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Low concentrations of large inedible particles reduce feeding rates of echinoderm larvae

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Abstract The planktonic larvae of many marine invertebrates must feed to develop to metamorphosis. The rate at which feeding larvae accumulate energy affects the amount of time they must spend in the plankton, which affects larval dispersal and mortality; it may also affect the amount of energy gained before metamorphosis, and thus limit growth or survivorship of early juveniles. Rates of energy acquisition are partly determined by the quantity of edible particles in the plankton. However, the plankton also contains many particles that are too large to be ingested. Prior studies suggest that high concentrations of such large inedible particles reduce larval feeding rates. This study examines whether the feeding rates of larvae of southern California echinoderms are reduced by lower, more frequently encountered concentrations of large inedible particles. Larvae of a holothuroid, two asteroids, and three echinoids were fed 6-µm beads alone or with large inedible beads at 25–500 inedible beads mL^{-1}. Five of the six species showed reduced clearance rates on 6-µm beads when exposed to as few as 25 inedible beads mL^{-1}. In similar experiments on an asteroid and an echinoid using natural large inedible particles (centric diatoms), larval clearance rates were reduced at 25 cells mL^{-1} and higher. Larval clearance rates were reduced by ~50% in treatments of 100 or 500 large inedible particles mL^{-1}. These results suggest that in nature, rates of food acquisition by larvae may depend not only on the abundance of food particles, but also on the abundance of potentially interfering non-food particles.

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Introduction

Many marine invertebrates have life cycles that include an obligately feeding planktonic larval stage. These larvae must capture food particles to fuel growth and development through metamorphosis. One challenge of this strategy is that suitable food particles often appear to be growth-limiting in concentration (e.g., Paulay et al. 1985; Fenaux et al. 1994; Reitzel et al. 2004; Pedersen et al. 2010). Food-limited larvae may have a prolonged planktonic period, increasing the risks of larval mortality by predation or advection away from suitable adult habitat (Olson and Olson 1989; Rumrill 1990; Morgan 1995). Surviving larvae may metamorphose with limited energy stores, producing low-quality juveniles that have reduced growth and survivorship (Hart and Strathmann 1994; Phillips 2002; Thiyagarajan et al. 2005; Pechenik 2006; Torres et al. 2016).

Another challenge for feeding larvae is that in addition to food, the plankton also contains particles that are not suitable as food but may interfere with the rapid capture and ingestion of food. For example, larvae may routinely encounter planktonic particles that are too large for them to ingest (Strathmann 1987). Larvae may respond to these inedible particles by altering their swimming behavior to avoid or disengage from them, or by capturing and subsequently rejecting them. Such interactions may reduce the amount of time that larvae could otherwise spend efficiently acquiring food particles, exacerbating the effects of low food concentrations.

We know of two studies that address the possibility that the presence of such large inedible particles may affect rates of larval feeding on suitable particles. Strathmann (1971) quantified clearance rates of three species of echinoderm larvae feeding on the dinoflagellate *Amphidinium* sp. (~25 µm maximum dimension) in suspensions that also contained the large diatom *Ditylum brightwellii* (~200 µm in length). Many (but not all) of the *D. brightwellii* cells were too large for the echinoderm larvae to consume. Strathmann found that diatoms at relatively high concentrations (1000 cells mL^{-1}) reduced clearance rates on *Amphidinium* sp. by plutei of the brittle star *Ophiopholis aculeata* and the sea urchin *Strongylocentrotus droebachiensis*, but not by bipinnariae of the seastar *Pisaster ochraceus*. This suggests that in at least some echinoderm larvae, the presence of large particles may reduce clearance rates on suitable food particles. However, larvae of all three species examined did consume some *D. brightwellii* cells, complicating interpretation of these results.

Hansen et al. (1991) examined feeding of veligers of the gastropod *Philine aperta* on *Isochrysis galbana* when exposed to varying concentrations of 45-µm diameter latex microspheres, which were too large for the veligers to ingest. Clearance rates on *I. galbana* were reduced in the presence of inedible particles at all concentrations tested (2500–20,000 microspheres mL^{-1}), in a dose-dependent manner. At the highest concentration of inedible particles offered, veligers cleared *I. galbana* at rates ~60% lower than when inedible particles were not present. Hansen et al. (1991) also observed larvae swimming in suspensions with and without inedible particles, and concluded that the reduced feeding rates in the presence of the inedible particles were due to a brief cessation of feeding each time a veliger encountered an inedible particle.

In both of these studies, concentrations of large particles were quite high. In nature, similar concentrations of large particles are likely to be found only in dense plankton blooms (Reid et al. 1970; Hansen et al. 1991; Moorthi et al. 2006; Degerlund and Eilertsen 2010), or in areas rich in suspended inorganic particles (Carriker 1986; Phillips and Shima 2006). Here we examine whether the feeding rates of larvae of six species of echinoderms (one holothuroid, two asteroids, and three echinoids) are affected by the presence of large inedible particles at low concentrations, a condition that larvae likely experience more frequently in nature.

Methods

Spawning and larval culturing

Adults of the holothuroid *Apostichopus parvimensis*, the asteroids *Astropecten armatus* and *Patiria miniata*, and the echinoids *Dendraster excentricus*, *Lytechinus pictus*, and *Strongylocentrotus purpuratus* were collected by SCUBA divers from subtidal sites near White Point and Point Fermin, Los Angeles County, California (33.7115N, −118.3049W) from 2014 to 2016. Adults were kept in recirculating seawater tanks at 16 °C until spawning. Spawning in *A. parvimensis* was induced by injection of 2–4 mL of 200 µM NGLWY-amide into the perivisceral coelom (Kato et al. 2009). Asteroids were induced to spawn by injection of 1–2 mL of 100 µM 1-methyladenine into the perivisceral coelom, and echinoids by injection of 1 mL of 0.53 M KCl into the perivisceral coelom (Strathmann 1987). Gametes from a single male and a single female of each species were used to start each culture. Spawned eggs were rinsed with 16 °C filtered seawater (FSW, pore size 0.2 µm), then fertilized with dilute sperm. Fertilized eggs were rinsed in FSW and held at 16 °C in a walk-in environmental chamber until larvae had hatched (at the blastula stage for the holothuroid and echinoids, and the gastrula stage for asteroids). Swimming embryos were decanted into clean beakers, and their concentration estimated from subsamples counted in a Bogorov tray. They were then diluted into 1-L glass beakers of FSW to yield a final concentration of 0.5 embryos mL^{-1}.

