Particle selection in the ribbed mussel *Geukensia demissa* and the Eastern oyster *Crassostrea virginica*: Effect of microalgal growth stage

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**A R T I C L E   I N F O**

Article history:
Received 22 August 2007
Accepted 26 February 2008
Available online 7 March 2008

Keywords:
pre-ingestive selection
particle sorting
bivalves
flow cytometry
microalgae
growth phase

**A B S T R A C T**

We studied particle selection in the ribbed mussel *Geukensia demissa*, an important suspension-feeding inhabitant of estuaries and intertidal zones of salt marshes along the Atlantic coast of North America. Adult mussels were fed on several mixtures of microalgal cultures (1) in exponential or (2) in stationary phase of growth, and the proportional occurrence of algal species in pseudofeces was examined by flow cytometry. The Eastern oyster, *Crassostrea virginica*, was chosen as a reference. Results showed that both mussels and oysters were able to selectively ingest or reject our experimental microalgae. Moreover, the pre-ingestive particle selection was affected by microalgal growth phase, particularly in mussels. For instance, the sorting efficiency index increased significantly in mussels fed with a blend made of *Nitzschia closterium*, *Isochrysis* sp. and *Tetraselmis suecica* harvested in stationary growth phase, as compared to the same blend made with microalgae in exponential growth phase. *Isochrysis* sp. and *T. suecica* were preferentially ingested by both bivalves whereas *N. closterium* was preferentially rejected in pseudofeces. These results demonstrate particle selection in ribbed mussel and underline the effect of algae growth phase on the sorting mechanisms.

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1. Introduction

The Atlantic ribbed mussel *Geukensia demissa* inhabits estuaries and intertidal zones of salt marsh along the Atlantic coast of North America. In wetlands, this suspension-feeding mollusk is often found in large populations (Jordan and Valiela, 1982; Franz, 2001) affecting benthic-pelagic coupling and marsh ecosystems. As a result, the ecological roles of *G. demissa* have been emphasized in many studies. Through suspension-feeding activities, the presence of dense ribbed mussel beds is known to reduce turbidity by filtering water and physically trapping and stabilizing substantial quantities of suspended particles (Bertness and Grosholz, 1985). Thus, large amounts of organic material can be deposited to the benthos or cycled into inorganic forms (Jordan and Valiela, 1982; Langdon and Newell, 1990). Suspension-feeders such as *G. demissa*, have been shown to strongly interact with phytoplankton and phytochoanthos populations (Kreeger and Newell, 2001). They remove a significant portion of microalgae as food, and in return, the grazing process cycles dissolved nutrients, reduces turbidity, increases light in the bottom and as a result stimulates microalgae growth (Dame, 1996). This species is well adapted to use resources available in salt marsh, where seston composition is extremely variable (Huang et al., 2003b). For instance, *G. demissa* has the ability to exploit a wide variety of food sources such as detrital cellulose from vascular plants (Charles and Newell, 1997), small-sized bacteria (Kemp et al., 1990; Newell and Krambeck, 1995; Kreeger and Newell, 1996), heterotrophic protists (Kreeger and Newell, 1996), microphytobenthos, cyanobacteria and phytoplankton (Riisgard, 1988; Kemp et al., 1990). Moreover, the digestive physiology of ribboned mussels can respond to seasonal variation in dietary composition of marsh water (Kreeger and Newell, 2001) and to the limited time available for feeding associated with living high in the intertidal zone (Charles and Newell, 1997).

