SELECTIVE INGESTION OF PELAGIC VERSUS BENTHIC ALGAE BY THE COCKLE *CERASTODERMA EDULE* (LINNÉ, 1758)

JOSE L. RUEDA[1]* AND AAD C. SMAAL[2]

[1]*Marine and Estuarine Ecology Unit, Department of Zoology and Entomology, The University of Queensland, Brisbane Qld 4072, Australia;* [2]*Netherlands Institute for Fisheries Research (RIVO), P. O. Box 77, 4400 AB Yerseke, The Netherlands*

ABSTRACT The pre-ingestive selection of microphytobenthic algae by the cockle *Cerastoderma edule* was studied in comparison with diets containing the pelagic diatom *Phaeodactylum tricornutum*. Treatments with the different diets covered a range of seston concentrations and organic content similar to field conditions. Rejection rates of *C. edule* exposed to the different treatments were significantly correlated with the concentration of total particulate matter. No significant differences in total rejection rates were found between pelagic and benthic diets. Organic rejection rate was significantly correlated with particulate organic matter of the treatments and no significant differences were found between both diets. Selection efficiency was significantly correlated with particulate organic matter concentration in both diets and no significant differences were found between the diets. Analysis of the pseudofeces composition by flow cytometry from cockles exposed to a mixed diet of microphytobenthic algae and *P. tricornutum*, showed a preferential ingestion of the pelagic diatom. Benthic species, such as small pennates and *Navicula* sp., were preferentially ingested in comparison to larger microphytobenthic species. The largest microphytobenthic species, *Cylindrotheca* sp., was significantly rejected. In general, *C. edule* is an opportunistic filter feeder that takes advantage of both pelagic and benthic algal cells.

KEY WORDS: Benthic diatoms, cockles, flow cytometry, rejection, selection efficiency, *Cerastoderma edule*

INTRODUCTION

Filter feeding bivalves are able to sort particles with their gills and labial palps (Kiørboe & Møhlenberg 1981, Newell & Jordan 1983, Newell et al. 1989, Ward et al. 1993) resulting in preferential ingestion of organic material and rejection of inorganic particles in pseudofeces. This preingestive selection has also been observed within phytoplankton with preferential ingestion of some groups of algal cells, both benthic and pelagic (Shumway et al. 1985, Bougrier et al. 1997, Baker et al. 1998). Preingestive selection of particles may be influenced by their chemical composition or by their size and shape.

Some studies about preingestive selection in bivalves have been carried out by using microscopic techniques to compare water and biodeposits or stomach contents in different species of deposit or filter feeder bivalves (Hummel 1985, Kamermans 1994, Shumway et al. 1987). Flow cytometry offers opportunities for the analysis of algal cells in experiments on the feeding behavior of bivalves (Shumway et al. 1985, Bougrier et al. 1997, Baker et al. 1998). Differential selection of various algal species has been demonstrated, using flow cytometric techniques by Peirson (1983) in the scallop *Argopecten irradians* (Lamarck 1819) and by Shumway et al. (1985) in *Ensis directus* (Conrad 1843), *Ostrea edulis* Linné, 1758, *Placopecten magellanicus* (Gmelin, 1791) and *Arctica islandica* (Linné, 1767). Shumway et al. (1985) showed that the flat oyster *Ostrea edulis* fed with a mixture of three algae of similar size (*Phaeodactylum tricornutum*, *Prorocentrum minimum*, and *Chroomonas salina*) preferentially ingested the dinoflagellate *Prorocentrum minimum*. In contrast, Cucci et al. (1985) have shown that individuals of *Mytilus edulis* Linné, 1758 fed with a mixed diet of the diatom *Phaeodactylum tricornutum*, the dinoflagellate *Prorocentrum*, and the cryptomonad flagellate *Chroomonas salina*, showed no differential ingestion of cells from suspension. Bougrier et al. (1997) found differences in the preingestive selection of different algal cells (mainly pelagic) by

Mytilus edulis and *Crassostrea virginica* (Gmelin, 1791) and related to differences in the shape of the algal cells. Recently Loret et al. (2000) have shown preferential ingestion of nanoflagellates and cryptophytes by the bivalve *Pinctada margaritifera* (Linné, 1758), by applying *in situ* HPLC techniques.

There is some controversy about the feeding behavior of bivalves, especially *C. edule*, exposed to benthic algae compared with pelagic algae, or the importance of microphytobenthos as a food source. Kamermans (1994) found microphytobenthic algae in the stomachs of the filter feeding bivalves *Mya arenaria* (Linné, 1758), *C. edule* and *M. edulis*, although the percentage of their abundance compared with pelagic algae, was higher in the deposit feeders *Scrobicularia plana* (Da Costa, 1778) and *Macoma balthica* (Linné, 1758). Recently two studies have been done measuring ratios of C and N isotopes in different primary producers and benthic invertebrates (Kang et al. 1999, Riera et al. 1999). These studies concluded that *C. edule* may prefer to ingest microphytobenthos and phytoplankton compared with detritus from fragments of benthic macro-algae and seagrasses or detritus from other origins. However, Herman et al. (2000), using also stable isotopes ratios, suggested that suspension feeders, such as *C. edule* and *M. arenaria*, depend almost exclusively on pelagic rather than microphytobenthic algae.

The aim of this study is to quantify the pre-ingestive selection of microphytobenthic algae in comparison with a pelagic species by the cockle *Cerastoderma edule*. A mixture of benthic and pelagic micro-algae occurs in the tidal flat environment of this filter feeder bivalve. The response of cockles exposed to the different diets was studied separately and also when both diets were mixed in the water column. In the latter case analysis were done by flow cytometry to analyze differential algal cell acceptance or rejection.

MATERIALS AND METHODS

Animals

In total 187 cockles (*Cerastoderma edule*) were used in the different experiments. Cockles had a shell length of 29.78 mm ±

---

*Corresponding author. E-mail: J.rueda@zen.uq.edu.au

1.27 (mean ± standard error) in experiments with microphytobenthos and 29.75 mm ± 0.46 in experiments with *Phaeodactylum tricornutum*); ash free dry weight was 255.75 mg ± 30.15 in experiments with microphytobenthos and 259.87 mg ± 20.55 in experiments with *P. tricornutum*. No significant differences were found in shell length or ash free dry weight of the animals used in both experimental treatments. All individuals were collected from an intertidal mudflat situated in the Oosterschelde estuary (SW Netherlands). Experiments were performed in spring 1997 and summer 1999, in a field laboratory at Jacobahaven (Oosterschelde estuary—SW Netherlands).

Shell length, dry tissue weight (DW) and ash-free dry tissue weight for each animal were determined. After measuring the shell length, animals were immersed in boiling water until the shell was opened. Soft tissues were excised, dried at 70°C for minimum 48 h in a drying oven and weighed (= dry weight). Ash-free dry weight is determined as the weight loss after ignition in a furnace at 520°C for 3 h.

