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Discrimination in ingestion of protistan prey by larval crabs

Shawn Hinz, Stephen Sulkin*, Suzanne Strom, Jill Testermann

Shannon Point Marine Center, 1900 Shannon Point Road, Anacortes, Washington 98221, USA

ABSTRACT: We determined the incidence of ingestion of 4 autotrophic dinoflagellates and 1 heterotrophic dinoflagellate by first stage larvae of 4 species of crabs. Crab species were 2 winter spawning brachyurans *Cancer magister* and *C. oregonensis*, 1 summer spawning brachyuran *Hemigrapsus oregonensis*, and 1 anomuran *Rhinolithodes wosnessenskii*. Autotrophic dinoflagellate prey were *Prorocentrum micans*, which sustain survival of crab larvae in laboratory culture, and 2 species of *Alexandrium* spp. that do not. *P. micans* were ingested by virtually all larvae of all 4 crab species, while both toxic and non-toxic strains of *Alexandrium* were almost never ingested. Results of rearing experiments generally confirmed that larvae were receiving no nutritional contribution from *Alexandrium* spp. prey. When brachyuran larvae were presented with mixtures of *P. micans* and *Alexandrium* spp. in defined ratios, virtually all larvae ingested both types of algal prey. Suspending *Alexandrium* cells in *P. micans* exudate did not enhance their ingestion nor did suspending *P. micans* in *Alexandrium* exudate reduce ingestion. Ingestion of plastic beads was low (<12%) except when offered in combination with *P. micans* cells (58%). *H. oregonensis* larvae ingested the heterotrophic dinoflagellate *Noctiluca scintillans* that had previously fed on either *P. micans* or one of the toxic *Alexandrium* strains, with no apparent preference. Results suggest the presence of a positive ingestion stimulus provided by *P. micans* and *N. scintillans*, but its absence in *Alexandrium* spp. Absence of ingestion of *Alexandrium* was not related to the presence of toxins. The ingestion stimulus appears to reside on the prey cell surface. Although crab larvae appear able to discriminate among algal prey, non-discriminate feeding seems likely to occur in mixed prey assemblages in which at least some prey possess the positive ingestion cue, perhaps permitting rapid ingestion of available particles when dense prey patches are encountered in an otherwise sparse prey environment.

KEY WORDS: Protists · Crabs · Larvae · Nutrition · Prey discrimination

INTRODUCTION

Larvae of most species of brachyuran crabs are planktotrophic; that is, they must feed on particulate sources of nutrition in the plankton to develop successfully to metamorphosis. In addition to needing a source of energy to sustain metabolism, most species must obtain specific nutrients from the diet to maximize survival and development rate (Sulkin & van Heukelem 1980, Levine & Sulkin 1984).

Obtaining a source of nutrition early during the first larval (zoeal) stage is particularly important in promoting good survival during later zoeal stages (e.g. Anger & Dawirs 1981, Staton & Sulkin 1991, Hartman & Sulkin 1999). Although brachyuran larvae have been thought to require relatively high densities of motile animal prey, Lehto et al. (1998) and Sulkin et al. (1998a,b) have shown that larvae of the crabs *Cancer* spp. and *Hemigrapsus oregonensis* ingest both autotrophic and heterotrophic protists and derive nutritional benefit from them. They speculated that while such diets alone may not be sufficient to sustain larval development, protists may be an important source of nutrition, sustaining first-feeding larvae until they encounter sufficient densities of suitable metazoan prey. This capacity to utilize the carbon

*Corresponding author. E-mail: sulkin@cc.wwu.edu

sources of the microbial food web may be particularly important since suitable metazoan prey are often patchy in distribution and may not be present in sufficient densities to sustain larvae (Damkaer 1977, Paul et al. 1979, 1989).

However, while protists such as the autotrophic dinoflagellate *Prorocentrum micans* and the heterotrophic dinoflagellate *Noctiluca scintillans* have proved effective at providing nutrition to crab larvae, not all protists are. For example, the ciliates *Euplotes vannus* and *Parauranema virginianum* did not sustain development of larval crabs (Sulkin 1975). Further, larvae that were fed the ciliates *Strombidinopsis acuminatum* and an *Uronema* sp., the dinoflagellates *Oxyrrhris marina*, and 2 species of *Alexandrium* did not show higher survival than did unfed larvae (Sulkin unpubl. data). It is not clear whether these differences among protists as prey for crab larvae are due to selective ingestion on the part of the larvae or to differences in biochemical composition of the various prey.

