Reference: *Biol. Bull.* **238:** 41–53. (February 2020)

DOI: 10.1086/707718

Particle Selection in Suspension-Feeding Bivalves: Does One Model Fit All?

JULIE JONES, BASSEM ALLAM, AND EMMANUELLE PALES ESPINOSA*

School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York 11794-5000

Abstract. Suspension-feeding bivalves are known to discriminate among a complex mixture of particles present in their environments. The exact mechanism that allows bivalves to ingest some particles and reject others as pseudofeces has yet to be fully elucidated. Recent studies have shown that interactions between lectins found in the mucus covering oyster and mussel feeding organs and carbohydrates found on the microalga cell surface play a central role in this selection process. In this study, we evaluated whether these interactions are also involved in food selection in bivalves with other gill architectures, namely, the clam *Mercenaria mercenaria* and the scallop *Argopecten irradians*. Statistical methods were used to predict whether given microalgae would be rejected or ingested depending on their cell surface carbohydrate profiles. Eight different microalgae with previously established surface carbohydrate profiles were grown and harvested during their exponential growth phase to be used in feeding experiments. Microalgae were then used in 17 feeding experiments where different pairs of microalgae were presented to clams and scallops to evaluate selection. Decision trees that model selection were then developed for each bivalve. Results showed that microalgae rich in mannose residues were likely to be ingested in both bivalves. N-acetylglucosamine and fucose residues also seem to play a role in food particle choice in scallops and clams, respectively. Overall, this study demonstrates the role of carbohydrate-lectin interactions in particle selection in suspension-feeding bivalves displaying different gill architectures, and it highlights the importance of mannose residues as a cue for the selection of ingested particles.

Received 25 July 2019; Accepted 14 December 2019; Published online 11 February 2020.

* To whom correspondence should be addressed. Email: emmanuelle.palesespinosa@stonybrook.edu.

Abbreviations: AUC, area under the receiver-operating characteristic curve; ConA, *Canavalia ensiformis* lectin; ECA, *Erythrina cristagalli* lectin; FITC, fluorescein isothiocianate; FL1–FL3, fluorescence channels 1–3; NIFL, normalized intensity of FITC-lectin; PEA, *Pisum sativum* agglutinin; PWM, *Phytolacca americana* lectin; SE, sorting efficiency; UEA, *Ulex europaeus* lectin; WGA, wheat germ agglutinin.

Introduction

Suspension-feeding bivalves thrive in coastal areas and provide many important ecosystem services (Asmus and Asmus, 1991; Dame, 1996). They contribute to many ecological functions, including building habitats for other species, removing phytoplankton and transferring energy to the benthos (*e.g.*, *via* biodeposition), and cycling dissolved nutrients (Cloern, 1982; Prins *et al.*, 1997; Newell, 2004). In their habitats, suspension-feeding bivalves interact with a large amount of diverse suspended particles (Alldredge and Silver, 1988); thus, they have evolved strategies to discriminate between these materials and to select their food from a complex surrounding environment (Allen, 1921; Fox, 1936; Ward and Shumway, 2004; Pales Espinosa *et al.*, 2008). This selection enhances the ingestion of nutritious particles and optimizes the bivalves' energy uptake. However, the exact mechanism that allows bivalves to reject certain particles in favor of higher-quality particles remains largely unknown (Ward and Shumway, 2004). This lack of knowledge limits the understanding of how energy flows through coastal ecosystems.

Previous studies have explored suspension feeding in bivalves, and several authors have highlighted the importance of ctenidium architecture in this process (Ward *et al.*, 1994; Beninger and St-Jean, 1997b; Beninger *et al.*, 2005). While the functional activity of the ctenidium is conserved among different bivalves, the organization of this organ has evolved into different architectures on the basis of the degree to which contiguous filaments are connected to each other (Atkins, 1936, 1937a, b; Galtsoff, 1964; Dufour, 1998; Pechenik, 2000; Gosling, 2015). For example, mussels (*e.g.*, genus *Mytilus*) harbor relatively simple filibranch ctenidia, where contiguous filaments are attached to each other by specialized ciliary junctions. In contrast, the pseudolamellibranch (*e.g.*, oysters) and

the eulamellibranch (*e.g.*, clams, such as members of the genus *Mercenaria*) have evolved a more complex gill architecture where contiguous filaments are joined by connective tissue (*i.e.*, interfilament junction). Ctenidium complexity is further magnified by the fact that, in some bivalves, the total surface area has increased by folding or plica. In this specific case, filaments are often organized into principal and ordinary filaments, and the ctenidia are then designated "heterorhabdic" (*e.g.*, oysters, scallops) as opposed to "homorhabdic" (one single type of filament, sometime designated as "flat gill," *e.g.*, mussels). The direction of the beating of the ciliary tracts on filaments is also complex (Ribelin and Collier, 1977; Beninger *et al.*, 2005). All of these different morphologies may have an impact on particle selection, and previous studies highlighted differential involvement of ctenidia in particle sorting (as opposed to labial palps) among different bivalve taxonomic groups (Ward *et al.*, 1998b).

Other criteria, such as the characteristics of suspended particles, have also been suspected as being involved in food sorting. For example, it was found that live cells are selectively ingested by bivalves, while mineral particles and detrital matter are rejected (Newell and Jordan, 1983; Pastoureaud *et al.*, 1996; Ward *et al.*, 1998b). In addition, when a mix of several species of microalgae is presented to some bivalves, they are able to select some species over others (Shumway *et al.*, 1985; Cognie *et al.*, 2001). Studies have also supported the idea that the selection process can be controlled by biochemical cues and that the physicochemical surface properties of food particles may also contribute to selection (Beninger and Decottignies, 2005; Pales Espinosa *et al.*, 2008, 2016a; Rosa *et al.*, 2013). Our recent investigations demonstrated that cell surface carbohydrates covering microalgae have an essential role in microalgal selection by oysters (*Crassostrea virginica*) and mussels (*Mytilus edulis*); and results showed that these organisms preferentially ingest microalgae rich in glucose and mannose residues (Pales Espinosa *et al.*, 2016a). In fact, a growing body of evidence suggests that mucus covering the feeding organs of bivalves is not a mere carrier for captured particles but that specific lectin-carbohydrate interactions take place between mucus and food particles, triggering selection (Pales Espinosa *et al.*, 2010a; Pales Espinosa and Allam, 2013). Despite these recent discoveries, the exact factors that determine whether a particle will be ingested or rejected by suspension-feeding bivalves are still not completely known. More importantly, our current knowledge of the role of food particle carbohydrates in sorting is limited to *C. virginica* and *M. edulis*, and it is unclear whether carbohydrate-protein interactions are involved in food selection in other bivalve species with different gill architectures.

The objective of the present study is to determine the role of cell surface carbohydrates in microalgal selection in the hard clam (*Mercenaria mercenaria*; also known as northern quahog) and the bay scallop (*Argopecten irradians*). Both of these bivalves present ctenidium architecture different from that of oysters and mussels (Table 1), and they have already been shown to be able to sort particles (Bricelj and Malouf, 1984; Shumway *et al.*, 1997; Grizzle *et al.*, 2001). These two bivalves also have different habitats, biology, and behaviors. The hard clam has a long life span and lives mostly buried in sediment, and it uses its siphon to pump water and food particles present along the surface of sediment (Kraeuter and Castagna, 2001). The bay scallop has a short life and is motile (Pohle *et al.*, 1991). When young, bay scallops attach themselves, often to eelgrass, to avoid predators. They then drop to the sediment surface as they grow and as they move on along the sediment.

Using statistical models, we evaluated whether cell surface carbohydrates covering microalgae play a role in the particle selection process of these two bivalves. Once confirmed, we further assessed whether these carbohydrates are similar to those involved in food sorting in oysters and mussels. The study also served to provide predictions as to whether a particular microalga will be ingested or rejected by a bivalve, based on its cell surface carbohydrate signature.