To enhance the nutritive value of consumed particles and to optimize energy gain, several suspension feeding bivalves, such as the eastern oyster *Crassostrea virginica* (Shumway et al., 1985), are able to select food, typically by passing the water over their gills and, from it, straining suspended matter and food particles. Through this mechanism, suspension Feeders preferentially ingest particles of interest while undesirable particles are rejected in pseudofeces (Loosanoff and Engle, 1947; Morton, 1960). In this manner, the impact of suspension-feeding bivalves on aquatic ecosystems is significantly amplified by their ability to select and ingest specific particles. Although *Geukensia demissa* is known to have developed physiological adaptations to address challenging environmental conditions, such as long emersion in high intertidal areas of salt marsh (Charles and Newell, 1997; Franz, 2001 and references therein; Kreeger and Newell, 2001), to our knowledge,
there is no prior investigation of ribbed mussel’s ability to sort its food particles. However, Kemp et al. (1990) suspected this ability by measuring filtration rates in *G. demissa* and finding that the mussels were able to remove some particle types with greatly differing effectiveness. This mytilid species is characterized by a filibranch homorhabdic ctenidia and other marine members of this family are known to sort particles, including *Mytilus edulis* (Cucci et al., 1982; Ward and Targett, 1989; Bougrier et al., 1997), *Mytilus trossulus* (Ward et al., 1998), *Mytilus chileniensis* (Velasco and Navarro, 2002), and *Perna viridis* (Ke and Wang, 2002). The freshwater mussels *Dreissena polymorpha* (Dreissenidae) (Baker et al., 1998), as well as *Margaritifera margaritifera*, *Ambeloma plicata* and *Pyganocon cataracta* (Unionidae) (Baker and Levinton, 2003), were found to select particles as well. However, saltwater and freshwater mussels are not closely related (separate subclasses) and have different gill structure, making inappropriate any extrapolation from one group to another.

Particle selection mechanism in suspension-feeders is controlled by diverse physical, chemical, and biological factors in the environment and many previous studies have shown that changes in size, density, electrostatic charges or concentration of particles can affect selection (Iglesias et al., 1996; Barilé et al., 1997; Bougrier et al., 1997; Cognie et al., 2001; Ward and Shumway, 2004). Some studies have also demonstrated that chemical cues represent important factors mediating particle selection mechanisms in bivalves (Shumway et al., 1985; Ward and Targett, 1989; Pales Espinosa et al., 2007, 2008). Chemical cues identified during these studies include extracellular phytoplankton metabolites (Ward and Targett, 1989; Pales Espinosa et al., 2007) and carbohydrates coating microalgae cell-surfaces (Pales Espinosa et al., 2008). Interestingly, both ectrines and cell-surface membrane markers change during the cell cycle of cultured microalgae, suggesting various outcomes for their interactions with suspension-feeders at different life stages. Thus, production and excretion of secondary metabolites (fatty acids, carbohydrates, pigments, or toxins) by microalgae often increased during stationary phase of algal growth and nutrient depletion (Targett and Ward, 1991; Harker et al., 1996; Fidalgo et al., 1998), as well as differing effectiveness. This mytilid species is characterized by different gill structure, making inappropriate any extrapolation from one group to another.

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Abbreviation</th>
<th>Class</th>
<th>Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isochrysis sp.</td>
<td>Is</td>
<td>Prymnesiophyceae</td>
<td>5–6</td>
</tr>
<tr>
<td>Nitzschia closterium</td>
<td>Nc</td>
<td>Diatom (penate)</td>
<td>17–20</td>
</tr>
<tr>
<td>Tetraselmis suecica</td>
<td>Ts</td>
<td>Prasinophyceae</td>
<td>10–18</td>
</tr>
<tr>
<td>Thalassiosira pseudonana</td>
<td>Tp</td>
<td>Diatom (centric)</td>
<td>5–6</td>
</tr>
<tr>
<td>Thalassiosira weissflogii</td>
<td>Tw</td>
<td>Diatom (centric)</td>
<td>25–27</td>
</tr>
</tbody>
</table>

2.2. Feeding experiments

The design of the feeding experiments was inspired from prior studies investigating particle selection in bivalves (Shumway et al., 1985; Ward et al., 1998; Pales Espinosa et al., 2007). *Geukensia demissa* (Dillwyn) were collected from the Maurice River estuary (Port Norris, NJ, USA). *Crasostrea virginica* (Gmelin), harvested in Delaware Bay, were obtained from a commercial source (Bivalve Packing Inc., Port Norris, NJ, USA). Bivalves were scrubbed to remove all epiphytes and encrusting organisms from their shells. All animals were acclimated in the laboratory for a minimum of 1 week (fed daily with cultured *Isochrysis* sp., 28 salinity, 21 °C), then purged in filtered (0.22 μm) seawater for a day prior to being used in the feeding experiments.