The present study was designed to determine whether crab larvae are selective in their ingestion of different protists. We compared the ingestion of an autotrophic dinoflagellate known to sustain survival of crab larvae *Prorocentrum micans* with others that do not *Alexandrium tamarense* and *A. fundyense*. Use of *Alexandrium* spp. as experimental prey also permitted examination of larval ingestion of both toxic and non-toxic strains of an autotrophic dinoflagellate.

MATERIALS AND METHODS

Experimental protocol. Experiments were conducted to determine whether newly hatched larvae of 4 species of crabs would ingest cells of target protists provided individually and in various combinations. Ingestion was determined by direct observation of larval guts as described below.

Diet treatments included 2 autotrophic dinoflagellates that, although different in shape and volume, are approximately the same diameter (35 to 50 µm). *Prorocentrum micans* (strain UTEX 1993), previously shown to sustain crab larvae in laboratory culture (Lehto et al. 1998, Sulkin et al. 1998b), was used as a control diet. Two species of *Alexandrium* (shown not to sustain larval development in preliminary tests) were used in the experiments: *A. fundyense* 1719 (Af1719), a strain shown to be toxic in mouse bioassays, and 2 strains of *A. tamarense*, 1 toxic (At118) and 1 non-toxic (At115) (Teegarden & Cembella 1996). All phytoplankton strains were obtained from the National Center for Collection of Marine Phytoplankton (Bigelow Laboratory, West Boothbay Harbor, ME). The toxicity of the strains used in the present study was confirmed by standard mouse bioassay procedures before and at the conclusion of the experiments.

Crab species tested were 3 brachyurans, the 2 winter spawners *Cancer magister* and *C. oregonensis* and the summer spawner *Hemigrapsus oregonensis*. An anomuran lithodid, *Rhinolithodes wosnessenskii*, was included because its larvae are facultative planktotrophs, able to feed but not requiring an external source of nutrition to survive.

Larvae were fed both single and mixed-species algal diets as well as combinations of algal cells and plastic beads, and algal cells immersed in the freshly prepared exudates of other phytoplankton species. In addition, larvae were fed the heterotrophic dinoflagellate *Noctiluca scintillans* that itself was cultured in the laboratory on either *Prorocentrum micans* or Af1719.

Experimental animals. All 4 crab species were collected in ovigerous state in the inland marine waters of the northern Puget Sound basin near the Shannon Point Marine Center in Anacortes, Washington. Larvae of *Cancer magister* and *C. oregonensis* hatch in early winter, with larvae of the latter species being generally more resistant to nutritional stress (Sulkin & McKeen 1999). Larvae of these species are present in the water column during a period of very low primary productivity and may be particularly dependent on a source of microbial nutrition during the critical period of early development (Sulkin et al. 1998a). Larvae of *Hemigrapsus oregonensis* hatch in the summer, a period of comparatively high primary productivity and of frequent blooms of toxic algae including *Alexandrium* spp. *Rhinolithodes wosnessenskii* larvae have not been studied extensively, but typically hatch locally in the early spring (Haynes 1984). Their ingestion of protists was of interest because their nutritional needs contrast with those of the 3 brachyuran species.

Preparation and maintenance of diet treatments. ***Autotrophic dinoflagellates:*** *Prorocentrum micans* was cultured in 1 l polycarbonate bottles in f/2 nutrient medium maintained at 22°C on a 12/12 h light/dark cycle. To maintain cells in growth phase and to normalize for the feeding experiments, cultures were maintained at an approximate cell density of 1000 ml^{-1}. Starter cultures were not axenic but contained no other algae. All 3 strains of *Alexandrium* were cultured using the same procedures.