Materials and Methods

Bivalves

Clams (*Mercenaria mercenaria* (Linnaeus, 1758)) (48.2 ± 2.5 mm shell length) were obtained from Frank M. Flower and Sons Oyster Company (Oyster Bay, NY), and scallops (*Argopecten irradians* (Lamarck, 1819)) (60.0 ± 4.2 mm shell length) were collected from Southold Bay (Southold, NY) in July 2017. All epibionts were cleaned from bivalve shells, and the bivalves were acclimated in the laboratory for one week. They were fed daily (5% dry weight) with DT's Live Marine Phytoplankton (Sustainable Aquatics, Jefferson City,

Table 1

Bivalve species in which particle selection has been modeled based on microalgae cell surface carbohydrates

Species	Common name	Filament connection	Filament type	Source
Mytilus edulis	Blue mussel	Filibranch	Homorhabdic	Pales Espinosa *et al.*, 2016a
Argopecten irradians	Bay scallop	Filibranch	Heterorhabdic	This study
Crassostrea virginica	Eastern oyster	Pseudolamellibranch	Heterorhabdic	Pales Espinosa *et al.*, 2016a
Mercenaria mercenaria	Hard clam	Eulamellibranch	Homorhabdic	This study

TN) (Pales Espinosa and Allam, 2006) and fresh algal cultures (*Isochrysis* sp.). A day prior to being used in feeding experiments, bivalves were held in filtered seawater (0.45 μm).

Microalgae

Microalgae species were selected using several criteria: a size range optimal for bivalve feeding, different taxonomic groups, and whether they were relatively easy to grow (Table 2). Selected species were grown in f/2 medium (Guillard, 1982) at 18 °C under a 12h : 12h dark : light photoperiod. Growth rate was determined daily by counting, and microalgae were harvested in the exponential phase of growth and were used immediately in feeding experiments. The carbohydrate signature of exponentially growing microalgae was previously determined using eight commercial lectins (Table 3; Pales Espinosa *et al.*, 2016a). Briefly, the procedure was done by separately incubating algae with fluorescently labeled (fluorescein isothiocianate [FITC]) lectins before fluorescent signal intensity was collected on the green fluorescence channel (FL1) of a BD BioSciences (San Jose, CA) FACSCalibur flow cytometer (see *Flow cytometry analysis*). Fluorescence signals were then corrected by subtracting fluorescence signals generated by unlabeled algae (*i.e.*, auto-fluorescence). Furthermore, fluorescence signals were then normalized to the size of each microalga (forward scatter), since larger algae species produce higher fluorescence even if the lectin affinity is similar to that of a smaller species. A final normalization was made to correct for differences in the amount of FITC molecules attached to each lectin molecule (provided by the manufacturer, EYLabs, San Mateo, CA). Accordingly, each microalga was characterized by eight normalized intensities of FITC-lectin (NIFL) (Table 3).

Feeding experiments

Experiments followed the general design described in detail in Pales Espinosa *et al.* (2016a). Microalgae were used in a series of feeding experiments to investigate potential relationships between microalgal cell surface carbohydrates and selection or rejection by bivalves. Clams and scallops (n = 10–12 per experiment, as well as an empty shell used as a negative control to account for possible particle settling) were transferred to individual 3-L aquaria and were used in a total of 17 discrete feeding experiments performed over a total period of 3 weeks in July 2017 (typically, three experiment days per week), using exponentially growing microalgal cultures to minimize changes in bivalve physiological status and in microalga cell surface characteristics due to algae growth phase. Microalgae were kept in suspension by slow stirring, using a micropipette every 10 minutes; and at the same time, water samples from each aquarium were taken to determine possible changes in the ratio between the two microalgae (possibly from sedimentation or different retention efficiencies).

Each experiment tested a different pairing containing equal concentrations (50% each) of two microalgae (*ca.* 10^5 cells mL^{-1} final concentration). The design of the feeding experiments is summarized in an interaction network analysis (Fig. 1; Cytoscape, ver. 3.0, Shannon *et al.*, 2003). For each diet, water samples (1 mL) were taken and used to determine the ratio of the two microalgae. About 15 minutes after the beginning of their production, pseudofeces were collected. Pseudofeces were then homogenized by vortexing and were filtered through a 50-μm nylon-mesh sieve to be immediately analyzed using flow cytometry.

Flow cytometry analysis

A FACSCalibur flow cytometer (BD BioSciences) was used to analyze pseudofeces composition. Excitation was achieved using a 488-nm argon laser, and a minimum of 2×10^4 events were analyzed. One or more of the following parameters were used to characterize, discriminate, and count microalgae: forward light scatter (FSC, particle size) and side light scatter (SSC, intracellular complexity), FITC fluorescence (FL1, 535 nm), and photosynthetic pigment auto-florescence (FL2, 585 nm for phycoerythrin; FL3, 670 nm for chlorophyll *a*).

Data treatment and statistical analysis

XLSTAT software (ver. 2015.1.02, Addinsoft, New York, NY) and Statgraphics (ver. 5.1, Statgraphics Technologies, The

Table 2

List of microalgae used in the feeding experiments

Class	Species	Strain (Milford Microalgal Culture Collection)	Cell length (μm)
Bacillariophyceae	*Amphora coffaeaformis*	A-ora	20
Chlorophyceae	*Chlamydomonas* sp.	11/35	10
Chlorophyceae	*Chlorella autotrophica*	580	2
Prymnesiophyceae	*Cricosphaera carterae*	961	10
Prymnesiophyceae	*Pavlova lutheri*	MONO	4
Prasinophyceae	*Prasinocladus marinus*	163/1B	10
Cryptophyceae	*Rhodomonas lens*	Rhodo	12
Prasinophyceae	*Tetraselmis chui*	PLY429	9

Table 3

Fluorescein isothiocianate-labeled lectins used to characterize microalgae cell surface carbohydrates

Name	Origin	Carbohydrate specificity
ECA	*Erythrina cristagalli*	N-acetyllactosamine, galactose
HPA	*Helix pomatia*	N-acetylgalactosamine
PWM	*Phytolacca americana*	Oligomers of β(1,4)-linked N-acetylglucosamine
ConA	*Canavalia ensiformis*	α-D-mannose, α-D-glucose, branched mannose
PEA	*Pisum sativum*	Methyl-D-mannopyranoside, D-mannose
PNA	*Arachis hypogaea*	Terminal β-galactose
WGA	*Triticum vulgaris*	Chitobiose, N-acetyl-glucosamine
UEA-I	*Ulex europaeus*	α-L-fucose

Plains, VA) were used in this study to conduct all statistical analyses. Two series of goodness-of-fit tests (G tests) were used to analyze data obtained from feeding experiments as described previously (Pales Espinosa *et al.*, 2009). A first series of tests ensured that, within each treatment, replicate samples of the diet and pseudofeces were homogeneous. The second series tested the null hypothesis that the proportion of each particle type in the diet or in the pseudofeces was not different between treatments. In addition to the comparison of raw counts, a sorting efficiency (SE) index was calculated to examine particle selection (Iglesias *et al.*, 1992). This index was calculated as

$$\mathrm{SE} = 1 - (p/D),$$

where p represents the proportion of a particular particle in the pseudofeces and D represents the proportion of a particular

