Title. Temporal variation in food limitation in larvae of the sand dollar *Dendraster excentricus*

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Running page head. Food limitation in echinoid larvae

Abstract. Rates of development of the feeding larvae of marine invertebrates may often be limited by inadequate food, extending the length of the larval period and increasing overall larval mortality. A better understanding of the frequency and importance of this phenomenon requires knowledge of the food concentration below which larvae are limited, and above which they are not, as well as estimates of how strongly food supply affects length of the planktonic period. We addressed these issues using larvae of the sand dollar *Dendraster excentricus* as a model and chlorophyll *a* (chl *a*) concentration as a metric of food abundance. We reared larvae in natural seawater collected from coastal southern California, as well as in reduced and supplemented food treatments created from this natural seawater, six times from 2017-2019 to take advantage of temporal variation in chl *a* concentration. Larvae showed morphological responses indicative of low food in nature in only one of six experiments and showed delayed time to 50% metamorphic competence in two of six experiments. Larvae appeared to be food limited below chl *a* concentrations of ~2.4-3.0 $\mu g \bullet L^{-1}$, but developed at maximal rates at higher food concentrations. Low natural food supplies delayed time to 50% competence by up to 1.25 days. An 11-year record of chl *a* concentration in waters of coastal southern California suggests that larvae of *D. excentricus* are likely food limited in developmental rate throughout much of the year except for late winter to late spring.

Key words: *Dendraster excentricus*, echinoid, food limitation, larval ecology, planktonic duration

1. INTRODUCTION

The planktonic feeding larvae of marine invertebrates usually develop from small eggs that do not contain sufficient materials or energy to produce a juvenile (Strathmann 1993, McEdward & Miner 2006). Such larvae must spend time in the plankton capturing, digesting, and assimilating food in order to make up this deficit and develop to metamorphic competence. Many studies suggest that planktonic larvae are subject to high instantaneous rates of mortality (Rumrill 1990, Morgan 1995, White et al. 2014), so any extensions of the length of the planktonic period may substantially increase overall mortality, with potential effects on recruitment and the dynamics of adult populations (Olson & Olson 1989, Morgan 1995), the evolution of life-history traits (Olive 1992), and the evolution of larval form and function (McAlister & Miner 2018, Pernet 2018). Understanding the factors that control time to competence have thus been of interest since larval ecology emerged as a discipline in the middle of the 20th century (Thorson 1950, Young 1990).

One environmental factor known to affect time to competence in feeding larvae is food availability. Many laboratory studies demonstrate that larvae grow or develop more slowly when food (usually cells of one or a few species of cultured phytoplankton) is sparse relative to when it is abundant (Strathmann 1987, Boidron-Métairon 1995). These results do not help, however, in addressing a critical question: how frequently and intensely are rates of larval growth or development limited by the abundance of food in nature? Attempts to answer this question typically use one of two approaches (Fenaux et al. 1994). Supplementation experiments are most common; here, rates of larval growth or development are compared between larvae reared on a natural diet and those reared on the natural diet supplemented with additional food. More rapid growth or development in the supplemented treatment indicates that the natural diet is limiting.

A second, less frequently used approach is to survey the characteristics of larvae captured directly from the plankton. Larvae of some taxa (notably echinoid echinoderms: McAlister & Miner 2018) display phenotypic plasticity in response to food level, and thus descriptions of the form of field-collected larvae may be used to make inferences about food levels those larvae have experienced in nature. Among taxa with ciliated larvae, such studies have been carried out on the larvae of annelids, molluscs, and especially echinoderms (Table 1). Together they suggest that ciliated larvae are frequently food limited in rates of growth or development.

Examination of these results, however, suggests several additional important questions. One of these is obvious: what food conditions permit maximal rates of larval growth and development? An answer to this question is clearly needed if we want to quantify the frequency of food limitation in nature. Answering it requires characterizing food abundance. An ideal description of food abundance would involve data on the concentrations of all particle types available, as well as their nutritional quality and availability to larvae (e.g., some phytoplankton are too large to be ingested, and others have escape responses and may be more difficult to capture for larvae: von Dassow et al. 2013, Lizárraga et al. 2017), all at the small spatial scales relevant to larvae (Pernet 2018). It might also include measurement of dissolved organic matter, uptake of which may contribute to larval energy budgets, though it does not appear to be sufficient to fuel growth in feeding larvae (e.g., Moran & Manahan 2004). Such measurements are challenging, and instead workers interested in food limitation or other aspects of larval performance have typically used simpler bulk measurements of food abundance – e.g., the concentration of chl *a*, particulate organic carbon, or particles (all considering only the size fraction of particles actually available to larvae as food). Though all of these bulk metrics are imperfect, as they ignore particle nutrient quality and availability, with few exceptions (e.g,

Olson et al. 1987, discussed below) they appear to be informative in studies of larval performance in relation to food abundance. There is no empirical evidence that any one of these simple metrics is superior to the others, and they may often covary. For example, simple multiplicative conversion factors are routinely used to convert between particulate organic carbon and chl *a* (e.g., Strickland 1960, Olson et al. 1987, Fotel et al. 1999), and Fenaux et al. (1994) found that particle and chl *a* concentrations in the edible size fraction were highly correlated. The concentration of chl *a* in the edible size fraction of particles is the only metric of food conditions reported in most studies of food limitation, and thus the only one for which comparative data exist. In addition, scientists have been measuring chl *a* levels over a range of spatial scales in diverse marine habitats for many decades, providing useful context for interpreting the results of food limitation experiments and surveys.