Cultures were maintained at 16 °C with continual stirring from a paddle system (Strathmann 1987). As soon as larvae were capable of ingesting food, cells of *Rhodomonas* sp. cultured in f/2 medium were added to cultures at a final concentration of 10,000 cells mL^{-1}. Prior to feeding, algal cells were concentrated by centrifugation, the growth medium decanted and discarded, and the cells resuspended in FSW. Concentrations of stock suspensions of algal cells were estimated using an Accuri C6 flow cytometer, except during experiments with *A. parvimensis*, when the flow cytometer was not available and a hemocytometer was used to estimate algal concentrations. Water in cultures was changed by reverse filtration every other day, and food was replenished after each water change. Larval age is reported in days post-fertilization (dpf).

Identifying the maximum size of edible particles

We identified the largest particles that could be consumed by larvae of each species by offering them polystyrene divinylbenzene microspheres (beads) of a range of diameters over their planktonic period, and later examining them to determine the maximum size ingested. Beads were particle counter size standards ranging 15–120 µm in diameter (Table 1). Beads of each size were incubated separately in 2% bovine serum albumin (BSA: Jackson ImmunoResearch catalog #001-000-161) in deionized water for 1–3 h prior to use to reduce clumping (Pace and Bailiff 1987; Hansen et al. 1991; Pernet and Strathmann 2011). After

BSA incubation, beads were concentrated by centrifugation and resuspended in deionized water, then centrifuged again and resuspended in FSW to make separate stock suspensions of BSA-coated beads of each size. Concentrations of 15- and 30-μm beads were estimated using the flow cytometer (or, for experiments with *A. parvimensis*, a hemocytometer); those of larger beads were estimated by averaging counts from three subsamples of known volume in a Bogorov tray.

One culture of larvae of each of the six species was established as described above. Larvae of *S. purpuratus* were surveyed for maximum edible particle size at three ages; larvae of the other five species were surveyed at four ages (Table 2). Larvae were starved in FSW for 1–3 h before experimentation to increase the likelihood that they would feed. After starvation, ten larvae were placed in each of 24 (eight bead diameters × three replicates per bead diameter) 20-mL glass scintillation vials, each containing 20 mL FSW. The only exceptions were larvae of *A. armatus* of two ages: 8 dpf larvae of *A. armatus* were offered only bead sizes up to 60 μm in diameter, and 14 dpf larvae of *A. armatus* were offered only bead sizes up to 90 μm in diameter. Preliminary observations showed that larvae of *A. armatus* of these ages were incapable of ingesting beads as large as 60 or 90 μm, respectively.

The appropriate volume of a stock suspension of BSA-coated beads of a specific size was added to each vial to yield a final concentration of 100 beads mL^{-1}. Vials were immediately placed on a slowly rotating (2.5 rpm) plankton wheel at 16 °C, where larvae were allowed to feed. After 20 min, feeding was terminated by addition of ~1 mL of 100% formalin to each vial. As many larvae as possible were retrieved from each vial (range = 4–10 larvae $vial^{-1}$; mean = 9.78; n = 584 vials) and the number of beads present in the stomach and intestine counted. Beads of a given size were considered edible if at least 10% of the larvae examined contained one or more beads of that size in the stomach or intestine. This 10% criterion was chosen to prevent exceptional larvae from defining whether a particle size was edible or not for a specific species/age. All beads larger than the largest edible bead size were considered inedible. Inedible beads were sometimes present in the mouth or the esophagus, and when present we enumerated these.

Table 1 Nominal bead diameter, mean bead diameter (±SD) as estimated by the vendor, and vendor catalog number for all sizes of polystyrene divinylbenzene beads used to identify the maximum size of edible particles

Nominal diameter (μm)	Mean diameter ± SD (μm)	Catalog number
15	14.92 ± 1.36	18328
30	30.39 ± 1.36	64170
45	45.60 ± 6.61	07314
60	59.48 ± 1.08	64200
75	76.10 ± 1.24	24049
90	90.00 ± 2.15	07315
100	100.00 ± 1.50	4K100
120	117.51 ± 2.63	64225

All beads were obtained from Polysciences Inc., except for 100 μm beads, which were obtained from ThermoFisher Scientific

On the same day as each of these surveys, we obtained morphometric data for at least nine larvae of each species (range = 9–19 larvae per survey; mean = 10.43; n = 23 surveys). Larvae were haphazardly drawn from the culture that served as the source of experimental larvae, placed on microscope slides in FSW, and (with the exception of *A. armatus*) relaxed by addition of an equal volume of 7.5% $MgCl_2$. Larvae of *A. armatus* were not relaxed prior to imaging. Larvae were oriented ventral side up (auriculariae, bipinnariae, and brachiolariae) or dorsal side up (plutei) to better image larval digestive structures, then gently restrained by pressure from a coverslip supported by modeling clay at its four corners. They were observed using differential interference contrast optics on an Olympus BX51 microscope, and images were recorded with a QImaging QIClick camera. We used ImageJ (Abràmoff et al. 2004) to estimate midline body length and the smallest inner diameter of the esophagus for each larva.

Do large inedible particles affect echinoderm larval feeding performance?