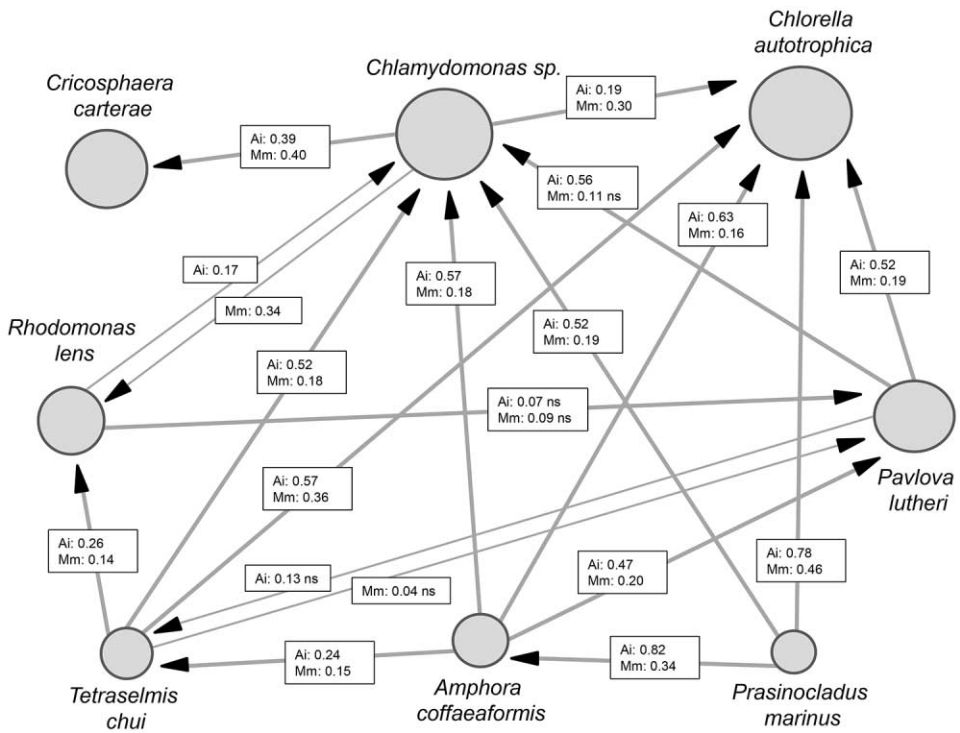

Figure 1. Interaction networks depicting the food choices of scallops (*Argopecten irradians*; Ai) and clams (*Mercenaria mercenaria*; Mm). Nodes associated with eight species of microalgae are drawn as gray circles. Node size is proportional to closeness centrality (*i.e.*, measurement that takes into account the number of times a microalga was used in feeding experiments and the number of times it was preferentially ingested). Links represent pairs of microalgae used in feeding experiments. Arrowheads point to the selected microalgae, and sorting efficiencies are boxed and presented on the arrows. Link width is proportional to sorting efficiency index, indicated in the Ai or Mn label. "ns" indicates that the sorting efficiency was not significant (G test, $n = 10$–12, $P > 0.05$).

particle in the diet. For a given particle type, a positive SE signifies that it is preferentially ingested by the bivalve, and a negative SE signifies that it is rejected. Zero signifies that active selection is absent.

Classification trees were then built to predict either the selection or the rejection of a microalga, based on lectin-binding patterns (Breiman *et al.*, 1984; De'ath and Fabricius, 2000; Elith *et al.*, 2008; Pales Espinosa *et al.*, 2016a). Each tree's complexity was held constant (maximum tree depth = 2), and different algorithms were tested (CHAID, exh CHAID, C&RT, and Quest) to simplify biological interpretation. Because each microalga was used in multiple feeding experiments and could be preferentially ingested in one experiment and rejected in another, depending on the second species composing the pair, the simple utilization of NIFLs in the model was inappropriate. Instead, new independent variables were calculated to take into account not the intrinsic value of the NIFL but the difference between the NIFLs of the ingested microalga and the NIFLs of the rejected microalga (*i.e.*, ΔNIFL; *e.g.*, ΔWGA [wheat germ agglutinin]). The performance of a model was examined by evaluating the area under the receiver-operating characteristic curve (AUC) (Hanley and McNeil, 1982). According to a scale established by Swets (1988), a model could be noninformative (AUC = 0.5), poorly accurate (0.5 < AUC < 0.7), moderately accurate (0.7 < AUC < 0.9), highly accurate (0.9 < AUC < 1), or perfect (AUC = 1). In this framework, the AUC was evaluated in order to determine the performance of a model. The robustness of each model was cross validated by removing 10% of the data (*i.e.*, one observation) and calculating a new model, using the remaining data (Ripley, 1996).

In addition, two classification tree models previously established for oysters (*Crassostrea virginica*) or mussels (*Mytilus edulis*) were evaluated for scallops and clams as new test data. Similarly, the two models developed in this study for scallops and clams were evaluated with results obtained previously with *C. virginica* and *M. edulis* to appraise the extent of conservation of carbohydrate cues used for food selection in different bivalve species. The observed values (results from the feeding experiments from a different bivalve) were compared with the expected values, using a confusion matrix and the calculation of the accuracy (XLSTAT ver. 2015.1.02), followed by the calculation of a binomial proportion confidence interval (Agresti and Coull, 1998), an interval estimate of a success probability *P* (R platform [R Foundation for Statistical Computing, Vienna], biconf from Hmisc package). Accuracies within the confidence limit indicate that the model fitted the new set of data.

Results

Results of the 17 feeding experiments are summarized in the interaction networks shown in Figure 1 and Table A1. The ratio of each microalga in all experimental diets hovered around 50% (47%–53%) and did not change throughout the duration of each experiment (chi-square goodness-of-fit tests, $P > 0.25$), suggesting that no differential settling and/or clearance of algae occurred.

In 15 of 17 feeding experiments, the 2 bivalves preferentially ingested the same microalga species but with different sorting efficiencies, with some being not significant (Fig. 1; Table A1). For example, this is the case for both the clams and the scallops when fed the pairings *Pavlova lutheri*/*Chlamydomonas* sp. and *P. lutheri*/*Rhodomonas lens*.

In one feeding experiment, bivalves did not select the same algae. For instance, in the pairing *Chlamydomonas* sp./*R. lens*, scallops preferentially ingested *Chlamydomonas* sp. ($P = 0.03$) while clams preferentially ingested *R. lens* ($P < 0.001$). Finally, when presented with the pairing *P. lutheri*/*Tetraselmis chui*, neither scallops nor clams showed selection. Interestingly, the two microalgae *Chlamydomonas* sp. and *Chlorella autotrophica* were very often preferentially ingested by both bivalves, whereas *Prasinocladus marinus* and *Amphora coffaeaformis* were more often rejected. To further evaluate whether the eight descriptive variables (*i.e.*, ΔNIFL) explain whether a microalga is ingested or rejected and to determine the rules that would govern the model, classification trees were crafted and considered (Breiman *et al.*, 1984; Ripley, 1996). The best model for scallops was obtained using the C&RT algorithm (Gini index, maximum tree depth = 2; Fig. 2). The AUC calculated for this model was 0.93, indicating a highly robust and predictive model. The outcome of the analysis predicted that if ΔPWM (*Phytolacca americana* lectin) is in the interval $[-1.957, -0.056[$ and ΔPEA (*Pisum sativum* agglutinin) is in the interval $[0, 3.653[$, then the corresponding microalga is preferentially ingested in 100% of cases. In other words, if ΔPEA is positive, then it is likely that the microalga will be preferentially ingested (86.7%). The cross validation showed that the AUC obtained for the validation samples ranged between 0.92 and 0.97.

The best model that fits clam sorting data was also generated using the C&RT algorithm and is represented in Figure 3 (maximum tree depth = 2). The AUC for this model was 0.84, suggesting an accurate predictive model. The analysis predicted that if ΔUEA (*Ulex europaeus* lectin) is in the interval $[0.09, 1.156[$ and ΔPEA is in the interval $[-0.199, 3.653[$, then the corresponding microalga is preferentially ingested in 100% of cases. As for scallops, this model establishes that if ΔPEA is positive, then it is likely that the microalga will be preferentially ingested (76.5%). The cross validation showed that the AUC obtained for the validation samples ranged from 0.75 to 0.92.

The models previously established for *C. virginica* and *M. edulis* (Pales Espinosa *et al.*, 2016a) and the two models established for *A. irradians* and *M. mercenaria* (this study) were also cross evaluated using the data obtained with different bivalves (Fig. 4). Statistical analysis (confusion matrices and binomial proportion confidence intervals) revealed that the first model