For echinoderms, the group with the most comparative data on food limitation, the chl *a* level sufficient to fuel maximal rates of growth and development is not well known. Food limitation has been detected in echinoderm larvae at chl *a* levels ranging from 0.1 to 2.3 $\mu g \cdot L^{-1}$ (Table 1). Only two published studies did not detect evidence for food limitation. In one of these, on the tropical seastar *Acanthaster planci*, the chl *a* level in the treatment meant to mimic natural conditions was extremely low (0.25 $\mu g \cdot L^{-1}$), so the result is surprising (Olson 1987). Subsequent work (Okaji 1996, Fabricius et al. 2010) suggested that chl *a* levels might have been elevated in Olson's (1987) experimental chambers, and that larvae of *A. planci* are indeed frequently food limited in nature. In the other, on the temperate sea urchin *Strongylocentrotus purpuratus*, chl *a* levels were not reported but may have been high, as the study was carried out during a period of upwelling (Miller & Emlet 1999). It is difficult to identify a chl *a* boundary between food-limited and food-unlimited growth for echinoderm larvae from these data, as chl *a* is not reported in the

only plausible documented case of food-unlimited growth, but at least for some species it appears to be >~2 μg•L^{-1} (Table 1). The boundary level may, of course, vary among species and habitats.

Another important question concerns the relationship of food limitation to the primary parameter of interest, time to competence. How strongly do food conditions experienced by larvae in nature affect time to competence? Few data are available to address that question, for several reasons. First, some studies of food limitation measure rates of larval growth or development, but do not attempt to quantify time to competence (e.g., Reitzel et al. [2004] on *Dendraster excentricus* and Paulay et al. [1985] on *Ophiopholis aculeata*). Second, in studies that do estimate time to competence, what is often reported is time to first settlement, a value that may not be representative of the average time to competence in a treatment (e.g., Paulay et al. 1985 on *D. excentricus*). A better way to assess this would be to attempt to induce metamorphosis using standardized cues over time, using data on the responses of all larvae in an experimental unit to make estimates of typical time to competence (e.g., the time it takes for a specific percentage of larvae in a given experimental unit to reach metamorphic competence).

In this study of food limitation in the sand dollar *D. excentricus*, we address both of these questions. We attempted to constrain estimates of the chl *a* boundary between food-limited and food-unlimited growth in nearshore coastal waters of southern California, which frequently have chl *a* levels >2 μg•L^{-1}. We reared larvae in the laboratory in natural seawater collected from the field, as well as in reduced and supplemented food treatments created from this natural seawater. To supplement this investigator-generated variation in food level, we repeated this experiment six times over the course of two years to take advantage of temporal variation in chl *a*. Within experiments, we assessed whether larvae were food limited by looking both for evidence of

food-level induced phenotypic plasticity, and by generating estimates of the median time to metamorphic competence.

2. MATERIALS & METHODS

2.1. Establishing and characterizing treatments

Treatment solutions were prepared using seawater collected each morning from a floating dock near the mouth of Alamitos Bay, in Long Beach, California, USA (33.7458°, -118.1151°). Seawater was collected from just below the surface in two 9-L carboys. Carboys were transported to the laboratory within the next 20 minutes. On arrival in the laboratory, water temperature (to the nearest 0.1 °C) and salinity (0.1) were measured immediately using an Ertco 1003-3S thermometer (Germany) and a portable refractometer (Area Inc., Homestead, Florida, USA).

Seawater was held in a 16 °C environmental chamber for no more than a few hours until processing. All collected seawater was first filtered through a 35-µm mesh into a single 20-L carboy; this removed large inedible particles (Lizárraga et al. 2017), as well as most potential predators and competitors of *Dendraster excentricus* larvae. This suspension was mixed, then divided into three 9-L carboys. One of them was designated the natural seawater (NS) treatment. The supplemented treatment (NS+) was created by adding cells of *Rhodomonas lens* (CCMP739) to another of the carboys to yield a final concentration of 1000 cells•mL^{-1} (except for the last of the six experiments, that of Aug 2019, when *R. lens* was added to yield a final concentration of 5000 cells•mL^{-1}). The supplementation level of 1000 cells• mL^{-1} was chosen with the aim of increasing chl *a* concentration in the treatment by ~1 µg•L^{-1} in the NS+ treatment relative to NS treatment; based on preliminary measurements of natural chl *a* levels at our site, we predicted

