3-14-2022

Determinants of food selection by bivalve larvae

Maria Rosa

Diane K. Padilla

Received: 19 November 2020 | Accepted: 6 February 2022

DOI: 10.1111/ivb.12366

ORIGINAL ARTICLE

Invertebrate Biology WILEY

Determinants of food selection by bivalve larvae

Maria Rosa[1,2] | Dianna K. Padilla[2]

[1]Department of Biology, Connecticut College, New London, CT, USA

[2]Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, USA

Correspondence
Maria Rosa, Department of Biology, Connecticut College, New London, CT 06320, USA.
Email: mrosa@conncoll.edu

Funding information
NSF, Grant/Award Number: DBI-1611997

Abstract

Selection of food particles for consumption by larvae impacts nutritional gain needed for growth, development, and metamorphosis. Past work has suggested that molluscan larvae are capable of collecting food within a narrow size range. Recent studies have found evidence of size-independent food selection in molluscan larvae, but relatively little is known about the characteristics of particles that larvae preferentially capture. Therefore, we conducted experiments with the larvae of two mussels, *Mytilus trossulus* and *Mytilus edulis*, to determine whether they are selective feeders, and if so, whether we could determine the characteristics of particles selected as food. We fed larvae microalgae and polystyrene microspheres of different sizes, nutritional content, surface charge, and hydrophobicity. We found that for both species, there was no effect of size on particle selection for particles 2–8 μm, but, surprisingly, these two congeners preferentially captured particles with different characteristics. Larvae of *M. trossulus* preferentially captured particles that were more hydrophilic and had a more negative surface charge, but there was no effect of nutritional content. The larvae of *M. edulis* showed a different pattern; they preferentially captured particles with low surface charge and greater food value, but hydrophobicity did not affect selection. Larvae of these two congeners are indeed selective in which particles they collect but appear to be using different rules for selection. More work is needed to determine whether there are any general patterns that govern particle selection for larvae and mechanisms that could produce the observed patterns. Such work is needed to help us to determine whether individual species use different rules or whether there are general patterns in the types of particles larvae select.

KEYWORDS
feeding, *Mytilus*, particle properties, selectivity

1 | INTRODUCTION

In marine systems, suspension feeding is a major feeding mode for a wide diversity of animals across different life stages and has evolved independently multiple times (e.g., Bullivant, 1968; M.F. Strathmann, 1987; R.R. Strathmann, 2020). Invertebrate examples include both mobile organisms, such as copepods, cladocerans, salps, and larvaceans, as well as sessile or sedentary organisms, such as ascidians, barnacles, and bivalves (Riisgård & Larsen, 2010). In addition, suspension feeding is used by the vast majority of free-living, feeding larval stages across phyla (Pernet, 2017; M.F. Strathmann, 1987). Even in many taxa in which the adults do not suspension feed, there is still a suspension-feeding larval stage, typically with a ciliary-based mechanism of particle capture (Emlet, 1990; Pernet, 2017; R.R. Strathmann, 1978).

The best studied group of adult suspension feeders is bivalve molluscs because of their important impacts on marine systems

through their feeding processes (Dame, 2012; Ferreira et al., 2019). These important impacts include controlling phytoplankton abundance, biomass, and diversity (Asmus & Asmus, 2005; Jørgensen, 1981; Newell, 1988); reducing harmful algal blooms (Harke et al., 2011); and pumping of organic matter to the benthos (Dame, 2012). Introductions and removal of bivalves can produce major shifts in planktonic and benthic communities (Padilla et al., 2011). Although some work has been done on the mechanics of ciliary feeding in invertebrate larvae and the importance of body form on the hydrodynamics of feeding (e.g., Emlet, 1990), surprisingly little is known about the selective feeding of larval stages (Olson & Olson, 1989; Pernet, 2017). At present, we do not have a general understanding of selective suspension feeding for invertebrate larvae.

For molluscs, the mode of ciliary feeding and the likely mechanism used for particle capture and selection is quite different between larvae and adults. The veliger larva is the hallmark of the Mollusca. Most bivalves produce small (~100–500 μm) veliger larvae that develop from nonfeeding trochophores. Although adult bivalves trap food on mucus on their ctenidium (gill), molluscan larvae do not have a gill and do not produce the mucus of adults. Veligers are characterized by the velum, a specialized ciliated structure used for swimming and food capture (Chan et al., 2013). Cilia on the velum come in contact with suspended particles, and there is evidence that direct interception by individual cilia is responsible for food capture (Romero et al., 2010); thus, veligers interact with food particles individually. The velum is shed at metamorphosis to the juvenile stage, when the ctenidia (gills) become the suspension-feeding organ.

Recent work has suggested that veligers of some species can selectively capture or feed on different microalgal food particles (e.g., Baldwin, 1995; Hansen, 1991; Liao et al., 2017; Rosa & Padilla, 2020), but generalizable patterns or mechanisms underlying this selection are yet to be determined. Our recent work indicates that veligers of the Pacific oyster *Crassostrea gigas* preferentially capture certain species of microalgae (Rosa & Padilla, 2020). The percent of polyunsaturated fatty acids (PUFAs) in an algal species has been shown to affect larval growth and time to metamorphosis, and is commonly used as a measure of nutritional quality (Hendriks et al., 2003). But, for *C. gigas*, food selection does not appear to be based on the size or nutritional quality of the microalgae. Because the veliger form is shared among all molluscs with feeding larvae, it is important to assess whether this larval form produces limits on selective suspension feeding and particle capture.

In this study, we quantified microalgal particle capture rates for the larvae of two mussel congeners, the blue mussel, *Mytilus edulis* Linnaeus 1758, which is found on the Atlantic Ocean coastlines in North America, Great Britain, and Europe, and *M. trossulus* A. Gould 1850, which is found on eastern shores of the Pacific Ocean in North America. Particle capture rates were determined for veligers fed four different microalgal species and two different sizes of polystyrene (PS) microspheres. We then tested whether known particle properties, such as size and surface charge, affected capture rate.

2 | METHODS

2.1 | Larval rearing and maintenance

Reproductive adults of *Mytilus trossulus* (35–55 mm) were collected from Argyle Lagoon, San Juan Island, WA (48.5201°N, 123.0146°W). Adults were kept in flow through ambient water (16°C) at the Friday Harbor Laboratories (University of Washington, Friday Harbor, WA) and supplemented with Shellfish Diet© (Reed Mariculture). Individuals were induced to spawn with standard techniques (warming and injecting 5% KCl solution into the gonad; see M.F. Strathmann, 1987). Gametes from three females (pooled) and two males (pooled) were combined, and successful fertilization was confirmed via microscopy. Larvae produced from this pooled mixture of gametes were maintained in 4-L batch cultures, in glass jars filled with 0.22-μm filtered seawater (FSW) at ambient temperature, at a density of 5 individuals/ml. Larvae were fed daily a combination (50:50 by biovolume) of *Tisochrysis lutea* El M. Bendif & I. Probert 2013 (strain T-iso) and *Diacronema lutheri* (Droop) Bendif & Véron 2011 (strain MONO) at 10^4 cells/ml. Given the culture density and feeding concentrations used, interference in feeding among individuals resulting in phenotypic changes in velum size (as per R.R. Strathmann et al., 1993) would not be expected. Culture water was changed three times per week, and cultures were maintained at ambient water temperature (16°C) until use. Larvae 3–5 days old and 155.0 ± 6.1 μm in size (mean ± *SD*) were used in the feeding experiments.