Noctiluca scintillans: The heterotrophic dinoflagellate *Noctiluca scintillans* was cultured in 2 l polycarbonate bottles filled with 0.2 µm filtered seawater and a dilute EDTA-trace metal mix (Gifford 1985). Stock cultures were fed weekly on *Prorocentrum micans*. Before the ingestion experiments, a stock culture was taken off the *P. micans* diet and starved for 5 d to clear its food vacuoles of cells. The starved *N. scintillans* cells were then divided into 2 groups, 1 fed for 48 h on *P. micans*

and the other on Af1719. Both algae were provided to *N. scintillans* at cell densities of 3000 ml^{-1} from cultures grown to exceed that density. Twenty *N. scintillans* were selected randomly from each treatment for examination of their food vacuoles to insure that the cultures were actively feeding on the respective algal diets. *N. scintillans* cells were concentrated by reverse filtration (80 µm sieve) for feeding to crab larvae.

Exudate preparations: To determine whether chemicals exuded by the various algae might have an influence on ingestion behavior, *Prorocentrum micans* cells were suspended in freshly prepared exudates of Af1719 (toxic) and At115 (non-toxic), while *Alexandrium* cells of both species were suspended separately in exudate from *P. micans*. All 3 cell types were gravity filtered from the growth medium (10 µm filter) to produce both exudate and isolated cells. *P. micans* cells were then suspended in either of the *Alexandrium* cell exudates and both *Alexandrium* cells were suspended separately in the *P. micans* exudate. Cell concentrations after suspension in exudate were approximately 1000 ml^{-1}. Because rapid lability of exuded *Alexandrium* toxin was reported by Hansen (1989), ingestion observations were made less than 1 min after exudate was isolated.

Combination treatments: To determine the effect of the presence of 1 algal species on the ingestion of the other, defined mixtures were fed to crab larvae. To produce the mixtures, stock cultures of target species were diluted to cell densities of 1000 ml^{-1} and appropriate volumes of each were combined to produce the desired ratio at a total cell density of 1000 ml^{-1}. For example, a 1:1 ratio of *Prorocentrum micans* to At118 was produced by manipulating each stock culture to 1000 ml^{-1}, then combining equal volumes of the 2 stock cultures to provide the experimental diet. These 1:1 ratios of *P. micans* to Af1719, At115, and At118 were fed to larvae of all 3 brachyuran species. In addition, larvae of *Hemigrapsus oregonensis* were fed *P. micans*: Af1719 ratios of 1:2, 1:5, 1:10, 1:15, 1:20, and 1:30.

Additional experiments used plastic beads (Fluorosprite 25 µm latex beads; Polysciences, Inc., Warrington, PA) either alone or in combination with *Prorocentrum micans* or Af1719 cells at a total cell (particle) density of 1000 ml^{-1}. Plastic beads were also suspended in freshly prepared exudate of either *P. micans* or Af1719 at a total particle count of 1000 ml^{-1}. Plastic microsphere experiments were carried out using larvae of *Hemigrapsus oregonensis* only.

Ingestion experiments. Ingestion was determined by examining the guts of selected larvae for presence of the target algal cells using an epifluorescence microscope. Individual larvae to be examined were removed by pipet from the feeding culture and placed on a microscope slide. Excess water was removed from the slide and the larvae were observed at 40×. Ingestion was deemed to have occurred when there were at least 3 individual algal cells in the gut. In the vast majority of cases, the guts either contained many cells or were empty. Ingestion tests were identical for all treatments. Larvae used in the experiments had hatched during the previous 12 h and had not been fed. For each observation, larvae from at least 2 broods were pooled before selection of individual larvae for the experiment. For each test, 100 larvae were arbitrarily selected from a large pool of at least several hundred, were placed in one 80 mm diameter glass bowl, and were fed the target diet. At specified observation times, 15 larvae were selected haphazardly and observed as described above for evidence of prey cells in their guts. Larvae were discarded after observations. This experiment was repeated with 3 different groups of larvae, with treatment comparisons based on the percentage of larvae feeding in each experiment (n = 3). Ingestion of single diet types and 1:1 ratios was typically observed at 3, 6, 12, 24, 48, and 96 h. Time intervals of observations for other experiments varied and are specified with the results.

In the *Noctiluca scintillans* experiment, a mixture of 10 cells fed *Prorocentrum micans* and 10 fed Af1719 was fed to each of 10 *Hemigrapsus oregonensis* larvae, each placed in a separate 10 mm diameter glass bowl for 8 h. At the end of 8 h, the remaining *N. scintillans* cells were examined and identified as to treatment. The experiment was repeated 3 times with different groups of larvae. The percentage of each type of *N. scintillans* cell ingested by each larva was determined and compared.

