Journal of Experimental Marine Biology and Ecology 446 (2013) 320–327

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Effects of particle surface properties on feeding selectivity in the eastern oyster *Crassostrea virginica* and the blue mussel *Mytilus edulis*

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ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form 16 April 2013
Accepted 17 May 2013
Available online 28 June 2013

Keywords:
Bivalve feeding
Crassostrea virginica
Mussel
Mytilus edulis
Oyster
Particle selection

ABSTRACT

The mechanisms by which bivalve molluscs differentiate among particles are not clearly understood. Recent evidence suggests that particle selection by bivalves can be mediated by interactions between carbohydrates attached to a particle's surface and lectins present in the mucus covering the feeding organs. The physicochemical surface properties of particles have also been proposed as factors contributing to particle selection. In this study, we examined the effects of surface charge and wettability of 10-μm spheres on particle selection by two species of suspension-feeding bivalves: eastern oysters (*Crassostrea virginica*) and blue mussels (*Mytilus edulis*). Microspheres were delivered to bivalves in particle-selection assays, and the proportions of spheres rejected as pseudofeces and egested as feces were determined using flow cytometry. Results suggest that when given a choice, both mussels and oysters rejected some types of microspheres (e.g., aluminum oxide) and ingested other types (e.g., polystyrene). In some assays, microspheres with very different surface charges or wettabilities were handled similarly, indicating that neither property alone was a qualifier for selection. The differences in surface properties between pairs of microspheres were also considered and used as variables in discriminant analyses. For oysters and mussels, the generated models explained ca. 25% and 72% of the variation in the data, respectively. In both models, wettability was more important than charge in classifying data into their correct group (rejected, preferentially ingested, or not selected). Results from this study support the idea that non-specific physicochemical interactions can play a role in mediating selection in suspension-feeding bivalves.

1. Introduction

In estuarine systems, macrobenthic biomass often is dominated by particle-feeding molluscs (see reviews by Dame, 1996; Russell-Hunter, 1983). Suspension-feeding bivalves in particular play a major role in nutrient cycling, seston modification, and the structuring of benthic food webs (Asmus and Asmus, 1991; Bayne and Newell R.C., 1983; Dame, 1996; Langdon and Newell R.I.E., 1990; Ward and Shumway, 2004). Bivalves are exposed to large amounts of particulate matter that includes both nutritious and non-nutritious particles (Newell R.C., 1965; Owen, 1966). Consequently, particular attention has been focused on the mechanisms of feeding in bivalve molluscs.

The particle-selection capabilities of bivalves that allow for maximal energy and nutrient uptake have been elucidated in several studies. For example, some bivalves have been shown to: a) discriminate between different algal species (Bougrier et al., 1997; Lesser et al., 1991; Shumway et al., 1985, 1997); b) exhibit strong selection in favor of living particles (Beninger et al., 2007; Kiørboe and Møhlenberg, 1981; Newell R.I.E. and Jordan, 1983; Ward et al., 1997); and c) preferentially ingest particles based upon nutrient content (Bayne et al., 1977; Kiørboe and Møhlenberg, 1981; Levinton et al., 2002; MacDonald and Ward, 1994; Newell R.I.E. and Jordan, 1983; Ward et al., 1997). The exact mechanisms used by suspension-feeding bivalves to determine which particles are ingested and which are rejected as pseudofeces, however, are still unknown.

The process of pre-ingestive particle sorting by suspension-feeding bivalves can be described as either passive or active (see reviews by Jørgensen, 1996; Ward and Shumway, 2004). Active selection would be dependent upon a chemosensory response by the cilia or feeding organs to feeding stimuli (Ward and Shumway, 2004), whereas, passive selection is dependent upon the physicochemical interactions between the particles and the feeding organs, with factors such as particle size and shape possibly serving as important contributors to sorting (e.g., larger particles preferentially selected over smaller particles, see Bayne et al., 1977; Riisgård, 1988). Recent work by Pales Espinosa et al. (2009, 2010a, 2010b) has provided evidence for passive selection in bivalves based upon the specific chemical interaction

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http://dx.doi.org/10.1016/j.jembe.2013.05.011

between lectins in the mucus of pallial organs and carbohydrates present on the surfaces of cells and particles in suspension.

Selection based upon the surface characteristics of natural particles and nonspecific physicochemical interactions with the feeding organs is another example of passive selection. Physicochemical properties, such as wettability and electrostatic charge, have been suggested to play possible roles in selection (Beninger, 1991; Newell C.R. et al., 1989). Wettability is a weak force dependent upon hydrophobic–hydrophilic interactions between a surface and a liquid, and refers to the ability of a surface to be wetted as a function of hydrogen bonding (Good and Van Oss, 1992). Generally, a non-wettable surface is hydrophobic, and a wettable surface is hydrophilic.