Reproductive adults of *M. edulis* (30–55 mm) were collected from Milford, CT (41.2307°N, 73.0640°W) and then maintained in flow through ambient seawater (18°C) at the NOAA Fisheries Milford Lab (Milford, CT). For conditioning, mussels were supplemented with additional cultured microalgae (*Chaetoceros* sp.) and Shellfish Diet© (Reed Mariculture). Larval rearing was the same as for *M. trossulus*, with the following differences: Gametes from three females (pooled) and seven males (pooled) were combined, and successful fertilization was confirmed via microscopy. Larvae produced from this pooled mixture of gametes were transferred to Stony Brook University and maintained in 4-L batch cultures. Given the culture density and feeding concentrations used, interference in feeding between individuals resulting in phenotypic changes in velum size (as per R.R. Strathmann et al., 1993) would not be expected. Cultures were maintained at 18°C until use. Larvae 3–5 days old and 123.6 ± 19.6 μm in size (mean ± *SD*) were used in the feeding experiments.

Although the two species were reared at different temperatures, they were reared at the ambient temperatures where they occur naturally and were at the same stage of development during tests. The optimal temperature for rearing larvae of *M. edulis* has been determined to be 18°C (Helm & Bourne, 2004; Galley et al., 2010). Rearing larvae of *M. edulis* at 16°C would have delayed development considerably. Rearing larvae of *M. trossulus* at 18°C results in very high mortality and abnormal development (M.F. Strathmann, 1987; personal observation). In both cases, larvae that were tested were from a genetically diverse cohort. All larvae that were tested were pooled from the different mass culture beakers prior to use in experiments.

This spread any possible effects of the beaker in which larvae were reared across replicates for each feeding trial. Experiments were conducted with d-stage veligers (3–5 days old), and there was no significant difference in size among the larvae within a species among experiments over the 3-day period.

2.2 | Particle capture experiments

We used a modified experimental design from a prior study (Rosa & Padilla, 2020). We used both microalgae and PS microspheres with different properties (Table 1). In feeding experiments, we used two different sizes of fluorescent PS microspheres (Fluoresbrite® YG, Polyscience), 3 μm (441,486 catalog #241735) and 6 μm (441,486 catalog #24157-1), and four different species of microalgae, *Rhodomonas lens* PASCHER & RUTTNER 1913 (Rhodo), *Nannochloropsis* sp. (FHL644), *Diacronema lutheri* (MONO), and *Tisochrysis lutea* (T-iso) as single particle types or in paired combinations. Prior to use, the PS microspheres were centrifuged at 1500 RCF, the supernatant decanted, and particles resuspended in 0.2-μm FSW two times to remove any residual surfactants and then diluted to achieve the required concentration for each experiment. Algae were grown at high concentrations in f/2 media and were then diluted (at least 1:1000) in 0.2-μm FSW to reach the required concentration for each experiment.

The microalgae selected for these experiments are commonly used for feeding and maintenance of larvae for both scientific and aquaculture use (Helm & Bourne, 2004). The microalgal species used differed in relative nutritional content (percentage of PUFAs; adopted from Helm & Bourne, 2004) and known physical surface properties (Rosa et al., 2017; Table 1). The combinations of algae and PS that were tested were preselected to present contrasting cell size (measured as cell length), nutritional quality (% PUFA), and cell surface properties (Table 1). The five combinations were: *Nannochloropsis* sp. and *Pavlova lutheri*; *Nannochloropsis* sp. and *T. lutea*; *Nannochloropsis* sp. and 3-μm PS; *T. lutea* and *P. lutheri*; and *T. lutea* and 6-μm PS.

In all cases, microalgal cell densities were determined with a flow cytometer (Guava InCyte, MilliporeSigma, at Friday Harbor Laboratories, WA; or C6 Plus, BD Sciences, at the NOAA lab in CT), which allowed us to determine cell densities when single or multiple species/particles were used in experiments. These two machines use lasers with the same excitation wavelengths; thus, the cell capture rates were comparable across experiments. For both instruments, samples were read at a fast speed (66 μl/min) for 1 min, using the following emission/excitation lasers to determine particle densities. For all single particle experiments using microalgae, cell populations were defined using the 584/40 versus 670LP lasers (FL2 vs. FL3). For all single particle experiments using PS microspheres, particle populations were defined using forward scatter versus 670LP laser (FSC vs. FL3). For all the paired particle experiments, particle populations were defined using FL2 versus FL3 lasers. Particle counts were then standardized to number per milliliter for calculating capture rates.

To determine particle capture rates, stock solutions were prepared by diluting algae and washed PS particles in 5 L of FSW to a total cell density of 10^4 cells/ml. Initial concentrations of algae and PS were then determined from these stock solutions with a flow cytometer. Each treatment was allocated to test beakers, mixed, and a 1-ml subsample taken to confirm initial particle concentration. Larvae were then added to the sample beakers (2 larvae/ml, 5 replicates per treatment) for each microalgal treatment. A 1-ml aliquot was collected hourly from the center of each beaker, without mixing, for 5 h. Control replicate beakers of the same size with the same volume (100 ml, no larvae, 3 or 4 replicates per treatment) were used to determine particle settling rates over the experimental time period and sampled similarly. Over the time course of the feeding trials, the algal settling

TABLE 1 Summary of traits of microalgae and polystyrene microspheres used in feeding experiments

Particle	Size (μm)	Cell volume (μm^3)	Surface charge (mV)	Contact angle (hydrophobicity)	Relative PUFA (% total cell)	Selection by *Mytilus trossulus*	Selection by *Mytilus edulis*	Selection by *Crassostrea gigas*
Nannochloropsis sp.	3	22	−12 ± 1.17	93 ± 6.08 (hydrophobic)	8%	–	+	
Pavlova lutheri	4	88	−16 ± 0.71	66 ± 3.56 (hydrophilic)	10%	+/=	–	+
Tisochrysis lutea (T-iso)	6	45	−6 ± 0.87	83 ± 3.08 (hydrophilic)	30%	=	–	–
Rhodomonas lens	8	69	−13 ± 0.96	93 ± 2.45 (hydrophobic)	45%	=		–
Polystyrene microsphere	3 & 6	14 & 113	−11 ± 1.05	113 ± 0.96 (hydrophobic)	0%	+/=	–	

