Vol. 474: 167–177, 2013
doi: 10.3354/meps10093

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published January 31

Particle selection and regulation of particle uptake by the slipper limpet *Crepipatella fecunda*

O. R. Chaparro[1,*], J. A. Montory[1], S. V. Pereda[1], R. J. Thompson[2], G. Rivera[1], S. J. A. Osores[1], C. J. Segura[1]

[1]Instituto de Ciencias Marinas y Limnológicas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile
[2]Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland A1C 5S7, Canada

ABSTRACT: We established the role of the pallial filter in the suspension-feeding slipper limpet *Crepipatella fecunda* (Gastropoda, Calyptraeidae), which inhabits coastal areas of southern Chile characterised by sediment resuspension resulting from tidal forces. We determined that this species is capable of qualitative and quantitative pre-ingestive selection of particles. We exposed individual limpets to diets composed of a mixture of sediment and microalgae and measured the particle concentrations in the inhalent region, the infrabranchial cavity and the exhalent region, allowing us to derive an electivity index individually for the pallial filter and the gill. At a particle concentration at which pseudofaeces are always produced, and regardless of the proportion of sediment in the diet, the pallial filter selectively removed sediment (inorganic) material from suspension and diverted it into the lateral canal of the mantle for rejection. Qualitative particle selection on the gill was observed when the proportion of microalgae in the diet was low and microalgae were preferentially retained. When the diet was composed entirely of sediment, the gill retained only 15% of the suspended particles. The gill is more a collector than a selector of particles, while the pallial filter regulates the concentration of particles entering the pallial cavity and increases the nutritional quality of the suspension available for removal by the gill, thereby partially compensating for the high proportions of inorganic matter in the resuspended sediment which the limpet encounters during the tidal cycle, especially in the periods immediately before exposure to air and after reimmersion.

KEY WORDS: Particle selection · *Crepipatella* · Seston

INTRODUCTION

Sessile suspension-feeders usually experience temporal variations in the quantity and quality of food available (Navarro et al. 1993, Chaparro et al. 2008). The density and composition of the seston is influenced in the long-term by factors such as seasonal changes in phytoplankton production and in the short-term by stochastic factors such as resuspension of sediments by tidal forces, intense rainfall and high winds (Yukihira et al. 1999, Chaparro et al. 2008). Thus the food intake, and hence the energy balance, of the suspension-feeder can fluctuate according to changes in the particle field (Armsworthy et al. 2001, Velasco & Navarro 2002, Fernández-Reiriz et al. 2004).

Many suspension-feeders capture food particles on the gills and transfer the material to the mouth for ingestion (e.g. Winter 1978). This mechanism is widespread in bivalve molluscs, but is less common in gastropods (Chaparro et al. 2002, Barillé et al. 2006). In the calyptraeid gastropods *Crepipatella dilatata* and *C. fecunda*, the radula and gill are both used for food collection, the importance of each depending on the stage of sexual development of the individual (Chaparro et al. 2002, 2004, Navarro & Chaparro 2002, Montiel et al. 2005). These calyptraeid species are protandric hermaphrodites, and when the individual

*Email: ochaparr@uach.cl

becomes female, and usually sedentary, the gill develops into the principal feeding structure. Many calyptraeids attach to rocks or boulders in the intertidal zone (Chaparro et al. 2004), often in places where mud and/or sand are readily resuspended (Navarro et al. 1993, Barillé et al. 1997). Resuspension is often associated with tidal flows, and results in changes in the particle field (Wildish & Kristmanson 1979, Anderson & Meyer 1986) and hence the nutritional environment of any suspension-feeder, although most of the information available is for bivalves (Berg & Newell 1986, Hawkins et al. 1998). The suspension-feeder must adapt to such variations in the food supply, especially to prevent overloading of the feeding structures when the seston concentration is very high, and most (including the gastropod *C. fecunda*; Chaparro et al. 2004) develop mechanisms to separate particles according to quality or size and reject the least desirable material as pseudofaeces, especially when there is an excess of particles in the water column. In bivalves, elimination of particles may occur as a result of their low nutritional value (MacDonald & Ward 1994, Navarro & Widdows 1997, Hawkins et al. 1998, Ward et al. 1998, Velasco & Navarro 2002, Fernández-Reiriz et al. 2004), large size (Defossez & Hawkins 1997), or toxicity of harmful algae (Wikfors 2005).

Pseudofaeces production is a well-known mechanism in bivalve molluscs, in which the gills and labial palps play central roles (Winter 1978, Ward & Shumway 2004), but it has not been extensively studied in suspension-feeding gastropods. Members of the family Calyptraeidae produce more than one type of pseudofaeces, each originating in a different anatomical region or structure of the animal. Chaparro et al. (2004) identified pseudofaeces from 3 sources in the slipper limpet *Crepipatella* (=*Crepidula*) *fecunda*: the gill, the food pouch and the lateral tract of the mantle (inhalent area). Those of branchial origin result from excessive production of mucous cords for ingestion rather than the rejection of indigestible material or particles of unsuitable size or quality. Pseudofaeces formed in the food pouch and the lateral canal of the mantle have a common origin (Chaparro et al. 2002), mainly from particles that arrive in the inhalent area and then encounter the pallial filter (Werner 1953, see also review by Fretter & Graham 1994). Although the mechanism is not well understood, the collection of particles in this area is apparently well developed in the calyptraeid *C. fecunda*, which lives in habitats characterised by frequent resuspension of sediment (Chaparro et al. 2004). This increase in suspended inorganic matter not only reduces the quality of the food available to the suspension-feeder, but may also overload the gill, so that any mechanism that limits entry of particles into the pallial cavity may be adaptive in maintaining gill function.

Crepipatella fecunda, a species from southern Chile, is common and abundant in the intertidal zone of shores dominated by muddy sand, and is therefore exposed to frequent resuspension of bottom sediments. The purpose of this study was to characterise the particle field created by resuspension events associated with the tidal cycle and to investigate the response of the limpet to the resulting variation in food availability by means of controlled experiments using prepared diets with different organic contents. We also examined the production of pseudofaeces and tested the hypothesis that particle selection can take place in the inhalent region, permitting the limpet to regulate the quality and/or quantity of particles that enter the mantle cavity and are available for capture by the gill.