Wettability has been demonstrated to be related to particle capture in the freshwater crustacean *Daphnia magna*, with wettable particles being retained at a higher proportion than non-wettable particles (Gerritsen and Porter, 1982). Charged particles are more readily captured than particles with a neutral charge by both the brittle star *Ophiopholis aculeate* (LaBarbera, 1978) and larval northern quahog (= hard clam) *Mercenaria mercenaria* (Solow and Gallager, 1990). Hernroth et al. (2000) examined the influence of the surface charge of *Salmonella typhimurium* cells upon selection by the blue mussel *Mytilus edulis* and found that cells with a lower net-negative charge are more likely to be captured than cells with a higher negative charge. Combined, these studies suggest that particle surface properties of cells may also play a role in particle capture and selection by suspension-feeding marine bivalved molluscs.

To investigate possible mechanisms by which bivalves select between particles, studies need to be designed to deconstruct the complex interactions between particles and feeding organs. Our previous work (Pales Espinosa et al., 2009, 2010a, 2010b) focused on biochemical interactions between particles and the ctenidia and labial palps independent of effects caused by differences in surface charge and wettability. In this study, experiments were designed to remove effects attributable to biochemical interactions and size of the particles, and focus only on the effects of surface charge and wettability upon pre-ingestive particle selection. Two commercially and ecologically important species of suspension-feeding bivalves were examined: the eastern oyster (*Crassostrea virginica*) and blue mussel (*Mytilus edulis*). The oyster (pseudolamellibranchiate) has a heterorhabdic, plicate gill structure and is able to select on both the ctenidia and labial palps (Ward et al., 1997). Mussels (filibranchiate) exhibit the more primitive condition, and have homorhabdic, flat ctenidia. No selection is known to occur on the ctenidia of mussels (Newell C.R. et al., 1989; Ward et al., 1993, 1998). Thus, a comparison of particle-selection capabilities in these two species can provide insight into the potential roles of surface properties in particle sorting in bivalves with different ctenidial architectures and loci of selection.

2. Materials and methods

2.1. Organisms

Eastern oysters, *C. virginica* (Gmelin, 1791) (30–60 mm shell height), were obtained from the Cornell Cooperative Extension Oyster Hatchery (Suffolk, NY) and transported to seawater facilities at the University of Connecticut Avery Point Campus (Groton, CT). Blue mussels, *Mytilus edulis* (Linneaus, 1758) (30–60 mm shell length), were collected from Long Island Sound, Connecticut. Bivalves were cleaned of fouling material and acclimated to, and maintained at, 18 °C in filtered seawater at a salinity of 30. They were fed laboratory-cultured microalgae, *Tetraselmis* sp., until used in the particle-selection assays.

2.2. Characterization of surface properties of microspheres

To examine the roles of particle-surface properties in feeding selectivity, the surface charge and wettability of six different types of microspheres were determined. Each microsphere type had a mean diameter of 10 μm and was composed of either: 1) aluminum oxide (= alumina, AlO_2), 2) fluorescent polystyrene with attached carboxyl groups (YG-C, DG; Polysciences, Inc.), 3) fluorescent polystyrene without attached carboxyl groups (YG; Polysciences, Inc.), 4) silica with attached amino groups ($SiNH_2$, Phenomenex Inc.), or 5) silica without attached amino groups (SiO_2, Sorbtech Inc.). Microspheres were characterized both before and after coating with a 0.5% methyl cellulose solution. The coating solution was prepared by dissolving high molecular weight methyl cellulose (4000 cP) in Milli-Q water (MQ, Millipore Corp.) with gentle heating and vigorous stirring for 20 min.

Zeta potential (= electric potential) was used as a proxy for particle surface charge, and determined by means of a Zetasizer Nano 2ZS instrument (Malvern Instruments Inc., UK). The instrument measures electrophoretic mobility using laser-Doppler electrophoresis. In short, an electric charge is passed through a capillary cell containing a suspension of particulate matter, and particle velocity is measured. Using the viscosity and dielectric constant of the suspension, as well as the measured electrophoretic mobility, a zeta potential is then calculated using the Smoluchowski equation (Abramson et al., 1942). The Zetasizer instrument used in these experiments functioned best when microspheres were suspended in media with low ionic strength. Measurements, therefore were made in seawater at a salinity of 15. Feeding trials were conducted at a salinity of 30; therefore, some zeta-potential measurements were made at this salinity and compared to measurements made at the lower ionic strength.

Wettability was determined by measuring the contact angle formed between a water droplet and a particle surface (Hiemenz, 1986). For these measurements, a particle pad of each microsphere type was made by either 1) suspending microspheres in MQ water, filtering the suspension through a 3-μm polycarbonate filter (2.5 cm diameter), and then drying overnight at 70 °C; or 2) pressing powdered microspheres onto an ~2 cm strip of double-sided tape. A drop of MQ water (4 μl) was placed on the pad and photographed using a digital camera attached to a side-mounted dissecting microscope (Mohammadi et al., 2003). The image analysis software Image J® was then used to measure the contact angle formed between the liquid and particle pad (Volpe et al., 2006). As per Pashley et al. (1985), any surface with a contact angle greater than 90° was classified as non-wettable (= hydrophobic). Conversely, any surface with a contact angle less than 90° was classified as wettable (= hydrophilic).