Note: Particle properties include size (maximum cell dimension), particle surface charge, hydrophobicity (contact angle), and percent content of polyunsaturated fatty acids (PUFA) in algae. – denotes rejection (low capture); +, ingestion (high capture); =, no selection observed. Blank cells indicate that the particle was not tested. Data on capture of these same species of microalgae by larvae of *Crassostrea gigas* (Rosa & Padilla, 2020) are provided for comparison. Size was directly measured in this study; algal surface charge (mean ± *SE*) and contact angle (mean ± *SE*) from Rosa et al. (2017); algal biovolume and PUFAs from Helm & Bourne, 2004.

rates in control chambers (without larvae) were negligible (0.001 ± 0.002 particles/ml). No differential settlement of the different particles during the timed trials was observed. Therefore, the loss of cells due to sinking in the controls was subtracted from test concentrations prior to determining capture rates. Experiments with *M. trossulus* were run at 16°C, ambient temperature at the time of the experiment, and experiments with *M. edulis* were run at 18°C, optimal temperature for *M. edulis* development.

To calculate capture rates, particle densities within each replicate were plotted over time. Only the sampling time frame with a clear linear decrease in cell density (in all cases between time 0 and 3 h) was used to calculate particle capture rate per larva per hour (as per Frost, 1972). Analysis of variance (ANOVA)was used to determine differences in capture rates among particles for each mussel species, and post hoc tests were used to determine differences among specific treatments (Statistica©). We also tested for correlations between capture rate and particle properties, including particle size, nutritional content (PUFAs), surface charge, and hydrophobicity.

For pairwise tests of particles offered for each species, one-way ANOVAs were used to determine whether there were significant differences in capture rates for each pair.

3 | RESULTS

3.1 | Single particle experiments

For larvae of *Mytilus trossulus*, there were significant differences in capture rates among tested particles when offered alone (ANOVA, $F(4,24) = 55.13$, $p < .001$; Figure 1, Table 2). A Tukey post hoc test determined that cells of *Diacronema lutheri* were captured at the highest rates (1067 ± 227 cells larva^{-1} h^{-1}). Cells of *Tisochrysis lutea* were captured at the second highest rate (294 ± 152 cells

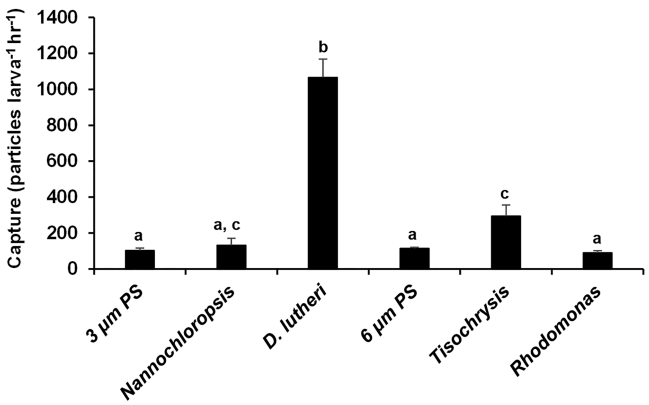


FIGURE 1 Number of particles captured larva^{-1} hr^{-1} for each species of microalga or size of polystyrene microsphere (PS) when presented alone to larvae of *Mytilus trossulus*. A two-way ANOVA found a significant effect of particle type on capture ($p < 0.001$). Data presented as mean ± standard error, n = 4 or n = 5. Lowercase letters denote significant differences in capture among particle types (Tukey HSD, $p < 0.05$)

TABLE 2 Clearance rates for feeding experiments

Single particle experiments									
	Particle type								
Bivalve species	3-µm microsphere		*Nannochloropsis* sp.		*Pavlova lutheri*	6-µm polystyrene		*Tisochrysis lutea*	*Rhodomonas lens*
Mytilus trossulus	0.03 ± 0.01		0.09 ± 0.05		0.18 ± 0.02	0.13 ± 0.01		0.31 ± 0.47	0.10 ± 0.01
Mytilus edulis	0.15 ± 0.04		0.08 ± 0.04		0.04 ± 0.03	0.24 ± 0.14		0.25 ± 0.01	NA
Mixed particle experiments									
	Particle pair								
Bivalve species	*Nannochloropsis* sp.	*T. lutea*	*Nannochloropsis* sp.	*P. lutheri*	*Nannochloropsis* sp.	3-µm microsphere	*T. lutea*	*P. lutheri*	*T. lutea*	6-µm microsphere
Mytilus trossulus	0.08 ± 0.05	0.09 ± 0.03	0.06 ± 0.04	0.11 ± 0.03	0.05 ± 0.02	0.03 ± 0.01	0.06 ± 0.04)	0.10 ± 0.02	0.08 ± 0.04	0.04 ± 0.04
Mytilus edulis	0.04 ± 0.02	0.03 ± 0.05	0.05 ± 0.02	0.01 ± 0.02	0.05 ± 0.04	0.20 ± 0.03	0.02 ± 0.02	0.01 ± 0.02	0.03 ± 0.03	0.04 ± 0.06

Note: Rates in ml larva^{-1} h^{-1}. Data shown as mean ± *SD*; NA indicates data were not collected; sample size was 4 or 5 replicates per treatment.

larva^{-1} h^{-1}), and cells of *Rhodomonas lens* were captured at the lowest rate (90 ± 26 cells larva^{-1} h^{-1}; Figure 1). There was no significant difference among capture rates of *R. lens* (90 ± 26 cells larva^{-1} h^{-1}), *Nannochloropsis* sp. (132 ± 88 cells larva^{-1} h^{-1}), and the 3-μm (104 ± 26 cells larva^{-1} h^{-1}) and 6-μm PS microspheres (114 ± 13 cells larva^{-1} h^{-1}).

For larvae of *Mytilus edulis*, significant differences in capture rates among particles tested were also found (ANOVA, $F(4,15) = 14.76$, $p < .001$; Figure 2, Table 2). Unfortunately, the culture of *R. lens* crashed, so this species was not available for tests with *M. edulis*. A Tukey post hoc test determined that for larvae of *M. edulis*, cells of *Nannochloropsis* sp. (65 ± 31 cells larva^{-1} h^{-1}) and *Tisochrysis lutea* (67 ± 3 cells larva^{-1} h^{-1}) were captured at the highest rates (Figure 2). The 3-μm PS microspheres were captured at the lowest rates (6 ± 3 particles larva^{-1} h^{-1}). There was no significant difference in capture rates of cells of *P. lutheri* (12 ± 8 cells larva^{-1} h^{-1}) and the 3-μm (12 ± 10 cells larva^{-1} h^{-1}) and 6-μm PS microspheres (12 ± 10 cells larva^{-1} h^{-1}).