MATERIALS AND METHODS

Resuspended particulate material in the intertidal

Water samples were taken at 30 min intervals throughout a semidiurnal tidal cycle during October spring tides (range approximately 5.5 m) from the intertidal zone of Yaldad Bay (43° 07′ S, 73° 44′ W), in the south of Chile, where a dense population of *Crepipatella fecunda* is located. Sampling was initiated just as the rising tide began to cover the individuals, and ended when the falling tide left them exposed to the air. The water was collected by gentle suction from a syringe through an aquarium air hose (diameter 5 mm) positioned 5 cm above the bottom, immediately adjacent to the limpets, with the tip inclined upwards at an angle of approximately 45° to minimise disturbance of the underlying sediment. Before each sample was taken, all seawater lying in the hose was removed with the syringe and discarded. Samples (100 to 200 ml, depending on the concentration of seston) were taken in triplicate for gravimetric analysis of the seston, and the volumes recorded. Further samples (100 ml in triplicate) were taken at the same time for determination of particle concentration and size distribution.

Water samples for gravimetric analysis were immediately passed under gentle vacuum through a prewashed, precombusted (450°C), weighed filter (Whatman GF/C, 25 mm diameter). For determination of total particulate matter (TPM), particulate organic matter (POM) and particulate inorganic matter (PIM),

loaded filters were gently washed with distilled water to remove salts, dried at 60°C for 24 to 48 h, cooled in a desiccator and weighed. They were then combusted for 5 h at 450°C, cooled in a desiccator and reweighed. The weight of uncombusted material (ash) represented PIM, POM being obtained by difference.

A Beckman Coulter Z2 particle counter fitted with a 50 µm diameter tube, calibrated with latex beads of known diameter, was used to determine the number of particles and their size distribution in the remaining water samples.

Collection and maintenance of limpets

Sessile female slipper limpets *Crepipatella fecunda* of shell length 40 to 50 mm were collected from the intertidal zone in Yaldad Bay, southern Chile. They were transported to the laboratory, detached from the natural substrate and allowed to adhere to transparent acrylic plates (each 10 × 6.5 × 0.3 cm, 1 specimen per plate). On the reverse side of each plate, 3 holes (diameter 2 mm) were drilled beneath the limpet in specific locations: (1) the inhalent zone of the pallial cavity, (2) the area between the inhalant zone and the gill (infrabranchial section of the pallial cavity; Fretter & Graham 1994) and (3) the exhalent (postbranchial) zone (Fig. 1). The reattached limpets were then returned to holding tanks with flowing seawater (temperature 12°C, salinity 30) and fed daily with cultured algae *Isochrysis galbana*. The holes were left open during observation periods, but plugged at all other times.

Preparation of diets and video recording of experimental animals

Seven diets were prepared with various proportions of sediment (inorganic matter) and microalgae (mostly organic matter): 100, 80:20, 60:40, 50:50, 40:60, 20:80 and 100% microalgae:sediment. Sediment was obtained at low tide from the uppermost

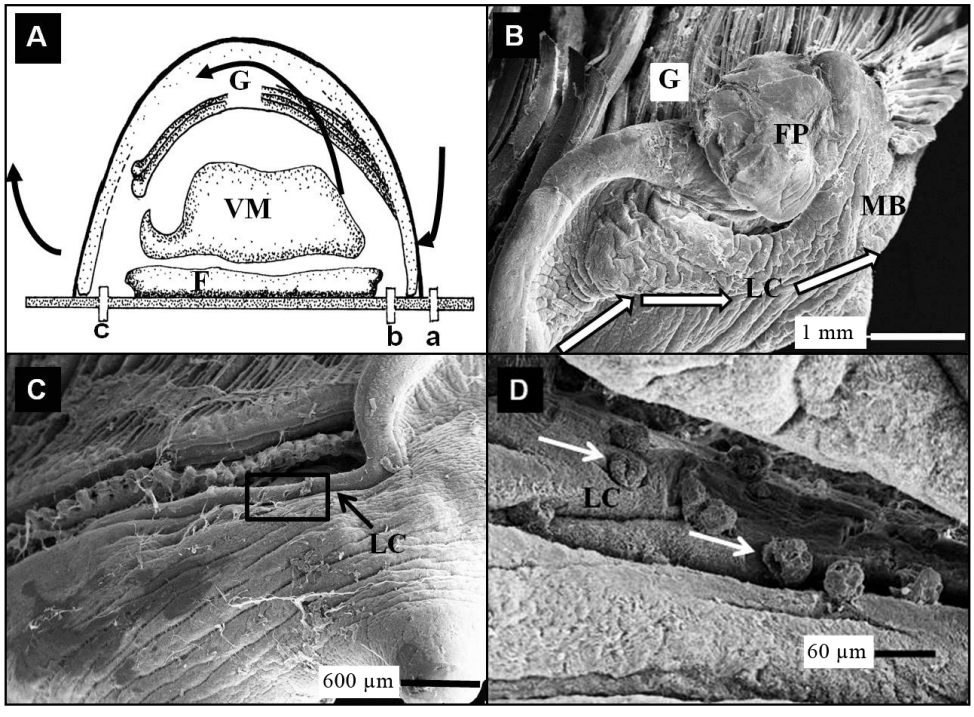

Fig. 1. *Crepipatella fecunda*. (A) Frontal view of a transverse section through an individual adhering to a plastic sheet, showing insertion points of canulae from which water samples were removed during experimental treatments. a: inhalent zone of the pallial cavity; b: area between the inhalant zone and the gill (infrabranchial section of the pallial cavity; Fretter & Graham 1994); c: exhalent (postbranchial) zone. Arrows: direction of water flow. (B,C) Scanning electron microscope (SEM) view showing the connection between the lateral canal and the food pouch area, where pseudofaeces originating in the pallial filter are eliminated from the mantle cavity. (D) Higher resolution image of the area within the rectangle in (C): SEM view of the lateral canal with particles (arrows) moving to the anterior region, probably to be discarded as pseudofeces. F: foot; FP: food pouch; G: gill; LC: lateral canal; MB: mantle border; VM: visceral mass. White arrows in (B): direction of pseudofaeces transport in the lateral canal towards the mantle margin for elimination to the exterior