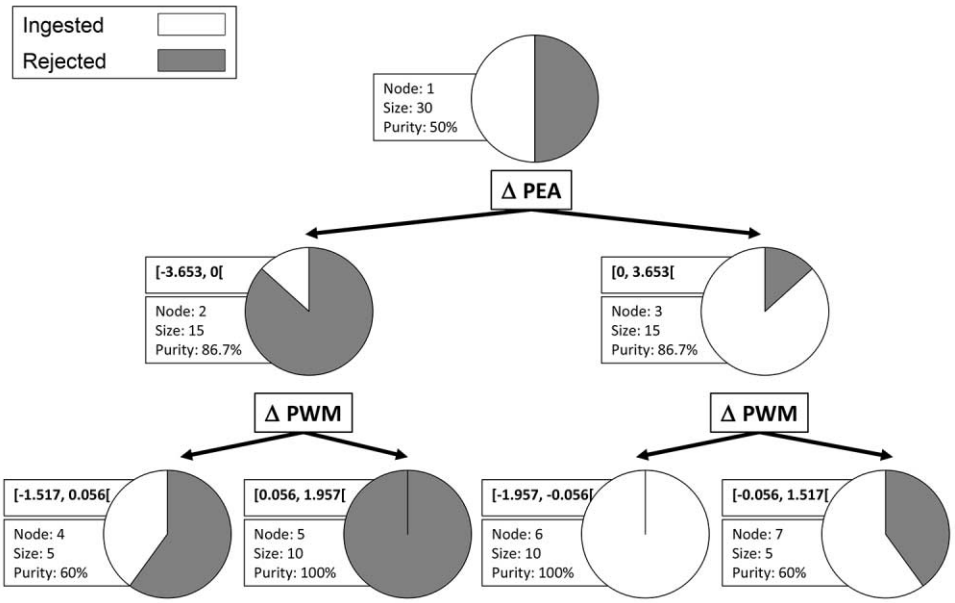

Figure 2. Classification tree model describing selection or rejection of a pair of microalgae based on differences in their lectin-binding patterns in the scallop *Argopecten irradians*. Rules were built using the C&RT algorithm (maximum tree depth = 2). Intervals for explanatory variables are given in brackets. Size indicates the number of objects at the node. Purity (also shown in pie graphs) is the percentage of objects corresponding to the dominating category of the dependent variable at the node. In this way, the rule for node 6 predicted that if ΔPWM is in interval [−1.957, −0.056[and ΔPEA is in interval [0, 3.653[, then the corresponding microalga is preferentially ingested in 100% of cases. Robustness (area under the receiver-operating characteristic curve [AUC]) of the model was 0.93, and the AUC of validation samples ranged from 0.92 to 0.97. PEA, *Pisum sativum* agglutinin; PWM, *Phytolacca americana* lectin.

established for *C. virginica*/*M. edulis* (CvMeModel1: C&RT algorithm, Gini index, maximum tree depth = 2, ΔPEA as first explanatory variable) led to a good prediction for the data obtained for both *A. irradians* and *M. mercenaria*. In this case, the model accuracy (0.78) was within the confidence interval [0.48–0.89] and was above the probability of success (0.73). The addition of the second explanatory variable (*i.e.*, ΔConA [*Canavalia ensiformis* lectin]) to the model led to a model accuracy of 0.83, which was still within the confidence interval [0.42–0.84], and a probability of success of 0.67.

The second model established for *C. virginica*/*M. edulis* (CvMeModel2: C&RT algorithm, Gini index, maximum tree depth = 2, Δ(PEA+ConA) as first explanatory variable) also established good prediction for the data from *A. irradians* and *M. mercenaria*. The model accuracy (0.81) was within the confidence intervals ([0.48–0.89] and [0.42–0.84]) and was close to the probability of success (0.73 and 0.67) for *A. irradians* and *M. mercenaria*, respectively. The addition of the second explanatory variable (*i.e.*, ΔECA [*Erythrina cristagalli* lectin]) to the model did not change the model accuracy (0.81), but it lowered the confidence intervals ([0.30–0.75] and [0.25–0.70]) and the probability of success (0.53 and 0.47) for *A. irradians* and *M. mercenaria*, respectively. As a consequence, the model accuracy after adding the second variable fell outside the confidence interval.

Further, the model established for *M. mercenaria* (Clam: C&RT algorithm, Gini index, maximum tree depth = 2, ΔPEA as first explanatory variable) gave a good prediction for the data obtained for *A. irradians* and *C. virginica*/*M. edulis*. The model accuracy (0.80) was within the confidence intervals ([0.62–0.96] and [0.63–0.92]) and was near the probability of success (0.87 and 0.81) for *A. irradians* and *C. virginica*/*M. edulis*, respectively. The addition of the second explanatory variable (*i.e.*, ΔUEA) to the model did not change the model accuracy (0.80), but it lowered the confidence intervals ([0.19–0.64] and [0.16–0.48]) and the probability of success (0.40 and 0.30) for *A. irradians* and *C. virginica*/*M. edulis*, respectively, making the model accuracy fall outside the confidence interval.

Finally, the model established for *A. irradians* (Scallop: C&RT algorithm, Gini index, maximum tree depth = 2, ΔPEA as first explanatory variable) also fit the data obtained for *M. mercenaria* and *C. virginica*/*M. edulis*. The model accuracy (0.87) was within the confidence intervals ([0.55–0.93] and [0.59–0.89]) and was above the probability of success (0.80 and 0.78) for *M. mercenaria* and *C. virginica*/*M. edulis*, respectively. The addition of the second explanatory variable (*i.e.*, ΔPWM) to the model did not change the model accuracy (0.87), but it lowered the confidence intervals ([0.15–0.58] and [0.18–0.52]) and the probability of success (0.33) for *M. mercenaria* and *C. virginica*/*M. edulis*, respectively. Consequently,

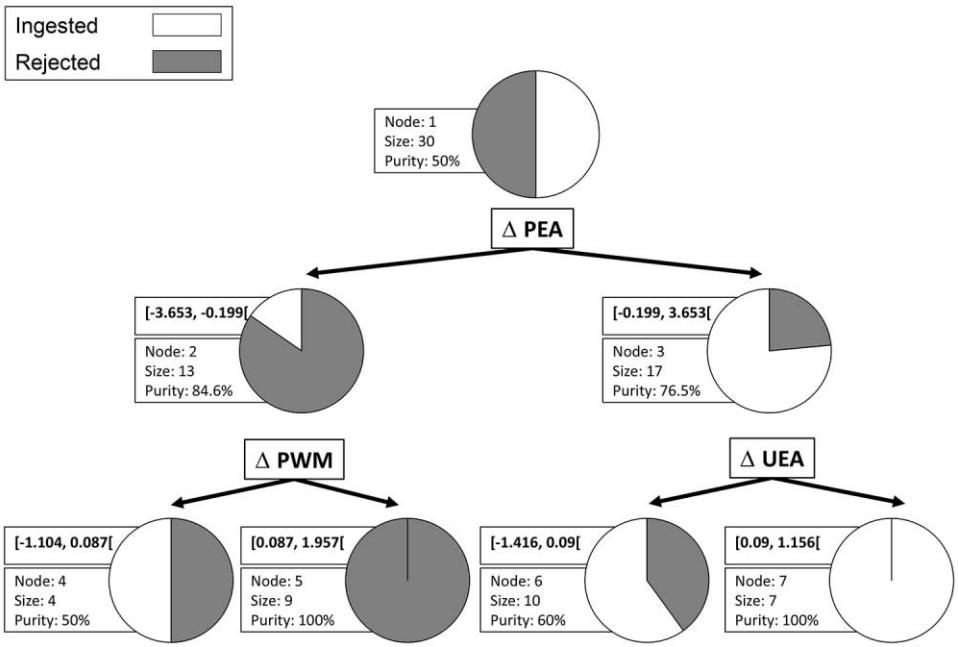

Figure 3. Classification tree model describing selection or rejection of a pair of microalgae based on differences in their lectin-binding patterns in the clam *Mercenaria mercenaria*. Rules were built using the C&RT algorithm (Gini, maximum tree depth = 2). Intervals for explanatory variables are given in brackets. Size indicates the number of objects at the node. Purity (also shown in pie graphs) is the percentage of objects corresponding to the dominating category of the dependent variable at the node. In this way, the rule for node 7 predicted that if ΔUEA is in interval [0.09, 1.156[and ΔPEA is in interval [−0.199, 3.653[, then the corresponding microalga is preferentially ingested in 100% of cases. Robustness (area under the receiver-operating characteristic curve [AUC]) of the model was 0.84, and the AUC of validation samples ranged from 0.75 to 0.92. PEA, *Pisum sativum* agglutinin; PWM, *Phytolacca americana* lectin; UEA, *Ulex europaeus* lectin.

the model accuracy after adding the second variable fell outside the confidence interval.