3.2 | Mixed particle experiments

Particle capture rates were determined for both mussel species feeding on pairs of microalgae or microspheres, for a total of five different combinations. For *Mytilus trossulus*, there was no significant difference in the capture rate among any of the pairs of particles tested (*Nannochloropsis* sp. and *Tisochrysis lutea*, $F(1,6) = 0.33$, $p = .58$; *Nannochloropsis* sp. and *Diacronema lutheri*, $F(1,8) = 1.63$, $p = .24$; *Nannochloropsis* and 3-μm microspheres, $F(1,8) = 4.442$, $p = .07$; *T. lutea* and *D. lutheri*, $F(1,6) = 0.18$, $p = .69$; *T. lutea* and 6-μm microspheres, $F(1,6) = 0.47$, $p = .53$; Figure 3).

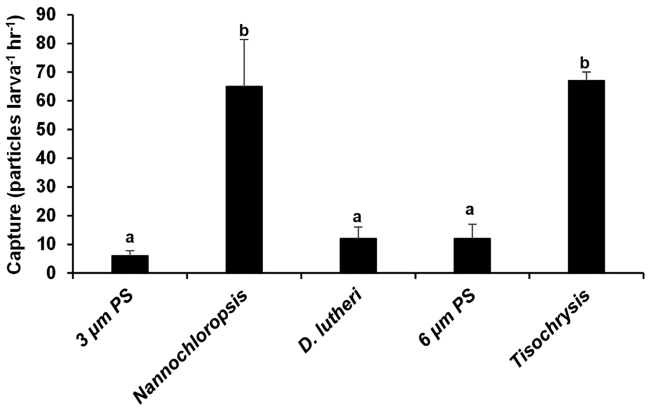


FIGURE 2 Number of particles captured larva^{-1} hr^{-1} for each species of microalga or size of polystyrene microsphere (PS) when presented alone to larvae of *Mytilus edulis*. A two-way ANOVA found a significant effect of particle type on capture ($p < 0.001$). Data presented as mean ± standard error, n = 4 or n = 5. Lowercase letters denote significant differences in capture among particle types (Tukey HSD, $p < 0.05$)

For larvae of *Mytilus edulis*, there was no significant difference in the capture rate of cells of *Nannochloropsis* sp. relative to *T. lutea* when the two species were offered in combination ($F(1,6) = 4.06$, $p = .09$; Figure 4). However, larvae captured more cells of *Nannochloropsis* sp. than of *D. lutheri* ($F(1,6) = 15.086$, $p < .01$) and more cells of *Nannochloropsis* sp. than of the 3-μm microspheres ($F(1,6) = 2.99$, $p = .013$) when offered in combination. There was no difference in the capture rate of cells of *T. lutea* and *D. lutheri* when they were offered together ($F(1,6) = 0.002$, $p = .97$). Similarly, there was no difference between the capture rate of cells of *T. lutea* and the 6-μm microspheres when offered together ($F(1,6) = 1.13$, $p = .33$).

3.3 | Particle properties and capture rate

Relationships between the different particle properties (size, algal nutritional value, percentage of PUFAs, hydrophobicity, contact angle, and surface charge) and capture rate were tested for both species. For larvae of *Mytilus trossulus*, there was no relationship between particle size and capture rate of particles ($F(1,37) = 2.696$, $p = .11$; Figure 5A). Similarly, there was no relationship between capture rate and nutritional content as measured by percentage of PUFAs ($F(1,37) = 2.343$, $p = .13$; Figure 5B). There was a significant negative relationship between particle charge and capture rate ($R^2 = .33$, $F(1,32) = 15.875$, $p < .001$; Figure 5C). There was also a significant negative relationship between capture rate and hydrophobicity, with the particles that were the most hydrophilic captured at the highest rates ($R^2 = .58$, $F(1,32) = 44.149$, $p < .001$; Figure 5D).

For larvae of *Mytilus edulis*, there was also no relationship between particle size and capture rate ($F(1,18) = 0.178$, $p = .68$; Figure 6A). There was a significant positive relationship between capture rate and % PUFAs ($R^2 = .42$, $F(1,18) = 12.758$, $p = .002$; Figure 6B), with particles having the highest concentration of nutritional fatty acids captured at the highest rate. There was also a positive significant relationship between particle capture rate and surface charge ($R^2 = .46$, $F(1,18) = 15.405$, $p < 0.001$; Figure 6C), the opposite of what was observed for *M. trossulus*. There was no relationship between capture rate and hydrophobicity ($F(1,18) = 1.287$, $p = .27$; Figure 6D).

4 | DISCUSSION

Factors affecting food selection and nutritional gain for a given species are important not only for growth and survival but also for understanding the evolution of organismal design and convergence across taxa. But, for suspension-feeding marine invertebrate larvae, surprisingly little is known about the characteristics, in addition to size, of particles that are selectively captured. Our results revealed that for two species of mussel, *Mytilus trossulus* and *M. edulis*, particle size alone, from 3 to 8 μm, had no effect on particle capture rates. But, other particle characteristics were correlated with capture rates when particles were offered alone, and those features differed