2.3. Characterization of surface properties of natural particles

To determine whether the surface properties of test microspheres were within the range of what bivalves encounter in the field, the surface charge and wettability of three species of microalgae (*Isochrysis* sp. [clone *T-iso*], *Tetraselmis maculata*, and *Nitzschia closterium*) and two natural particles commonly found in coastal marine environments (clay and dead, ground marsh grass, *Spartina alterniflora*) were determined (Table 1). Zeta potentials of the natural particles were determined using the methods described above. Microalgal cultures were grown in media at a salinity of both 15 and 30 so that zeta potential values obtained at the two ionic strengths could be compared.

Wettability of microalgae was determined by contact angle measurements as described above. Surface pads of live microalgae were produced by vacuum-filtering 2–4 mL of culture through a 3 μm polycarbonate filter. Concentrations of the suspension ranged from 3.5 to 8×10^6 cells per mL depending on the microalgal species used. To remove salts, 5 mL of isotonic ammonium formate was added and allowed to drip through the filter for 2 min. Vacuum was then applied briefly to settle the material, and the pads were dried overnight at 70 °C. Control pads were also produced by filtering

Table 1
Surface properties of microspheres used in the assays and natural particles used for comparison. In all cases but the polystyrene with carboxyl group (DG), coating particles with methyl cellulose reduced the surface charge and made the wettable particles (<90°) non-wettable (>90°). Data presented as means ± standard deviation in parentheses (n = 5–11).

Particle type	Zeta potential (mV)		Contact angle (degrees)		Density g/cm^{-3}
	SW	Methyl cellulose	SW	Methyl cellulose	
Polystyrene (YG)	−11.3 (1.1)	n/a	112.9 (3.6)	n/a	1.05
Polystyrene-carboxyl I (YG-C)	−8.0 (0.9)	−1.1 (0.2)*	111.9 (3.3)	112.6 (1.3)	1.05
Alumina (AlO_2)	−6.0 (1.8)	n/a	0 (0)	n/a	3.90
Polystyrene-carboxyl II (DG)	−3.8 (0.4)	−3.8 (0.7)	108.3 (2.7)	104.3 (5.3)	1.05
Silica amino ($SiNH_2$)	4.3 (1.3)	n/a	9.6 (0.8)	n/a	2.60
Silica (SiO_2)	−11.6 (0.6)	−1.8 (0.5)*	7.2 (1.3)	92.7 (8.3)*	2.60
Isochrysis sp (clone *T-iso*)	−6.9 (0.9)	n/a	83.7 (3.1)	n/a	1.58
Tetraselmis maculata	−11.0 (0.4)	n/a	92.9 (7.9)	n/a	1.58
Nitzschia closterium	−9.4 (1.0)	n/a	93.2 (6.1)	n/a	1.58
Kaolin clay	−14.6 (0.6)	n/a	9.5 (0.9)	n/a	2.16
Spartina alterniflora	−11.4 (0.5)	n/a	92.9 (6.2)	n/a	0.70

* Denotes significant changes in the surface property of a sphere type after coating with methyl cellulose (P < 0.01, paired t-test).

only the ammonium formate to ensure that measured contact angles were not a result of this washing treatment.

2.4. Particle selection bioassays

For each experiment, mussels and oysters were placed individually in containers (1 L) filled with a seawater suspension containing a 50:50 ratio by particle number of two of the previously-described microspheres. Each bivalve was glued to a craft stick, and the stick secured to the lip of the container with a wooden clip such that animals were oriented ventral side up. A stir bar was added, and the container was placed on an electromagnetic stir plate. Prior to the start of a selection assay, an electronic particle counter (Coulter Mulitsizer IIe) was used to enumerate microspheres and establish beginning concentrations. In all assays, mussels were delivered microspheres at a total concentration of ca. 1.0×10^4 spheres · mL^{-1}, whereas oysters were delivered microspheres at a concentration of 1.5×10^4 spheres · mL^{-1}. Each assay ran for 1 h, with T_0 for a given individual starting when the shell valves opened. A 10 mL sample of the suspension was collected at the beginning (T_0) and then 30 min (T_{30}) and 60 min (T_{60}) after the start of the assay to calculate changes in microsphere ratios in suspension over time. All pseudofeces and feces produced by each individual throughout the assay were collected, by gently suctioning the biodeposits using glass pipettes as they were being expelled, and placed in separate Falcon tubes.

Microsphere pairs were selected to test several hypotheses regarding the effects of surface properties on particle selection (Table 2). For example, choosing microspheres with a similar surface charge and different wettabilities tested the null hypothesis that wettability has no effect on selection. Choosing microspheres with similar wettabilities and different surface charges tested the null hypothesis that charge has no effect on selection. Particles with similar surface properties were also paired to determine if selection was occurring independently of the two measured properties.