Synthetic particles

We addressed this question by assessing the clearance rates of echinoderm larvae on edible beads (6 μm in diameter) alone or in the presence of various concentrations of inedible beads. Echinoderm larvae clear 6-μm beads at high rates (see "Results" section, below). Based on the results of the surveys described above, we used 100-μm beads as model inedible particles for holothuroid and asteroid larvae, and 75-μm beads for echinoid larvae.

For these experiments, unique cultures of larvae of five of the studied species were established as described above. For the sixth species, *S. purpuratus*, larvae from a single culture were used both to determine maximum edible particle size and in these experiments. Larvae of a given species were starved in FSW for 1–3 h before experimentation. After starvation, 11 larvae were placed in each of 32 (auriculariae, bipinnariae, brachiolariae: four treatments × eight replicates per treatment) or 40 (plutei: five treatments × eight replicates per treatment) 20-mL glass scintillation vials in 20 mL FSW. Within a species, all treatments contained the same initial concentration of edible 6-μm fluorescent beads (ex. 441 nm, em. 486 nm, Polysciences

catalog #17156): 400 beads mL^{-1} (auriculariae, bipinnariae, and brachiolariae) or 650 beads mL^{-1} (plutei). Treatments differed in the concentration of inedible beads. Larvae of all species were exposed to four concentrations of inedible beads: 0, 25, 50, and 100 beads mL^{-1}. Additionally, plutei were exposed to a fifth treatment, 500 beads mL^{-1}, to account for the smaller size of plutei (compared to auriculariae, bipinnariae, and brachiolariae) and their potentially lower encounter rates with large inedible beads. To determine an appropriate concentration for this extra treatment for plutei, we estimated the concentration of inedible beads required so that the encounter rate of plutei with inedible beads (ER_{plutei}), would be similar to the encounter rates of bipinnariae with inedible beads ($ER_{bipinnariae}$) at a concentration of 50 beads mL^{-1} (a concentration at which both *A. armatus* and *P. miniata* had significantly lower clearance rates on edible particles compared to treatments containing no inedible particles). We modeled encounter rate as

$$\mathrm{ER}\left(\text{beads s}^{-1}\right) = \mathrm{LCA}\left(\mu\mathrm{m}^2\right) \cdot \mathrm{LV}\left(\mu\mathrm{m\ s}^{-1}\right) \cdot \mathrm{PC}\left(\text{beads } \mu\mathrm{m}^{-3}\right),$$

where ER is encounter rate with inedible beads; LCA is larval cross sectional area; LV is larval swimming velocity; and PC is the concentration of inedible beads. We estimated $LCA_{bipinnariae}$ as that of a circle with diameter of the mean body length of 22 dpf *A. armatus* larvae, and LCA_{plutei} as that of a circle with diameter of the mean distance between the tips of the postoral arms of 11 dpf larvae of *D. excentricus* (measured in ImageJ as previously described). We assumed that auriculariae, bipinnariae, brachiolariae, and plutei had equivalent swimming velocities, and solved for the PC_{plutei} that would yield an ER_{plutei} equivalent to $ER_{bipinnariae}$ at 50 beads mL^{-1}. The additional treatment of 500 beads mL^{-1} may have elevated encounter rates of some plutei with inedible particles beyond that of bipinnariae at 50 beads mL^{-1}, because the adjustment was based on measurements from the species with the smallest pluteus (*D. excentricus*) and the species with the largest bipinnaria (*A. armatus*).

Stock suspensions of BSA-incubated edible (6-μm) and inedible (75 μm for pluteus larvae, and 100 μm for auricularia, bipinnaria, and brachiolaria larvae) beads were prepared as described above. Concentrations of stock suspensions of edible beads were counted using the flow cytometer (or hemocytometer, for experiments with *A. parvimensis*), and those of larger beads using a Bogorov tray.

Experiments commenced by addition of BSA-incubated 6-μm edible beads to yield a final concentration of 400 (auriculariae, bipinnariae, and brachiolariae) or 650 beads mL^{-1} (plutei), immediately followed by addition of the appropriate volume of BSA-incubated inedible beads to yield the treatment concentration. Vials were immediately placed on the plankton wheel at 16 °C. After 10 min, feeding was terminated by addition of ~1 mL of 100% formalin. At the concentrations of edible beads we used, 10 min is not sufficient time for larvae to become satiated (Lizárraga et al. unpubl. data), and is not sufficient time for ingested beads to be lost to defecation for most species (Strathmann 1971; Rassoulzadegan et al. 1984; Podolsky 1994).

Larvae were retrieved from each vial (range = 5–11 larvae $vial^{-1}$; mean = 9.97; n = 195 vials), and the number of 6-μm beads present in the stomach and intestine of each larva counted using fluorescence microscopy. We converted ingestion rate (number of beads ingested per unit time) to clearance rate (volume of water cleared of particles per unit time) by dividing ingestion rate by the initial concentration of edible beads. Vial means were obtained by averaging the clearance rates of all larvae retrieved in each vial. For all treatments, we included larvae that had not ingested any edible beads (clearance rates of zero) in the overall vial means in the event that the presence of large inedible particles had prevented some larvae from ingesting any particles during the experiment. Data were examined for normality and equality of variances using Shapiro–Wilk and Levene tests, respectively. We used one-way ANOVA to test for treatment differences in vial mean clearance rates, and post hoc Tukey's tests for pair-wise comparisons. All statistical tests were carried out using R v3.1.1 (R Core Team 2016).

Natural particles

To determine if natural large inedible particles affect echinoderm larval clearance rates, we carried out similar experiments using a naturally occurring phytoplankter instead of synthetic particles. We obtained a culture of the centric diatom *Coscinodiscus radiatus* (CCMP312) from the National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences. This diatom, which ranges in cell diameter from ~35 to 180 μm (Olenina et al. 2006), is common in the eastern Pacific. We poured diatom cells onto Nitex meshes and collected the retained cells to obtain diatoms larger than the previously determined inedible size for bipinnariae (using a 110-μm mesh) and plutei (80-μm mesh). To reduce contamination by smaller diatom cells, which might be edible, we repeated this sieving process three times. We verified the effects of our size fractionation by obtaining a concentrated subsample of sieved diatoms, imaging them in valve view on a compound microscope, and measuring the smallest diameter of each cell using ImageJ.

