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Allometric relationships in feeding and digestion in the Chilean mytilids *Mytilus chilensis* (Hupé), *Choromytilus chorus* (Molina) and *Aulacomya ater* (Molina): A comparative study

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ABSTRACT

As a first attempt to explain the functional basis of the co-occurrence of three sympatric species of native mussels frequently found at the cold-temperate coasts of Chile, we tested the existence of potential morpho-functional differences operating at the feeding and digestive levels of *Mytilus chilensis*, *Choromytilus chorus* and *Aulacomya ater*. Clearance rate (CR ($l\ h^{-1}$)), gill area (GA: mm^2), dry weight of labial palps (W_{LP}: mg), gut contents (GC: mg), weight of digestive gland (W_{DG}: mg), specific (mg of maltose or tyrosine mg $prot^{-1}\ h^{-1}$) and total (mg of maltose or tyrosine digestive $gland^{-1}\ h^{-1}$) amylase, cellulase, laminarinase, xylanase and protease activities were measured in different size individuals of the three species collected from Corral Bay (Chile). Hydrolytic activity of digestive extracts towards natural substrates elaborated from different macrophyte was also tested for the 3 species. No significant differences between species were found for mass-exponents (b) scaling CR (b = 0.744) and GA (b = 0.803). However, size-specific CR and GA were found to vary between species, ranking as follows: *M. chilensis* > *C. chorus* > *A. ater*. W_{LP} fitted to an inter-specific common function (b = 0.77). Regarding digestive parameters, no significant inter-specific differences were found between mass-exponents for GC (b = 0.434), W_{DG} (b = 1.067) and total enzyme activities (common b ranged from 0.904 to 1.212), except total amylase which was significantly higher in *A. ater* (b = 1.838). Specific activities of digestive enzymes were size-independent except *A. ater* amylase, which increased with body-size, and protease in the three species which decreased with size. For a common body-size, GC and carbohydrase activities were significantly higher in *M. chilensis* than in *C. chorus* and *A. ater*. Inter-specific differences were found in the composition of carbohydrase pool: amylase was the prevalent enzyme in *M. chilensis* (up to 44.5% of total carbohydrase) whereas the three carbohydrases had a similar contribution in *C. chorus* and *A. ater*. Sugar release from vascular plants was significantly higher in *C. chorus*.

1. Introduction

Dense communities of mussels constitute a universal ecological component of coastal environments. A characteristic feature of these extensive mussel beds is the coexistence of a certain number of different mussel species that compete for space and food. Among them, those belonging to the genera *Mytilus*, *Chroromytilus*, *Aulacomya* and *Perna* have been found to be the most frequently dominant species.

There have been a few comparative studies analyzing possible inter-specific differences in the physiological energetics of mussels. Results obtained from comparing several of the most abundant species in South African coasts (*Choromytilus meridionalis*, *Perna perna*, *Aulacomya ater* and the recently introduced *Mytilus galloprovincialis*) led to the following main conclusions: *M. galloprovincialis* achieves the highest filtering activity and scope for growth, attaining the highest growth rates and highest fecundity values (Van Erkom Schurink and Griffiths, 1991, 1992, 1993), whereas ii) *A. ater* behaves as a slow food consumer and grower (Griffiths and King, 1979; Van Erkom Schurink and Griffiths, 1993) presenting 4–5 times lower rates of growth than *Mytilus* and *Choromytilus* (Van Erkom Schurink and Griffiths, 1993).

Similar inter-specific differences have been observed in comparative studies performed with mussels from New Zealand: Gardner (2000, 2002) and Helson et al. (Helson and Gardner, 2007; Helson

Abbreviations: CR, clearance rate; GA, gill area; W_{LP}, dry weight of labial palps; GC, gut content; W_{DG}, dry weight of digestive gland; p_c, protein content in the digestive extract; A_{spc}, specific amylase activity in the digestive gland; C_{spc}, specific cellulase activity in the digestive gland; L_{spc}, specific laminarinase activity in the digestive gland; X_{spc}, specific xylanase activity in the digestive gland; P_{spc}, specific protease activity in the digestive gland; A_{tot}, total amylase activity in the digestive gland; C_{tot}, total cellulase activity in the digestive gland; L_{tot}, total laminarinase activity in the digestive gland; X_{tot}, total xylanase activity in the digestive gland; P_{tot}, total protease activity in the digestive gland.

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et al., 2007) reported that *M. galloprovincialis* and *Perna canaliculus* attain significantly highest clearance rates and scopes for growth than *Aulacomya maoriana* over a broad range of seston concentrations and compositions.

In the cold-temperate coasts of Chile, three native mussel species, the Chilean blue mussel *Mytilus chilensis* (chorito) the black mussel *Choromytilus chorus* (choro) and the ribbed mussel *A. ater* (cholga) form dense beds in intertidal and subtidal sedimentary and rocky bottoms, where their cultivation or direct harvesting constitutes one important sea resource (Carranza et al., 2009). Although there is scanty information on growth rates and maximum attainable size in these species, evidence suggests a similar relationship to that found for South African and New Zealander mussels as regards inter-specific comparisons: namely individuals belonging to the genus *Mytilus* behave as fast growers whilst those belonging to *Aulacomya* are slow growers (Winter et al., 1984). For instance *M. chilensis* attains a mean shell-length of 83 cm in 3 years, whereas it takes 5 years to *A. ater* to achieve the commercial size of 70 cm, which roughly represents a 2 fold difference in growth rate.