2.5. Microsphere enumeration

Biodeposits (feces and pseudofeces) were digested in 10% sodium hydroxide (NaOH) for 7 days or in 70% nitric acid for 2 days depending upon the microsphere type (e.g., NaOH dissolves silica spheres) to disrupt the biodeposit matrix and disperse the spheres. After digestion, samples were washed (2×) in MQ, centrifuged at 1500 *g* for 10 min, and resuspended in 10 mL of MQ. Resuspended microspheres were enumerated using a FACScan flow cytometer (BD BioSciences, San Jose, CA). A fluorescence detector (FL2) and forward scatter detector (FSC) were used to enumerate the fluorescent polystyrene particles. The silica and aluminum particles were enumerated using the FSC and side-scatter (SSC) detectors.

2.6. Data analyses

A modified electivity index (EI) was calculated based upon the number of microspheres in each fecal and pseudofeces sample to determine if selection was occurring (Jacobs, 1974; MacDonald and Ward, 1994). This index was defined as:

$$\mathrm{EI} = \frac{(S - W)}{(S + W) - 2(S \times W)},$$

Table 2
Surface properties of microsphere pairings used in the feeding assays. Shaded lines show the zeta potential (mV) of each microsphere type. Un-shaded lines represent the contact angle (°) of each microsphere type, where an angle >90° is a wettable (=hydrophilic) surface, and an angle <90° is a non-wettable (=hydrophobic) surface. See Table 1 for microsphere designations.

YG vs alumina	
–11	–6
113°	0°
YG vs silica amino	
–11	4
113°	10°
YG vs silica	
–11	–11
113°	7°
YG vs silica (mc)	
–11	–2
113°	93°
YG vs YG-C	
–11	–8
113°	112°
YG vs YG-C (mc)	
–11	–1
113°	112°
YG-C vs alumina	
–8	–6
112°	0°
YG-C vs silica amino	
–8	4
112°	10°
DG vs silica amino	
–4	4
108°	10°

where *S* is the proportion of the particular microsphere type in the post-capture sample (pseudofeces or feces) and *W* is the proportion of that microsphere in the suspension at the beginning of the assay. A negative EI indicates that the particular microsphere type was depleted in the sample (pseudofeces or feces) compared to the initial suspension; whereas, a positive EI indicates that the microsphere type was enriched in the sample.

Calculated EI for each microsphere type tested were arcsine transformed (Zar, 1984) and compared to zero using a one-sample t-test (as per Ward and Targett, 1989). As a result of biological variation (i.e., non-feeding, spawning events), the *n* for each assay varied from 4 to 13. Indices that were not significantly different from zero indicated that no selection between the two microsphere types had occurred. Significantly positive or negative EI values indicated that selection had occurred. Regression analyses were also performed to examine the dependence of EI on surface property variables.

A discriminant analysis (DA) was performed to determine if particles that were rejected, preferentially ingested, or not selected (based on the significance of EI values) could be classified on the basis of both surface charge and wettability. Two analyses were run on the data obtained from each bivalve species. The first analysis used the measured surface property values for each microsphere tested. The second analysis used the difference in surface properties between pairs of microspheres used. The generated variables included delta surface charge (absolute value) and delta wettability. A classic linear model (Systat 13©) was applied and Wilks' Lambda statistic used to determine significance of the models. Canonical correlations and discriminant functions were calculated to determine the proportion of total variability explained by the models and determine which of the surface properties (= factors) was the most important in classifying the microsphere types into their respective groups. In all statistical tests a significance level of $\alpha = 0.05$ was used (Table 3).

3. Results

3.1. Particle surface properties

The surface properties of six different types of microspheres were measured, with three different types (YG-C, DG, and SiO_2) being analyzed both before and after being coated with 0.5% methyl cellulose (Table 1). With the exception of the silica-amino microspheres (+4.3 mV), all spheres had negative surface charges ranging from −3.8 mV for polystyrene with carboxyl groups to −11.3 mV for the polystyrene without the carboxyl groups. Contact angles ranged from 0° (wettable) for the alumina microspheres to 112° (non-wettable) for the polystyrene microspheres. For microspheres coated with methyl cellulose, surface charges ranged from −0.5 mV to −3.8 mV and contact angles ranged from 92° to 117°. Correlation analyses showed no significant relationship between the surface charges and contact angles of the microspheres ($n = 42$, $r = 0.19$, $P > 0.05$). A two-sample t-test showed no significant difference between the zeta potential of microspheres in seawater at a salinity of 15 and those at a salinity of 30 ($n = 6$, $P > 0.05$). Zeta potential measurements taken at a salinity of 15 were, thus, representative of zeta potentials measured at 30, the salinity at which the experiments were conducted.

Surface charges measured for the three species of microalgae (*Isochrysis* sp., *T. maculata*, and *N. closterium*) and two natural particles commonly found in estuarine environments (clay and dead, ground *S. alterniflora*) ranged from −6.9 mV to −14.6 mV. Contact angles of these particles ranged from 9° to 93° (wettable and non-wettable surfaces; Table 1). These data demonstrate that surface properties of experimental microspheres were similar to the properties of particles encountered by suspension-feeders in natural habitats.