We conducted experiments using methods identical to our experiments listed above (with the exception of inedible particle type) with bipinnariae of the asteroid *A. armatus* (28 dpf) and plutei of the echinoid *D. excentricus* (11 dpf). Inspection of the data showed slight departures

from assumptions of normality and equality of variances. Despite this, we used one-way ANOVA to test for treatment effects, as ANOVA is robust to slight violations of these assumptions (Zar 1996). We used post hoc Tukey's tests for pairwise comparisons.

Results

Identifying the maximum size of edible particles

The maximum sizes of edible beads and the minimum sizes of inedible beads for larvae of all six species and ages tested are shown in Table 2. Larvae of all species and ages could capture and ingest the smallest beads offered (15-µm diameter). Within each species, the maximum size of edible beads increased with age. With the exception of the larvae of *A. armatus*, no larvae ingested beads >45-µm diameter, at any age. At 8 dpf, larvae of *A. armatus* could not ingest beads >15-µm diameter, but the maximum size of edible beads increased to 45 µm at 14 dpf, and to 90 µm at 22 dpf. Brachiolariae of *P. miniata* reached lengths almost as great as those of *A. armatus* bipinnariae, but never ingested beads >45-µm diameter.

With the exception of *D. excentricus*, larvae of all species were observed with inedible beads in the mouth at two or more ages. In some cases, the largest inedible beads observed in the mouth were the same size as the smallest inedible beads for that species/age (e.g., both were 30 µm for 11 dpf *L. pictus*). In other cases, larvae could capture and move beads much larger than the smallest inedible bead size to the mouth. For example, 28 dpf brachiolariae of *P. miniata* never had particles >60-µm diameter in their stomach or intestine, but sometimes held inedible beads up to 120-µm diameter in their mouths. Inedible beads were occasionally observed in the esophagus of 22 and 28 dpf *A. armatus* and 7 and 14 dpf *P. miniata*.

Table 2 Size (as midline body length) and feeding capabilities of larvae of the six species of echinoderms studied here

Species	Age (dpf)	Mean body length ± SEM (µm)	Largest edible bead (µm)	Smallest inedible bead (µm)	Largest inedible bead in mouth (µm)
Apostichopus parvimensis (auricularia)	5	525.7 ± 12.1	15	30	45
	8	731.6 ± 13.6	15	30	45
	12	945.7 ± 25.6	30	45	45
	16	1099.5 ± 19.8	30	45	60
Astropecten armatus (bipinnaria)	8[a]	549.5 ± 11.7	15	30	–
	14[b]	1190.0 ± 28.9	45	60	90
	22	1811.8 ± 63.4	90	100	120
	28	1966.4 ± 64.1	90	100	120
Patiria miniata (bipinnaria/brachiolaria)	7	554.6 ± 19.5	30	45	–
	14	839.3 ± 40.4	45	60	90
	21	1284.2 ± 70.1	45	60	100
	28	1570.3 ± 68.7	45	60	120
Dendraster excentricus (pluteus)	4	284.3 ± 2.1	30	45	–
	7	353.1 ± 1.8	30	45	–
	11	461.2 ± 4.6	45	60	–
	14	477.4 ± 6.0	45	60	–
Lytechinus pictus (pluteus)	5	309.1 ± 5.0	15	30	–
	11	461.8 ± 11.6	15	30	30
	16	640.0 ± 18.3	30	45	75
	21	683.7 ± 20.1	30	45	–
Strongylocentrotus purpuratus (pluteus)	7	304.4 ± 6.4	15	30	–
	14	495.0 ± 5.8	30	45	90
	20	623.7 ± 6.0	45	60	100

At each age listed (in days post-fertilization, dpf), larvae were exposed to polystyrene divinylbenzene beads ranging in diameter from 15–120 µm (see "Methods" section; Table 1). Only particles found in the stomach or intestine were considered edible; others were considered inedible. Dashes indicate no inedible particles were observed in the larval mouth

[a] Larvae of this age were only offered beads up to 60 µm in diameter

[b] Larvae of this age were only offered beads up to 90 µm in diameter

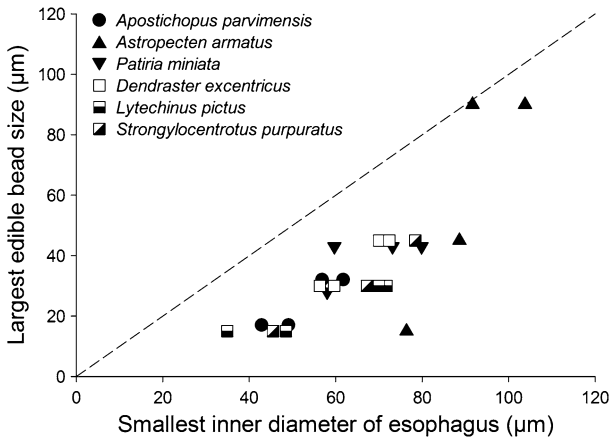

Fig. 1 Largest edible bead size as a function of mean smallest inner diameter of the esophagus for six species of echinoderm larvae tested at different ages. The dashed line represents the line of identity ($x = y$). Data for *Apostichopus parvimensis* and *Patiria miniata* are slightly offset for visibility

To better understand the relationship between larval morphology and the maximum size of edible particles, we plotted the largest edible particle diameter versus the mean smallest inner diameter of the esophagus for larvae of all species and ages (Fig. 1). All values fell to the right of the line of identity, indicating that the maximum edible particle size was smaller than the smallest inner diameter of the esophagus for all species/ages of larvae examined in this study.