The occurrence of fast and slow growing sympatric mussel species reveals a great plasticity and provides an excellent opportunity to analyze the adaptability of the morpho-functional traits participating in food acquisition, digestion and absorption in related species of bivalves. Certainly, the comparative frame has been unevenly developed in the literature concerning feeding and digestive processes in bivalves. Comparisons of feeding activities for two or more species submitted to the same food conditions have been performed in many occasions (Bacon et al., 1998; Cranford and Hill, 1999; Hawkins et al., 1998; MacDonald and Ward, 2009; Navarro et al., 2011; Smaal et al., 1997; Velasco and Navarro, 2003, and above referred literature regarding co-occurring mussels) and inter-specific differences in clearance rate linked in some cases to relative ctenidial surface area (Hawkins et al., 1990; Navarro et al., 2011). On the contrary, information about inter-specific differences in parameters related to digestive performance is very poor. Experimental comparison of gut contents has been undertaken only twice (Bayne et al., 1984; Hawkins et al., 1990). Concerning enzyme activities, there are many early surveys on the distribution of digestive enzymes (reviews Purchon, 1978; Vonk and Western, 1984), but only a few quantitative studies comparing the composition of the digestive pool and its possible contribution to the feeding strategies of species (Albentosa and Moyano, 2009; Brock and Kennedy, 1992; Labarta et al., 2002; Lucas and Newell, 1984; Seiderer et al., 1982; Simon and Mcquaid, 1999). Conclusions regarding environmental aspects of the comparative biochemistry of digestive processes are difficult to achieve from above literature since the studies include heterogeneous taxonomic groups and also because computation of allometric relationships of parameters, a factor which is essential for inter-specific comparison, is absent in most of the cases.

Here we present part of the results obtained in a series of experiments designed to analyze the feeding and digestive behaviour of three mussel species *M. chilensis*, *C. chorus* and *A. ater*, co-occurring in Corral bay (Chile). The aim of the present contribution was to achieve inter-specific comparisons concerning i) clearance rates and gut contents measured under the same feeding conditions as well as the relative size of the organs participating in food uptake (gills and labial palps) and digestion (digestive gland), in order to evaluate the capacity of the different mussel species to acquire and allocate food, and ii) the activity of the main digestive enzymes (amylase, cellulase, laminarinase, xylanase, and unspecific proteases) in extracts of the digestive gland, combined with the assessment of the activity of these extracts towards natural substrates obtained from different plant tissues (digestibility). With the exception of digestibility of natural substrates, all these measurements were performed in specimens largely differing in size, so that allometric relationships could be computed and statistically compared among species.

2. Material and methods

2.1. Experimental set-up and manipulation of mussels

Groups of individuals of *M. chilensis*, *C. chorus* and *A. ater* measuring 30, 50 and 70 mm mean shell-length were sampled from subtidal populations at Corral Bay (39º53′S; 73º25′W) by scuba diving during January 2008. Specimens of *M. chilensis* and *A. ater* were taken from rocky substratum, whereas those of *C. chorus* were collected from an adjacent gravel-sandy substratum. Although *C. chorus* can colonize rocky substrates (Carranza et al., 2009), the distribution of this species in Corral Bay appears limited to the gravel-sandy and stony bottoms of the bay.

15 individuals of each species (5 per size group) were placed in a feeding tank and fed for 2 days a diet consisting of cells of the microalgae *Isochrysis galbana* mixed with ashed particles of superficial sediment collected from an intertidal mudflat. The sediment was added to achieve an organic percentage in the diet of approximately 70%. Concentrated stocks of the diet were dosed into the experimental tank at rates set to provide for a stable concentration between 1.5 and 2 mg l^{-1}. After 2 days mussels were placed in filtering chambers and clearance rate was individually measured. Subsequently, mussels were dissected to determine the dry weight of soft tissues.

Another 15 individuals of each species (5 per size group) were placed in an experimental tank, fed for 2 days the same diet used in the feeding experiment and subsequently submitted to gut evacuation for the analysis of allometric relationship of gut contents. Following gut evacuation mussels were dissected and their gill area (mm^2) and dry weight of their labial palps (mg) were determined (see Section 2.4) to compute the allometric relationship of these structures involved in food acquisition.

A different group of 21 individuals (7 per size group) from each species was used to perform *in vitro* assays of digestive enzyme activities. The digestive gland of individual mussels was dissected and used to measure the activities of i) the four main carbohydrases (amylase, cellulase, laminarinase and xylanase), ii) unspecific protease and iii) *in vitro* digestibility of organic particle obtained from various vascular plants and macroalgae (Section 2.5).

2.2. Feeding experiments

2.2.1. Characteristics of experimental diet

Composition of the diet was determined in duplicated water samples taken at regular intervals from the feeding tanks, that were filtered onto ashed pre-weighed GF/C glass-fiber filters and subsequently processed to determine concentrations (mg l^{-1}) of total particulate matter (TPM), and particulate inorganic (PIM) and organic matter (POM). TPM and PIM were estimated, respectively, as the dry and ash weight increment of the filters, and POM as the difference between TPM and PIM.

2.2.2. Experimental determination of clearance rate

Clearance rate (CR: l h^{-1}) of individual mussels were measured after placing each mussel in a flow-through feeding chamber and leaving them to resume normal feeding. CR was calculated as $CR = F(Ci - Co)/Ci$, being F the water flow rate through each experimental feeding chamber, Ci the concentration of particles in the water entering the chambers (measured at the outflow of a control chamber with an empty mussels-shell placed in it), and Co the concentration of particles measured from the outflow of each experimental feeding chamber. Particle concentrations were always determined as the average of at least two replicate samples, measured with a particle size analyzer Elzon 180 XY equipped with a 120 μm aperture diameter counting tube. Water samples used for determination of suspended particle concentration (in weight) and organic content were collected every hour for a period of 5–6 h.