Bivalves were placed in individual trays, each supplied with 3 L of filtered seawater containing one of the tested diets. A control tray with empty oyster shells was used to measure microalgal sedimentation. Microalgae were kept in suspension using a magnetic stirrer (gentle stirring to avoid spreading pseudofeces). Experiments lasted for 2 h during which water samples were taken for flow cytometry analyses at 0, 60 and 120 min to determine sedimentation. Pseudofeces were collected from each individual and their algal composition was determined using flow cytometry. Prior to the analysis, pseudofeces samples were vortexed to disrupt particle aggregates and filtered through a Nitex net (64 μm).

2.3. Flow cytometry analysis

Flow cytometry was used to discriminate and enumerate different microalgal species in diets and pseudofeces. Prior to the experiment, each algal species was processed by the flow cytometer to identify its distinguishing characteristics. All particles were discriminated based on their optical and autofluorescence properties by means of a Coulter EPICS C flow cytometer/ sorter equipped with a 2000 mW, 488 nm argon ion excitation laser and interfaced with the standard computer. The algae were detected by the simultaneous measurements of their forward light scatter (FLS), which is correlated to the size of each cell, and the fluorescence of their chlorophyll pigments (log red fluorescence or RFL collected at 660 nm). Gains and photomultiplier high voltage settings were adjusted to include all cells on the two-parameter display plot. List-mode data were collected for at least 5000 particles in each sample. The proportion of each algae species was calculated using bitmaps (electronic outlining) on the two-parameter plots.
2.4. Data analysis

A series of goodness-of-fit tests ($G$ test) was performed using raw counts to compare the proportion of each type of microalga in diet and pseudofeces samples. The null hypothesis was that the proportion of each type of microalga was the same in diet and pseudofeces. In addition to the comparison of raw data, a sorting efficiency (SE) index was calculated in order to examine particle selection (Iglesias et al., 1992). This index was defined as:

$$SE = 1 - (P/D)$$

where $P$ and $D$ represent the proportion of the particle of interest in the pseudofeces and diet, respectively. A positive SE for a given particle type indicates that it is preferentially ingested (particle type is depleted in the pseudofeces, compared to diet), a negative SE indicates rejection (particle type is enriched in the pseudofeces compared to diet), and zero indicates the absence of active selection. After confirming their normal distributions, calculated SEs were compared to zero using a one sample t-test (two-tailed). The null hypothesis was that the selection efficiencies were equal to zero (i.e. no selection). In all statistical tests, results were considered significant if $p < 0.05$.

3. Results

Although the targeted proportion of each microalga was 33%, $a$ posteriori counts revealed the following proportions in the feeding blends: Diet 1, 39.8 ± 1.1% (mean ± SD) of Nitzschia closterium, 29.1 ± 1.1% of Isochrysis sp. and 31.1 ± 2.2% of Tetrastemis suecsa; Diet 2, 37.3 ± 2.1% of N. closterium, 28.8 ± 2.3% of Thalassiosira pseudonana and 33.8 ± 1.2% of Thalassiosira weissflogii; Diet 3, 33.9 ± 2.4% of N. closterium, 29.4 ± 3.6% of Isochrysis sp. and 37.2 ± 3.9% of T. suecsa (Fig. 1). These proportions remained stable over the entire duration of the experiment indicating that differential settling of microalgae did not occur.