Do large inedible particles affect echinoderm larval feeding performance?

Synthetic particles

Experiments were designed with eight replicates per treatment, but in some treatments 1–3 replicates were lost due to laboratory error; the final number of replicates in each treatment is shown in Fig. 2. Estimated clearance rates of larvae in control treatments (no large inedible beads present) were high for five of six species, ranging from ~160 to 310 µL h^{-1} (Fig. 2). Larvae of the holothuroid, *A. parvimensis*, were the exception, with a mean clearance rate of only 60.1 µL h^{-1} (±4.3 SEM, $n = 8$). With the exception of one bipinnaria of *A. armatus* whose stomach contained seven 100-µm beads, no inedible beads were observed in the stomachs of any larvae in these experiments.

Larvae of five of the six species examined showed significant, dose-dependent reductions in clearance rate on edible particles in the presence of large inedible particles (Fig. 2). Again, larvae of *A. parvimensis* were the sole exception to this pattern; for that species, the effect of treatment on clearance rate was not significant ($p = 0.054$, Fig. 2a), though the overall pattern was similar to that of the other species. Clearance rates of bipinnariae exposed to as few as 25 (*P. miniata*) or 50 (*A. armatus*) large inedible beads mL^{-1} were significantly lower than those of controls (Fig. 2b, c). At a concentration of 100 large inedible beads mL^{-1}, clearance rates of bipinnariae of *A. armatus* and brachiolariae of *P. miniata* were ~65 or 50% lower (respectively) than those of larvae in the control treatment. Plutei of all three echinoid species showed significant reductions in clearance rates on edible particles at concentrations of 100 or more inedible beads mL^{-1} (Fig. 2d–f). At 500 inedible beads mL^{-1}, pluteus clearance rates were ~50–70% lower than those of controls.

Natural particles

In the experiment with *A. armatus*, the mean diameter of diatoms after sieving was 109.06 µm (±1.27 SEM, $n = 100$). Only 5% of diatoms obtained from our sieving treatments were ≤90 µm in diameter (the largest edible size previously determined for *A. armatus* of this age: Table 2), and in our experiments, we observed only one bipinnaria with a diatom in its stomach. For the experiment with *D. excentricus*, the mean diameter of diatoms after sieving was 100.21 µm (±1.43 SEM, $n = 100$); all diatoms measured were larger than the previously determined largest edible size for this species (Table 2), and we observed none that ingested diatoms.

In these experiments, larvae of both species had high estimated clearance rates in the control treatments (Fig. 3a, b). Compared to controls, both bipinnariae and plutei showed reduced clearance rates in treatments containing large inedible diatoms. Larvae of both species had significantly lower clearance rates at all concentrations of diatoms tested, even the lowest concentration (25 cells mL^{-1}). Clearance rates at the highest concentrations of diatoms offered (100 cells mL^{-1} for bipinnariae and 500 cells mL^{-1} for plutei) were much lower than those at the same concentrations of synthetic particles (Fig. 2b, d).

Discussion

The planktonic feeding larvae of marine invertebrates must collect sufficient net energy (in the form of particulate food) to develop to metamorphic competence. The more rapidly they are able to acquire energy the less likely they are to die in the plankton (Rumrill 1990; Morgan 1995) and the more energy they acquire the more likely they are to produce a high-quality juvenile at metamorphosis (e.g., Phillips 2002; Thiyagarajan et al. 2005). One determinant of the rate of energy acquisition by larvae is the quality of the feeding environment. Most previous work describing larval feeding has focused on the