Table 3
Results of the discriminant analysis (DA) model. There was no notable difference observed in the model predictions based on whether the model was run with either the absolute particle surface characteristics or the differences between particle properties (delta). Both models were significant ($P < 0.01$) for the oyster and mussel data. This indicates that the model can discriminate between no selection and selection. The discriminant functions were low, indicating there is no functional difference between surface charge and wettability alone as qualifiers of selection. The mussel model was stronger, correctly explaining 72% of the observed variance in the data.

	Oysters		Mussels	
	Absolute values	Delta values	Absolute values	Delta values
R^2	0.256	0.26	0.719	0.719
Canonical correlation	26%	26%	72%	72%
Angle function	0.024	0.024	0.04	0.04
Charge function	0.024	0.016	0.07	0.09
Wilk's Lambda	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

3.2. Bioassay parameters

Based upon the calculated surface properties, nine different microsphere combinations (= assays) were offered to both oysters and mussels (Table 2). These combinations reflected a broad range of surface properties that were similar to those of natural particles. The theoretical proportion of the two microsphere types in each assay was 50:50; however, *a posteriori*, flow-cytometric counts showed that this theoretical ratio often was not achieved in the exposure water. Measured ratios of the two microsphere types remained stable during each assay (water samples taken at T_{30} and T_{60}; data not shown), indicating that there was little differential settling of the microspheres (<5%). Nonetheless, because of the cumulative effect of small differences in starting ratios, settling rate and capture efficiency, microsphere proportions in the assay water at T_0 were not used as "*W*" in the EI equation (see Section 2.6). Instead, the total number of microspheres captured (i.e., total in pseudofeces plus feces) was used to calculate proportions (Ward and Targett, 1989). For each bivalve, this ratio was a more reliable and conservative estimate of the proportion of microspheres that were captured. *A posteriori* counts of the number of microspheres in the biodeposits and the number not captured after an assay ended (T_{60}), revealed that ~95% of all offered microspheres were recovered. This high recovery rate suggests that few ingested microspheres were lost.

3.3. Differential handling by the pallial organs

Electivity indices (EI) for the pseudofeces of oysters demonstrated that pre-ingestive selection of several microsphere types occurred. When given in combination with YG microspheres, mean EI for only AlO_2 was significantly greater than zero (rejected; $\bar{x} = 0.61 \pm 0.2$ SD, $n = 5$; Fig. 1A). Mean EI for no other microsphere type was significantly different from zero when given in combination with YG. When delivered in combination with YG-C microspheres, EIs for both AlO_2 and $SiNH_2$ were significantly greater than zero in the pseudofeces (rejected; $\bar{x} = 0.53 \pm 0.3$ SD, $n = 8$; $\bar{x} = 0.48 \pm 0.3$ SD, $n = 8$; respectively; Fig. 1B). When given in combination with DG microspheres, however, mean EI for $SiNH_2$ was not significantly different from zero (no selection; $\bar{x} = 0.02 \pm 0.2$ SD, $n = 5$). In all assays, the opposite result was found for microspheres in the feces. Additionally, when delivered in combination with YG microspheres, EI for methyl-cellulose-coated YG-C was significantly less than zero in the feces (rejected; $\bar{x} = 0.34 \pm 0.4$ SD, $n = 9$).

Electivity indices for the pseudofeces of mussels also demonstrated that pre-ingestive selection of several microsphere types occurred. When given in combination with YG microspheres, mean EI for AlO_2 was significantly greater than zero in the pseudofeces (rejected; $\bar{x} = 0.44 \pm 0.2$ SD, $n = 4$). In contrast, EIs for YG-C and methyl-cellulose-coated SiO_2 and YG-C were significantly less than zero in the pseudofeces (preferentially ingested; $\bar{x} = -0.37 \pm 0.3$ SD, $n =$