Larval survival and development rate experiments. To further assess the value of toxic and non-toxic strains of *Alexandrium* to crab larval development, larval cultures of the 2 *Cancer* spp. were maintained from hatching to molt to zoeal stage 2 on Af1719 and At115. An unfed treatment and a fed control consisting of freshly hatched nauplii of the brine shrimp *Artemia* spp. (Argentemium, Argent Chemical Laboratory, Redmont, WA) were also tested simultaneously. Algae were fed to the larvae at a concentration of 1000 cells ml^{-1} and *Artemia* spp. at 8 ml^{-1}.

Newly hatched larvae from at least 2 broods were pooled. Larvae were haphazardly selected from the pool and placed in plastic trays containing 12 individual cell wells, 1 larva well^{-1}. Each well contained approximately 4 ml of *Alexandrium* culture medium containing the algal cells. The unfed larvae were placed into culture medium with no prey present. Three trays (each containing 12 larvae) were haphazardly assigned to each of the 4 treatments and fed accordingly. Larvae were transferred daily to clean cell wells containing fresh medium with the appropriate diet. Mortality and evidence of molting to zoeal stage

2 were noted daily. Treatment comparisons were based on whether the diet supported development to zoeal stage 2 and, if so, whether there was a difference in duration of the first zoeal stage. In treatments where there was no molting to zoeal stage 2, mean days of death were calculated and compared.

Because *Rhinolithodes wosnessenskii* larvae can develop to zoeal stage 2 without feeding, the experimental design for survival and development rate differed from that of the 2 *Cancer* spp. Mortality and percentage of larvae molting on unfed, freshly hatched *Artemia* spp. nauplii and Af1719 (1000 cells ml^{-1}) treatments were compared on Day 5 of the culture. This day was selected as providing a sufficient feeding time for dietary effects to be evident, while providing an endpoint before 100% molting of unfed larvae. Larvae were obtained from 1 female and distributed among 9 trays, each containing 12 larvae. Three trays were haphazardly assigned to each diet treatment. Cultures were transferred daily to fresh medium containing the appropriate diet treatment.

Larvae of *Hemigrapsus oregonensis* were raised from hatching on the following diet treatments: unfed, AF1719 at 1000 cells ml^{-1}, 1:1 combination ratio of *Prorocentrum micans*:Af1719 at 500 cells ml^{-1} each (total density of 1000 ml^{-1}), and *P. micans* only at the reduced dosage of 500 cells ml^{-1}. Larvae were obtained from 1 female crab and distributed among 3 cell well trays for each diet treatment (n = 36 larvae $treatment^{-1}$). Larvae were transferred daily to fresh medium and fed the appropriate diet. Survival was monitored, with mean days of death compared among the treatments.

Table 1. Mean percentage of larvae that show ingestion of indicated prey types at each observation time. Af1719: *Alexandrium fundyense* 1719 (toxic); At115: *A. tamarense* 115 (non-toxic); At118: *A. tamarense* 118 (toxic); Pm: *Prorocentrum micans*. n = 3 for each observation time

Crab species / Time interval	Diet treatment: Pm	At118	At115	Af1719
Cancer oregonensis				
3	90	0	0	71
6	100	0	0	100
12	100	0	0	75
24	100	0	0	34
48	95	0	0	0
96	100	0	0	4
Cancer magister				
3	95	0	0	0
6	100	2	0	0
12	100	0	0	0
24	100	3	3	2
48	100	0	0	0
96	100	0	0	0
Hemigrapsus oregonensis				
3	100	0	0	0
6	100	0	0	0
12	100	0	0	0
24	100	0	0	0
48	100	0	0	0
96	100	0	0	0
Rhinolithodes wosnessenskii				
3	100	–	0	0
6	100	–	0	0
12	100	–	0	0
24	100	–	0	0
48	100	–	0	0
96	100	–	0	0

RESULTS

Ingestion of individual diets

Prorocentrum micans were ingested by virtually all larvae at all observation times in all 4 species tested (Table 1). Cells were present in 90 to 100% of the larvae at the first (3 h) measurement and guts remained filled with *P. micans* throughout the 96 h period of the experiment.