Diets and Treatments

Two different diets were used in the different experiments: microphytobenthic algae (Benthic) and cells of *Phaeodactylum tricornutum* (Pelagic). Microphytobenthic algae were collected in the Oosterschelde estuary. The top layer of sediment (2–3 mm) was sampled and after sampling, coarse sediment and microphytobenthos were separated by mixing them in filtered seawater. After sedimentation of the large particles the water containing mainly benthic algae (based on observations with microscopy) was used for the experimental treatment. The microphytobenthic species were benthic diatoms, including species from the genus *Nitzschia* (length 61 ± 11 μm; width 7 ± 1 μm), *Navicula* (length 46 ± 11 μm; width 22 ± 3 μm), *Cylindrotheca* (length 81 ± 5 μm; width 23 ± 2 μm), and small pennate cells (length 30 ± 2 μm; width 6 ± 1 μm). The pelagic diatom *P. tricornutum* was cultured outdoors in 100-l reservoir tanks.

Pelagic and Benthic algae were added separately to filtered seawater in different concentrations (treatments) for both diets (Table 1). Each treatment was completed by adding silt particles collected in the same location as cockles and microphytobenthos. This silt was dried and burned at 520°C during 4 h for removing the organic content. A coulter counter was used to determine particle concentration, which was monitored during the experiment for differences of the particle concentration from the experimental treatments. Total particulate matter in the different experiments ranged from 40.2 mg.l⁻¹ to 161.2 mg.l⁻¹ and the organic content ranged from 9.08%–58.91% (see Table 1).

Analysis of Particulate Matter

Water samples of the different treatments were collected from the control chamber (without animal) at the start of the experiment and before collection of the biodeposits. Total particulate matter (TPM: mg.l⁻¹) was determined by filtering one liter sample onto pre-weighed and pre-ashed Whatman GF/C filters, rinsing with ammonium formate and drying at 70°C (minimum 24 h). Particulate inorganic matter (PIM: mg.l⁻¹) was estimated from the same filters as the weight after ignition during 3 h at 450°C. Particulate organic matter (POM) resulted from the difference of TPM and PIM. In all weight measurements an electronic balance was used which determined the nearest 0.01 mg. Dietary composition was

TABLE 1.

Characteristics of the different experiments for each diet and treatment. Concentrations of total particulate matter (TPM: mg · l⁻¹), particulate organic matter (POM: mg · l⁻¹), particulate inorganic matter (PIM: mg · l⁻¹) and percentage of organic matter from the seston (% OM).

Treatment code	TPM (mg · l⁻¹)	POM (mg · l⁻¹)	PIM (mg · l⁻¹)	OM (%)
Diet with *Phaeodactylum tricornutum*				
1p	48.90	4.44	44.46	9.08
2p	55.32	29.56	25.76	53.43
3p	58.82	8.18	50.64	13.91
4p	83.64	24.88	58.76	29.75
5p	92.40	17.00	75.40	18.40
6p	95.28	32.32	62.96	33.92
7p	125.84	15.04	110.80	11.95
8p	152.34	17.66	134.68	11.59
Diet with Microphytobenthos				
1m	48.68	28.68	20.00	58.92
2m	53.92	10.10	43.82	18.73
3m	72.52	13.76	58.76	18.97
4m	76.44	30.72	45.72	40.19
5m	80.96	28.04	52.92	34.63
6m	87.48	34.24	53.24	39.14
7m	98.40	20.98	77.42	21.32
8m	161.20	37.92	123.28	23.52
Mixed diet				
1mx	51.56	20.80	30.76	40.34
2mx	59.52	17.42	42.10	29.27
3mx	28.05	5.87	22.18	20.92

characterized in terms of organic content by weight (%OM = POM × 100/TPM), POM and TPM.

Experiments with Separate Diets

A flow-through system was used in all experiments. The water containing the different treatments was pumped to the experimental chambers from a diet-reservoir (300 L) in which a mixer and an air pump promoted the resuspension of particles. Peristaltic pumps (Watson Marlow) were used for pumping the water to the individual experimental chambers. The animals were placed individually in chambers of 300-ml volume and one chamber was used as a control for sedimentation of particles. The flow in all chambers during the experiments was between 3 and 4 l/h, and the concentration of particles in the outflow of the chambers containing animals was always more than 70% compared with the control chamber (without animals).

Collection of pseudofeces started after an acclimation period of 3 h to each treatment. Pseudofeces rejected during 3-h periods were collected 2 to 3 times separately for each individual. Pseudofeces samples were filtered on preweighed Whatman GF/C filters and measured according to the same procedure as described for samples of the diets. Rejection rates (mg.h⁻¹) of total (RR), organic (ORR) and inorganic (IRR) particulate matter were calculated. Rates were standardized to an equivalent 500 mg ash free dry tissue cockle by calculating the expression Ys = Ye (0.5/We)ᵇ, where Ys: rate of standard-sized cockle; Ye: uncorrected physiologic rate; We: measured ash free dry weight of experimental animal; b: allometric coefficient for clearance rate of cockles (b = 0.57; Urrutia 1997, Smaal et al. 1997).

Selection efficiency (SE) was calculated using the values for organic fraction of seston (f = POM/TPM) and pseudofeces (p = ORR/RR) from the different experiments. Values were calculated as: SE = (1-(p/f)) × 100.

Experiments with a Mixed Diet

In these series of experiments, cockles were exposed to a mixed diet and the same flow through system described in the previous paragraph was used. The diet contained a mixture of microphytobenthic algae and the diatom *P. tricornutum* (size 17 ± 2 μm). The different size of this pelagic species, and its fluorescence characteristics make it clearly distinguishable from the microphytobenthic species used (sizes between 30 ± 2 μm and 81 ± 11 μm). Individuals were acclimated 3 h to the mixed diet and pseudofeces produced by each animal were collected during periods of 1 h. Sample analysis by flow cytometry was done directly after the experiment. Another series of samples of pseudofeces was collected in the experiment for selection efficiency measurements.

Algal composition of diet and pseudofeces was analyzed using a flow cytometer (EurOPA: European Optical Plankton Analyser). Standard beads (1.07 μm, Duke Scientific, USA) were used for calibration and optical adjustments of the EurOPA instrument. A 529 nm and 633 nm laser were used for excitation. Laser light is scattered when a particle traverses the laser beam and is measured in forward and perpendicular (PLS) direction. Laser light irradiating autotrophic phytoplankton is partly emitted as fluorescence. Fluorescence emission excited by the green laser is measured in the red (FGR) and orange (FGO) bandwidth. The number of particles processed in the flow cytometric analysis was 5,000 and 20,000. Only data derived by FGR-triggering (i.e., fluorescent particles) were used to distinguish between groups of particles with different optical characteristics. The grouping or clustering of data was calculated by using the software program Matlab version 1.0.

The algal species studied are easy to distinguish in the scatter plots of the graph representing FGR and PLS data. The star-shaped form of *Phaeodactylum tricornutum* occupied a large area with values of PLS, between 1,500 and 2,200, and values of fluorescence FGR in general below 2,200 and above 1,900. Microphytobenthos were composed with mainly four subgroups (related to the most abundant species). The benthic diatom species with lower FGR and lower PLS was *Navicula* sp., together with a group of small pennate cells, which could not be identified with values of PLS (1,800–2,300) and FGR (1,900–2,200). *Nitzchia* species had a higher PLS value (2,400–2,500) and higher FGR (2,500–2,700). *Cylindrotheca* species displayed the highest PLS (almost 2,500) and the highest FGR (almost 3,000).