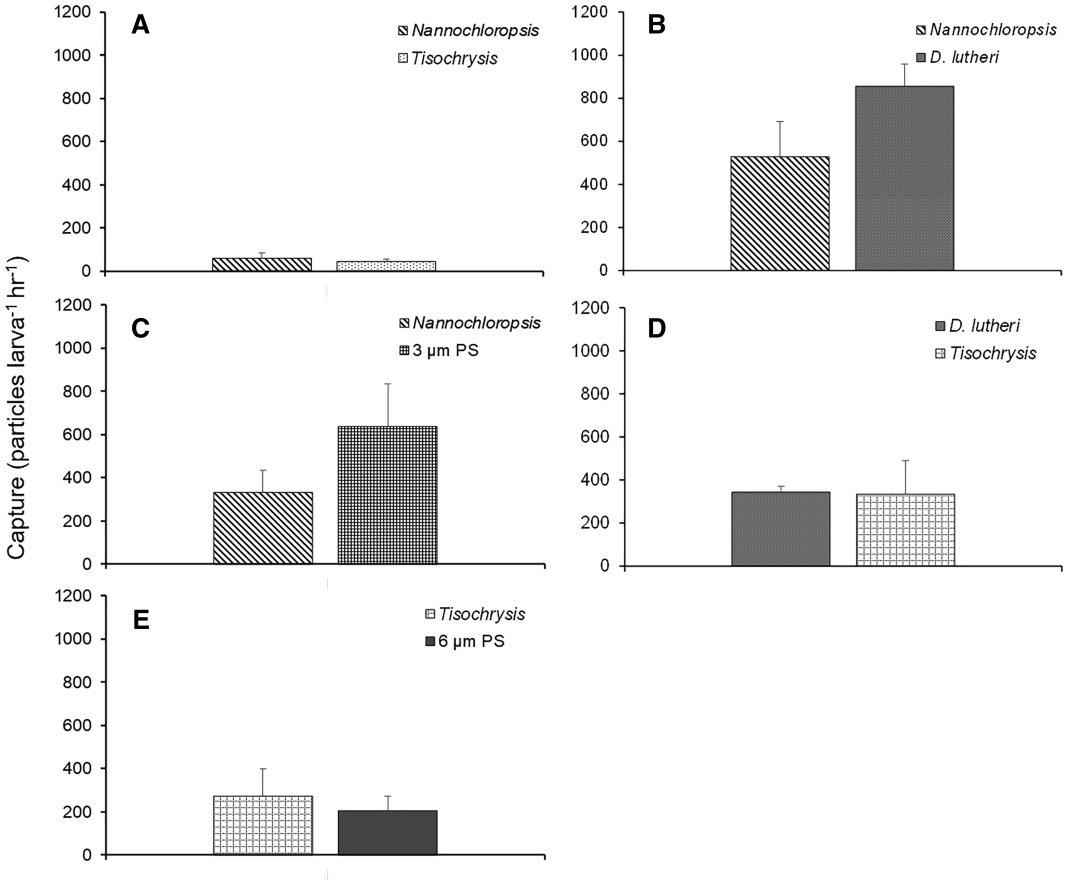


FIGURE 3 Number of particles captured larva^{-1} hr^{-1} by larvae of *Mytilus trossulus* when presented in choice experiments. Mixed particle diets represent cells of: (A) *Nannochloropsis* sp. and *Tisochrysis lutea*, (B) *Nannochloropsis* sp. and *Diacronema lutheri*, (C) *Nannochloropsis* sp. and 3-µm polystyrene microspheres (PS), (D) *Diacronema lutheri* and *T. lutea*, and (E) *T. lutea* and 6-µm polystyrene microspheres. Data presented as mean ± standard error, n = 4 or n = 5

for larvae of these two closely related species. For larvae of *M. trossulus*, preferentially captured particles that were more hydrophilic, and had a more negative surface charge, primarily cells of *Pavlova lutheri*. For *M. edulis*, a different pattern emerged. Larvae of this species preferentially captured particles with low surface charge, and hydrophobicity did not affect selection. These two species also differed in the importance of nutritional value on particle selection. From aquaculture studies, it is known that PUFAs play an important role in affecting adult growth and gonad development (Delaporte et al., 2005; Hendriks et al., 2003; Utting & Millican, 1997), as well as for larval growth, survivorship, and post-metamorphic growth and survivorship (Ronquillo et al., 2012; Utting & Millican, 1997; Vanderploeg et al., 1996). Larvae of *M. edulis* preferentially captured particles with greater PUFA content, but, for larvae of *M. trossulus*, there was no effect of PUFA content on particle capture rates.

Given the properties of the algae tested, it is difficult to separate whether the correlations with particle properties are indeed causal or whether other unknown properties of the alga could affect capture rates. For example, larvae of *M. trossulus* greatly preferred the alga *Diacronema lutheri* and captured it at higher rates than all other particles. This alga has a low surface charge and is hydrophilic. More tests are needed that will allow a separation of these surface characteristics and species properties; experiments with other algal species or artificial particles that have contrasting characteristics are needed to directly test the role of surface properties and particle selection. When particles were offered in combination, relative capture rates of the two particle types were often different than when they were offered alone. Although larvae of *M. trossulus* consumed cells of *D. lutheri* at a much higher rate when offered alone, the capture rate of *Diacronema* did not differ from that of other particles when offered as pairs. Larvae of *M. edulis* captured significantly more cells of *Nannochloropsis* sp. and *Tisochrysis lutea* than other particles when offered alone. When offered particles in pairs, cells of *Nannochloropsis* sp. were consumed more than other particles, but cells of *T. lutea* were consumed at the same rate as other particles offered. A more complete set of tests, including all pairs of particles and combining all particles together, are needed to gain a fuller picture of how to interpret these data.

Size-independent feeding preferences are well documented in adults of suspension-feeding bivalves (Rosa et al., 2018). For larval molluscs, however, few studies have examined selective feeding,

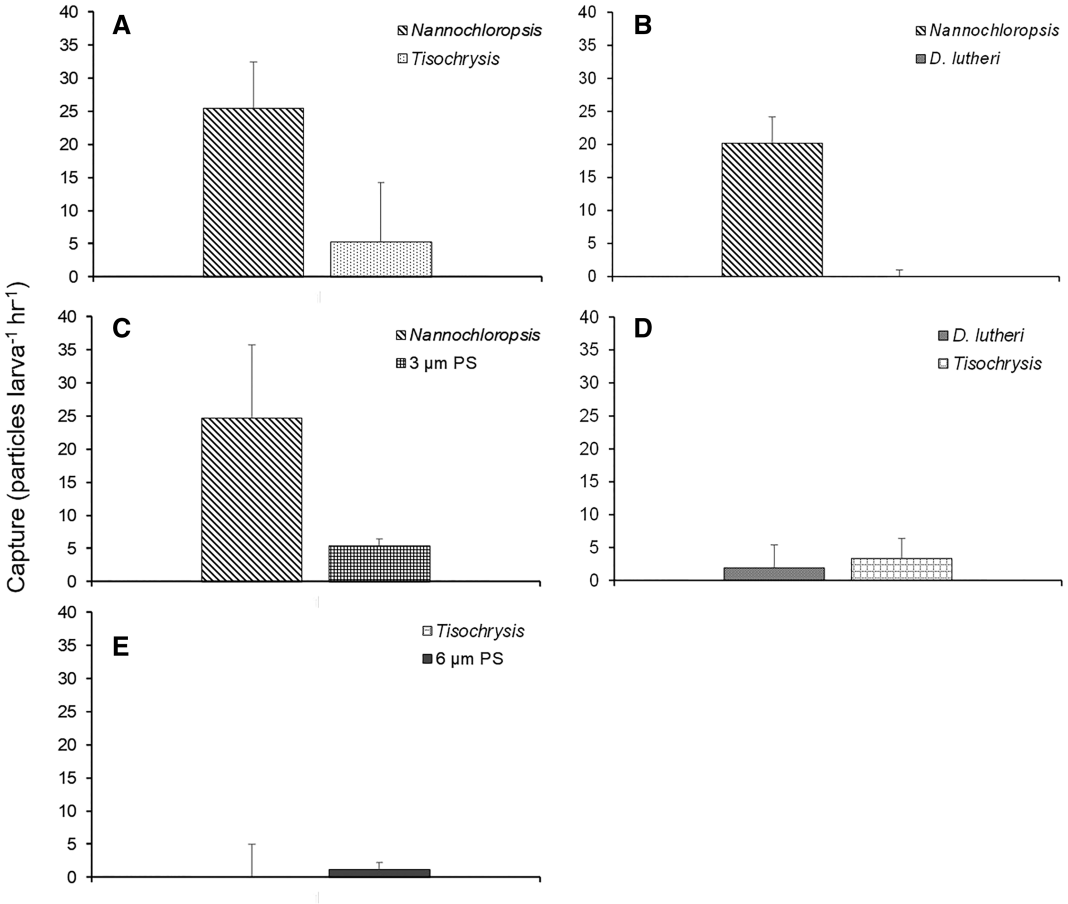
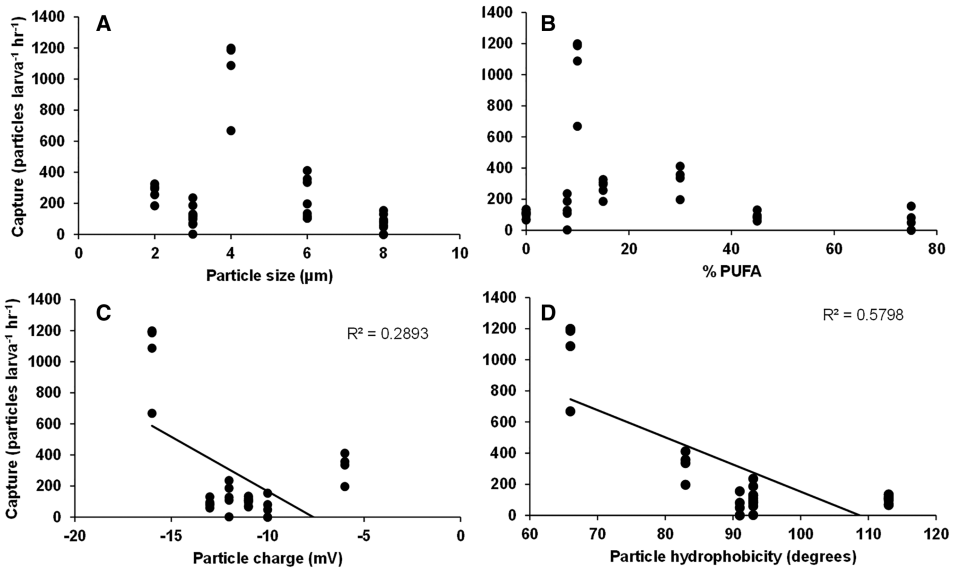