By contrast, both non-toxic (At115) and toxic (At118, Af1719) strains of *Alexandrium* were almost never ingested by any species at any observation time (Table 1). Only in *Cancer oregonensis* was ingestion of *Alexandrium* cells noted and only of the toxic Af1719 strain (Table 1). In this case, there was initial ingestion of cells, with 100% of the larvae feeding by 6 h, followed by a rapid decline in ingestion such that by 48 h, no larvae were found to have Af1719 cells in their guts.

The effects of raising *Cancer oregonensis* and *C. magister* larvae on *Prorocentrum micans* and *Alexandrium* strains generally confirmed the ingestion results. *C. oregonensis* larvae fed the control diet of freshly hatched *Artemia* spp. nauplii showed 93% survival to zoeal stage 2 with a mean stage duration of 10.9 ± 1.1 d (mean ± SE). Unfed larvae and those fed the non-toxic At115 or the toxic Af1719 all died before molting to zoeal stage 2. No significant differences in mean days of death were found among these 3 treatments (Table 2; ANOVA, $p > 0.05$), suggesting that the *Alexandrium* cells were not contributing nutritionally. Although the ingestion experiments suggested that larvae did feed

Table 2. *Cancer* spp. Mean ± SE days of death for first stage larvae of the 2 species fed the indicated diets. No significant differences were found among treatments for either species (ANOVA, $p > 0.05$). Initial sample size = 36 for each treatment. See Table 1 for definitions of diet treatments

Diet	Crab species: *C. oregonensis*	*C. magister*
Af1719	12.8 ± 0.56	5.6 ± 0.54
At115	13.8 ± 0.73	7.8 ± 0.30
Unfed	11.6 ± 0.24	5.4 ± 0.19

briefly on Af1719 cells, there is no evidence that such feeding resulted in a delay in mortality.

Results with *Cancer magister* larvae were similar. Larvae fed the brine shrimp nauplius control developed successfully to zoeal stage 2 (60% survival; mean ± SE stage duration 8.4 ± 1.0 d). There was no significant difference in mean days of death among the unfed and 2 *Alexandrium*-fed treatments (Table 2; ANOVA, $p > 0.05$). Again, there is no evidence that sustained exposure to either *Alexandrium* diet resulted either in nutritional benefit or toxicity to the larvae.

Results from the facultative planktotroph *Rhinolithodes wosnessenskii* provided an interesting contrast with the 2 *Cancer* spp. On Day 5, there were significant differences among treatments in percentage mortality (Table 3; ANOVA on arcsine transformed data, $p < 0.05$). Results of a Tukey's honestly significant difference (HSD) test (Table 3) show a higher mortality for unfed larvae than for larvae fed either *Artemia* nauplii or AT1719. There were also significant differences among treatments in mean percentage molting on Day 5 (Table 3; ANOVA on arcsine transformed data, $p < 0.05$), with feeding on brine shrimp nauplii accelerating development, while feeding on Af1719 delaying it compared with the unfed treatment (Tukey's HSD test, $p = 0.05$). These results imply that at least a few of the larvae were feeding on Af1719 either throughout the experiment in contrast to the direct observations on ingestion (Table 1) or after 96 h (e.g. Day 5).

Table 3. *Rhinolithodes wosnessenskii.* Mean percentage mortality (A) and percentage molt (B) of stage 1 larvae on Day 5 on indicated diet treatments. Shared letter indicates no significant difference ($p = 0.05$) for each measurement

A			
Diet	Mortality (%)	SE	Tukey's HSD
Unfed	26	0.47	a
Artemia spp.	10	0.20	b
Af1719	8	0.61	b
B			
Diet	Molt (%)	SE	Tukey's HSD
Artemia spp.	65	0.12	a
Unfed	32	0.29	b
Af1719	17	0.43	c

Combination diets

When *Prorocentrum micans* was presented to larvae of all 3 species of brachyuran crabs in a 1:1 ratio with any of the 3 *Alexandrium* strains, nearly 100% of the larvae ingested algal cells at all observation times (Table 4). Furthermore, direct observations revealed that in all cases *both* algal species were being ingested.

To further elucidate this observation, larvae of *Hemigrapsus oregonensis* were fed diets consisting of a range of *Prorocentrum micans*:Af1719 ratios as described earlier. Incidence of ingestion was observed at 8 h. The percentage of larvae feeding began to decline at a ratio of 1:10 (Fig. 1), dropping to less than 50% feeding at a ratio of 1:20, with less than 5% feeding at a ratio of 1:30. Again, in all cases where ingestion was noted, both algal species were present in the guts.