3.1. Selection of algae in exponential phase of growth

The proportion of Nitzschia closterium and Tetrastemis suecsa in mussel pseudofeces fed with Diet 1 decreased slightly from 39.8 ± 1.1% in the diet to 35.6 ± 2.8% and from 31.1 ± 2.2% to 30.4 ± 4.9%, respectively (Fig. 1). At the same time, the percentage of Isochrysis sp. increased from 29.1 ± 1.1% in Diet 1 to 34.0 ± 5.0% in mussel pseudofeces. Differences in the proportions of each algal species between Diet 1 and mussel pseudofeces were statistically significantly ($G = 150$, $n = 10$, $p < 0.0001$). Results obtained with oysters were more pronounced ($G = 1904$, $n = 12$, $p < 0.0001$). The proportion of N. closterium increased significantly from 39.8 ± 1.1% in Diet 1 to 50.1 ± 2.7% in oyster pseudofeces (Fig. 1). The percentage of Isochrysis sp. decreased from 29.1 ± 1.1% in Diet 1 to 18.5 ± 4.4% in oyster pseudofeces, while the proportion of T. suecsa did not change.

Results obtained using Diet 2 were slightly different. For instance, the proportion of Nitzschia closterium increased significantly from 37.3 ± 2.1% in the diet to 43.4 ± 1.1% in mussel pseudofeces (Fig. 1). The proportions of Thalassiosira pseudonana and Thalassiosira weissflogii decreased slightly from 28.8 ± 2.3% to 25.9 ± 2.1% and from 33.8 ± 1.2% to 30.7 ± 1.6%, respectively. These changes in the proportions of each algal species were significantly different ($G = 317$, $n = 12$, $p < 0.0001$). Changes were more dramatic in oyster pseudofeces, as the proportion of N. closterium increased significantly from 37.3 ± 2.1% in the diet to 55.8 ± 1.1%. At the same time, the proportions of T. pseudonana and T. weissflogii decreased significantly from 28.8 ± 2.3% in diet to 17.2 ± 1.1% in pseudofeces and from 33.8 ± 1.2% to 27.0 ± 1.9%, respectively ($G = 477$, $n = 11$, $p < 0.0001$).

Calculated selection indices highlighted differences in microalgal processing between mussels and oysters. For instance, sorting efficiencies were generally lower in mussels than those determined for oysters when fed with microalgae in exponential phase (Fig. 2). Considering both diets, indices varied from −0.17 (Isochrysis sp.) to 0.10 (Nitzschia closterium) for Geukensia demissa whereas indices calculated for Crassostrea virginica varied from −0.50 (N. closterium) to 0.40 (Thalassiosira pseudonana). Selection indices were not statistically significant in mussels fed Diet 1 and were only significant for N. closterium (rejection) and T. pseudonana (acceptance) in Diet 2 (Student’s t-test, $p < 0.05$). In contrast, oysters significantly rejected N. closterium both in Diet 1 and Diet 2 whereas Isochrysis sp. (Diet 1), T. pseudonana and Thalassiosira weissflogii (Diet 2) were preferentially accepted.

3.2. Selection of algae in stationary phase of growth

Diet 3 had the same species composition as Diet 1 but only cultures in stationary phase of growth were used. Results show a significant impact of algae phase of growth on particle selection. For instance, the proportion of Nitzschia closterium increased from 33.4 ± 2.4% in Diet 3 to 72.8 ± 5.1% in mussel pseudofeces and to 64.4 ± 6.9% and in oyster pseudofeces (Fig. 1). Similarly, the percentages of both Isochrysis sp. and Tetrastemis suecsa decreased significantly in mussel and oyster pseudofeces when compared to their proportions in the diet. From 29.4 ± 3.6% in the diet, the proportion of Isochrysis sp. decreased to 17.3 ± 2.7% and 15.3 ± 5.8% in mussel and oyster pseudofeces, respectively. Similarly, the proportion of T. suecsa decreased from 37.2 ± 3.9% to 9.9 ± 4.1%.
the marshgrass *Spartina alterniflora* in Delaware Bay (Langdon and Newell, 1990). Under the same conditions, bacteria also supplied 71% of the mussel's metabolic nitrogen requirements. This characteristic allows ribbed mussels to inhabit environments, such as high intertidal salt marshes, that might be considered inhospitable to a filter-feeding bivalve. Their ability to use a wide range of trophic resources raised the question about whether they have the same ability to select their food particles as other filamentous homorhabdic mussel species and what benefit this might offer. Results obtained in this study show that the ribbed mussel *G. demissa* is able to sort food particles, especially when fed with microalgae in stationary growth phase. Algal sorting was significantly less marked when mussels were fed with microalgae in exponential growth phase.