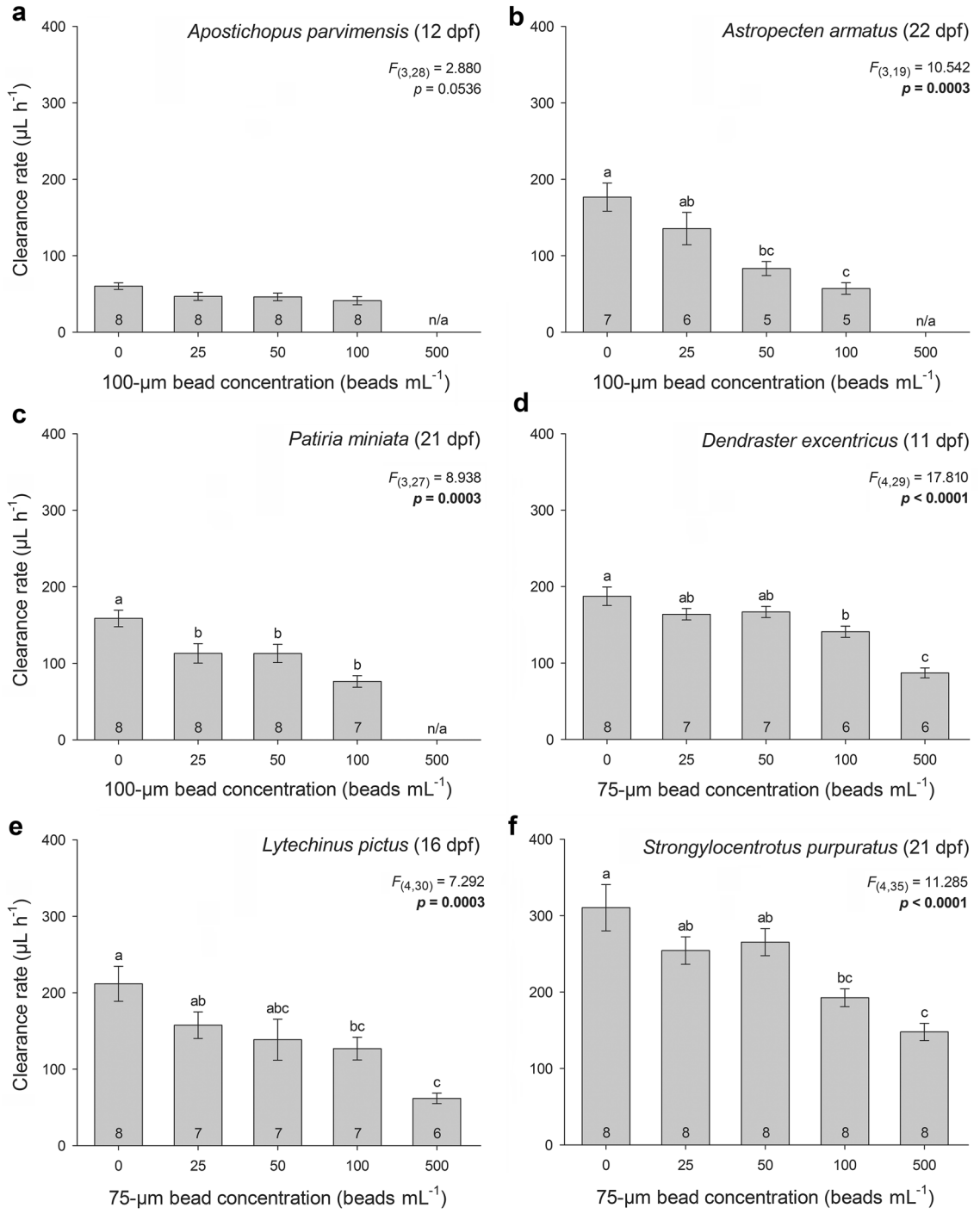

Fig. 2 Mean clearance rates (±SE) of larvae of **a** *Apostichopus parvimensis*, **b** *Astropecten armatus*, **c** *Patiria miniata*, **d** *Dendraster excentricus*, **e** *Lytechinus pictus*, and **f** *Strongylocentrotus purpuratus* exposed to various concentrations of large inedible beads. Different letters above bars indicate significant differences between pairs (post hoc Tukey's HSD, $p < 0.05$). The number of replicates for each treatment is given at the bottom of each bar

quantity of edible particles and its relationship to larval growth and development (reviewed by Olson and Olson 1989). Many but not all of these studies suggest that in nature, the abundance of food is so low as to limit larval growth. Here, we provide evidence that another aspect of the feeding environment—the abundance of large inedible particles—also affects the feeding performance of larvae.

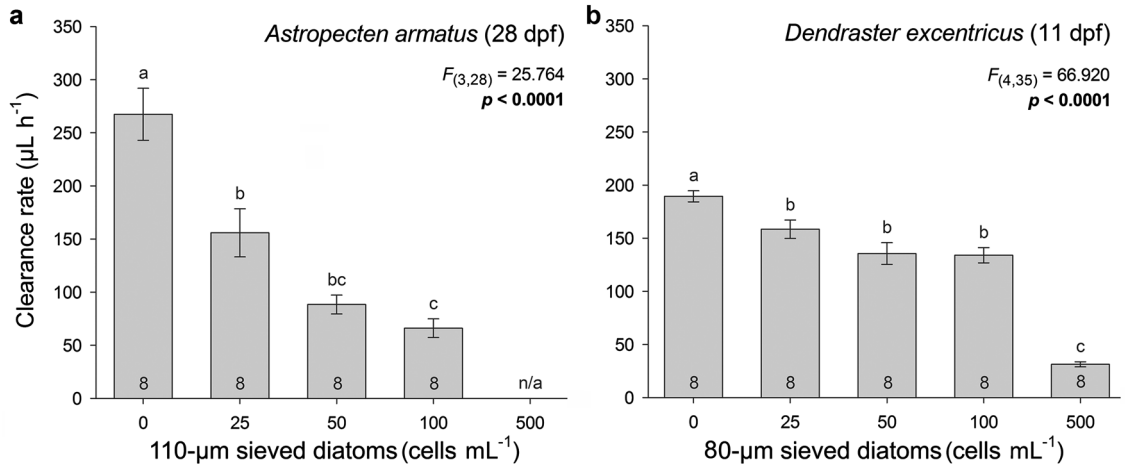

Fig. 3 Mean clearance rates (± SE) of larvae of **a** *Astropecten armatus* and **b** *Dendraster excentricus* exposed to various concentrations of *Coscinodiscus radiatus*. Diatoms have been passed through either an 110-µm (for *A. armatus*) or 80-µm (*D. excentricus*) Nitex mesh. Different letters above bars indicate significant differences between pairs (post hoc Tukey's HSD, $p < 0.05$). The number of replicates for each treatment is given at the bottom of each bar

The maximum size of edible particles

Larvae of the six species of echinoderms we studied almost never ingested particles larger in diameter than the mean smallest inner diameter of the esophagus (Fig. 1). This is consistent with the results of Strathmann (1971) on larvae of an asteroid, an echinoid, a holothuroid, and an ophiuroid. It is possible that particles whose minimum dimension is larger than the smallest inner diameter of the esophagus simply cannot fit into the esophagus. We occasionally observed particles that were slightly larger than the mean smallest inner diameter of the esophagus in the esophagus (e.g., older larvae of *A. armatus* had a mean smallest inner diameter of the esophagus of 90 µm, but a few individuals were found with 100-µm beads in the esophagus). Each of these individuals may simply have had an esophagus that was slightly larger than average. Note that, our study included only spherical and disk-shaped particles. Strathmann (1971) found that echinoderm larvae did not ingest rod-shaped cells or chains of cells that were larger than 30 µm in diameter and ~200 µm long, suggesting that there are also upper size limits to edibility for elongate particles.