When *Hemigrapsus oregonensis* larvae were raised in laboratory culture on the 1:1 ratio of *Prorocentrum micans*:Af1719, there was a significant delay in mortality compared with an unfed treatment or with larvae fed only Af1719 (Table 5; ANOVA, $p < 0.05$; Tukey's HSD, $p = 0.05$). As anticipated from the ingestion observations, there was no delay in mortality on the latter diet compared with the unfed control. Larvae fed the *P. micans* diet at 500 cells ml^{-1} did not survive to zoeal stage 2 in this experiment. However, adding Af1719 at 500 cells ml^{-1} to *P. micans* at 500 cells ml^{-1} neither accelerated nor delayed mortality.

Exudate experiments

To determine whether the differences in incidence of ingestion between the *Prorocentrum micans* diet and the *Alexandrium* strains were due to chemical stimuli produced by the latter, *P. micans* cells were suspended

Table 4. Mean percentage of larvae that show ingestion of indicated 1:1 ratio combinations of prey types for specified crab species. See Table 1 for definitions of diet treatments

Crab species	Diet Treatment		
Time interval	Pm/At115	Pm/Af1719	Pm/At118
Cancer oregonensis			
3	90	100	100
6	90	95	90
12	90	90	90
24	95	90	90
48	100	88	90
96	100	100	90
Cancer magister			
3	100	100	100
6	100	95	90
12	85	90	90
24	100	90	90
48	100	88	90
96	100	100	90
Hemigrapsus oregonensis			
3	90	100	90
6	90	100	100
12	90	100	90
24	95	100	100
48	100	90	100
96	100	100	100

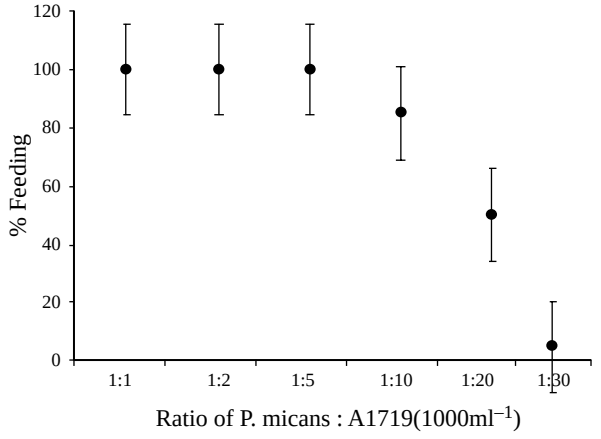

Fig. 1. Percentage of *Hemigrapsus oregonensis* larvae feeding on indicated ratios of *Prorocentrum micans*:*Alexandrium fundyense* 1719 (Af1719) cells. Total cell density is 1000 ml^{-1}

Table 5. Mean ± SE day of death for *Hemigrapsus oregonensis* larvae fed the indicated diets. There were significant differences among treatments (ANOVA, $p < 0.05$). Shared letters indicate no significant differences (Tukey's HSD, $p = 0.05$). See Table 1 for definitions of diet treatments. n: numbers of larvae. Total cell counts at 1000 ml^{-1} except Pm

Diet	Mean day of death	n	Tukey's HSD
Af1719	5.3 ± 0.15	36	a
Unfed	5.8 ± 0.25	36	a
Pm/Af1719 (1:1)	8.2 ± 0.46	36	b
Pm (500 ml^{-1})	9.2 ± 0.47	34	b

Table 6. Mean percentage of larvae that showed evidence of ingestion of indicated prey-exudate combinations at each observation time. See Table 1 for definitions of diet treatments. Ex: exudates. n = 3 in each case

Crab species		Diet		
Time interval	At115/ Pm Ex	Af1719/ Pm Ex	Pm/ At115 Ex	Pm/ Af1719 Ex
Cancer oregonensis				
12	0	0	100	90
24	0	0	100	90
Cancer magister				
12	0	0	100	100
24	0	0	100	90

in freshly prepared exudate of the non-toxic At115 and the toxic Af1719 as described above. In both *Cancer* spp., incidence of *P. micans* ingestion was close to 100% in the exudates of the 2 *Alexandrium* strains (Table 6). When Af1719 and At115 cells were suspended in *P. micans* exudate, there was no incidence of ingestion (Table 6). Thus, the stimulus to feed on *P. micans* is not affected by the presence of *Alexandrium* exudates, nor are *Alexandrium* cells rendered more palatable by the presence of *P. micans* exudate.