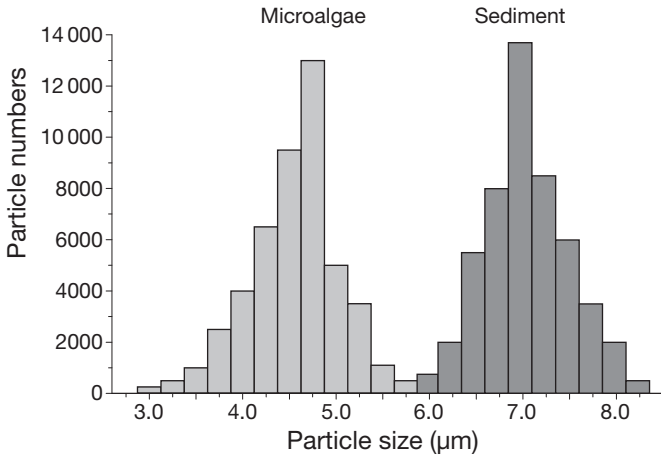

Fig. 2. Size distribution (equivalent spherical diameter, ESD) of the mixed suspension of microalgal cells (mean = 4.7 µm, SD = 1.02) and sediment particles (mean = 7.1 µm, SD = 1.03) used for preparing experimental diets. In this case, the diet is 50% microalgae and 50% sediment (50:50). Particle concentration approximately 1×10^5 ml^{-1}

layers of the beach from which the limpets were collected and passed through a series of screens with decreasing porosity. The fraction between 5 and 10 µm, which contained most of the suspended particles, was retained, dried for 48 h at 60°C and combusted in a muffle furnace (450°C, 4 h). The inorganic residue was sonicated, yielding a suspension of particles with a mean equivalent spherical diameter (ESD) of 7.1 ± 1.03 µm, mean ± SD (Fig. 2). For the organic fraction, a monoculture of *Isochrysis galbana* (mean ESD = 4.75 ± 1.02 µm) was maintained in f/2 medium (Fig. 2). Particle numbers were determined with a Beckman Coulter Z2 particle counter, as previously described. A concentration of approximately 1×10^5 particles ml^{-1} was maintained in each experimental diet, a level at which pseudofaeces are always produced by *Crepipatella fecunda* (Chaparro et al. 2004).

Table 1. Proportions of microalgae:sediment and organic: inorganic content in the experimental diets (mean ± SD, total n = 42)

Microalgae: sediment diet (%)	Fraction (%) Organic	Inorganic
100:0	75 (1.86)	25 (2.68)
80:20	26.8 (1.45)	73.2 (1.45)
60:40	14.8 (0.49)	85.2 (0.49)
50:50	10 (0.63)	90 (0.63)
40:60	7.5 (0.67)	92.5 (0.75)
20:80	2.5 (0.20)	97.5 (0.31)
0:100	1.5 (0.15)	98.4 (0.16)

A known volume of each diet suspension was passed under gentle vacuum through a Whatman GF/C filter (diameter 47 mm). PIM and POM were estimated as described above for seston samples. Organic material comprised 0 to 78% of TPM, depending on the diet (Table 1).

Each diet suspension was diluted with 20 l filtered seawater (0.5 µm, salinity 30) in a header tank maintained at 16 ± 1°C. Particles were kept in suspension by recirculating the water with a pump and by continuous aeration. The diluted suspension was fed by gravity to a series of 250 ml chambers in which the experimental limpets were held, one per chamber. Each specimen, affixed to an acrylic plate, was positioned so that the ventral surface was uppermost, allowing the investigator to observe the mantle cavity structures through a dissecting microscope fitted with a charge coupled device (CCD) camera connected to a video recorder and monitor. Recordings of feeding behaviour were made for periods of up to 4 h and included a size scale for reference. Of particular importance is the lateral canal of the mantle, formed by a ciliated depression which connects the pallial filter with the anterior region and through which particles destined for elimination as pseudofaeces are transported (Chaparro et al. 2004) (Fig. 1B–D). The activity of the lateral canal during periods of particle rejection, expressed as the time in which the canal contained material as a proportion of total time, was observed from video sequences. Images were captured with an ATI 'All in Wonder' frame-grabbing card and processed using Scion Image Pro Plus 3.0 software.

Diet quality and production of pseudofaeces

Before observations were made, a canula was placed in each of the holes previously drilled in the acrylic plate to which the limpet was affixed. After the specimen had been ventilating for at least 30 min, the first water samples were taken from the canulae through pasteur pipettes. Three samples (10 ml each) were carefully removed from each canula during the experiment: one at the beginning, one near the midpoint and one at the end, such that disruption of the pallial filter was minimal.

The density of particles in each sample and their size distribution were determined with a Beckman Coulter Z2 particle counter, as described above, allowing us to estimate the proportions of algae and sediment material in the 3 regions sampled, the inhalent, the infrabranchial cavity and the exhalent,

and thus identify qualitatively and quantitatively the material retained in each (Fig. 1A). Retention percentages for sediment particles and for microalgae were estimated with reference to the diet offered (in the case of retention by the pallial filter) or with reference to the post-pallial filter particle concentration (in the case of retention by the gill).

Electivity index

An electivity index (EI) (Jacobs 1974, modified by Baker & Levinton 2003) was calculated for both the pallial filter and the gill to express the degree of acceptance or rejection of inorganic and organic particles by each. The index was calculated as follows:

$$EI = -\{(S - P) / [(S + P) - (2 \times P \times S)]\} \quad (1)$$

where EI = electivity index; P = proportion of particles of interest in pseudofaeces (number of the specific particle/total particles); S = proportion of particles of interest in suspension (number of the specific particle/total particles).

For calculation of an EI for the pallial filter, pseudofaeces were defined as those particles that were retained by the pallial filter in the inhalent region. In the case of the gill EI, pseudofaeces were defined as those particles not retained by the gill (comparing the number of particles arriving on the gill, i.e. in the infrabranchial cavity, and leaving the gill, i.e. in the exhalent). Values for EI lie between −1 and +1. Depending on the EI under consideration, a positive value indicates that the specific particle is preferentially retained for ingestion (e.g. gill), whereas a negative value means that the particle retained is rejected by the organism (e.g. pallial filter). In each case, an EI of zero indicates that there is no selection of particles at that level.

Statistical analysis

A 1-way repeated measures ANOVA was used to identify significant differences in the concentrations of TPM, POM and PIM in the water column at various times and the mean diameter of the suspended particles. One-way ANOVA was used to compare among treatments (diets) the proportion of total particles retained by the pallial filter and by the gill, as well as the time that particles were observed leaving the lateral tract as a proportion of total time.

Two-way ANOVA was employed to examine differences among diet treatments in the proportions of microalgae and sediment retained by the pallial filter and the gill. Single sample *t*-tests were used to determine whether EI values for the pallial filter and the gill were significantly different from zero.