FIGURE 4 Number of particles captured larva^{-1} hr^{-1} by larvae of *Mytilus edulis* when presented in choice experiments. Mixed particle diets represent cells of: (A) *Nannochloropsis* sp. and *Tisochrysis lutea*, (B) *Nannochloropsis* sp. and *Diacronema lutheri*, (C) *Nannochloropsis* sp. and 3-µm polystyrene microspheres (PS), (D) *D. lutheri* and *T. lutea*, and (E) *T. lutea* and 6-µm polystyrene microspheres. Data presented as mean ± standard error, n = 4 or n = 5

FIGURE 5 Number of particles captured by larvae of *Mytilus trossulus* larva^{-1} hr^{-1} versus surface properties of particles. Graphs show the relationships between the number of particles captured and: (A) particle size (µm), (B) PUFA (%), (C) charge (mV), and (D) hydrophobicity (contact angle in degrees) for each particle type; n = 15 or n = 16. Regression lines and R^2 values are provided for significant relationships (Pearson correlation, $p < 0.05$)



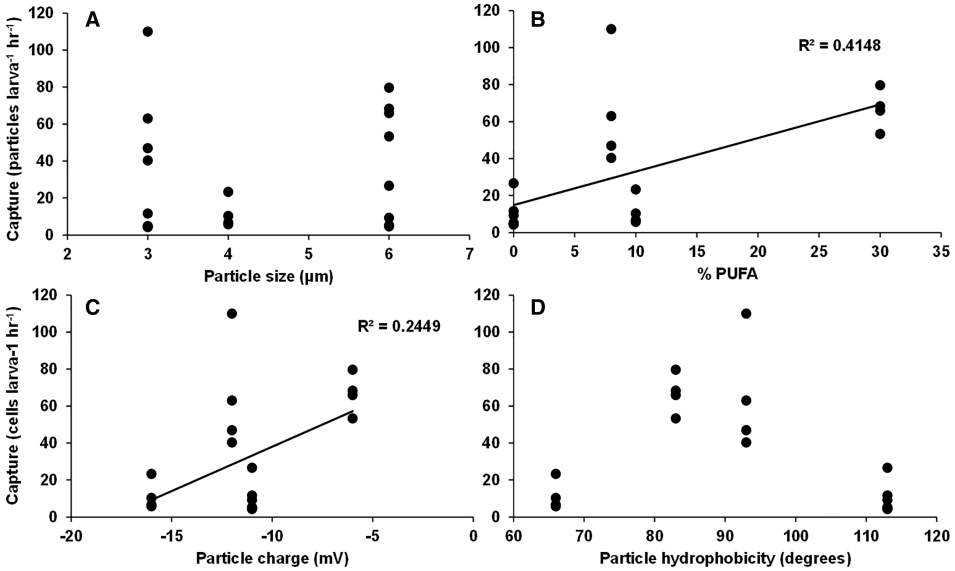


FIGURE 6 Number of particles captured by larvae of *Mytilus edulis* larva^{-1} hr^{-1} versus surface properties of particles. Graphs show the relationships between the number of particles captured and: (A) particle size (µm), (B) PUFA (%), (C) charge (mV), and (D) hydrophobicity (contact angle in degree)s for each particle type; n = 15 or n = 16. Regression lines and R^2 are provided for significant relationships (Pearson correlation, $p < 0.05$)

although there is some evidence of selectivity (e.g., Baldwin, 1995; Rosa & Padilla, 2020). When given combinations of multiple algae, larvae of the gastropod *Philine aperta* consume significantly more cells of *Cryptomonas* ("*Rhodomonas*") *baltica* than of *Nannochloropsis* sp. or *Isochrysis galbana* (Hansen, 1991). Similarly, larvae of the oyster *C. gigas* have been shown to preferentially ingest certain types of particles over others (Cole & Galloway, 2015; Rosa & Padilla, 2020). The results presented here indicate that larvae of both species of *Mytilus* show particle selectivity, with some particles preferentially captured over others, but the two species selected different types of particles. We found no significant relationship between particle sizes and capture rate for either mussel species used in this study.