Discussion

Particle selection in suspension-feeding bivalves must be fully understood in order to improve our understanding of and prediction of bivalve growth and to better define the roles of bivalves in their dynamic ecosystems. Although it is clear that suspension feeders are able to discriminate among particles of varying sizes and chemical compositions, the mechanism by which they are able to select some and reject others needs additional research. Our recent findings demonstrated that interactions between carbohydrates present on microalgal cell surfaces and lectins present in the mucus coating bivalve feeding organs are central in particle selection by oysters and mussels (Pales Espinosa *et al.*, 2016a; Pales Espinosa and Allam, 2018). The architecture of and the contribution of the ctenidia and labial palps in particle sorting are variable between different bivalve species (Ward *et al.*, 1997; Cognie *et al.*, 2003; Dutertre *et al.*, 2007). Whether these divergent ctenidial and palp types operate *via* similar mechanisms to affect particle sorting is an intriguing and unexplored question. Therefore, the extension of our working hypotheses of a central role of lectin-carbohydrate interactions in food sorting across suspension-feeding molluscs into two different species of bivalves, the hard clam *Mercenaria mercenaria* (homorhabdic eulamellibranch) and the bay scallop *Argopecten irradians* (heterorhabdic filibranch), was of significant importance. In this study, we tested the hypothesis that particle selection in suspension-feeding bivalves is directed by some carbohydrates more than others and that these carbohydrate residues are similar, regardless of the bivalve species.

If the overall post-ingestion processing of particles in suspension-feeding bivalves (*i.e.*, capture, transport on pallial organ, rejection of pseudofeces) is species dependent (Beninger *et al.*, 1992, 1997; Riisgard *et al.*, 1996; Beninger and St-Jean, 1997a, b; Ward *et al.*, 1998a; Beninger and Veniot, 1999), our findings support that the cues used for particle selection are, in contrast, conserved. Results indicate that in a pair of two algal species, the species with the highest lectin binding affinity (*i.e.*, higher levels of target cell surface carbohydrates) is preferentially ingested, as compared to another species with lower lectin binding signals. The latter species can still be preferentially ingested when paired with a third microalgae species having yet lower lectin binding affinity.

Together with previous work, the results of this study support the role of carbohydrate-lectin interactions in particle

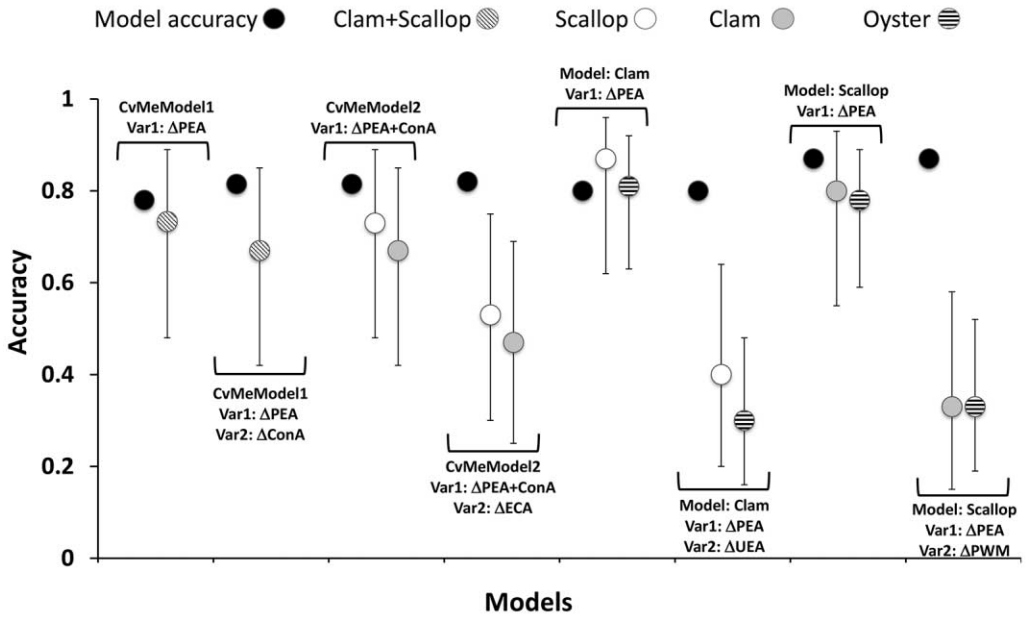

Figure 4. Performance of four classification tree models (C&RT algorithm, confusion matrices), using data from other bivalve species. The confusion matrices of each of the four models built for *Crassostrea virginica* (CvMeModel1), mussel (CvMeModel2), *Mercenaria mercenaria* (Clam), and *Argopecten irradians* (Scallop), using either the first explanatory variable (Var1) or both variables (Var1 and Var2), were used to determine the model accuracies. Binomial proportion confidence intervals (including probability of success; lower and upper 95% intervals) were calculated using new tested data (*i.e.*, data from bivalve species not used to build the given model). Model accuracies within the confidence limit indicate that the model can explain the new set of data. ConA, *Canavalia ensiformis* lectin; ECA, *Erythrina cristagalli* lectin; PEA, *Pisum sativum* agglutinin; PWM, *Phytolacca americana* lectin; UEA, *Ulex europaeus* lectin.

selection in at least four suspension-feeding bivalves, with each species presenting one of the four major ctenidial architectures, as well as a different way of life and biology. More interestingly, this study highlights the central role of mannose residues associated with microalgae cell surface in particle sorting, in at least the four tested bivalves.

Indeed, results indicate that for all species tested and models built, PEA binding affinity is identified as the main predictor of feeding choices. Further, the cross evaluation of the different models showed that all can explain new test data (*i.e.*, data from different bivalve species) only when the first explanatory variable, PEA, was considered. These findings strongly support the same carbohydrate cues mediating selection across all tested species. Since PEA is specific to mannose residues (Cheong *et al.*, 1999; Table 3), our findings underline that mannose associated with microalgae cell surface represents the main cue for selection in a broad range of suspension-feeding bivalves. Interestingly, other marine organisms, such as members of the heterotrophic Dinophyceae, use the same mechanism to prey. Thus, mannose and glucose residues present on microalgae cell surfaces are used by some Dinophyceae to select and capture their food particles (Ucko *et al.*, 1999; Wootton *et al.*, 2007; Martel, 2009).

The binding affinities of the secondary explanatory variables ConA (specific to glucose and mannose residues; observed in CvMeModel1), ECA (specific to N-acetyllactosamine, galactose; observed in CvMeModel2), PWM (specific to N-acetylglucosamine residues; observed in the Scallop model), and UEA (specific to fucose residues; observed in the Clam model) also seem to play a role in the mechanism of selection, even though they are not as important as that of PEA, which alone can drive all of the proposed models. Further, the addition of the secondary explanatory variables in the model's cross evaluation accentuates the difference between the model accuracy and the confidence interval, including the probability of success, highlighting again the central role of PEA in particle sorting.

If there is a consensus regarding the first explanatory variable (*i.e.*, PEA), the disparity between the second explanatory variables could be due to several factors, including bias due to size of the sample, overfitting of the models (*e.g.*, one explanatory variable might be sufficient), or real biological differences between the bivalve species. In fact, proteomic analysis of oyster and mussel pallial mucus revealed a high diversity of mucosal lectins (Pales Espinosa *et al.*, 2016b). If, for example, mannose- and galactose-binding lectins have been confirmed to be present in oyster pallial mucus (Vasta *et al.*, 1984; Pales Espinosa *et al.*, 2016b), the carbohydrate affinity of many of these lectins has not yet been identified. Differences in the bivalve mucosal "lectinome," qualitatively and quantitatively,

could explain some of the differences observed here for the secondary variables identified. Differences in the lectin profiles in mucus associated with the feeding organs of the different bivalve species would not be surprising and could be due to multiple factors, including their habitat characteristics (*e.g.*, hydrology, primary production, turbidity, and other environmental constraints), way of life (endofaunal *vs.* epifaunal species), and internal energy demands. Indeed, it was shown that later stages of gametogenesis or food depletion are highly correlated with the production of lectins and food sorting in oysters and mussels (Pales Espinosa and Allam, 2013).

Overall, the results presented here are in agreement with previous data demonstrating that the use of dicer substrate small interfering RNA (DsiRNA) targeting two mannose-binding mucosal lectins (*i.e.*, CvML3912 and CvML3914) reduces the abundance of the cognate transcripts and significantly decreases food-sorting ability among silenced oysters as compared with control animals, thus highlighting, once more, the central role of mannose residues associated with microalgae in particle selection in oysters (Pales Espinosa and Allam, 2018).