In bivalves, gills and labial palps are the principal structures involved in particle selection. *Geukensia demissa* has a nonplicate (flat) and homorhabdic (one type of filament) ctenidium (Morton, 1979). This gill architecture is common to several other Mytilidae, which have the ability to select particles (Bougrier et al., 1997; Ward et al., 1998). Interestingly, visual observations (endoscopy associated or not with histology) led to the conclusion that in *Mytilus edulis* (Beninger and St Jean, 1997) and *Mytilus trossulus* (Ward et al., 1998), the main particle selection role in the mussels' labial palps could be the main sorting organ. Even though we did not find a description of the structure of *G. demissa* labial palps, it is likely that their morphology and function are closely related to those of *M. edulis* palps, as described by Beninger et al. (1995) and Beninger and St Jean (1997). Thus, based on taxonomic classification and morphological resemblance, *G. demissa* was expected to be able to select particles like other Mytilidae members (i.e. *M. edulis*). However, the fact that particle selection in mussels and oysters varies with microalgae growth phase was unexpected, and to our knowledge, a finding that has not been reported before in adult bivalves.

Although it is now shown that many bivalve mollusks can select among different types of particulate matter, the criteria used to differentiate between particles remain undefined. Among several theories, some studies have supported the idea that bivalves can use chemical cues to discriminate among particles (Kiørboe and Møhlenberg, 1981; Newell and Jordan, 1983; Shumway et al., 1985; Ward and Targett, 1989; Pales Espinosa et al., 2007). In fact, chemical communication is widely distributed in the marine environment and thousands of organic metabolites have been identified in seawater. In numerous physiological processes, marine organisms interact with each other using chemical signals (Hay, 1996 and references therein). Microalgae species are known to produce (Hodgson et al., 1991; Fidalgo et al., 1998) and excrete (Schmidt and Hansen, 2001; Fistarol et al., 2005; Uronen et al., 2005) metabolites during their life cycle. Metabolites released by microalgae, including polysaccharides, nitrogenous substances, amino acids, fatty acids and vitamins (Shimizu, 1996) can be recognized by grazers and influence their feeding behavior (Shaw et al., 1995; Tillmann and John, 2002; Leising et al., 2005 and numerous references therein). In the case of the interactions between suspension-feeding bivalves and microalgae, Ward and Targett (1989) demonstrated that mussels, *M. edulis*, are able to select and preferentially ingest synthetic beads coated with metabolites produced by microalgal species at the end of their exponential growth phase. Several authors, using blends of algae similar in size, have assumed that chemoselection in bivalves is based on microalgal exudates (Shumway et al., 1985; Baldwin, 1995). More recently, we demonstrated that microalgae selection in oysters (*Crassostrea gigas* and *Crassostrea virginica*) involves extracellular metabolites (Pales Espinosa et al., 2007), as well as glycoconjugates coating algal cells (Pales Espinosa et al., 2008).

Extracellular metabolites and glycoconjugates on microalgal cell surfaces are not constant in quality and quantity during different

**Fig. 2.** Sorting efficiency (mean ± SD) of microalgae by *Geukensia demissa* and *Crassostrea virginica*. See Table 1 for abbreviations. Positive values indicate a microalgal preference (Student’s t-test, p < 0.05). Sorting efficiencies in Diet 3 are significantly different from those calculated in Diet 1 for all algae species in both bivalves except for *Isochrysis* sp. in oysters.

(mussel pseudofeces) and 20.3 ± 3.6% (oyster pseudofeces). Changes in the proportions of each algae species between Diet 3 and mussel and oyster pseudofeces were significantly different (p < 0.0001; G = 4165, n = 11 and G = 7782, n = 10, respectively).