To examine the degree of acceptance or rejection of particle types (pelagic or benthic algae), we calculated an electivity index (EI) (Jacobs et al. 1974), modified by Baker et al. (1998), as follows:

$$EI = -[(P - S) / ((P + S) - (2 * P * S))]$$

where P is the particle ratio in the pseudofeces and S is the particle ratio in the suspension. Electivity index can range from −1.0 to 1.0. A positive EI for a given particle type indicates preferential ingestion (depletion of the particle type in the pseudofeces compared with the suspension), and a negative EI indicates rejection (enrichment of a particle type in the pseudofeces compared with the suspension).

Subsamples of the diet and pseudofeces were also fixed in Lugol's and the algal composition was determined using microscopic techniques for determination in addition to the flow cytometer results.

Statistics

All statistical tests were performed using the program SYSTAT for PC version 9.0. Multiple stepwise regression analyses were used to test for significant relationships between physiologic rates and parameters of the treatments. Analysis of covariance was used for comparing the rejection rates and selection efficiencies of cockles with the different diets. Electivity indices were compared with zero using a one-sample, two-tailed, nonparametric Wilcoxon signed-rank test. These analyses test the null hypothesis that selectivity of a particular type is equal to zero (no sorting). A t-student test was used to compare concentrations of the different algal species in samples of the diet and pseudofeces.

RESULTS

Response to Diets

Data about quality and quantity of the experimental treatments are shown in Table 1. All treatments were above pseudofeces threshold and the obtained physiologic values with the different diets and treatments have been listed in Table 2. Multiple stepwise regression analyses indicated a significant positive relationship between rejection rate (RR) and total particulate matter (TPM) from the treatments of both diets (Fig. 1; Table 3). The model rejected parameters POM and %OM. Analysis of covariance in-

TABLE 2.

Physiological response of cockles with the different diets and treatments: Selection efficiency (SE: %), rejection rate of pseudofeces (RR: mg · h⁻¹), organic rejection rate (ORR: mg · h⁻¹) and inorganic rejection rate (IRR: mg · h⁻¹). Mean value ± standard error. Number of measurements (n).

Treatment code	SE (%)	RR (mg · h⁻¹)	ORR (mg · h⁻¹)	IRR (mg · h⁻¹)	n
Diet with *Phaeodactylum tricornutum*					
1p	4.9 ± 6.5	29.3 ± 1.7	2.6 ± 0.3	26.8 ± 1.4	6
2p	46.1 ± 4.3	38.3 ± 7.2	10.8 ± 2.0	27.5 ± 5.5	10
3p	13.9 ± 9.4	22.3 ± 6.2	2.4 ± 0.7	19.9 ± 5.5	6
4p	36.8 ± 9.3	24.3 ± 5.0	5.9 ± 1.9	18.4 ± 3.1	12
5p	9.4 ± 2.5	54.3 ± 5.5	8.9 ± 0.7	45.3 ± 4.9	16
6p	41.9 ± 2.2	45.8 ± 2.7	9.1 ± 0.6	36.7 ± 2.3	12
7p	26.1 ± 3.4	96.4 ± 8.7	8.4 ± 0.6	88.0 ± 8.1	6
8p	14.5 ± 2.6	80.3 ± 5.5	8.0 ± 0.7	72.3 ± 4.9	10
Diet with Microphytobenthos					
1m	31.9 ± 4.1	34.7 ± 2.9	13.8 ± 1.1	20.9 ± 2.2	12
2m	18.7 ± 9.4	17.2 ± 3.1	2.6 ± 0.3	14.6 ± 2.9	6
3m	13.1 ± 9.6	27.4 ± 4.9	4.3 ± 0.6	23.2 ± 4.4	6
4m	34.9 ± 2.4	42.7 ± 1.6	11.1 ± 0.4	31.6 ± 1.5	15
5m	34.3 ± 2.5	70.9 ± 4.5	16.2 ± 1.1	54.7 ± 3.7	12
6m	43.1 ± 3.5	47.9 ± 6.4	10.1 ± 0.9	37.7 ± 5.7	10
7m	22.5 ± 1.9	50.8 ± 6.8	8.2 ± 1.0	42.7 ± 5.8	16
8m	37.5 ± 7.7	91.0 ± 13.2	13.6 ± 0.2	77.4 ± 13.0	6
Mixed diet					
1mx	25.1 ± 4.4	–	–	–	10
2mx	15.6 ± 3.5	–	–	–	6
3mx	6.6 ± 1.6	–	–	–	8

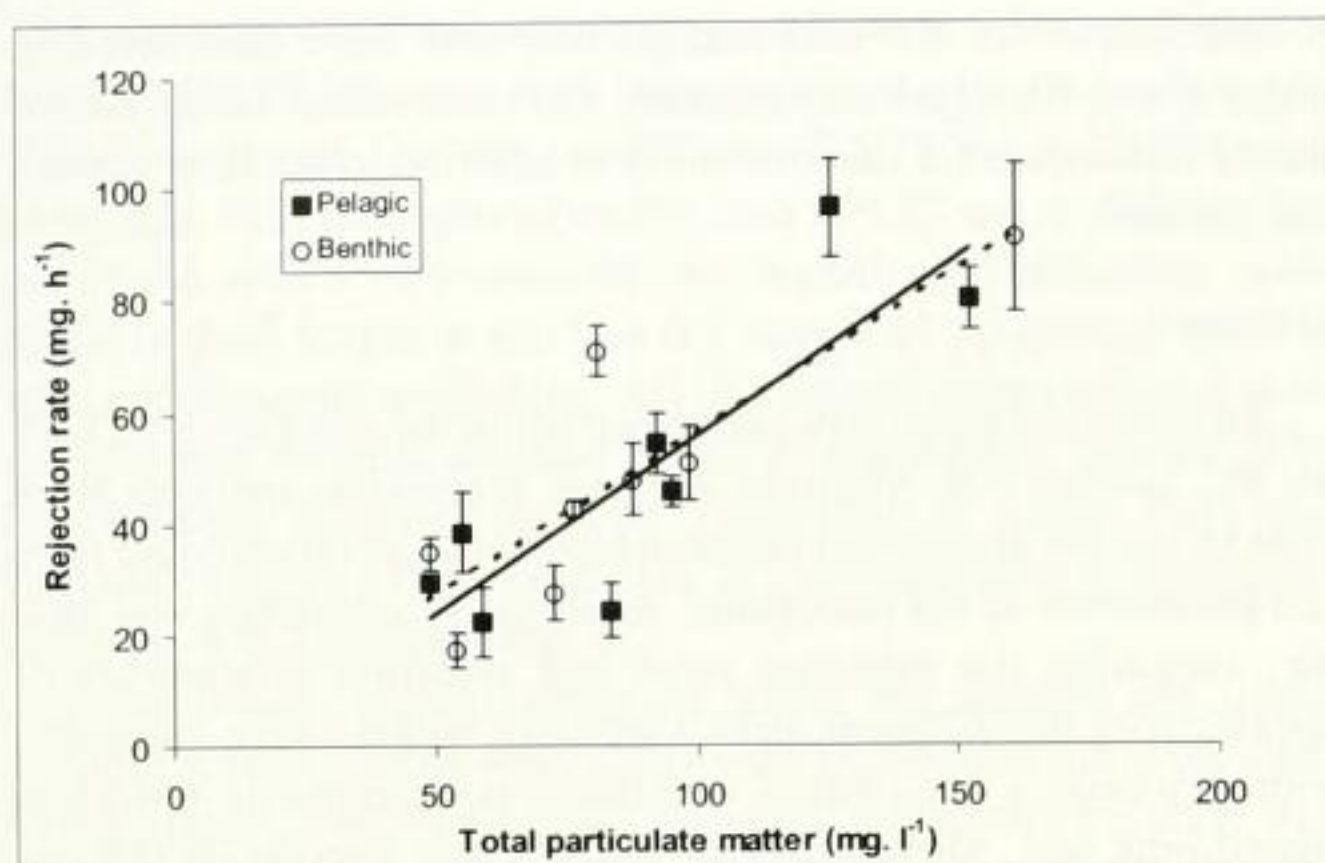**Figure 1. Rejection rate (mg · h⁻¹) of pseudofeces as a function of the seston concentration in both diets. Pelagic: Results for treatments with *Phaeodactylum tricornutum* (line). Benthic: Results for treatments with microphytobenthos (dotted line). Vertical bars indicate standard error. See Table 2 for number of measurements.**