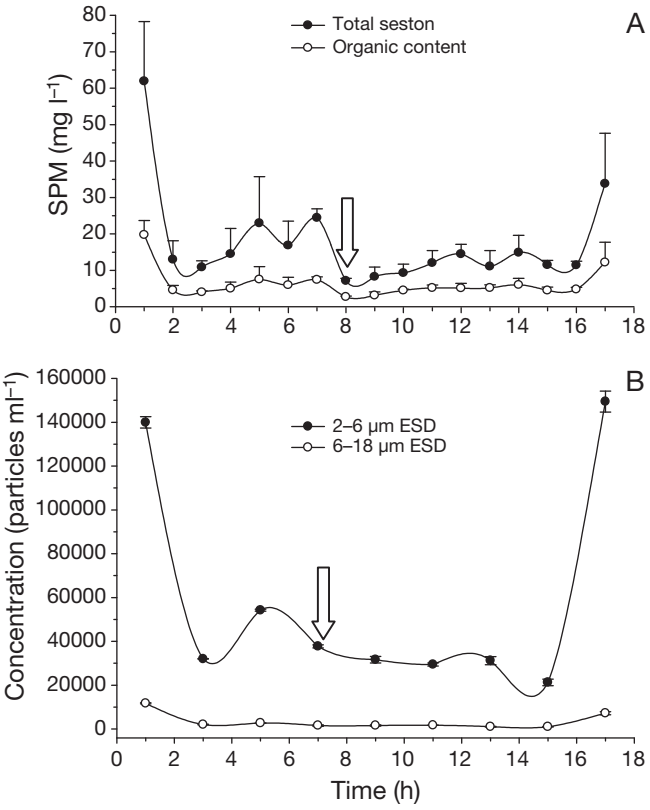

Fig. 3. Temporal variation during a tidal cycle of (A) suspended particulate matter (SPM), showing total and organic particulate matter for near-bottom samples and (B) particle concentration for 2 particle size classes: 2 to 6 and 6 to 18 µm equivalent spherical diameter (ESD). Arrow indicates high tide. Values are means ± SD

RESULTS

Suspended particulate matter (SPM)

Temporal differences were recorded in TPM, POM and PIM in water samples taken close to the limpets (1-way repeated measures ANOVA, $F_{(32,68)} = 9.97$, $p < 0.0001$). Highest values were observed at the beginning of the immersion period, when the tide was flooding (mean values = 62, 20 and 42 mg l^{-1} for TPM, POM and PIM, respectively), and at the end, when the limpets were emerging during the ebbing tide (mean values 34, 12 and 22 mg l^{-1}, respectively) (Fig. 3A). Values generally remained constant, especially for POM, during the rest of the tidal cycle.

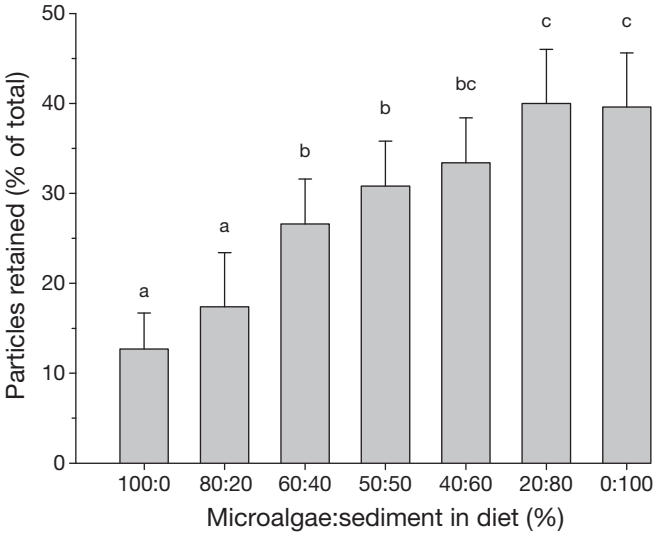

Fig. 4. *Crepipatella fecunda*. Number of particles captured by the pallial filter for limpets fed diets with varying proportions of organic and inorganic matter (mean ± SD, n = 10 for each treatment). Numbers are expressed as a percentage of total particles entering the inhalent region at a concentration of 1×10^5 particles ml^{-1}. Treatment means marked with the same letter are not significantly different, and vice-versa

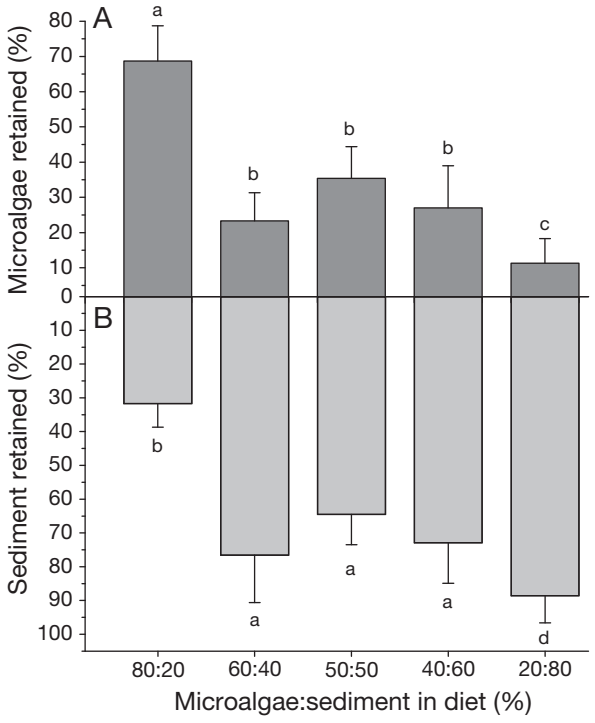

Fig. 5. *Crepipatella fecunda*. Effect of diet treatment on the proportion of (A) organic (microalgal) and (B) inorganic (sediment) particles retained by the pallial filter and channeled into the lateral canal for expulsion as pseudofaeces (mean ± SD, n = 10 for each treatment). Treatment means marked with the same letter are not significantly different, and vice-versa

For both size classes of particles (2 to 6 µm and 6 to 18 µm ESD), significant differences were observed in the concentration of particles in water samples taken at different points in the tidal cycle (1-way repeated measures ANOVA, $F_{(8,19)} = 1219.36$, $p < 0.0001$). The former were more numerous (ca. 140×10^3 particles ml^{-1}) than the latter, but in both classes concentrations were highest at the beginning of the period of immersion of the limpets on the flooding tide and at the moment of emersion at low tide (Fig. 3B). During the rest of the tidal cycle values were 3 or 4 times lower.

Inhalent region: retention of particles by the pallial filter according to diet quality

The percentage of total particles retained in the inhalent region was an increasing function of the proportion of inorganic matter in the diet, and varied from 13% in a pure suspension of algae to 40% in a suspension of sediment with no algae present (Fig. 4; 1-way ANOVA, $F_{(6,60)} = 34.17$, $p < 0.001$). Conversely, the proportion of particles that entered the infrabranchial cavity decreased at higher sediment concentrations.