Calculated selection indices clearly show the differences in microalgal processing by mussels and oysters between exponentially growing algae and those in the stationary stage. Both mussels and oysters selectively ingested *Isochrysis* sp. (sorting efficiencies of 0.41 and 0.48 respectively) and *Tetraselmis suecica* (0.73 and 0.45 respectively) in stationary phase (Fig. 2). In addition, both mussels (−1.18) and oysters (−0.93) significantly rejected *Nitzschia closterium* in pseudofeces. Significant differences were observed for both bivalves and for all algal species between sorting efficiencies obtained with Diet 3 and those calculated with Diet 1, except for *Isochrysis* sp. in oysters (Student t-test, p < 0.01).

### 4. Discussion

The estuarine environment exhibits huge temporal and spatial variation in concentration and biochemical composition of suspended particulate food resources (Langdon and Newell, 1990; Huang et al., 2003a). During periods of low phytoplankton abundance or in areas with short periods of immersion (Charles and Newell, 1997), detrital cellulose, bacteria or protists can be an important source of food for omnivore species such as *Geukensia demissa* (Kreeger and Newell, 2001). Thus, the direct utilization of cellulose and bacteria can account for 40% of the summer metabolic carbon requirement of mussels inhabiting marshes dominated by

**Image 1**

**Image 2**
growth stages. Thus, ectocrines are produced and excreted by microalgae especially in stationary phase of growth when media are depleted in nutrient (Fidalgo et al., 1998; Uronen et al., 2005). Moreover, it has been shown that glycoconjunct composition of microalgal cell surface changes significantly between different growth stages (Waite et al., 1995; Aguilera and Gonzalez-Gil, 2001), sometimes in relation to nutrient depletion (Kremp and Anderson, 2004). Thus, the amount of glycoconjuncts on the cell surface of the diatom *Thalassiosira pseudonannia* increased in early stationary phase under nutrient depletion (Waite et al., 1995). In the present study, the sorting efficiency of *Geukensia demissa* and *Crasostrea virginina* increased dramatically when fed with microalgae in the stationary growth phase, probably due to a variation in quality or quantity of compounds involved in the (positive or negative) selection process, including cell surface glycoconjuncts.

Our results show that *Isochrysis* sp. and *Tetraselmis suecica* were preferentially ingested whereas *Nitzschia clasterium* was preferentially rejected in pseudofeces of both bivalves. These results are supported by previous studies, describing *T. suecica* as a good diet for *Crasostrea gigas*, the flat oyster, *Ostrea edulis* and the Manila clam, *Ruditapes decussatus*, especially in combination with other algal species (Walne, 1970; Langdon and Waldock, 1981; Robert et al., 2001; Pales Espinosa et al., 2007). Moreover, *Isochrysis* spp. are considered as the industry standard for supporting bivalve growth (Ponis et al., 2003) as well as positive controls in numerous feeding studies (Bricelj and MacQuarrie, 2007; Padilla et al., 2006). Thus, *Isochrysis galbana* induced the best growth and lowest mortality rates in juvenile hard clams *Mercenaria mercenaria* when compared to several commercial diets (Pales Espinosa and Allam, 2006). In contrast, the diatom *N. cisterium* is known to produce high rates of biodeposition by *Crasostrea gigas*, the flat oyster (Beninger and St Jean, 2003; Pales Espinosa et al., 2003). Under environmental conditions where the quality of available food items is similar (i.e., no abundance of high-quality food), it was suggested that selection costs may outweigh any advantages of selective feeding, thus favoring the success of non-selectors (Sierszen and Frost, 1992). However, *G. demissa*, seems to be well adapted to low food availability and, in fact, grows better in intertidal than in subtidal zones where food is always accessible (Gillmor, 1982). Our results suggest that when the nutritional value of food particles is high enough to overcome the costs of selection process, *G. demissa* is able to become “selective”. The role of ectocrines produced by microalgae during their life cycle and the carbohydrate moieties present at their cell surface should be further investigated since they are suspected to play an important role in the selection mechanism.

**References**


References


**5. Conclusion**

**References**