Particle size may influence edibility for a variety of reasons: for example, large particles may be difficult for echinoderm larvae to capture, transport to the mouth, move into the esophagus, or pass into the stomach. We often observed large inedible particles in the mouths of larvae of five of the six species studied here (Table 2). This suggests that capture and transport to the mouth do not limit edibility, at least within the size ranges studied here. It seems more likely that larvae cannot move very large particles into the esophagus, or that large particles in the esophagus cannot pass through the cardiac sphincter separating the esophagus and stomach (Strathmann 1971; Burke 1981). The smallest inner diameter of the larval esophagus appears to be a good predictor of the maximum size of edible particles for diverse echinoderm larvae.

A diversity of particles larger than the maximum size of edible particles for any given species of echinoderm larva occur in coastal marine waters. How might these large inedible particles affect feeding rates of marine invertebrate larvae on edible, nutritious particles?

Low concentrations of large inedible particles reduce larval clearance rate

Our results clearly show that the feeding rates of larvae of five species of echinoderms were strongly reduced in the presence of large inedible particles—synthetic or natural—at relatively low concentrations. For those species, clearance rates in control treatments were similar to maximum rates previously recorded for other echinoderm larvae (Strathmann 1971; Hart 1996). Addition of large inedible particles yielded a roughly dose-dependent response in clearance rate: the higher the concentration of large inedible particles, the lower the larval clearance rate (Fig. 2). Larvae of *A. parvimensis* did not demonstrate this response, but clearance rates in the control treatment in this experiment were unexpectedly low (Fig. 2a), suggesting that the larvae were not healthy. Repeating the experiment with another batch of larvae might yield results consistent with those of the other studied species. Our results suggest that reduction in clearance rate in the presence of large inedible

particles may be widespread among the obligately feeding larvae of echinoderms.

Two previous studies have also indicated that large particles may interfere with feeding by larvae. Strathmann (1971) found that a large centric diatom present at 1000 cells mL^{-1} reduced clearance rates of plutei of an echinoid and an ophiuroid on smaller, edible dinoflagellates. Interpretation of this result is difficult since many of the diatoms were also edible, with larvae of both echinoderms clearing them at relatively high rates. The reduction in clearance of dinoflagellates may have been due to a preference for the diatom. Hansen et al. (1991) carried out a study similar to ours on veligers of the gastropod *P. aperta*. These had reduced clearance rates at concentrations of large inedible particles (45-μm latex beads) as low as 2500 beads mL^{-1}, with ~60% reductions in clearance rate seen at concentrations of 20,000 beads mL^{-1}. Though both of these studies demonstrate effects of large particles on larval feeding rates, the concentrations of large particles used by these investigators were high (≥1000 particles mL^{-1}). In nature, larvae probably only rarely encounter large inedible particles at these concentrations. Our results show that the clearance rates of echinoderm larvae can be dramatically reduced by large inedible particles at concentrations that are presumably much more frequently realized in nature.

What mechanism(s) underlie the observed reductions in larval clearance rate in the presence of large inedible particles? Strathmann (1971) suggested that echinoderm larvae might capture large particles and move them to the mouth, but then reject them. Frequent rejection behavior might reduce the amount of time available for feeding on edible particles. Hansen et al. (1991) observed veligers swimming in suspensions of edible particles, with or without large inedible particles. In the presence of large inedible particles, the veligers swam more slowly and with a different helical pattern than they did in the absence of such particles. Further, after a veliger encountered two or three large particles, it would typically retract its velum and sink before resuming swimming and feeding. In these cases, larval reactions to large particles reduced the efficiency of feeding or the amount of time larvae allocated to feeding.

Our preliminary observations of asteroid and echinoid larvae suggest another mechanism by which large inedible particles may interfere with feeding. When free-swimming larvae of *A. armatus* (14 dpf) and *S. purpuratus* (21 dpf) were incubated with 60- or 75-μm beads (respectively), >80% of the larvae rapidly captured and moved one or more inedible beads to their mouths, where the beads were held for at least 10 min (and up to 1 h) before rejection (Lizárraga et al. unpubl. data). While large particles were held in the mouth, larvae seemed to capture edible particles at normal rates. However, large particles held in the mouth may interfere with the handling or swallowing of captured edible particles, reducing the efficiency of larval feeding.

If large inedible particles must fit in the mouth to have an effect on clearance rates on edible particles by echinoderm larvae, as suggested by Strathmann (1971) and our preliminary observations above, then particles too large to fit in the larval mouth may not affect larval clearance rates. Thus, the range of sizes of inedible particles that affect larval feeding performance may be quite small (Strathmann 1971). Alternatively, particles too large to fit in the larval mouth may affect larval feeding by different mechanisms. Additional studies are required to clarify the mechanism(s) by which diverse large inedible particles affect feeding by echinoderm larvae.

Our results suggest that different types of larvae may vary in their sensitivity to large inedible particles. Clearance rates of larvae of the two species of asteroids we studied fell by ~50% at concentrations of large inedible beads of 50–100 beads mL^{-1}; for echinoid plutei, 50% declines in clearance rate were only reached at concentrations of 500 beads mL^{-1}. This apparent difference in sensitivity might be due to different encounter rates of larvae with large particles. Asteroid larvae were generally much larger than echinoid larvae (Table 2); if rates of water movement past the larval ciliary band were similar among all larvae, then at a given concentration of large inedible particles, asteroid larvae would likely encounter large particles more frequently than echinoid larvae. We attempted to correct for encounter rate by including a 500 beads mL^{-1} treatment for plutei; at this higher concentration, plutei should have encountered large inedible particles at the same rate as bipinnariae in the 50 beads mL^{-1} treatment. Our results are consistent with the apparent difference in sensitivity being due to differential encounter rate. An alternative possibility is that echinoid larvae may be more efficient at rejecting large inedible particles than asteroid larvae. As described by Strathmann (1971), echinoderm larvae have numerous means of rejecting particles. Some of these mechanisms are specific to members of each echinoderm class, and may result in among-class variation in the efficiency of particle rejection.