There was a significant effect of diet on the proportion of microalgae retained by the pallial filter (2-way ANOVA, $F_{(5,102)} = 108.61$, $p < 0.0001$). For the 80:20 (microalgae:sediment) diet, microalgae represented approximately 70% of the material retained for subsequent expulsion as pseudofaeces, decreasing to 10% in the 20:80 diet (Fig. 5A). Conversely, the proportion of sediment in the material retained in the inhalent area was greater in diets containing more sediment (2-way ANOVA, $F_{(5,102)} = 11.188$, $p < 0.002$), increasing from 32% in the 80:20 diet to 87% in the 20:80 diet (Fig. 5B). This preferential retention of sediment by the pallial filter in all diet treatments resulted in an enhancement in the quality of the food entering the infrabranchial cavity that differed among diet treatments (1-way ANOVA, $F_{(4,48)} = 7.63$, $p < 0.0001$, Fig. 6) from a mean of 2% enrichment for the 80:20 diet to 8–12% for the others (no significant difference among diets from 60:40 to 20:80).

Release of particles from the lateral canal

At the particle concentration used in these experiments, the lateral canal, a ciliary tract on the mantle wall extending anteriorly from the inhalent region,

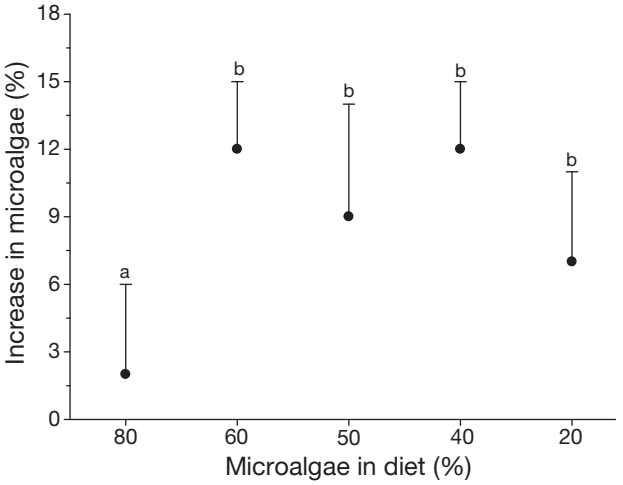

Fig. 6. *Crepipatella fecunda*. Effect of diet treatment on the percentage increase in numbers of organic particles (microalgae) after the suspension has traversed the pallial filter and reached the gill (mean ± SD, n = 10 for each treatment). Treatment means marked with the same letter are not significantly different, and vice-versa

was always active in transporting mucus-bound material. The period of time in which particles were released from the lateral canal per hour of observation time depended on the relative concentrations of microalgae and sediment (1-way ANOVA, $F_{(6,17)}$ = 3.94, p < 0.01; Fig. 7), and increased from approximately 30 s h^{-1} of observation for the 80:20 diet to 12 min for the 20:80 diet.

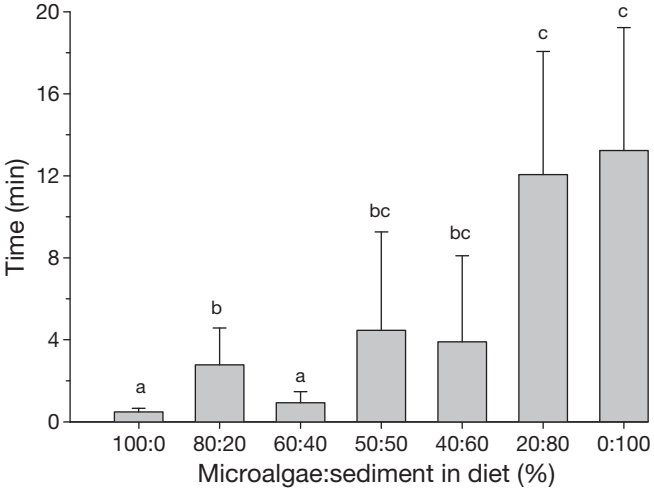

Fig. 7. *Crepipatella fecunda*. Effect of diet treatment on the period of time in which particles were recorded leaving the lateral canal of the mantle during 1 h of observation (mean ± SD, n = 8 for each treatment). Treatment means marked with the same letter are not significantly different, and vice-versa

Gill

The proportion of particles entering the pallial cavity that was retained by the gill differed among diet treatments (1-way ANOVA, $F_{(6,60)}$ = 26.93, p < 0.0001; Fig. 8). At 100:0 (pure microalgae), 56% of the particles were captured by the gill, and for diets from 80:20 to 40:60 the mean varied from 43 to 60%. Particle retention was significantly lower for the 2 diets least rich in microalgae (31% for 20:80 and 13% for 0:100). The percentage of particles retained was always higher for microalgae than for sediment (2-way ANOVA, $F_{(4,95)}$ = 10.28, p < 0.0001), but diet quality had no influence on percentage retention of either particle type (2-way ANOVA, $F_{(4,95)}$ = 7.04, p = 0.57; Fig. 9).

Electivity index

The EI for microalgae in the inhalent region was not significantly different from zero for all diets (*t*-test, p = 0.83; Fig. 10A), demonstrating no preferential retention or rejection of these particles. In contrast, the mean EI for sediment across all diet treatments was −0.43 ± 0.2 SD, and EI was significantly less than zero for all diets (*t*-test, p < 0.001),

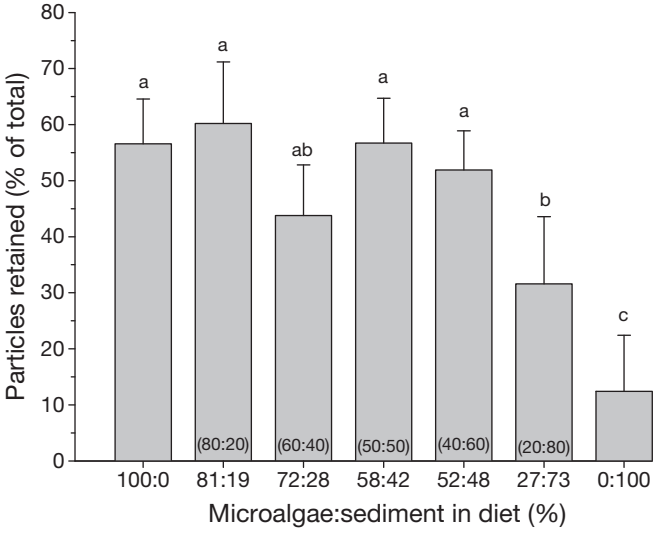

Fig. 8. *Crepipatella fecunda*. Effect of diet treatment on the percentage of total particles present that were retained by the gill (mean ± SD, n = 10 for each treatment). Values on the *x*-axis refer to the composition of the suspension in the infrabranchial cavity, i.e. pre-branchial condition. Corresponding values in parentheses refer to the original diet offered to the limpet, i.e. condition before the particles encountered the pallial filter. Treatment means marked with the same letter are not significantly different, and vice-versa