dicated that there were no significant differences between the diets ($/t/ = 0.256$; $P > 0.05$).

Organic rejection rates (ORR) were only significantly correlated to the particulate organic matter (POM) (Fig. 2, see Table 3), with TPM and %OM as nonsignificant parameters related to ORR. According to the regressions obtained in each case no significant differences were found between both diets ($/t/ = 1.136$; $P > 0.05$).

Multiple stepwise regression analysis indicated a significant positive relationship between selection efficiency (SE) and particulate organic matter (POM) (after log transformation) in both diets (Fig. 3 see Table 3). Although lower values of SE were

obtained with the benthic diet, analysis of covariance indicated that there were no significant differences between diets ($/t/ = 0.791$; $P > 0.05$).

Response to a Mixed Diet

Selection efficiency values as a function of POM concentration (see Fig. 3) were generally lower for the mixed diet of pelagic and benthic cells. Higher values of SE were registered at higher values of particulate organic matter.

Flow cytometer results showed significantly lower cell concentrations (expressed as %-age) in pseudofeces in comparison to the diet composition (Fig. 4), and therefore significant positive electivity indices (EI) (acceptance). For both diets, differences of algal concentration in pseudofeces and diet were significant using a t-student test (Pelagic: DF = 12, t = 14.09, $P < 0.001$; Benthic: DF = 12, t = 4.86, $P < 0.01$). However, the EI of cockles was significantly higher for pelagic ($EI_{Pelagic} = 0.31 \pm 0.01$, mean ± standard error) than for benthic cells ($EI_{Benthic} = 0.20 \pm 0.03$, mean ± standard error), indicating a preferential acceptance of the Pelagic rather than the Benthic component of the mixed diet. A lower EI for the benthic species was due to differences in the acceptance or rejection of the different benthic species within the Benthic group (Fig. 5). The smallest benthic species, such as *Navicula* sp. and the group of small pennates, were significantly ingested, displaying higher EI values than larger species such as *Nitzchia* sp. However, *Cylindrotheca* sp., the largest species, was significantly rejected, resulting in a negative EI value.

DISCUSSION

Production of pseudofeces of cockles was significantly related to the seston concentration in both diets. This relationship between

TABLE 3.

Stepwise multiple regression analyses of physiological parameters of *Cerastoderma edule* with total particulate matter (TPM: mg · l⁻¹), particulate organic matter (POM: mg · l⁻¹), particulate inorganic matter (PIM: mg · l⁻¹) and percentage of organic matter from the different diets (% OM). se: standard error.

	Pelagic diet				Benthic diet			
	Coefficient	se	r²	P	Coefficient	se	r²	P
Rejection rate (RR)								
Terms retained			0.73	<0.01			0.72	<0.01
Constant	7.97			<0.01	−1.67			<0.01
TPM	0.64	0.16		<0.01	0.58	0.14		<0.01
Terms rejected								
POM								
% OM								
TPM × POM								
Organic rejection rate (ORR)								
Terms retained			0.63	<0.05			0.68	<0.01
Constant	2.45			<0.01	−0.27			<0.01
POM	0.24	0.08		<0.05	0.41	0.11		<0.01
Terms rejected								
TPM								
% OM								
TPM × POM								
Selection efficiency (SE)								
Terms retained			0.67	<0.01			0.82	<0.005
Constant	−28.85			<0.01	−33.49			<0.005
Log POM	19.92	5.46		<0.01	19.94	3.85		<0.005
Terms rejected								
Log TPM								
Log % OM								

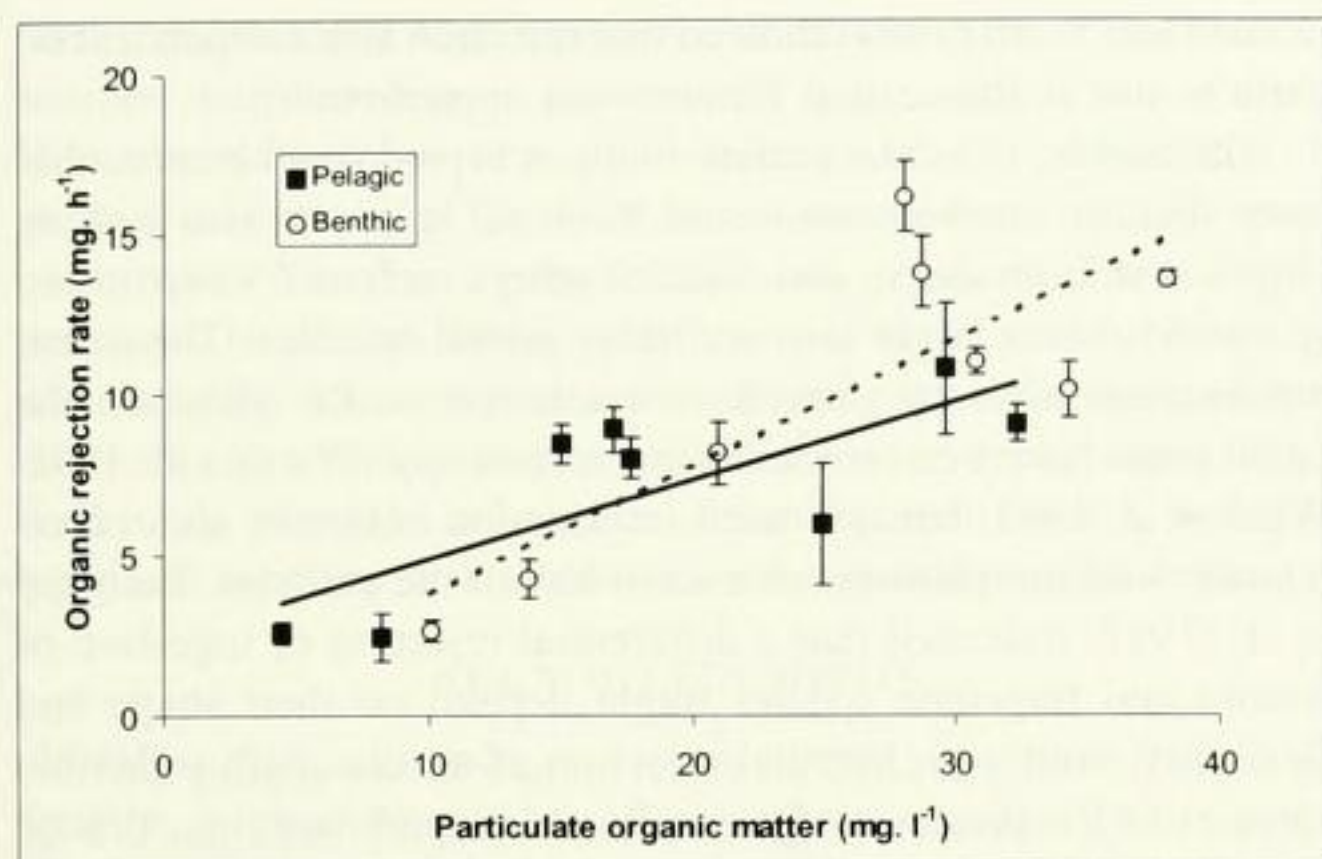**Figure 2.** Organic rejection rate of pseudofeces (mg · h⁻¹) as a function of the organic seston concentration in both diets. Pelagic: Results for treatments with *Phaeodactylum tricornutum* (line). Benthic: Results for treatments with microphytobenthos (dotted line). Vertical bars indicate standard error. See Table 2 for number of measurements.