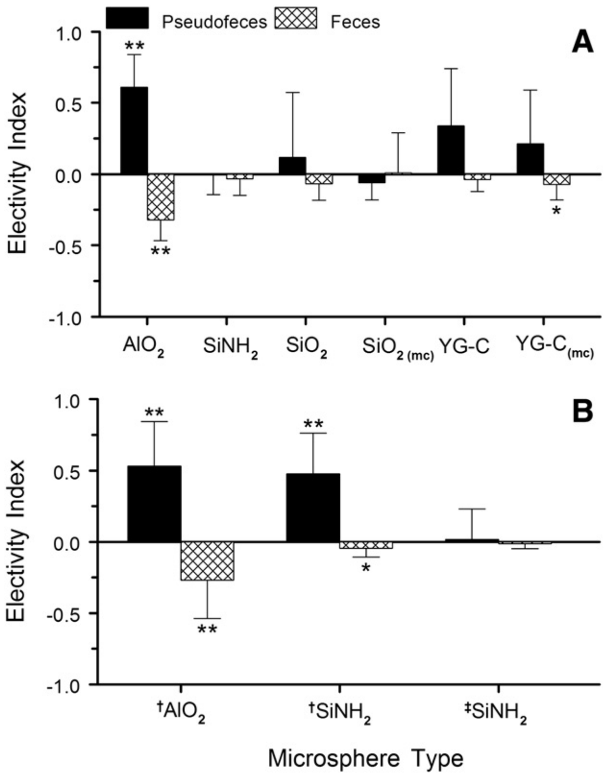

Fig. 1. Electivity indices exhibited by oysters for each of the feeding assays. An index of zero indicates no selection occurred. A negative EI indicates depletion of the microsphere type, whereas a positive EI indicates enrichment of the microsphere type in the sample. A) Microspheres tested against fluorescent polystyrene (YG). B) Microspheres tested against one of two fluorescent polystyrene microspheres with attached carboxyl groups (each with unique surface properties; see Table 1). † denotes AlO_2 and $SiNH_2$ tested against YG-C; ‡ denotes silica-amino tested against DG (see text for sphere descriptions), mc denotes microspheres treated with methyl-cellulose. Data presented as means ± standard deviation (n = 5–12). ** and * denote indices significantly different from zero at $P < 0.01$ and $P < 0.05$, respectively (one-sample t-test).

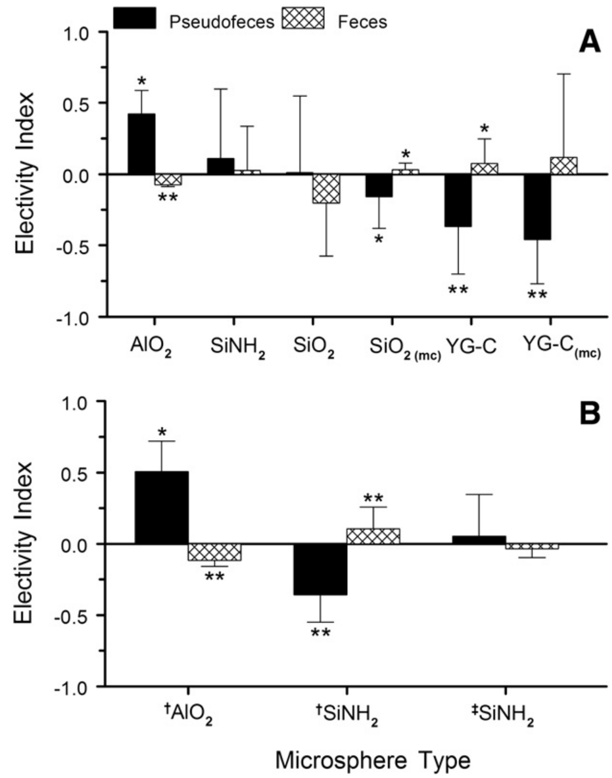

Fig. 2. Electivity indices exhibited by mussels for each of the feeding assays. An index of zero indicates no selection occurred. A negative EI indicates depletion of the microsphere type, whereas a positive EI indicates enrichment of the microsphere type in the sample. A) Microspheres tested against fluorescent polystyrene (YG). B) Microspheres tested against one of two fluorescent polystyrene microspheres with attached carboxyl groups (each with unique surface properties; see Table 1). † denotes AlO_2 and $SiNH_2$ tested against YG-C; ‡ denotes silica-amino tested against DG (see text for sphere descriptions), mc denotes microspheres tested with methyl-cellulose. Data presented as means ± standard deviation (n = 4–13). ** and * denote indices significantly different from zero at $P < 0.01$ and $P < 0.05$, respectively (one-sample t-test).

13; $\bar{x} = -0.41 \pm 0.42$ SD, $n = 9$; $\bar{x} = -0.46 \pm 0.3$ SD, $n = 11$; respectively; Fig. 2A). Mean EI for no other microsphere type was significantly different from zero when given in combination with YG. When delivered in combination with YG-C microspheres, mean EI for AlO_2 was significantly greater than zero in the pseudofeces (rejected; $\bar{x} = 0.55 \pm 0.3$ SD, $n = 4$), whereas EI for $SiNH_2$ was significantly less than zero (preferentially ingested; $\bar{x} = -0.36 \pm 0.2$ SD, $n = 8$; Fig. 2B). When given in combination with DG microspheres, however, mean EI for $SiNH_2$ was not significantly different from zero (no selection; $\bar{x} = 0.05 \pm 0.3$ SD, $n = 10$). In all assays, the opposite result was found for microspheres in the feces, except for methyl-cellulose-coated YG-C microspheres, the EI of which was not significantly different from zero (no selection; $\bar{x} = 0.12 \pm 0.59$ SD, $n = 11$).

3.4. Selection model

Regression analyses were performed on the mean EI of samples of pseudofeces and feces versus the surface properties of each microsphere type. For the oyster data, there was no significant relationship between EI values and surface charge or density ($P > 0.05$). There were weak, but significant positive relationships between the pseudofeces and feces EI and contact angle ($R^2 = 0.06$ and $R^2 = 0.07$, respectively, $P < 0.05$). Regression analyses of the mussel data showed no significant relationship between pseudofeces or feces EI and charge, angle or density ($P > 0.05$).