To complement our experimental results highlighting the role of mannose residues in particle selection in all bivalve species investigated here, we screened genomic databases for the identification of putative mannose-binding lectins. Even though genomic information for the different bivalve species is incomplete, public *Mytilus edulis* and *Argopecten irradians* protein and EST databases available at the National Center for Biotechnology Information (Bethesda, MD), as well as *Mercenaria mercenaria* genome (BA, unpubl. data) and transcriptomes (Wang *et al.*, 2016), revealed the presence of at least one putative C-type lectin with similarities (e-values ranging from $9e^{-24}$ to $1e^{-18}$ and identities ranging from 35.7% to 30.8%) with the sequence of the two mannose-binding lectins (*i.e.*, CvML3912 and CvML3914) identified in the oyster *Crassostrea virginica* (Fig. 5).

The identified lectins display signature features for C-type lectins, with AiCTL5, MeML, and MmML revealing four consensus cysteine residues and two optional cysteine residues that are expected to form disulfide bonds (Tasumi *et al.*, 2002). They also present two consensus WND/WYD and ENC/DNC/EHC triplet motifs considered to be the essential features for calcium binding (Drickamer, 1988, 1993). Additionally, the proteins contain another important QPN/QPS/QPD triplet motif, which is determinant for calcium binding and sugar specificity. Typically, EPN and QPD triplet motifs have been found in vertebrate C-type lectin carbohydrate recognition domains, and they bind mannose and galactose, respectively (Drickamer, 1992); but in molluscs, this motif is highly variable (Jing *et al.*, 2011; Mu *et al.*, 2012; Huang *et al.*, 2015) and its carbohydrate specificity often not assigned. Based on previous observations of

	A. irradians	*M. edulis*	*M. mercenaria*
Sequence code	ADL27440.1	ADI71474.1	comp165621_c0_seq1
Protein name	**AiCTL5**	**MeML**	**MmML**
References	Mu et al., 2012	Pales Espinosa et al., 2010	Wang et al., 2016; This study
e-value	9e-24	5e-18	1e-19
Identities	35.7%	32.5%	30.8%

```
AiCTL5          ---------MMFCI-------GLVILAFVQAQASCPHGWT-LEGTSCYHI-GREELTWTDAQRMC
MeML            -----FVYKITALI-------IVFCLFDYAATYTCSIGWHHGYRDNCYYF-SRFSATFWSAMSFC
MmML            NKLKSKLQDVDNIT-------VQLQKNIQELEMRCPTGWH-KHKHSCYFL-SSSTATWDNVMNWC
CvML3912        ---------MKTQI-------IILLAVLTAVLATCPADWQ-SYGDKCFFF-SRENETFADALKLC
CvML3914        ---------MKTEI-------IIFFAALTAVLATCPADWN-HYRDKCFFL-SRENETFASALKLC

AiCTL5            EHHKNSYLAR-----VETEVEDKAIQEMIRAQ----GHHSHKFWLGATDWTVEGEWQWEPEGSA
MeML              KTVGGKLIE------IDNYWEFQVLSRMARHR----R--FPDFWIGITDMYSEGAWQKATTQE-
MmML              KKNGGQLVE------YSDRGELKFIVDLVKVVSNWDNNIWTGFWTGADDTKHEGRWKWSKSGK-
CvML3912          EMIGSQYRRVASLATIDDAGTQKFLANFMRST----G--VRAFYFGATDIVHEGTWVWVSTGK-
CvML3914          EVIGSQYGRVASLATIDDAGTQNHIANLIKRS----G--FVTFYIGATDIVHEDTWVWVSTGK-

AiCTL5          SFTYSNWAHHQPNDHGGNDNCMSME----GESMFHWYDDNCSNKKKYICETAPSVDMTTVEY
MeML            QQTYFNWYESQPSNSGGHENCVEVY----TKLGMKWNDRHGDHRLRFVCEK-----------
MmML            SFTFTNWKAHQPDNGSGTEHCMVTL----VNSNGQWNDEQCYVRYKFVCERVL---------
CvML3912        VFTYTNWGPQQPNNRGGDENCAVLRDSADQVFHMQWGDSPCTTTINYICEMAAAEIHSPIVG
CvML3914        NATYTNWGPQQPNNRDGAENCAVLNYLPDEGFDMKWSDDPCASYFNYICEMASAEVHSPLVG
```

Figure 5. Alignment of the protein sequences of AiCTL5 (*Argopecten irradians*), MeML (*Mytilus edulis*), and MmML (*Mercenaria mercenaria*) with CvML3912 and CvML3914 C-type lectins (*Crassostrea virginica*). Cysteines are indicated in black. Conserved DNS, ENC, and EHC triplets, WxD (x = Y, N, G, and S) triplets, and QPx (x = N, S, and D) motifs are boxed in gray. The table indicates the National Center for Biotechnology Information code, the name, and the similarity (e-value and identity) of the putative C-type lectins to the ones already described in *C. virginica*.

CvML3912 (Pales Espinosa and Allam, 2018), it is possible that the QPN motif also found in AiCTL5 (*A. irradians*) displays specificity for mannose residues. Nevertheless, these findings must be carefully considered because no information on the specific location of these proteins in the animals is available, except for MeML, a mannose-binding lectin that was clearly detected in the mucosal tissue of the feeding organs of *M. edulis* (Pales Espinosa *et al.*, 2010b).

Overall, our new findings expand our understanding of the mechanisms governing selection in bivalve molluscs; and they provide a useful tool that could predict particle fate, based on surface carbohydrate signature, thus allowing for a better assessment of bivalve performance and benthic-pelagic coupling under various phytoplankton landscapes. Results from the different models indicate that the lectin PEA is a main predictor of feeding choices in a broad range of suspension-feeding bivalves. These findings strongly support the same carbohydrate cues (mannose and its derivatives) mediating selection across all tested species. Consequently, a model using only the explanatory variable ΔPEA appeared to be sufficient to explain microalgae choice in four suspension-feeding bivalve species with contrasting ecological and anatomical features.

However, it should be noted that experiments reported in the current study were performed during the summer; thus, they reflect specific physiological conditions for both bivalves. As described in previous studies (Bayne and Svensson, 2006; Pales Espinosa and Allam, 2013), selection in suspension-feeding bivalves is highly correlated with both exogenous factors (seasonal differences in carbon and nitrogen availability) and endogenous factors (cycles of reproduction and growth). Consequently, these models must be validated by using bivalves with different physiological conditions. Additional work is also needed to identify mucosal lectins present in *M. edulis*, *A. irradians*, and *M. mercenaria* and their precise roles in the feeding process.

Acknowledgments

We thank Prof. Stephen T. Tettelbach and Joseph Hinton (Cornell Cooperative Extension, NY) and Frank M. Flower and Sons (Oyster Bay, NY) for providing live animals. Microalgae used in this study were graciously provided by the National Oceanic and Atmospheric Administration Milford Lab. We are grateful to Prof. Robert Cerrato for his valuable help with statistics analysis. We are also thankful to the National Science Foundation (IOS-1656753) for funding this work. JJ was a research intern supported by the Simons Foundation.

Literature Cited

Agresti, A., and B. A. Coull. 1998. Approximate is better than "exact" for interval estimation of binomial proportions. *Am. Stat.* **52:** 119–126.

Alldredge, A. L., and M. W. Silver. 1988. Characteristics, dynamics and significance of marine snow. *Prog. Oceanogr.* **20:** 41–82.

Allen, W. R. 1921. Studies of the biology of freshwater mussels: experimental studies of the food relations of certain Unionidae. *Biol. Bull.* **40:** 210–241.

Asmus, R. M., and H. Asmus. 1991. Mussel beds: limiting or promoting phytoplankton? *J. Exp. Mar. Biol. Ecol.* **148:** 215–232.

Atkins, D. 1936. On the ciliary mechanisms and interrelationships of lamellibranchs. I. New observation on sorting mechanisms. *Q. J. Microsc. Sci.* **2:** 181–308.

Atkins, D. 1937a. On the ciliary mechanisms and interrelationships of lamellibranchs. II. Sorting devices on the gills. *J. Cell Sci.* **2:** 339–373.

Atkins, D. 1937b. On the ciliary mechanisms and interrelationships of lamellibranchs. III. Types of lamellibranch gills and their food currents. *J. Cell Sci.* **2:** 375–421.