Figure 4. Percentages of the different algal cells, from the total number of particles, both in the diet and the pseudofeces. Pelagic: *Phaeodactylum tricornutum*. Benthic: Microphytobenthic algae Vertical bars indicate standard error. In all measurements $n = 14$.

RR and the seston concentration has been observed in several studies and rates were comparable with our outcomes (Iglesias et al. 1992, Navarro & Widdows 1997, Urrutia 1997). Similarly, the rejection rate of organic matter increased with organic content of seston. At a constant clearance rate, filtration rate increases at increasing seston concentrations and the digestive system reaches maximum capacity, hence pseudofeces production increases.

Together with the production of pseudofeces, selection of organic material occurs prior to ingestion. Selection efficiency (SE) values found in our experiments were very similar to values documented for *C. edule* under comparable conditions of food quality (Iglesias et al. 1992, Urrutia 1997). The response by the cockle to the pelagic diet did not differ significantly from the benthic diet. The SE maximizes in *C. edule* as a response of an increment in the organic content in the diet (Iglesias et al. 1992, Urrutia 1997) reaching a maximum value in our study of 40%. This maximum value of gross SE (measurements based on the organic content of pseudofeces) for the cockle is similar to those obtained in previous
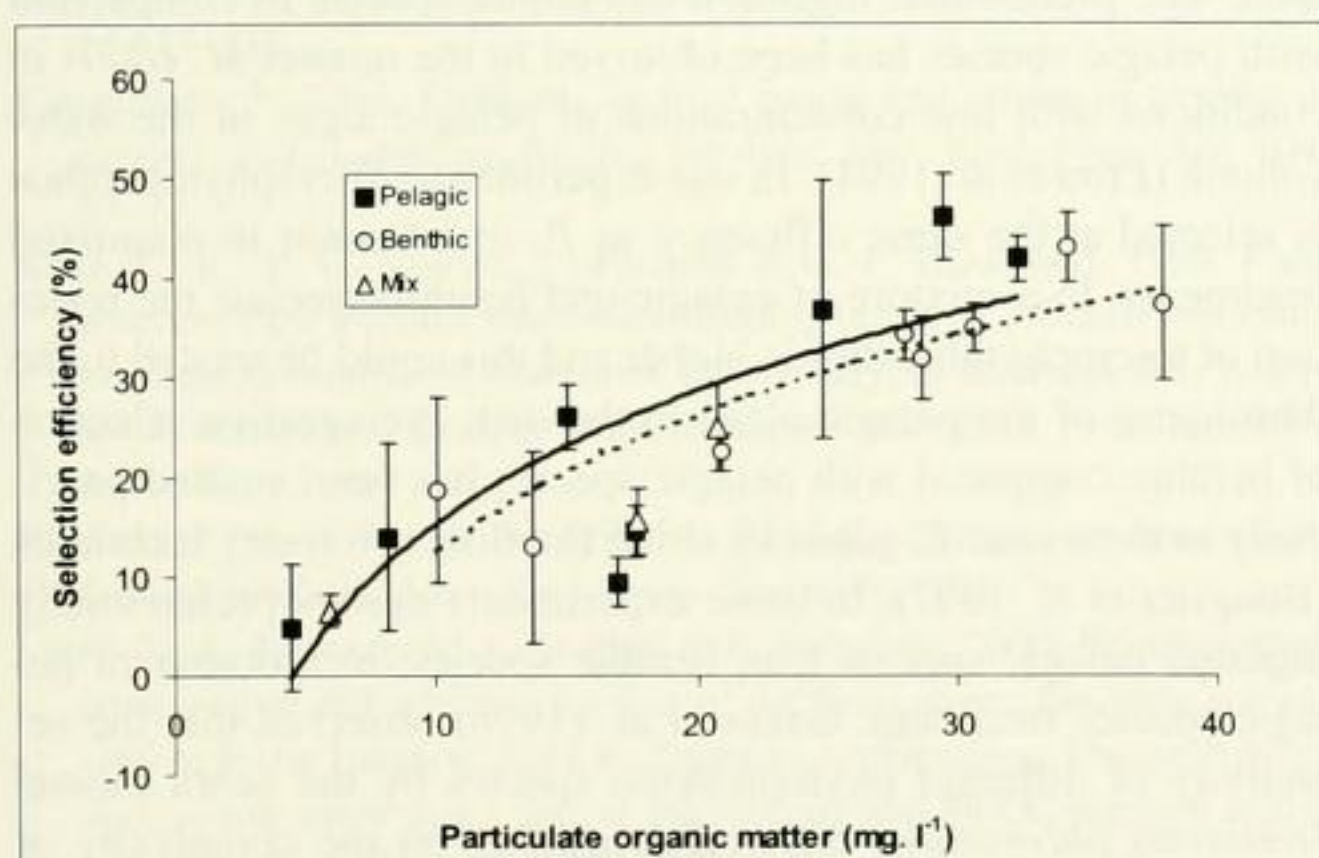
studies using pelagic algae as a food source (Iglesias et al. 1992, Urrutia 1997). The percentage of organic matter in the different treatments covered a range between 10%–60%. Several authors (Iglesias et al. 1992, Urrutia 1997) have described a decrease of the SE in *C. edule* exposed to treatments above 50% organic content. The decrease of SE values obtained in treatments containing Benthic algae could be related to a lower acceptance or the preferential rejection of some benthic species as it has been found with *Nitzchia* sp. and *Cylindrotheca* sp.