Regression analyses were also performed on the mean EI of pseudofeces and feces samples versus the absolute difference in surface properties between pairs of microsphere types. For the oyster data, there were weak, but significant positive relationships between pseudofeces and feces EI and delta charge ($R^2 = 0.12$ and $R^2 = 0.19$, respectively, $P < 0.05$). There was no significant relationship between pseudofeces or feces EI and delta angle ($P > 0.05$). For the mussel data, the only significant relationship was between pseudofeces EI and delta angle ($R^2 = 0.53$, $P < 0.05$). There was no significant relationship between pseudofeces EI and delta charge, or feces EI and delta charge or delta angle ($P > 0.05$).

Discriminant analysis (DA), using charge and contact angle as predictor variables, produced models with significant discriminant functions (Wilks' Lambda, $P < 0.01$). There was no notable difference observed in the results of models run using measured particle surface characteristics or the difference (delta) in surface properties between pairs of microspheres. For the oyster data, the models explained only ca. 26% of the total variability. Although the models correctly classified 100% of the rejected microsphere types, overall only 64% of all types could be classified into their correct groups (rejected or not selected; Fig. 3A). For the mussel data, the models explained ca. 72% of the total variability. Again, the models correctly classified 100% of the rejected microspheres, and overall 75% of all types were classified into their correct groups (rejected, preferentially ingested or not selected; Fig. 3B).

4. Discussion

The ability of suspension-feeding bivalves to select among captured particles has been studied extensively and is well documented

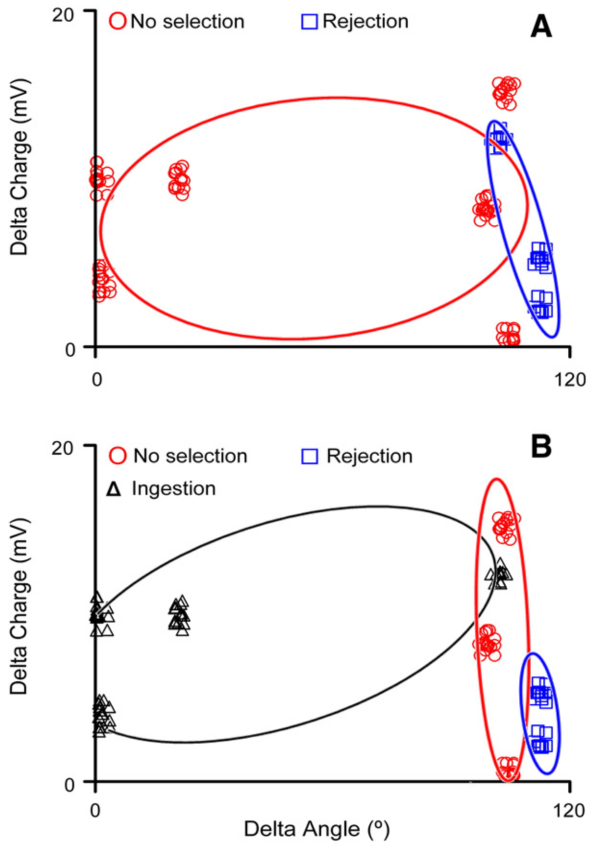

Fig. 3. Scatter plot matrix (SPLOM) of discriminant analysis models. Ellipses show areas of predicted selection (e.g. rejection, ingestion, no selection) based on surface-property values. Plotted data are absolute differences in surface characteristics (delta charge and delta angle), between the "counter" microsphere and the nine microspheres of interest. Data presented with random jitter to show data points with the same surface properties. A) Oyster selection model (describes 26% of variability). B) Mussel selection model (describes 72% of variability).

(see Bayne and Newell R.C., 1983; Beninger, 1991; Jørgensen, 1996; Ward and Shumway, 2004 for review). The mechanisms that underlie the selection process, however, remain largely unknown. Isolating individual potential cues that may influence particle selection allowed us to measure the subtle effects of surface properties on selectivity. Results of the present study demonstrate that the oyster, *C. virginica*, and the mussel, *Mytilus edulis*, can discriminate between particles of the same size based upon surface properties, rejecting some types, such as those composed of aluminum oxide, at higher proportions than other types, such as those composed of polystyrene with and without carboxyl groups. These findings suggest that non-specific physicochemical interactions play a role in mediating a passive selection mechanism. This conclusion also points to the need to characterize the surface properties (e.g. wettability and charge) of organic and inorganic particles that are used in selection experiments to understand fully the roles that particle physicochemical characteristics may play in the feeding process.

Although selective feeding occurred in ca. 50% of the assays, neither of the two surface-property variables alone could explain the observed patterns of particle selection. Electivity indices were weakly dependent on the magnitude of surface charge and wettability (linear regression). In some assays, microspheres with very different surface charges were handled in the same manner (e.g., oysters rejected both AlO_2 [−6.0 mV] and $SiNH_2$ [4.3 mV]); whereas, in other assays microspheres with similar surface charges were handled differently (e.g., mussels preferentially ingested YG-C [−8.0 mV] and rejected AlO_2 [−6.0 mV]). In some assays, highly-wettable microspheres were either rejected (e.g., oysters and mussels delivered AlO_2), preferentially ingested (e.g., mussels delivered $SiNH_2$), or not selected (e.g., oysters and mussels delivered SiO_2).