Bayne, B. L., and S. Svensson. 2006. Seasonal variability in feeding behaviour, metabolic rates and carbon and nitrogen balances in the Sydney oyster, *Saccostrea glomerata* (Gould). *J. Exp. Mar. Biol. Ecol.* **332:** 12–26.

Beninger, P. G., and P. Decottignies. 2005. What makes diatoms attractive for suspensivores? The organic casing and associated organic molecules of *Coscinodiscus perforatus* are quality cues for the bivalve *Pecten maximus*. *J. Plankton Res.* **27:** 11–17.

Beninger, P. G., and S. D. St-Jean. 1997a. Particle processing on the labial palps of *Mytilus edulis* and *Placopecten magellanicus* (Mollusca: Bivalvia). *Mar. Ecol. Prog. Ser.* **147:** 117–127.

Beninger, P. G., and S. D. St-Jean. 1997b. The role of mucus in particle processing by suspension-feeding marine bivalves: unifying principles. *Mar. Biol.* **129:** 389–397.

Beninger, P. G., and A. Veniot. 1999. The oyster proves the rule: mechanisms of pseudofeces transport and rejection on the mantle of *Crassostrea virginica* and *Crassostrea gigas*. *Mar. Ecol. Prog. Ser.* **190:** 179–188.

Beninger, P. G., J. E. Ward, B. A. Macdonald, and R. J. Thompson. 1992. Gill function and particle-transport in *Placopecten magellanicus* (Mollusca, Bivalvia) as revealed using video endoscopy. *Mar. Biol.* **114:** 281–288.

Beninger, P. G., S. C. Dufour, and J. Bourque. 1997. Particle processing mechanisms of the eulamellibranch bivalves *Spisula solidissima* and *Mya arenaria*. *Mar. Ecol. Prog. Ser.* **150:** 157–169.

Beninger, P. G., R. Cannuel, and S. Jaunet. 2005. Particle processing on the gill plicae of the oyster *Crassostrea gigas*: fine-scale mucocyte distribution and functional correlates. *Mar. Ecol. Prog. Ser.* **295:** 191–199.

Breiman, L., J. Friedman, C. J. Stone, and R. A. Olshen. 1984. *Classification and Regression Trees*. CRC Press, Boca Raton, FL.

Bricelj, V., and R. Malouf. 1984. Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. *Mar. Biol.* **84:** 155–165.

Cheong, Y., G. Shim, D. Kang, and Y. Kim. 1999. Carbohydrate binding specificity of pea lectin studied by NMR spectroscopy and molecular dynamics simulations. *J. Mol. Struct.* **475:** 219–232.

Cloern, J. E. 1982. Does the benthos control phytoplankton biomass in south San Francisco Bay? *Mar. Ecol. Prog. Ser.* **9:** 191–202.

Cognie, B., L. Barille, and E. Rince. 2001. Selective feeding of the oyster *Crassostrea gigas* fed on a natural microphytobenthos assemblage. *Estuaries* **24:** 126–134.

Cognie, B., L. Barillé, G. Massé, and P. G. Beninger. 2003. Selection and processing of large suspended algae in the oyster *Crassostrea gigas*. *Mar. Ecol. Prog. Ser.* **250:** 145–152.

Dame, R. F. 1996. *Ecology of Marine Bivalves: An Ecosystem Approach*. CRC Press, Boca Raton, FL.

De'ath, G., and K. E. Fabricius. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* **81:** 3178–3192.

Drickamer, K. 1988. Two distinct classes of carbohydrate-recognition domains in animal lectins. *J. Biol. Chem.* **263:** 9557–9560.

Drickamer, K. 1992. Engineering galactose-binding activity into a C-type mannose-binding protein. *Nature* **360:** 183–186.

Drickamer, K. 1993. Evolution of Ca^{2+}-dependent animal lectins. *Prog. Nucleic Acid Res. Mol. Biol.* **45:** 207–232.

Dufour, S. 1998. Étude des cils et des mucocytes de la surface abfrontale des branchies de bivalves. M.S. dissertation, Université de Moncton, Montcon, New Brunswick, Canada.

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

Elith, J., J. R. Leathwick, and T. Hastie. 2008. A working guide to boosted regression trees. *J. Anim. Ecol.* **77:** 802–813.

Fox, D. L. 1936. The habitat and food of the California sea mussel. *Bull. Scripps Inst. Oceanogr.* **4:** 1–64.

Galtsoff, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. *Fish. Bull.* **64:** 1–480.

Gosling, E. 2015. *Marine Bivalve Molluscs*. Wiley, Chichester, United Kingdom.

Grizzle, R. E., V. M. Bricelj, and S. E. Shumway. 2001. Physiological ecology of *Mercenaria mercenaria*. Pp. 305–382 in *Biology of the Hard Clam*, J. N. Kraeuter and M. Castagna, eds. Elsevier Science, Amsterdam.

Guillard, R. R. L. 1982. Culture of phytoplankton for feeding marine invertebrates. Pp. 108–132 in *Culture of Marine Invertebrate Animals*, W. L. Smith and M. H. Chanley, eds. Plenum Press, New York.

Hanley, J. A., and B. J. McNeil. 1982. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* **143:** 29–36.

Huang, M., H. Zhang, S. Jiang, L. Wang, R. Liu, Q. Yi, and L. Song. 2015. An EPD/WSD motif containing C-type lectin from *Argopectens irradians* recognizes and binds microbes with broad spectrum. *Fish Shellfish Immunol.* **43:** 287–293.

Iglesias, J. I. P., E. Navarro, P. A. Jorna, and 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.

Jing, X., E. Pales Espinosa, M. Perrigault, and B. Allam. 2011. Identification, molecular characterization and expression analysis of a mucosal C-type lectin in the eastern oyster, *Crassostrea virginica*. *Fish Shellfish Immunol.* **30:** 851–858.

Kraeuter, J. N., and M. Castagna. 2001. *Biology of the Hard Clam*. Elsevier Science, Amsterdam.

Martel, C. M. 2009. Conceptual bases for prey biorecognition and feeding selectivity in the microplanktonic marine phagotroph *Oxyrrhis marina*. *Microb. Ecol.* **57:** 589–597.

Mu, C., X. Song, J. Zhao, L. Wang, L. Qiu, H. Zhang, Z. Zhou, M. Wang, L. Song, and C. Wang. 2012. A scallop C-type lectin from *Argopecten irradians* (AiCTL5) with activities of lipopolysaccharide binding and gram-negative bacteria agglutination. *Fish Shellfish Immunol.* **32:** 716–723.

Newell, R. I. E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *J. Shellfish Res.* **23:** 51–62.

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

Pales Espinosa, E., and B. Allam. 2006. Comparative growth and survival of juvenile hard clams, *Mercenaria mercenaria*, fed commercially available diets. *Zoo Biol.* **25:** 513–525.

Pales Espinosa, E., and B. Allam. 2013. Food quality and season affect gene expression of the mucosal lectin MeML and particle sorting in the blue mussel *Mytilus edulis*. *Mar. Biol.* **160:** 1441–1450.

Pales Espinosa, E., and B. Allam. 2018. Reverse genetics demonstrate the role of mucosal C-type lectins in food particle selection in the oyster *Crassostrea virginica*. *J. Exp. Biol.* **221:** jeb174094.

Pales Espinosa, E., M. Perrigault, S. E. Shumway, J. E. Ward, G. Wikfors, and B. Allam. 2008. The sweet relationship between microalgae and *Crassostrea virginica*: implication of carbohydrate and lectin interactions in particle selection in suspension feeding bivalves. *J. Shellfish Res.* **27:** 1006–1007.

Pales Espinosa, E., M. Perrigault, J. E. Ward, S. E. Shumway, and B. Allam. 2009. Microalgal cell surface carbohydrates as recognition sites for particle sorting in suspension feeding bivalves. *J. Shellfish Res.* **28:** 695–695.

Pales Espinosa, E., D. Hassan, J. E. Ward, S. E. Shumway, and B. Allam. 2010a. Role of epicellular molecules in the selection of particles by the blue mussel, *Mytilus edulis*. *Biol. Bull.* **219:** 50–60.

Pales Espinosa, E., M. Perrigault, and B. Allam. 2010b. Identification and molecular characterization of a mucosal lectin (MeML) from the blue mussel *Mytilus edulis* and its potential role in particle capture. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **156:** 495–501.