In this study, we have documented the selection and acceptance of microphytobenthos as a benthic food source by the cockle. However, pelagic algae were accepted preferentially when mixed with microphytobenthic species. Moreover, only some microphytobenthic species were preferentially accepted by *C. edule*. Micro-

Figure 3. Mean values of selection efficiency (%) as a function of the organic seston concentration in each dietary condition. Pelagic: Results for treatments with *Phaeodactylum tricornutum* (line). Benthic: Results for treatments with microphytobenthos (dotted line). Mix: Results for treatments with a mixed diet. Vertical bars indicate standard error. See Table 2 for number of measurements.
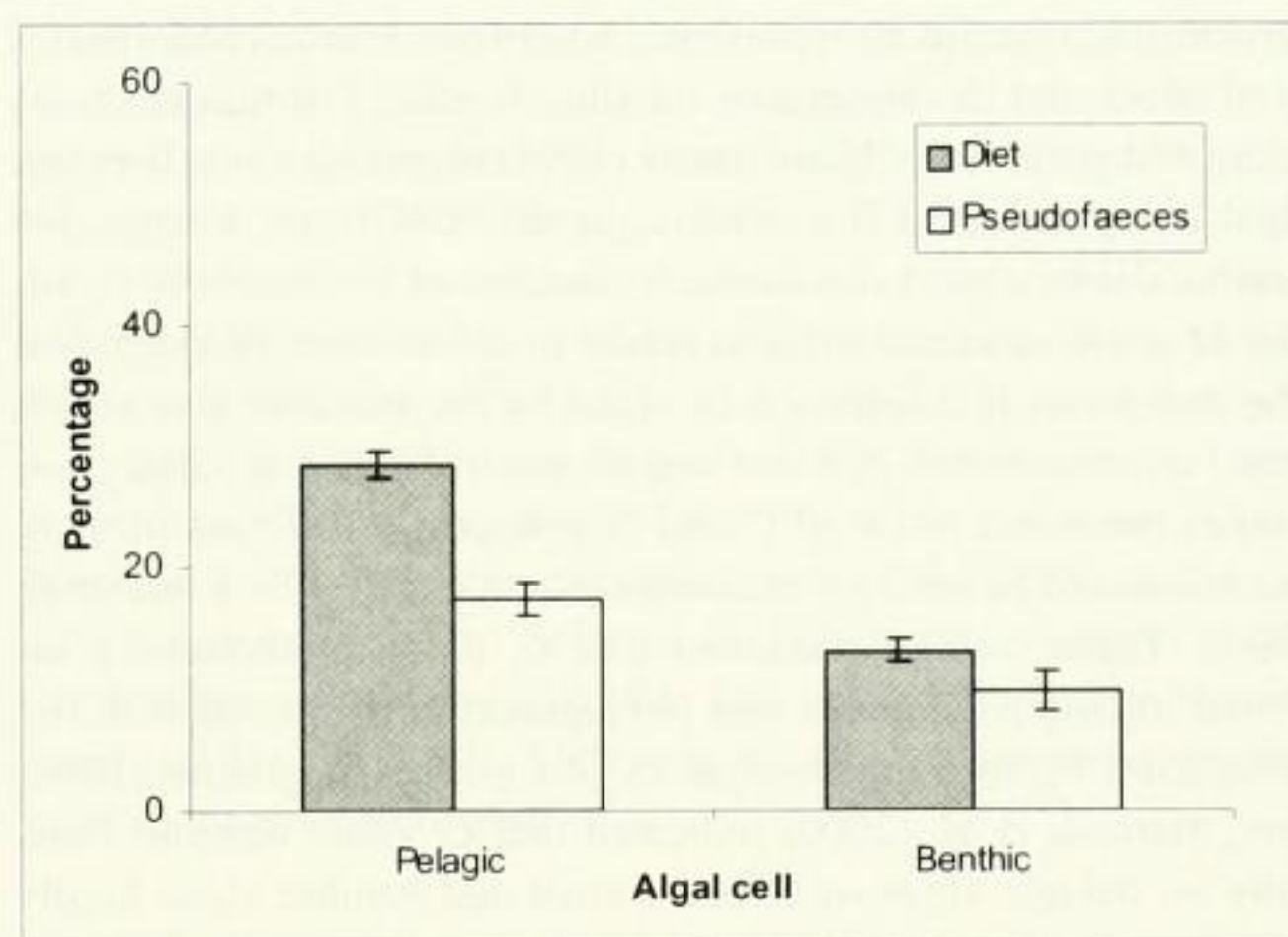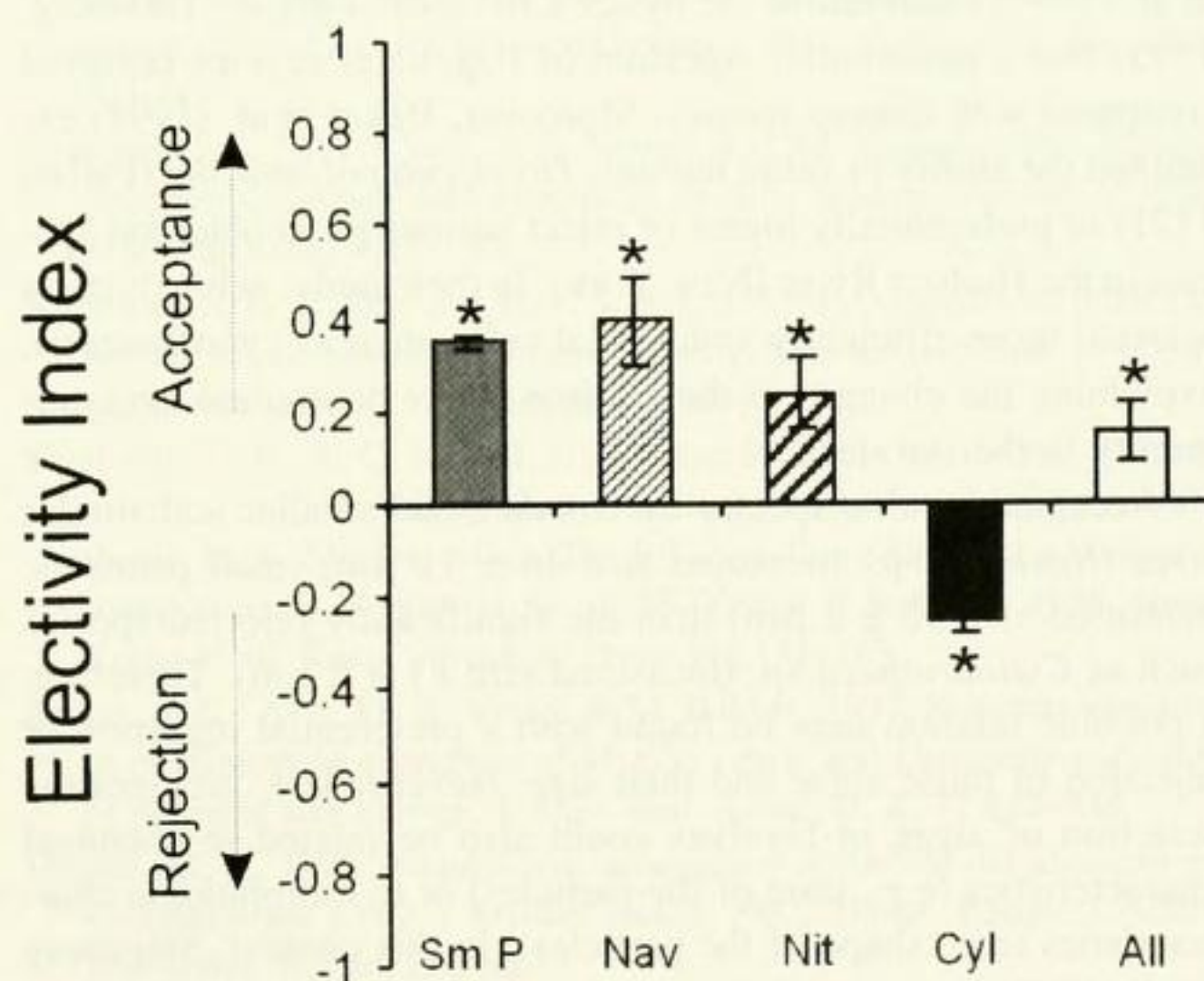