In addition to differences in selection based on nutritional content (% PUFA concentration), these two species also differed in the surface properties of particles that were selected (Table 1). Surface properties of particles, such as charge and hydrophobicity, can affect the stickiness of cells (sensu Ozkan & Berberoglu, 2013a). Generally, microalgal particles that are more hydrophobic tend to more readily adhere to each other (e.g., flocculation) and to other surfaces (Ozkan & Berberoglu, 2013b). As a mechanism of passive capture, cells that are stickier could more readily be captured by veligers. Romero et al. (2010) directly observed particle capture by larvae of the gastropod *Lacuna vincta* with high-speed videography. They found that the prototrochal cilia on the velum directly intercept particles. In those veligers, particle capture appears to occur via adhesion between individual cilia and algal cells. They also reported that within a species not all encounter events resulted in interception and capture of the particle. If such surface properties are used by bivalve veligers to preferentially capture cells, there should be a strong relationship between capture and surface properties. For larvae of *M. trossulus*, we found a significant negative relationship between capture and hydrophobicity, as well as capture and surface charge. Interestingly, particles that share these surface properties have generally been found to have a lower organic content and tend to have a high mineral content (e.g., kaolin clay and dead cord grass; Rosa et al., 2013, 2017). Larvae of this species ingested an average of 80 cells larva^{-1} h^{-1}, slightly higher but similar to our findings. Data collected from another cohort of *M. edulis* (data not shown) resulted in similar particle capture rates. In addition, particle capture rates for *M. edulis* were lowest when offered combinations of particles. Differences in capture rates could be related to temperature: Experiments were run at 16°C for *M. trossulus* and 18°C for *M. edulis*. This 16°C is at the high natural temperature range for *M. trossulus* development in the field, which could either increase metabolic rates (and feeding) or, if it is a stressful temperature, reduce feeding. Although larvae of *M. edulis* can experience higher temperatures in the field, according to recommendations by the Food and Agriculture Association of the United Nations (FAO 2008) and Galley et al. (2010), the optimal temperature for their development is 18°C. We did not observe any differences in growth, development, or survivorship between these two species in our experiments, but the larvae of *M. edulis* were smaller than those of *M. trossulus*, even though they were at the same developmental stage and age. This size difference could affect feeding rates. In addition, it has been noted that larvae of *M. edulis* have smaller velar lobes than larvae of *M. trossulus* (Sprung, 1984; Widdows, 1991), which could also affect particle capture rates. Further studies are needed to determine whether there are physiological or morphological differences that could account for the observed differences in capture rates.

In this study, we found evidence that veligers of congener mussels preferentially capture different foods when offered alone. Most importantly, the apparent selection criteria for these food choices reflected different particular surface characteristics. Observed patterns of preferential particle selection here were also different than those found for veligers of the Pacific oyster *C. gigas* (Rosa & Padilla, 2020). Combining our results found here with those for

C. gigas indicate that not all veligers, even congeners, select food based on the same characteristics. Combinations of food may also alter selection preferences. More species of bivalve larvae need to be examined to determine taxon-level differences in selection as well as potential mechanisms of selection, plasticity in selection, and environmental effects (legacies) on physiology that impact food selection by larvae.

Determining generalizable rules for selective suspension feeding among molluscan larvae (if there are any) will help to determine the constraints or opportunities that this mechanism has imposed on veliger larvae. Once these rules are determined, they can then be tested across closely related taxa in the Lophotrochozoa and other metazoan taxa with feeding larvae that use cilia for particle capture and selection.

ACKNOWLEDGMENTS

The authors would like to thank Drs. Emily Carrington (U. Washington) and Gary H. Wikfors (NOAA/NMFS) for use of their flow cytometers. Our thanks to Steve Pitchford (NOAA/NMFS) for technical assistance. Thanks also go to the Director and staff of the Friday Harbor Laboratories and the staff of the NOAA Milford laboratory. This work was funded by NSF grant DBI-1611997 to MR. This is contribution number 1260 of the Department of Ecology and Evolution, Stony Brook University.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this work.

ORCID

Maria Rosa https://orcid.org/0000-0002-8140-8256

REFERENCES

Asmus, H., & Asmus, R. M. (2005). Significance of suspension-feeder systems on different spatial scales. In R. F. Dame & S. Olenin (Eds.), *The Comparative Roles of Suspension-Feeders in Ecosystems* (Vol. 47, pp. 199–219). Springer-Verlag. https://doi.org/10.1007/1-4020-3030-4_12

Baldwin, B. S. (1995). Selective particle ingestion by oyster larvae (*Crassostrea virginica*) feeding on natural seston and cultured algae. *Marine Biology*, *123*(1), 95–107. https://doi.org/10.1007/BF00350328

Bullivant, J. (1968). A revised classification of suspension feeders. *Tuatara*, *16*(2), 151–159.

Chan, K. Y. K., Jiang, H., & Padilla, D. K. (2013). Swimming speed of larval snail does not correlate with size and ciliary beat frequency. *PLoS ONE*, *8*, e82764.

Cole, M., & Galloway, T. S. (2015). Ingestion of nanoplastics and microplastics by Pacific oyster larvae. *Environmental Science & Technology*, *49*(24), 14625–14632. https://doi.org/10.1021/acs.est.5b04099

Dame, R. F. (2012). *Ecology of Marine Bivalves: An Ecosystem Approach* (2nd ed.). CRC Press.

Delaporte, M., Soudant, P., Moal, J., Kraffe, E., Marty, Y., & Samain, J.-F. (2005). Incorporation and modification of dietary fatty acids in gill polar lipids by two bivalve species *Crassostrea gigas* and *Ruditapes philippinarum*. *Comparative Biochemistry and Physiology Part A*, *140*, 460–470. https://doi.org/10.1016/j.cbpb.2005.02.009

Emlet, R. B. (1990). Flow fields around ciliated larvae: Effects of natural and artificial tethers. *Marine Ecology Progress Series*, *63*(2/3), 211–225. JSTOR. https://doi.org/10.3354/meps063211

Ferreira, J. G., Grant, J., Petersen, J. K., Smaal, A. C., & Strand, Ø. (Eds.). (2019). *Goods and Services of Marine Bivalves* (1st ed., Vol. 2019). Springer. https://doi.org/10.1007/978-3-319-96776-9

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. 1: Feeding behavior of *Calanus*. *Limnology and Oceanography*, *17*(6), 805–815. https://doi.org/10.4319/lo.1972.17.6.0805

Galley, T. H., Batista, F. M., Braithwaite, R., King, J., & Beaumont, A. R. (2010). Optimisation of larval culture of the mussel *Mytilus edulis* (L.). *Aquaculture International*, *18*, 315–325.