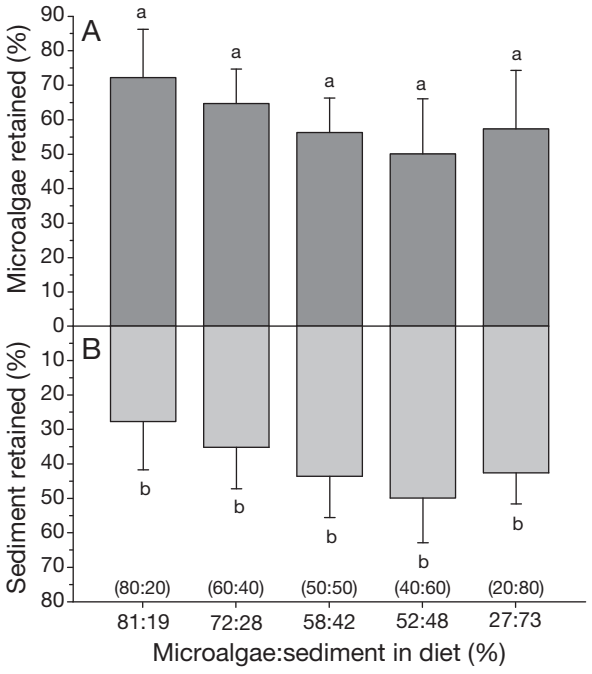

Fig. 9. *Crepipatella fecunda*. Effect of diet treatment on the percent retention of (A) microalgae and (B) sediment particles by the gill (mean ± SD, n = 10 for each treatment). Values on the *x*-axis refer to the composition of the suspension in the infrabranchial cavity, i.e. pre-branchial condition. Corresponding values in parentheses refer to the original diet offered to the limpet, i.e. condition before the particles encountered the pallial filter. Treatment means marked with the same letter are not significantly different, and vice-versa

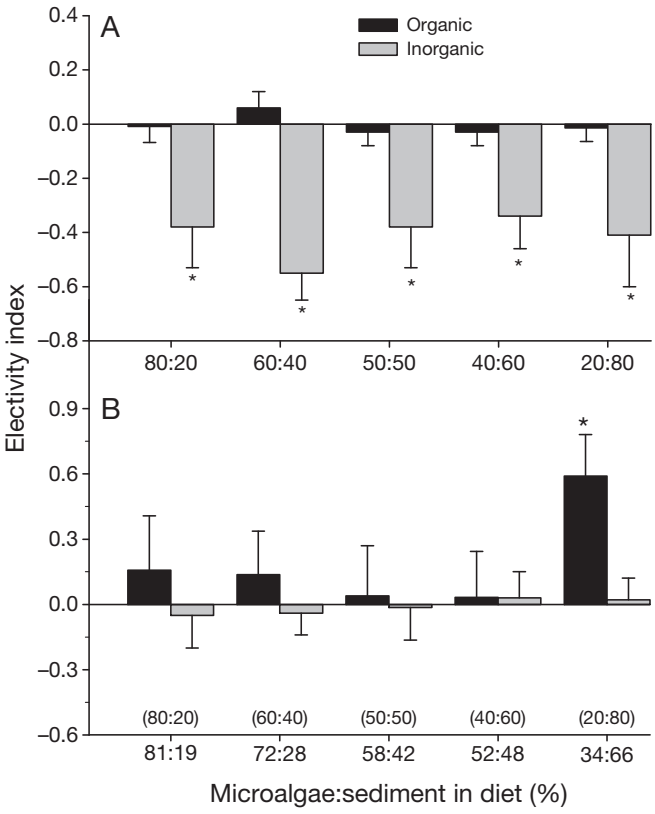

Fig. 10. *Crepipatella fecunda*. Effect of diet treatment on the electivity index for organic particles (microalgae) and inorganic particles (sediment) encountering (A) pallial filter (mean ± SD, n = 10 for each treatment) and (B) gill (mean ± SD, n = 10 for each treatment). In (B) values on the *x*-axis refer to the composition of the suspension in the infrabranchial cavity, i.e. pre-branchial condition. Corresponding values in parentheses refer to the original diet offered to the limpet, i.e. condition before the particles encountered the pallial filter. *Significantly different from zero

showing that sediment was selectively removed by the pallial filter and rejected as pseudofaeces.

In the case of the gill, the EI for microalgae was not significantly different from zero in diets containing less than 50% sediment (*t*-test, $p = 0.069$; Fig. 10B), but was significantly greater than zero for the 20:80 diet treatment (*t*-test, $p < 0.003$), demonstrating preferential retention of microalgae in the poorest diet. The EI for sediment was not significantly different from zero in any diet treatment (*t*-test, $p = 0.67$), indicating no selection by the gill for or against sediment.

DISCUSSION

Our data demonstrated substantial increases (up to 6 times) in seston concentrations associated with sediment resuspension during rising and falling tides in Yaldad Bay. Short-term variations in seston (concentration ranges of more than 10-fold) have been recorded in other marine environments, especially close to the bottom (Muschenheim & Milligan 1998). In closed bays such as Yaldad, the resuspended material from tidal and wind forces is mostly inorganic (Velasco & Navarro 2002). Similar patterns in TPM associated with the tidal cycle have been described at various locations (Great Sound, New Jersey, USA: Fegley et al. 1992; Kat O, Hong Kong: Wong & Cheung 2001). According to Velasco & Navarro (2002, 2003), most of the resuspended material in Yaldad Bay is composed of particles smaller than 3 to 15 µm ESD, although more than 80% of the particles are less than 7 µm ESD and within the size range utilised by the slipper limpet *Crepipatella fecunda*, which is abundant there.