Figure 5. Electivity indices (EI) for the different microphytobenthic species, as separate species or as a group, relative to the total concentration of particles. The studied species are small pennate cells (SmP), *Navicula* sp. (Nav), *Nitzchia* sp. (Nit), *Cylindrotheca* sp. (Cyl) and the benthic group (All), which includes all microphytobenthic species. Vertical bars indicate standard error. * Indices significantly different than zero ($P < 0.05$). In all measurements $n = 14$.

phytobenthos seems to represent a food source in several species of bivalves and its importance for filter feeding bivalves has been addressed previously. Kamermans (1994) found relations between algal composition of the water column (30% were resuspended benthic diatoms) and the stomach contents of the bivalves *C. edule*, *Mya arenaria* and *Mytilus edulis* in the western Wadden Sea. She concluded that selection of algae by the bivalves was absent based on comparison of water and stomach samples. Recently two studies measured ratios of C and N isotopes in different primary producers and benthic invertebrates (Kang et al. 1999, Riera et al. 1999). These studies concluded that *C. edule* preferentially ingested microphytobenthos and phytoplankton compared with detritus from benthic macro-algae or sea grasses fragments. However, Herman et al. (2000) indicated that *C. edule* depends basically on pelagic algae as a food source and benthic algae hardly contribute to the metabolism. Our experiment showed a differential pre-ingestive selection by *C. edule* of the different benthic species. This may explain the actual controversy about microphytobenthos as a food source for suspension feeders. More detailed information about acceptance and/or rejection of single microphytobenthic algal species is needed to clarify the actual controversy.

Results obtained in this study, using flow cytometry techniques, showed a preferential ingestion of Pelagic over Benthic species and a differential selection of the benthic species. Within the Benthic group, some species such as *Cylindrotheca* sp. was rejected in significantly higher concentrations than small pennates or *Navicula* sp. The mixed composition of the benthic diet is a feature that resembles the natural conditions where *C. edule* lives and a preferential selection of some species may occur also in the field. Studies on sorting of food in bivalves using flow cytometry to determine the preferences within mixed samples are scarce (Cucci et al. 1985, Shumway et al. 1985, Chrétiennot–Dinet et al. 1991, Bougrier et al. 1997, Baker et al. 1998). Cucci et al. (1985) did not observe preingestive selection in mussels fed with a diet of a diatom, a dinoflagellate and a cryptomonad. However, Bougrier et al. (1997) observed in the oyster *Crassostrea gigas* (Thunberg, 1793) that a preferential ingestion of flagellates species occurred compared with diatom species. Moreover, Baker et al. (1998) examined the ability of zebra mussels *Dreissena polymorpha* (Pallas, 1771) to preferentially ingest or reject various phytoplankton species in the Hudson River (New York). In their study, zebra mussels selected more efficiently small algal cells, such as cyanobacteria, explaining the changes in the Hudson River phytoplankton community in the last decade.

Accepted benthic species by *C. edule* had smaller and similar sizes (*Navicula* sp.: measured size 46 ± 11 µm; small pennates: measured size 30 ± 2 µm) than the significantly rejected species such as *Cylindrotheca* sp. (measured size 81 ± 5 µm). Therefore, a possible relation may be found with a preferential ingestion or rejection of those algae and their size. Nevertheless, preingestive selection of algae in bivalves could also be related to chemical characteristics (e.g., taste of the particles) or to morphologic characteristics (e.g., shape of the particles). In this context, Shumway et al. (1990) discussed that not only size is an important factor in the preingestive selection of particles but also mucoid trapping and chemoreceptors. Chrétiennot–Dinet et al. (1991) reported that the relative ingestion or rejection was not dependent on the size of the algae in *C. gigas* and *M. edulis*. Bougrier et al. (1997) reported similar results on those bivalves and no relation was found between preingestive selections of algal species with their size. Mac-

Donald and Ward (1994) showed that rejection is not dependent on particle size in the scallop *Placopecten magellanicus*.

The cockle, *C. edule*, preferentially accepted small benthic diatoms such as small pennates and *Navicula* sp., both also with an elliptical smooth shape, and rejected others such as *Cylindrotheca* sp., which has a larger size and large lateral spicules. The actual mechanisms whereby particles are selected on the gills and the labial palps have been studied using endoscopy (Ward et al. 1991, Ward et al. 1993) but not much information is known about their relation with morphologic characteristics of the particles. Bougrier et al. (1997) indicated that a differential rejection or ingestion of diatom and flagellate species might depend on their shape and flexibility, with a preferential rejection of species with inflexible spicules or the presence of a non-flexible membrane (i.e., silicate frustule), which may hamper the pass through the gill filter. Some authors (Shumway et al. 1985, Bougrier et al. 1997) have also detected a preferential ingestion of flagellates in comparison to diatoms and have speculated that the presence of the silicate frustules from the diatoms may appear as mineral particles to sensory receptors of the labial palps. According to this hypothesis the preferential selection of the pelagic diatom *P. tricornutum* could be influenced because benthic species are, generally, attached to silt particles and it may be possible that some species will have more chance to be rejected as inorganic particles by *C. edule*.

In this study, similar values of selection efficiency were registered when *C. edule* was exposed to Pelagic and Benthic treatments, and lower values obtained in mixed treatments. Riera and Richard (1995) have reported in *C. gigas* a preferential ingestion of benthic diatoms and detritus compared with phytoplankton in shallow estuarine waters. But they also reported that *C. gigas* had a preferential ingestion of phytoplankton, when compared with benthic diatoms and detritus, in deeper waters from the same bay. Shumway et al. (1987) found a preferential ingestion of benthic algae by *P. magellanicus* collected from deep-water populations, however individuals from shallow water populations seemed to ingest more pelagic than benthic algae. Gut contents of *P. magellanicus* generally reflected available organisms in the immediate habitat. The preingestive selection of algae in some filter feeding bivalves, such as *C. edule*, could be adapted to the composition and the percentages of the different species available in the water column. The preferential ingestion of benthic species in comparison with pelagic species has been observed in the mussel *M. edulis* in conditions with low concentrations of pelagic algae in the water column (Prou et al. 1994). In our experiments, microphytobenthos is selected at the same efficiency as *P. tricornutum* in nonmixed treatments. In a mixture of pelagic and benthic species, the rejection of microphytobenthos is higher and this could be related to the dominance of the pelagic algae in the diet. Preingestive selection of benthic compared with pelagic species has been studied previously in the oyster *C. gigas* by using the flow cytometry technique (Bougrier et al. 1997). In those experiments oysters preferentially ingested pelagic species than benthic species, in a dominant pelagic species treatment. Baker et al. (1998) observed that the selectivity of different phytoplankton species by the zebra mussel *Dreissena polymorpha* differed depending on the complexity of the offered suspension.