Ingestion of plastic beads

When *Hemigrapsus oregonensis* larvae were given plastic beads alone, very few fed on them (Table 7). However, when the beads were provided in combination with *Prorocentrum micans* cells at a ratio of 1:1 (total particle concentration of 1000 ml^{-1}), 58% of the larvae ingested both particle types. Furthermore, when beads were presented to larvae in combination with Af1719 cells or suspended in either *P. micans* or Af1719 exudate, there was low incidence of ingestion. As was the case with *Alexandrium* cells, plastic beads were ingested most readily when combined with *P. micans* cells.

Ingestion of *Noctiluca scintillans*

Noctiluca scintillans cultured on either *Prorocentrum micans* or Af1719 were readily ingested by *Hemigrapsus oregonensis* larvae. When the 2 diet treatments were offered to larvae in combination, 90% of the Af1719-fed and 93% of the *P. micans*-fed *N. scintillans* were ingested, with no significant difference between treatments (Student's *t*-test on arcsine transformed data, $p > 0.05$)

DISCUSSION

Virtually all larvae of all 4 species of crabs ingested *Prorocentrum micans*, while there was almost universal failure of larvae to ingest any strain of *Alexandrium*. This indicates some level of discrimination in feeding on algal cells by crab larvae.

For larvae to discriminate among prey, they must respond to prey-associated cues, presumably either chemical or mechanical stimuli. The mechanism by which crab larvae ingest captured protist cells is not well described. Sulkin et al. (1998a) described brachyuran larvae as 'encounter feeders'; that is, larvae grasp prey particles, both living and non-living (detritus), as both predator and prey move through the

Table 7. Mean percentage of *Hemigrapsus oregonensis* larvae feeding on plastic beads and algal mixtures after 8 h of exposure to the specified diets. See Table 1 for definitions of diet treatments. Ex: exudate. n = 3 in each case

Diet treatment	Feeding (mean %)	SE
Beads alone	5	0.11
Beads + Pm	58	0.23
Beads + Af1719	3	0.26
Beads + Pm Ex	12	0.43
Beads + Af1719Ex	8	0.21

water. Prey capture by crab larvae does not appear to require use of their well-developed eyes (Harvey & Epifanio 1997, Sulkin et al. 1998a) and occurs over a wide range of prey sizes (from at least 5 to 250 µm; Lehto et al. 1998). Larger prey typically are grasped by the endites of the maxillules and pushed toward the mandibles where they are masticated and ingested (Crain 1999). It is possible that the protist *Noctiluca*, being as large as the largest metazoan prey typically used in laboratory culture of crab larvae, are handled in a similar way. Crain (1999) described capture and handling of the smaller *Prorocentrum micans* cells by the anomuran crab *Placetron wosnessenskii* as a 'fling and clap' motion (as described by Koehl & Strickler 1981) in which the mouthparts first are swung rapidly outward, drawing the cell toward the mouth, then are rapidly drawn in, enclosing the cell. A similar activity was noted in the present study when larvae ingested *P. micans*, but not when they were presented with *Alexandrium* spp. In the latter case, larvae pushed the *Alexandrium* cell toward the mandibles, which pulled the cell in toward the mouth, then pushed it away.

Thus, larvae appeared to require intimate contact with the target cell before ingestion was stimulated. This is consistent with the lack of stimulus provided by the exudate and with the speculation by Teegarden & Cembella (1996) that copepods make prey ingestion decisions by recognizing cues either on the surface of the cell or in an associated fluid microzone around the cell. Both *Alexandrium tamarense* and *Prorocentrum micans* have lectin molecules on the cell surface (Hori et al. 1996). It is possible that a lectin or other molecule(s) associated with the cell surface play a role in prey discrimination on the part of larvae.