Hansen, B. (1991). Feeding behaviour in larvae of the opisthobranch *Philine aperta*. *Marine Biology*, *111*(2), 263–270. https://doi.org/10.1007/BF01319708

Harke, M. J., Gobler, C. J., & Shumway, S. E. (2011). Suspension feeding by the Atlantic slipper limpet (*Crepidula fornicata*) and the northern quahog (*Mercenaria mercenaria*) in the presence of cultured and wild populations of the harmful brown tide alga, *Aureococcus anophagefferens*. *Harmful Algae*, *10*(5), 503–511. https://doi.org/10.1016/j.hal.2011.03.005

Helm, M. M., & Bourne, N. A. (2004). Hatchery culture of bivalves: A practical manual. In A. Lovatelli (Ed.), *FAO Fisheries Technical Paper 471* (pp. 1–177). Food and Agricultural Organization of the United Nations.

Hendriks, I. E., van Duren, L. A., & Herman, P. M. J. (2003). Effect of dietary polyunsaturated fatty acids on reproductive output and larval growth of bivalves. *Journal of Experimental Marine Biology and Ecology*, *296*(2), 199–213. https://doi.org/10.1016/S0022-0981(03)00323-X

Jørgensen, C. B. (1981). A hydromechanical principle for particle retention in *Mytilus edulis* and other ciliary suspension feeders. *Marine Biology*, *61*(4), 277–282. https://doi.org/10.1007/BF00401566

Liao, K., Chen, W., Zhang, R., Zhou, H., Xu, J., Zhou, C., & Yan, X. (2017). QPCR analysis of bivalve larvae feeding preferences when grazing on mixed microalgal diets. *PLoS ONE*, *12*(6), e0180730. https://doi.org/10.1371/journal.pone.0180730

Newell, R. I. E. (1988). Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American oyster. In M. P. Lynch & E. C. Krome (Eds.), *Understanding the estuary: Advances in Chesapeake Bay research* (pp. 536–546). Chesapeake Bay Research Consortium.

Olson, R. R., & Olson, M. H. (1989). Food limitation of planktotrophic marine invertebrate larvae: Does it control recruitment success? *Annual Review of Ecology and Systematics*, *20*, 225–247.

Ozkan, A., & Berberoglu, H. (2013a). Cell to substratum and cell to cell interactions of microalgae. *Colloids and Surfaces. B, Biointerfaces*, *112*, 302–309. https://doi.org/10.1016/j.colsurfb.2013.08.007

Ozkan, A., & Berberoglu, H. (2013b). Physico-chemical surface properties of microalgae. *Colloids and Surfaces B: Biointerfaces*, *112*, 287–293. https://doi.org/10.1016/j.colsurfb.2013.08.001

Padilla, D. K., McCann, M. J., & Shumway, S. E. (2011). Marine invaders and bivalve aquaculture: Sources, impacts, and consequences. In S. E. Shumway (Ed.), *Shellfish Aquaculture and the Environment* (pp. 395–424). Wiley-Blackwell. https://doi.org/10.1002/9780470960967.ch14

Pernet, B. (2017). Larval feeding: Mechanisms, rates, and performance in nature. In T. Carrier, A. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae*. Oxford University Press. https://oxford.universitypressscholarship.com/view/10.1093/oso/9780198786962.001.0001/oso-9780198786962-chapter-7

Riisgård, H. U., & Larsen, P. S. (2010). Particle-capture mechanisms in suspension-feeding invertebrates. *Marine Ecology Progress Series*, *418*, 255–293. https://doi.org/10.3354/meps08755

Romero, M. R., Kelstrup, H. C. P., & Strathmann, R. R. (2010). Capture of particles by direct interception by cilia during feeding of a gastropod veliger. *Biological Bulletin*, *218*, 145–159.

Ronquillo, J. D., Fraser, J., & McConkey, A. J. (2012). Effect of mixed microalgal diets on growth and polyunsaturated fatty acid profile of European oyster (*Ostrea edulis*) juveniles. *Aquaculture*, *360-361*, 64–68. https://doi.org/10.1016/j.aquaculture.2012.07.018

Rosa, M., & Padilla, D. K. (2020). Changes in food selection through ontogeny in *Crassostrea gigas* larvae. *The Biological Bulletin*, *238*(1), 54–64. https://doi.org/10.1086/706821

Rosa, M., Ward, J. E., Frink, A., & Shumway, S. E. (2017). Effects of surface properties on particle capture by two species of suspension-feeding bivalve molluscs. *American Malacological Bulletin*, *35*(2), 181–188. https://doi.org/10.4003/006.035.0212

Rosa, M., Ward, J. E., Shumway, S. E., Wikfors, G. H., Pales-Espinosa, E., & Allam, B. (2013). Effects of particle surface properties on feeding selectivity in the eastern oyster *Crassostrea virginica* and the blue mussel *Mytilus edulis*. *Journal of Experimental Marine Biology and Ecology*, *446*, 320–327. https://doi.org/10.1016/j.jembe.2013.05.011

Rosa, M., Ward, J. E., & Shumway, S. E. (2018). Selective capture and ingestion of particles by suspension-feeding bivalve molluscs: A review. *Journal of Shellfish Research*, *37*(4), 727–746. https://doi.org/10.2983/035.037.0405

Sprung, M. (1984). Physiological energetics of mussel larvae (*Mytilus edulis*). IV. Efficiencies. *Marine Ecology Progress Series*, *18*(1/2), 179–186. JSTOR. https://doi.org/10.3354/meps018179

Strathmann, M. F. (1987). *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press; JSTOR. http://www.jstor.org/stable/j.ctvcwnh8b

Strathmann, R. R. (1978). The evolution and loss of feeding larval stages of marine invertebrates. *Evolution*, *32*(4), 894–906. https://doi.org/10.1111/j.1558-5646.1978.tb04642.x

Strathmann, R. R. (2020). Multiple origins of feeding head larvae by the Early Cambrian. *Canadian Journal of Zoology*, *98*, 761–776.

Strathmann, R. R., Fenaux, L., Sewell, A. T., & Strathmann, M. F. (1993). Abundance of food affects relative size of larval and postlarval structures of a molluscan veliger. *The Biological Bulletin*, *185*(2), 232–239. https://doi.org/10.2307/1542003

Utting, S. D., & Millican, P. F. (1997). Techniques for the hatchery conditioning of bivalve broodstocks and the subsequent effect on egg quality and larval viability. *Aquaculture*, *155*(1), 45–54. https://doi.org/10.1016/S0044-8486(97)00108-7

Vanderploeg, H. A., Liebig, J. R., & Gluck, A. A. (1996). Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena polymorpha*): The importance of size and polyunsaturated fatty acid content. *Journal of Great Lakes Research*, *22*(1), 36–45. https://doi.org/10.1016/S0380-1330(96)70932-2

Widdows, J. (1991). Physiological ecology of mussel larvae. *Aquaculture*, *94*(2–3), 147–163. https://doi.org/10.1016/0044-8486(91)90115-N

How to cite this article: Rosa, M., & Padilla, D. K. (2022). Determinants of food selection by bivalve larvae. *Invertebrate Biology*, *141*(2), e12366. https://doi.org/10.1111/ivb.12366