Pales Espinosa, E., R. M. Cerrato, G. Wikfors, and B. Allam. 2016a. Modeling food choice in suspension-feeding bivalves. *Mar. Biol.* **163:** 2–13.

Pales Espinosa, E., A. Koller, and B. Allam. 2016b. Proteomic characterization of mucosal secretions in the eastern oyster, *Crassostrea virginica*. *J. Proteomics* **132:** 63–76.

Pastoureaud, A., M. Heral, J. Prou, D. Razet, and P. Russu. 1996. Particle selection in the oyster *Crassostrea gigas* (Thunberg) studied by pigment HPLC analysis under natural food conditions. *Oceanol. Acta* **19:** 79–88.

Pechenik, J. 2000. *Biology of the Invertebrates*. McGraw-Hill, Boston.

Pohle, D. G., V. M. Bricelj, and Z. Garcia-Esquivel. 1991. The eelgrass canopy: an above-bottom refuge from benthic predators for juvenile bay scallops *Argopecten irradians*. *Mar. Ecol. Prog. Ser.* **74:** 47–59.

Prins, T., A. Smaal, and R. Dame. 1997. A review of the feedbacks between bivalve grazing and ecosystem processes. *Aquat. Ecol.* **31:** 349–359.

Ribelin, B. W., and A. Collier. 1977. Studies on the gill ciliation of the American oyster *Crassostrea virginica* (Gmelin). *J. Morphol.* **151:** 439–449.

Riisgard, H. U., P. S. Larsen, and N. F. Nielsen. 1996. Particle capture in the mussel *Mytilus edulis*: the role of latero-frontal cirri. *Mar. Biol.* **127:** 259–266.

Ripley, B. D. 1996. *Pattern Recognition and Neural Networks*. Cambridge University Press, Cambridge.

Rosa, M., J. E. Ward, S. E. Shumway, G. H. Wikfors, E. Pales Espinosa, and B. Allam. 2013. Effects of particle surface properties on feeding selectivity in the eastern oyster *Crassostrea virginica* and the blue mussel *Mytilus edulis*. *J. Exp. Mar. Biol. Ecol.* **446:** 320–327.

Shannon, P., A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski, and T. Ideker. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13:** 2498–2504.

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

Shumway, S. E., T. L. Cucci, M. P. Lesser, N. Bourne, and B. Bunting. 1997. Particle clearance and selection in three species of juvenile scallops. *Aquacult. Int.* **5:** 89–99.

Swets, J. A. 1988. Measuring the accuracy of diagnostic systems. *Science* **240:** 1285–1293.

Tasumi, S., T. Ohira, I. Kawazoe, H. Suetake, Y. Suzuki, and K. Aida. 2002. Primary structure and characteristics of a lectin from skin mucus of the Japanese eel *Anguilla japonica*. *J. Biol. Chem.* **277:** 27305–27311.

Ucko, M., R. P. Shrestha, P. Mesika, D. Bar-Zvi, and S. M. Arad. 1999. Glycoprotein moiety in the cell wall of the red microalga *Porphyridium* sp. (Rhodophyta) as the biorecognition site for the *Crypthecodinium cohnii*-like dinoflagellate. *J. Phycol.* **35:** 1276–1281.

Vasta, G. R., T. C. Cheng, and J. J. Marchalonis. 1984. A lectin on the hemocyte membrane of the oyster (*Crassostrea virginica*). *Cell. Immunol.* **88:** 475–488.

Wang, K., E. P. Espinosa, and B. Allam. 2016. Effect of "heat shock" treatments on QPX disease and stress response in the hard clam, *Mercenaria mercenaria*. *J. Invertebr. Pathol.* **138:** 39–49.

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

Ward, J. E., R. I. E. Newell, R. J. Thompson, and B. A. MacDonald. 1994. *In vivo* studies of suspension-feeding processes in the eastern oyster, *Crassostrea virginica* (Gmelin). *Biol. Bull.* **186:** 221–240.

Ward, J. E., J. S. Levinton, S. E. Shumway, and T. Cucci. 1997. Site of particle selection in a bivalve mollusc. *Nature* **390:** 131–132.

Ward, J. E., J. S. Levinton, S. E. Shumway, and T. Cucci. 1998a. Particle sorting in bivalves: *in vivo* determination of the pallial organs of selection. *Mar. Biol.* **131:** 283–292.

Ward, J. E., L. P. Sanford, R. I. E. Newell, and B. A. MacDonald. 1998b. A new explanation of particle capture in suspension-feeding bivalve molluscs. *Limnol. Oceanogr.* **43:** 741–752.

Wootton, E. C., M. V. Zubkov, D. H. Jones, R. H. Jones, C. M. Martel, C. A. Thornton, and E. C. Roberts. 2007. Biochemical prey recognition by planktonic protozoa. *Environ. Microbiol.* **9:** 216–222.

Appendix

Table A1

Sorting efficiencies (SE) of Argopecten irradians *and* Mercenaria mercenaria *fed a diet made with equal proportions of two microalgae*

Bivalves	Ingested	Rejected	SE	*P*-value
	Chlorella autotrophica	*Pavlova lutheri*	0.52	<0.001
	Tetraselmis chui	*Amphora coffaeaformis*	0.24	<0.001
	Chlorella autotrophica	*Tetraselmis chui*	0.57	<0.001
	Amphora coffaeaformis	*Pavlova lutheri*	0.47	<0.001
	Chlamydomonas sp. 1	*Amphora coffaeaformis*	0.57	<0.001
	Chlamydomonas sp. 1	*Tetraselmis chui*	0.52	<0.001
	Chlamydomonas sp. 1	*Pavlova lutheri*	0.56	<0.001
	Tetraselmis chui	*Pavlova lutheri*	0.13	0.15
Argopecten irradians	*Chlorella autotrophica*	*Amphora coffaeaformis*	0.63	<0.001
	Chlamydomonas sp. 1	*Rhodomonas lens*	0.17	0.03
	Rhodomonas lens	*Tetraselmis chui*	0.26	<0.001
	Pavlova lutheri	*Rhodomonas lens*	0.07	0.66
	Chlorella autotrophica	*Prasinocladus marinus*	0.78	<0.001
	Chlorella autotrophica	*Chlamydomonas* sp. 1	0.19	<0.001
	Chlamydomonas sp 1.	*Prasinocladus marinus*	0.52	<0.001
	Cricosphaera carterae	*Chlamydomonas* sp. 1	0.39	<0.001
	Amphora coffaeaformis	*Prasinocladus marinus*	0.82	<0.001
	Chlorella autotrophica	*Pavlova lutheri*	0.19	<0.001
	Tetraselmis chui	*Amphora coffaeaformis*	0.15	<0.001
	Chlorella autotrophica	*Tetraselmis chui*	0.36	<0.001
	Amphora coffaeaformis	*Pavlova lutheri*	0.2	<0.001
	Chlamydomonas sp. 1	*Amphora coffaeaformis*	0.18	<0.001
	Chlamydomonas sp. 1	*Tetraselmis chui*	0.18	0.03
	Chlamydomonas sp. 1	*Pavlova lutheri*	0.11	0.07
	Pavlova lutheri	*Tetraselmis chui*	0.04	0.92
Mercenaria mercenaria	*Chlorella autotrophica*	*Amphora coffaeaformis*	0.16	<0.001
	Rhodomonas lens	*Chlamydomonas* sp. 1	0.34	<0.001
	Rhodomonas lens	*Tetraselmis chui*	0.14	0.046
	Pavlova lutheri	*Rhodomonas lens*	0.09	0.45
	Chlorella autotrophica	*Prasinocladus marinus*	0.46	<0.001
	Chlorella autotrophica	*Chlamydomonas* sp. 1	0.3	<0.001
	Chlamydomonas sp. 1	*Prasinocladus marinus*	0.19	<0.001
	Cricosphaera carterae	*Chlamydomonas* sp. 1	0.4	<0.001
	Amphora coffaeaformis	*Prasinocladus marinus*	0.34	<0.001

SE shown are for the preferentially ingested species (*i.e.*, a lower proportion in the pseudofeces as compared to diet). Probability values <0.05 (*G* test, n = 10–12) indicate sorting efficiencies that are significant.