During the tidal cycle, we observed 2 peaks in seston concentration (2 to 6 µm ESD class) in water samples taken adjacent to the limpets: one during the rising tide, just as the advancing wavelets reached the exposed individuals, the other during the falling tide, just as the water was receding from the immersed

limpets. Particles smaller than 10 µm frequently dominate estuaries and coastal areas (Widdows et al. 1979). The mean particle diameter of the seston in Great Sound, a tidal lagoon in New Jersey, USA, is 9 µm and most of the particles are smaller than 20 µm (Fegley et al. 1992). According to Chaparro et al. (2008), the water column in the Quempillén estuary, Chiloé island, southern Chile, is also dominated by small particles (<10 µm; mean = 3 to 6 µm)

During periods of resuspension, the food quality of the seston available to the suspension-feeder can be diluted by inorganic material, resulting in physiological responses such as the production and release of pseudofaeces. Most of the information available is for bivalves (e.g. *Perna viridis*: Wong & Cheung 2001; *Mytilus edulis*: Hawkins et al. 1997; *Cerastoderma edulis*: Urrutia et al. 1996, Navarro et al. 1998; *Crassostrea gigas*: Barillé et al. 1997). The calyptraeid gastropod *Crepidula fornicata* maintains a constant clearance rate when exposed to an increase in the concentration of suspended particles and a concomitant decrease in particle quality (Barillé et al. 2006). These authors recorded an increase in biodeposits, including pseudofaeces, which is consistent with our observations of *Crepipatella fecunda*, but they found no evidence for or against pre-ingestive particle selection, either qualitative or quantitative. Navarro & Chaparro (2002) found that *C. fecunda* has the capacity to graze the substrate with the radula and select particles for ingestion. Sessile adults of this species possess 3 mechanisms for producing pseudofaeces: the lateral canal of the mantle wall, the food pouch and mucous cords from the gill (Chaparro et al. 2004). Chaparro et al. (2004) also observed that pseudofaeces production was associated with high algal concentrations, not particle quality. As the algal concentration approached 1×10^5 cells ml^{-1} *Isochrysis galbana*, mucus-bound particles entered the lateral canal via the pallial filter, which under these conditions regulated the number of particles reaching the pallial cavity. This was reflected in an increase in the percentage of time during which the canal contained mucus-bound particles and released them to the exterior. In our study, the lateral canal was active at the high particle concentration we used (1×10^5 particles ml^{-1}), regardless of diet quality, but the percentage of the time spent releasing particles to the exterior increased with the proportion of sediment in the diet. The canal was active more than 30% of the time at the highest sediment level, and approximately 40% of the particles were retained by the pallial filter. Sediment particles were preferentially retained in all diets (combinations of microalgae and sediment) tested, allowing a suspension of higher quality to enter the pallial cavity for capture by the gill. This enhancement of food quality ranged from 2 to 12% with respect to the suspension entering the inhalent cavity, higher values being associated with greater concentrations of sediment.

Thus, in *Crepipatella fecunda*, the pallial filter and lateral canal regulate the number of particles entering the pallial cavity under high seston loads, whether the material be primarily organic (e.g. microalgae, Chaparro et al. 2004) or inorganic, but this is more evident when the proportion of sediment is high. This observation suggests that there may also be a mechanism for qualitative particle selection at this level, and we found preferential retention of sediment in the diet with the lowest proportion of microalgae. Furthermore, the EI data demonstrate unequivocally that the pallial filter selectively removes sediment (inorganic material) from the system, regardless of the proportions of inorganic and organic matter present in the suspension, and transfers it to the lateral canal. This material is normally rejected as pseudofaeces from the antero-lateral region of the mantle edge, but at low particle concentrations many or all of the mucus-bound particles in the lateral canal reach the food pouch, from which they are removed by the radula, either for ingestion or rejection (Chaparro et al. 2004). The second function of the pallial filter is, therefore, to increase the proportion of organic material in the suspension arriving at the gill.

Pseudofaeces production and elimination contribute to the success of the suspension-feeder in highly turbid environments (Navarro & Widdows 1997), including those in which *Crepipatella fecunda* is found (Chaparro et al. 2002), and, when combined with qualitative selection of particles, enable the individual to compensate for the severe dilution of the organic component of the seston associated with high levels of resuspension of inorganic material (MacDonald & Ward 1994, Hawkins et al. 1998).

In our experiments, 55 to 60% of the particles arriving on the gill of *Crepipatella fecunda* were retained when 40% or more of the particles in suspension were microalgae, but at high sediment concentrations a much lower proportion was captured. When the suspension was composed only of sediment, less than 15% of the material was retained by the gill, the rest being released through the exhalant area. The EI indicated that there was selection by the gill in favour of organic particles in the diet suspension having the greatest proportion of sediment, but not in the other diet treatments. Thus, unlike the pallial filter, the gill behaves more as a collector than a selector of

particles, although it can select organic particles from a suspension dominated by inorganics. This non-specific retention of particles is consistent with the mechanism by which the particles are trapped, a mucous sheet that overlies the branchial filaments in *C. fecunda* (Chaparro et al. 2002) and *C. fornicata* (Werner 1953, Fretter & Graham 1994). In contrast, bivalve molluscs that possess a heterorhabdic gill often exhibit selection of particles on the branchia, enriching the material that is directed towards the mouth, e.g. *Pecten maximus* (Beninger et al. 2004), *Crassostrea virgina* and *Crassostrea gigas* (Ward et al. 1998) and *Crassostrea gigas* (Dutertre et al. 2007).

Particulate material that accumulates in the mucous cord on the tips of the branchial filaments in *Crepipatella fecunda* may be eliminated as pseudofaeces when there is too much material to ingest (Chaparro et al. 2002), but most of it is directed towards the mouth for ingestion. No particle selection occurs at this level. Selective removal of inorganic material by the pallial filter and its elimination via the lateral canal, therefore, increases the quality of the ingested material and partially compensates for the high inorganic content of resuspended particulates available to this species in turbid environments such as Yaldad. Thus, the pallial filter plays 2 important roles in the feeding behaviour and physiology of *C. fecunda* in an environment characterised by high particulate loads: regulation of the particle density of the suspension entering the mantle cavity and concentration of organic particulates for capture by the gill.

Acknowledgements. This research was supported by FONDECYT-Chile Grant No. 1100335 to O.R.C.

LITERATURE CITED

Anderson FE, Meyer LM (1986) The interaction of tidal currents on a disturbed intertidal bottom with a resulting change in particulate matter quantity, texture and food quality. Estuar Coast Shelf Sci 22:19–29

Armsworthy SL, MacDonald BA, Ward JE (2001) Feeding activity, absorption efficiency and suspension feeding processes in the ascidian, *Halocynthia pyriformis* (Stolidobranchia: Ascidiacea): responses to variations in diet quantity and quality. J Exp Mar Biol Ecol 260:41–69

Baker SM, Levinton JS (2003) Selective feeding by three native North American freshwater mussels implies food competition with zebra mussels. Hydrobiologia 505: 97–105

Barillé L, Prou J, Héral M, Razet D (1997) Effects of high natural seston concentrations on the feeding, selection, and absorption of the oyster *Crassostrea gigas* (Thunberg). J Exp Mar Biol Ecol 212:149–172

Barillé L, Cognie B, Beninger P, Decottignies P, Rincé Y (2006) Feeding responses of the gastropod *Crepidula fornicata* to changes in seston concentration. Mar Ecol Prog Ser 322:169–178