Though our study was not intended to compare the effects of different types of particles, cells of *C. radiatus* seemed to have stronger negative effects on clearance rate in the two species tested than did similarly sized beads (compare Figs. 2, 3). Additional studies comparing the effects of different types of natural particles on larval clearance rates are needed.

Implications for larvae in nature

A diversity of large particles are routinely found in marine plankton. These include phytoplankters, invertebrate eggs

and embryos, marine snow, sediment, and even anthropogenic microplastics (Cole et al. 2011). Concentrations of each type of large particle are probably typically low. For example, Reid et al. (1970), studying the phytoplankton off La Jolla in the summer of 1967, found concentrations of large phytoplankton species were generally ~0.1 cells mL^{-1} (their Table V-1). When the concentrations of the many uncommon species of large phytoplankters are summed, however, the total may be ~10–100 cells mL^{-1}. In bloom conditions, the concentrations of large cells can be much higher. Reid et al. (1970) reported that the dinoflagellate *Lingulodinium polyedrum* (as *Gonyaulax polyedra*), which reaches sizes of ~50 μm (Olenina et al. 2006) and is likely inedible to most species/ages of echinoderm larvae we studied (Table 2), reached concentrations of 40 cells mL^{-1} in weak red-tide conditions; in more intense blooms common in spring and summer in southern California, that species reaches 13,000 cells mL^{-1} (Moorthi et al. 2006). In nature, then, larvae may often be faced with large particles in sufficient concentrations to reduce clearance rate. How might this affect their growth and development?

Our experiments showed that large particles in concentrations as low as 25 particles mL^{-1} could dramatically reduce larval clearance rate. Clearance rates were lower by >50% when 50–500 particles mL^{-1} were present (Figs. 2, 3). If larval growth and development are limited by the concentration of edible particles (reviewed by Olson and Olson 1989), then their rates are determined in part by clearance rates. If clearance rates are reduced by 50%, then the length of the larval precompetent period [the minimum planktonic larval duration (PLD)] may be extended. We know of no direct tests of the effects of large inedible particles on PLD. However, a reasonable analog might be found in studies that examine PLD in echinoderms as a function of food availability. For example, McAlister and Moran (2013) found that a 66% reduction in food availability (from 300 to 100 cells mL^{-1}) extended development time by ~15–30% in the echinoids *Echinometra lucunter* and *E. viridis*. An 80% reduction in food level extended development time of the echinoid *D. excentricus* from 27 (for larvae reared in field-collected seawater) to 47 days (for larvae reared in diluted natural seawater), an extension of ~74% (Paulay et al. 1985). If large inedible particle-mediated reductions in clearance rate mimic the effects of reduced food rations, then the presence of large inedible particles may extend PLD, possibly leading to increased mortality (from predation or advection away from suitable habitat: Rumrill 1990; Morgan 1995) and increased dispersal of surviving larvae (Shanks 2009).

Even if the length of time larvae spend in the plankton is not affected by large inedible particle-mediated reductions in feeding rates, it is possible that reduced feeding rates may lead to lower quality juveniles at metamorphosis. A 75% reduction in larval food availability (from 2000 to 500 cells mL^{-1}) was associated with smaller size at metamorphosis and slower juvenile growth rates in the mussel *Mytilus galloprovincialis* (Phillips 2002). A 50% reduction in larval food availability (from 100,000 to 50,000 cells mL^{-1}) was associated with reduced cyprid energy reserves and reduced juvenile survival and growth in the field in the barnacle *Balanus amphitrite* (Thiyagarajan et al. 2005). If large inedible particle-mediated reductions in clearance rate mimic the effects of reduced food rations, then the presence of large inedible particles in the larval stage may reduce juvenile quality and performance in nature.

These arguments are predicated in part on the common (but not universal) finding that the quantity of suitable food particles in nature limits larval growth and development rates (e.g., Paulay et al. 1985; Fenaux et al. 1994; Fotel et al. 1999; Reitzel et al. 2004; Pedersen et al. 2010; but see Olson 1987; Hansen 1999). Spatial and temporal variation in the abundance of food may be sufficient to explain the varying results of experiments on food limitation, but the (usually unmeasured) abundance of large inedible particles in experiments may also be an important factor. If large inedible particles have a strong negative effect on clearance rate, then some cases of apparent food limitation may be caused not by the actual rarity of food particles, but by the presence of large inedible particles reducing larval feeding performance. Our results suggest that when designing food limitation experiments, it is not only necessary to manipulate the concentration of potential food particles, but also to control (or at least measure) the concentrations of large inedible particles.

A further complication of food limitation experiments is that in laboratory conditions, the naturally patchy distribution of phytoplankton is disrupted, leading to treatments that may represent only averaged (low) food concentrations. In nature, larvae may increase rates of energy acquisition by spending as much time as possible in or near food patches, such as thin phytoplankton layers, where concentrations of phytoplankton may be an order of magnitude or more above background levels (Durham and Stocker 2012). Our results, however, suggest that not all "food patches" may be trophic hotspots for larvae. High concentrations of phytoplankton are typically identified indirectly, by chlorophyll fluorescence or absorbance (e.g., Dekshenieks et al. 2001; Durham and Stocker 2012). It is often not clear if the phytoplankton cells present in dense patches can even be consumed by larvae. For example, Ryan et al. (2010) inferred that thin layers of phytoplankton in Monterey Bay were dominated by the dinoflagellate *Akashiwo sanguinea*; by virtue of their size (~50 μm), cells of this species are likely inedible for most of the species/ages of larvae we studied (Table 2). Further, even if "food patches" do include high concentrations of edible particles, they may

also include high concentrations of potentially interfering large inedible particles. Description of the types and sizes of particles present in phytoplankton patches is thus critical to determine their potential value to feeding larvae of marine invertebrates.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights Animals used in the study were collected under the authority of a scientific collecting permit issued to Bruno Pernet, and treated according to all applicable national and institutional guidelines.

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