The nature of a prey-associated ingestion cue might be positive (stimulating ingestion) or negative (stimulating rejection). The preponderance of evidence suggests that a positive stimulus was controlling ingestion of protists in the present study. This conclusion is supported by the observation that while *Alexandrium* spp. cells were not ingested, both *Prorocentrum micans* and *Noctiluca scintillans* were, the latter whether it had previously been fed either *P. micans* or Af1719. Furthermore, larvae ingested all 3 strains of *Alexandrium*, as well as inert plastic beads when they were combined with comparatively few *P. micans* cells. Thus, *Alexandrium* cells did not appear to produce a cue that induced rejection on the part of the larval predator, since they were ingested when combined with *P. micans* cells or when inside the food vacuoles of *N. scintillans*. The ingestion of both *Alexandrium* cells and plastic beads when each was combined with *P. micans* further suggests that once feeding is induced by a positive stimulus, such as that produced by *P. micans*, larvae cease to discriminate between cell types. While this scenario seems the most likely, the results do not rule out the possibility that *Alexandrium* cells do produce a rejection cue that is readily overridden by the presence of a positive cue produced by another alga. In either case, however, the positive stimulus dominates the process.

Clearly, the failure of larvae to ingest non-toxic as well as toxic strains of *Alexandrium* precludes the possibility that *Alexandrium*-produced toxin prevented ingestion of cells by larvae. Furthermore, placing larvae in exudate freshly prepared from either a favorable or an unfavorable prey had no effect on their ingestion behavior. The results thus do not support the role of a water-borne chemical stimulus, either positive or negative. It is possible, however, that the presumed cue on the prey cell surface is chemical in nature.

Larvae of *Cancer oregonensis* did ingest *Alexandrium fundyense* cells, at least initially. Because *C. oregonensis* did not ingest either non-toxic or toxic strains of *A. tamarense* and because other crab species, including the congener *C. magister*, did not ingest *A. fundyense*, this result appears to be a species-specific phenomenon for either the predator, the prey, or both. *Cancer oregonensis* has been shown to be more nutritionally flexible than its congener (Sulkin & McKeen 1999) and may be able to consume a wider variety of prey. It is interesting to note, however, that the percentage of larvae that showed evidence of having ingested this toxic strain declined over time. Presumably, the presence of toxin or some other factor resulted in larvae ceasing to ingest this prey after a period of exposure. Unfortunately, there is no non-toxic strain of *A. fundyense* available to rigorously test the role played by the toxin in this behavior.

Given the absence of ingestion of *Alexandrium* cells by the larvae, it is not surprising that this diet did not support development and that there was no delay in mortality compared with unfed controls in either *Cancer* species. Inducing *Hemigrapsus oregonensis* larvae to ingest the toxic strain of *A. fundyense* by combining it with *Prorocentrum micans* neither accelerated nor delayed mortality compared with presenting the larvae with a sub-optimal dosage of *P. micans* alone. This suggests that ingestion of toxic strains of an alga that does not itself stimulate predation had no deleterious effect on the larval predator. Only in the anomuran facultative planktotroph *Rhinolithodes wosnessenskii* did extended exposure to toxic *Alexandrium* have an effect. However, even in this case, results were mixed; a reduction in mortality was combined with a delay in development compared with the unfed control.

Larvae in nature are almost always faced with a complex mixture of prey. Feeding is likely to occur if the mixture includes sufficient quantities of prey that produce positive stimuli for ingestion. Moreover, if the

mixture does include sufficient quantities of such prey, larvae are also likely to ingest prey particles that do not provide this positive stimulus. This lack of discrimination in prey selection when favorable prey are present may promote rapid ingestion of available particles when larvae encounter dense patches of prey in an otherwise sparse prey environment.

It also seems likely that larvae do not feed when they find themselves in dense aggregates of prey dominated by unfavorable and possibly toxic blooms of algae. However, even harmful algal blooms, although consisting of only a few species at the bloom center, do sustain a more diverse community, including heterotrophic protists, at the periphery (Turner & Tester 1997). Thus, a prey field may develop at the periphery of such an algal bloom that is likely to be a favorable feeding environment for crab larvae.

Crab larvae, while they are omnivores that ingest a wide variety of prey types including metazoans, protists, and microbially colonized detrital particles, nevertheless appear to discriminate among prey. At least for such small prey as protists, the discriminatory behavior seems to require testing of individual cells. It has yet to be determined whether there is a relation between the presence of a positive prey-associated cue and the qualitative value of that prey as a nutritional source.

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