Beninger PG, Decottignies P, Rincé Y (2004) Localization of qualitative particle selection sites in the heterorhabdic filibranch *Pecten maximus* (Bivalvia: Pectinidae). Mar Ecol Prog Ser 275:163–173

Berg JA, Newell RIE (1986) Temporal and spatial variations in the composition of seston available to the suspension feeder *Crassostrea virginica.* Estuar Coast Shelf Sci 23: 375–386

Chaparro OR, Thompson RJ, Pereda SV (2002) Feeding mechanisms in the gastropod *Crepidula fecunda.* Mar Ecol Prog Ser 234:171–181

Chaparro OR, Segura CJ, Navarro JM, Thompson RJ (2004) The effect of food supply on feeding strategy in sessile female gastropods *Crepidula fecunda.* Mar Biol 144: 79–87

Chaparro OR, Segura CJ, Montiel YA, Thompson RJ, Navarro JM (2008) Variations in the quantity and composition of seston from an estuary in southern Chile on different temporal scales. Estuar Coast Shelf Sci 76: 845–860

Defossez JM, Hawkins AJS (1997) Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis, Ruditapes philippinarum* and *Tapes decussatus.* Mar Biol 129:139–147

Dutertre M, Barillé L, Haure J, Cognie B (2007) Functional responses associated with pallial organ variations in the Pacific oyster *Crassostrea gigas* (Thunberg, 1793). J Exp Mar Biol Ecol 352:139–151

Fegley SR, MacDonald BA, Jacobsen TR (1992) Short-term variation in the quantity and quality of seston available to benthic suspension feeders. Estuar Coast Shelf Sci 34: 393–412

Fernández-Reiriz MJ, Labarta U, Navarro JM (2004) Feeding and digestive response of *Argopecten purpuratus* to short-term variation in food quality and quantity. Aquaculture 237:347–364

Fretter V, Graham A (1994) British prosobranch molluscs. Their functional anatomy and ecology. The Ray Society, London

Hawkins AJS, Smith RFM, Bougrier S, Bayne BL, Héral M (1997) Manipulation of dietary conditions for maximal growth in mussels, *Mytilus edulis*, from Marennes-Oléron Bay France. Aquat Living Resour 10:13–22

Hawkins AJS, Smith RFM, Tan SH, Yasin ZB (1998) Suspension-feeding behaviour in tropical bivalve molluscs: *Perna viridis, Crassostrea belcheri, Crassostrea iradelei, Saccostrea cucculata* and *Pinctada margarifera.* Mar Ecol Prog Ser 166:173–185

Jacobs J (1974) Quantitative measurement of food selection. Oecologia 14:413–417

MacDonald BA, Ward JE (1994) Variation in food quality and particle selectivity in the sea scallop *Placopecten magellanicus* (Mollusca, Bivalvia). Mar Ecol Prog Ser 108:251–264

Montiel YA, Chaparro OR, Segura CJ (2005) Changes in feeding mechanisms during early ontogeny in juveniles of *Crepidula fecunda* (Gastropoda, Calyptraeidae). Mar Biol 147:1333–1342

Muschenheim DK, Milligan TG (1998) Benthic boundary layer processes and seston modification in the Bay of Fundy. Vie Milieu 48:285–294

Navarro E, Urrutia MB, Iglesias JIP, Ibarrola I (1998) Tidal

variations in feeding, absorption and scope for growth of cockles (*Cerastoderma edule*) in the bay of Marennes-Oleron (France). Vie Milieu 48:331–340

Navarro JM, Chaparro OR (2002) Grazing-filtration as feeding mechanisms in motile specimens of *Crepidula fecunda* (Gastropoda:Calyptreaidae). J Exp Mar Biol Ecol 270:111–122

Navarro JM, Widdows J (1997) Feeding physiology of *Cerastoderma edule* in response to a wide range of seston concentrations. Mar Ecol Prog Ser 152:175–186

Navarro JM, Clasing E, Urrutia G, Asencio G, Stead R, Herrera C (1993) Biochemical composition and nutritive value of suspended particulate matter over a tidal flat of southern Chile. Estuar Coast Shelf Sci 37:59–73

Urrutia MB, Iglesias JIP, Navarro E, Prou J (1996) Feeding and absorption in *Cerastoderma edule* under environmental conditions in the Bay of Marennes-Oleron (Western France). J Mar Biol Assoc UK 76:431–450

Velasco LA, Navarro JM (2002) Feeding physiology of infaunal (*Mulinia edulis*) and epifaunal (*Mytilus chilensis*) bivalves under a wide range of concentrations and qualities of seston. Mar Ecol Prog Ser 240:143–155

Velasco LA, Navarro JM (2003) Energetic balance of infaunal (*Mulinia edulis* King, 1831) and epifaunal (*Mytilus chilensis* Hupé, 1854) bivalves in response to wide variations in concentration and quality of seston. J Exp Mar Biol Ecol 296:79–92

Ward JE, Shumway SE (2004) Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J Exp Mar Biol Ecol 300:83–130

Ward JE, Levinton JS, Shumway SE, Cucci T (1998) Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Mar Biol 131:283–292

Werner B (1953) Über den Nahrungserwerb der Calyptraeidae (Gastropoda Prosobranchia). Morphologie, Histologie und Funktion der am Nahrungserwerb beteiligten Organe. Helgol Wiss Meeresunters 4:260–315

Widdows J, Fieth P, Worrall CM (1979) Relationship between seston, available food and feeding activity in the common mussel *Mytilus edulis.* Mar Biol 50:195–207

Wikfors GH (2005) A review and new analysis of trophic interactions between *Prorocentrum minimum* and clams, scallops, and oysters. Harmful Algae 4:585–592

Wildish DJ, Kristmanson DD (1979) Tidal energy and sublittoral macrobenthic animals in estuaries. J Fish Res Board Can 36:1197–1206

Winter JE (1978) A critical review on some aspects of filter-feeding in Lamellibranchia. Haliotis 7:71–87

Wong WH, Cheung SG (2001) Feeding rhythms of the green-lipped mussel, *Perna viridis* (Linnaeus, 1758) (Bivalvia: Mytilidae) during spring and neap tidal cycles. J Exp Mar Biol Ecol 257:13–36

Yukihira H, Klumpp DW, Lucas JS (1999) Feeding adaptations of the pearl oysters *Pinctada margaritifera* and *P. maxima* to variations in natural particulates. Mar Ecol Prog Ser 182:161–173

Editorial responsibility: Roger Hughes, Bangor, UK

Submitted: April 17, 2012; Accepted: October 8, 2012
Proofs received from author(s): January 20, 2013