Using both surface-property variables in discriminant analyses (DA) produced models that could classify microsphere types as either being rejected, preferentially ingested or not selected with varying degrees of accuracy. Although the discriminant functions of both models were significant, the oyster model was much weaker (explaining 26% of the variability) than the mussel model (explaining 72% of the variability. Examination of the scatterplot matrices (SPLOM; Fig. 3) produced by the analyses suggested that the most-wettable particles (e.g., alumina, silica) were more likely rejected by oysters and mussels than non-wettable particles. Natural inorganic particles (e.g., Kaolin), which have little nutritional value, also tend to be very wettable and are often rejected in the pseudofeces (Kiørboe et al., 1980; Newell R.I.E. and Jordan, 1983). Overall, our data suggest that when two particles with an intermediate difference in surface charge (between 2 mV and 5 mV), and large differences in contact angle (>100°), were fed to bivalves the more-wettable particle tended to be rejected. When particles of similar wettabilities were fed, the particle with the more negative or more positive charge was often rejected. A broader library of particles with different surface properties is needed, however, before more-robust, predictive models can be developed describing the surface characteristics that elicit rejection or preferential ingestion in bivalves.

Differences in density between some of the microspheres used in the study presented a confounding factor in the analyses of the data. For example, alumina spheres, which were consistently rejected by oysters and mussels, had the highest density, but lowest contact angle of any particle tested. In fact, the density of all microspheres was highly correlated with their contact angle measurements (Pearson correlation coefficient = −0.8). Whether particle density plays a role in selection has been the source of debate for many years (Bernard, 1974; Newell R.I.E. and Jordan, 1983; Ward and Targett, 1989; Yonge, 1923). In the current study, we argue that density was not an important factor in particle selection by oysters and mussels because capture and transport of particles by the feeding organs of bivalves occurs at very low Reynolds numbers (e.g., Ward, 1996). By applying Stokes' Law, we calculated the settling velocity of the most-dense microsphere used in this study (alumina, diameter = 10 μm, density = 3.9 g cm^{-3}). Using standard values for seawater at a temperature of 20 °C and salinity of ca. 32, settling velocity for alumina spheres was calculated to be about 0.5 μm h^{-1}. This velocity is orders of magnitude lower than pre-capture particle approach velocity (ca. 6×10^6 μm h^{-1}) and post-capture particle handling velocities (range ca. 0.8 to 3.8×10^6 μm h^{-1}) measured for the pallial organs of several bivalve species (Ward, 1996). Accordingly, the effect of different densities on the movement of particles and hence selection was very likely negligible. Additionally, different microspheres with equal densities were rejected or preferentially ingested, suggesting that other characteristics were more important in the observed particle discrimination.

The recent findings by Pales Espinosa et al. (2009, 2010a, 2010b) on the role of lectins in particle selection, and results of the present study imply an expanded role of mucus in the feeding process of bivalves (e.g., selection, capture, and transport). Results of previous studies support this contention. For example, Beninger and Decottignies (2005) examined the effects of the organic "casing" of the phytoplankton *Coscinodiscus perforatues* on feeding selectivity of the king scallop *Pecten maximus*. Exposing the bivalve to empty senescent cells, the authors found that the intact frustules and associated organic molecules elicited preferential ingestion of empty cells, which was similar to that of living cells. A follow-up study examining the same effects on feeding selectivity by the oyster *Crassostrea gigas* found a different result. Oysters were able to differentiate between the empty and living cells (Beninger et al., 2008), suggesting that the growth phase of the diatom (e.g., stationary vs senescent) was a quality factor in selection for this bivalve species. These findings suggest that interspecific differences in feeding selectivity can occur, and that the surface properties of cells and their interaction with mucus produced by the feeding structures can mediate selection. Similar conclusions have been drawn from research on the adhesion of bacteria to the epithelial mucus of rainbow trout. In these studies (Amaro et al., 1995; Balance et al., 2002; Krovacek et al., 1987; Swanson et al., 2003), adhesion of different types of bacterial cells was affected by the specific qualities of mucus produced by the trout.

In bivalves, both specific (e.g., lectin-sugar) and non-specific interactions (e.g., surface-charge, wettability) seem to contribute to particle discrimination. These interactions are likely mediated by the type of mucus and mucus constituents produced by the feeding organs, and act in concert to produce a biologically significant selection response. The results also support the idea that particle selection in bivalves may be variable, e.g., particles with given surface characteristics may be preferentially ingested under some conditions and rejected under others.

Acknowledgements

The authors would like to thank Dr. Devendra Kalonia for use of laboratory space and equipment, Gregg Rivara at Cornell Cooperative Oyster Hatchery for providing oysters, Bridget Holohan and Jennifer Alix for technical support, and Dr. Lauren Stefaniak and Kelley Bostrom for helpful comments on the manuscript. This study was supported by NSF Grant# IOS-0718820 and IOS-1147122 to JEW and SES and IOS-0718453 and IOS-1146920 to EPS and BA, with additional support to MR from the National Shellfisheries Association, the American Malacological Society, and the Department of Marine Sciences, University of Connecticut. **[RH]**

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