Summarizing, the pre-ingestive selection of benthic algae by *C. edule* is similar and nonsignificantly lower than the pre-ingestive selection of pelagic species, when benthic and pelagic species are not mixed. This situation of maximum presence of benthic diatoms in the seston could be similar to environmental characteristics of

tidal flat areas with high resuspension rates of benthic diatoms, or seasons with low pelagic productivity (e.g., autumn and winter). However, acceptance of benthic algae is lower, compared with pelagic, and not equal for the different components of the benthic group when mixed in a pelagic dominant treatment. These conditions may be present during warm periods of the year (e.g., spring and summer) when phytoplankton blooms occur in their natural environment. *C. edule* may be considered as an opportunistic filter feeder that may take advantage of certain algal species, both pelagic and benthic, in relation to their availability in the field.

ACKNOWLEDGMENTS

LITERATURE CITED

Baker, S. M., J. S. Levinton, J. P. Kurdziel & S. E. Shumway. 1998. Selective feeding and biodeposition by zebra mussels and their relation to changes in phytoplankton composition and seston load. *J. Shellfish. Res.* 17:1207–1213.

Bougrier, S., A. J. S. Hawkins & M. Héral. 1997. Preingestive selection of different micro algal mixtures in *Crassostrea gigas* and *Mytilus edulis*, analysed by flow cytometry. *Aquaculture* 150:123–134.

Chrétiennot–Dinet, M. J., D. Vaulot, R. Galois, A. M. Spano & R. Robert. 1991. Analysis of larval oyster grazing by flow cytometry. *J. Shellfish. Res.* 10:457–463.

Cucci, T. L., S. E. Shumway, R. C. Newell, R. Selvin, R. R. L. Guillard & C. M. Yentsch. 1985. Flow cytometry: a new method for characterization of differential ingestion, digestion and egestion by suspension feeders. *Mar. Ecol. Prog. Ser.* 24:201–204.

Herman, P. M. J., J. J. Middelburg, J. Widdows, C. H. Lucas & C. H. R. Heip. 2000. Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. *Mar. Ecol. Ser.* 204:79–92.

Hummel, H. 1985. Food intake of *Macoma balthica* (Mollusca) in relation to seasonal changes in its potential food on a tidal flat in the Dutch Wadden Sea. *Neth. J. Sea Res.* 19:52–76.

Iglesias, J. I. P., E. Navarro, P. Alvarez–Jorna & I. Armentia. 1992. Feeding, particle selection and absorption in cockles *Cerastoderma edule* (L.) exposed to variable conditions of food concentration and quality. *J. Exp. Mar. Biol. Ecol* 162:177–198.

Jacobs, J. 1974. Quantitative measurement of food selection. *Oecologia* 14:413–417.

Kamermans, P. 1994. Similarity in food source and timing of feeding in deposit- and suspension-feeding bivalves. *Mar. Ecol. Prog. Ser.* 104: 63–75.

Kang, C. K., P. G. Sauriau, P. Richard & G. F. Blanchard. 1999. Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes–Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. *Mar. Ecol. Prog. Ser.* 187: 147–158.

Kiørboe, T. & F. Møhlenberg. 1981. Particle selection in suspension-feeding bivalves. *Mar. Ecol. Prog. Ser.* 5:291–296.

Loret, P., A. Pastoureaud, C. Bacher & B. Delasalle. 2000. Phytoplankton composition and selective feeding of the pearl oyster *Pinctada margaritifera* in the Takapoto lagoon (Tuamotu Archipielago, French Polynesia): *in situ* study using optical microscopy and HPLC pigment analysis. *Mar. Ecol. Prog. Ser.* 199:55–67.

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

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

Newell, R. I. E. & S. J. Jordan. 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 13:47–53.

Newell, C. R., S. E. Shumway, T. L. Cucci & R. Selvin. 1989. The effects of natural seston particule size and type on feeding rates, feeding selectivity and food resource availability for the mussel *Mytilus edulis* Linnaeus, 1758 at bottom culture sites in Maine. *J. Shellfish Res.* 8: 187–196.

Peirson, W. M. 1983. Utilization of eight algal species by the bay scallop *Argopecten irradians concentricus* (Say). *J. Exp. Mar. Biol. Ecol.* 68: 1–11.

Prou, J., L. Barillé, M. Héral, G. Rastikol, P. Soletchnik, S. Bougrier, D. Razet & P. Geairon. 1994. Influence du cycle semi-diurne et vives-eaux mortes-eaux sur la disponibilité particulaire et son utilisation par une population de *Mytilus edulis*. *Haliotis* 23:139–153.

Riera, P. & P. Richard. 1995. Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine bay of Marennes–Oleron. *Estuar. Coast. Shelf Sci.* 42:347–360.

Riera, P., L. J. Stal, J. Nieuwenhuize, P. Richard, G. Blanchard & F. Gentil. 1999. Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen stable isotopes: importance of locally produced sources. *Mar. Ecol. Prog. Ser.* 187: 301–307.

Shumway, S. E., T. L. Cucci, R. C. Newell & C. M. Yentsch. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. *J. Exp. Mar. Biol. Ecol* 91:77–92.

Shumway, S. E., R. Selvin & D. F. Schick. 1987. Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin, 1791): A qualitative study. *J. Shellfish Res.* 6:89–95.

Shumway, S. E., R. C. Newell, D. J. Crisp & T. L. Cucci. 1990. Particle selection in filter feeding bivalve molluscs: A new technique on an old theme. In: B. Morton, editor. The Bivalvia, Proceedings of a Memorial Symposium in Honour of Sir C. M. Yonge, Edinburgh, 1986. Hong Kong: Hong Kong University Press. pp. 151–165.

Smaal, A. C., A. P. M. A. Vonck & M. Bakker. 1997. Seasonal variation in physiological energetics of *Mytilus edulis* and *Cerastoderma edule* of different size classes. *J. Mar. Biol. Assoc. U. K.* 77:817–838.

Urrutia, M. B. 1997. Adquisición, selección y absorción del alimento en *Cerastoderma edule* y *Mytilus edulis*. PhD. Thesis. Basque Country University, Bilbao. 202 pp.

Ward, J. E., P. G. Beninger, B. A. Mac Donald & R. J. Thompson. 1991. Direct observations of feeding structures and mechanisms in bivalve molluscs using endoscopic examinations and video image analysis. *Mar. Biol.* 111:287–291.

Ward, J. E., B. A. MacDonald, R. J. Thompson & P. G. Beninger. 1993. Mechanisms of suspension-feeding in bivalves: resolution of current controversies by means of endoscopy. *Limnol. Oceanogr.* 38:265–272.