 15 min. The feces and pseudofeces of each mussel were collected throughout the experiment with a pipette. All samples (water, feces and pseudofeces) were filtered separately through washed Whatman GF/C filters (25 mm Ø) and rinsed with ammonium formate. In the laboratory, filters with water samples were dried at 60 °C for 48 h and weighed to obtain the dry weight, which accounted for the total particulate matter (TPM). Afterwards, filters were ashed at 450 °C for 4 h before the final weighing to obtain the particulate inorganic matter (PIM). The particulate organic matter (POM) was calculated as the weight loss between TPM and PIM. The organic content of the bay water (f) was calculated as the average fraction between POM and TPM.

2.4. Physiological feeding parameters

Mussels were placed in the individual chambers of the feeding-devices and allowed to rest to recover from any stress associated with handling. The individual chambers were cleaned before the beginning of the experiment. The feces and pseudofeces of each mussel were then collected separately with a pipette as soon as they were produced. During approximately 2 h we collected enough biodeposits to end the experiment. Feces and pseudofeces produced by each mussel ($n = 16$) were filtered individually and processed for organic and inorganic matter (as indicated for the water samples) in order to compute the total, organic and inorganic rates of egestion and rejection respectively. The physiological components of the absorptive balance (Table 1) were then calculated according to the biodeposition method (Iglesias et al., 1998).

The ingestion rates of the total matter (TIR: mg/h) and organic matter (OIR: mg/h) were obtained as the difference between the filtration and rejection rates of either the total or organic matter. In order to quantify the preingestive selection of food through pseudofeces production, we expressed rejection as a percentage of

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