that this would increase food levels in NS+ by ~30-50% over NS treatments, an increment that we thought likely to yield measurable differences in larval form or development rate if they were food-limited in nature. We increased the supplementation level to 5000 cells• mL^{-1} in Aug 2019 in an effort to determine if the greater increment might lead to a larger, more easily detected signal in larval form or development. *Rhodomonas* spp. have been shown to be excellent food sources for echinoid larvae (e.g., Hinegardner 1969, Strathmann 2014, Hodin et al. 2019), and we routinely rear larvae of *D. excentricus* to metamorphic competence on a unialgal diet of this strain of *R. lens* (unpubl. data). The *R. lens* was cultured in f/2 medium and used for experiments while in exponential growth phase. Before use, algal cells were pelleted by centrifugation, the growth medium decanted, and the cells resuspended in seawater filtered through a 0.2-µm (pore size) filter (filtered seawater, FSW) (Hodin et al. 2019). The concentration of algal cells in this stock suspension was counted using an Accuri C6 flow cytometer (BD Biosciences, San Jose, California, USA). The reduced food treatment (NS-) was created by diluting the 35-µm filtered seawater suspension 1:1 with FSW, reducing chl *a* in that treatment to ~50% that of the NS treatment. Based on preliminary measurements of natural chl *a* levels at our site, and the results of prior experiments on food limitation in echinoderm larvae (Table 1), we thought it likely that the NS- treatment would be food limiting for larvae of *D. excentricus*. After making the treatments, three replicate measurements of chl *a* fluorescence in each treatment were made with an Aquafluor Handheld Fluorometer (Turner Designs, San Jose, California, USA). Absolute chl *a* concentrations of 1-L samples of treatment solutions were also estimated frequently throughout the study (at least six times for each of the six experiments) using the spectrophotometric method of Strickland & Parsons (1968, pp. 193-194). An ordinary least squares regression between relative fluorescence units (mean of the three replicates; RFU) and absolute chl *a* concentration

was generated from these data (chl *a* = 0.232*RFU, R^2 = 0.87, N = 40), and later used to convert daily RFU measurements to absolute chl *a* concentrations.

2.2 Obtaining larvae and establishing treatments

Adults of *D. excentricus* were collected from the intertidal and shallow subtidal zones near San Pedro, California (33.7078°, -118.2766°) and maintained in recirculating seawater tanks at 16 °C in the laboratory for up to one month before use. We obtained gametes from adults by injecting ~1 mL of 0.53M KCl into the perivisceral coelom (Strathmann 1987). Eggs were rinsed once with FSW, resuspended in ~50 ml of FSW, then fertilized using diluted sperm. Fertilized eggs were then transferred to 1 L of FSW, and held unstirred in an environmental chamber at 16 °C. By 24 h after fertilization (1 day post-fertilization, dpf), blastulae had hatched and congregated at the water surface. Swimming blastulae were then decanted into a clean beaker. We estimated the concentration of blastulae in this stock suspension in five 500-µl subsamples (mixing prior to taking each subsample) and used the mean of these estimates to calculate the volume of stock suspension needed to deliver 250 larvae to each replicate beaker (0.25 larvae•mL^{-1}). We added this volume to each of the 12 experimental 1-L beakers (three treatments, each replicated four times), each filled with the appropriate treatment solution (in the Jul 2017 experiment, there were only three replicates per treatment). Beakers were then placed in a fully randomized arrangement in an environmental chamber maintained at 16 °C and stirred four times per minute using a paddle stirrer (Strathmann 2014).

Beginning in the Apr 2018 experiment, we used the methods above to add an estimated 250 larvae to each of two or three additional 1-L beakers of FSW to which 10,000 cells•mL^{-1} of *R. lens* (a growth saturating food level: unpub. data) had been added. These “count control” beakers

were maintained on a paddle stirrer at 16 °C for 3-4 days with no water changes, during which time larvae grew substantially and became much easier to count. The larvae in each of these beakers were then concentrated onto a 65-µm mesh, resuspended in ~50 mL FSW, killed with formalin, then counted in a Bogarov tray. Our expectation was that most mortality in our treatment beakers was associated with the stress of water changes; in the absence of water changes, as in the count control beakers, we expected very little mortality. This method allowed us to characterize how closely we approached our target initial concentrations of larvae in each experiment, and to generate more accurate estimates of mortality in each treatment beaker.

2.3. Daily maintenance

Treatments were established at 1 dpf (~24 h after fertilization). Because at this stage of development larvae had short feeding ciliary bands and thus could only capture food particles from suspension at low rates (Strathmann 1971; Hart 1991), we did not change water and re-establish treatment conditions until 3 dpf, but after 3 dpf we carried out water changes daily until termination of the experiment. On each of these days, we first estimated the amount of chl *a* remaining in each beaker using the fluorometer. We then did a complete water change in each beaker by "forward filtration" (Hodin et al. 2019). Larvae in each beaker were concentrated on to a 120-µm mesh and their original beaker cleaned using hot freshwater. Larvae were then rinsed back into their original beaker using the appropriate treatment suspension and the beaker was replaced on the paddle stirrer at 16 °C until the next day's water change. Because treatments were based on natural seawater passed through a 35-µm mesh, but daily water changes involved discarding all beaker contents that passed through a much larger mesh (120 µm), any competitors

(primarily rotifers) that were seeded into beakers on a given day were removed the next day, minimizing their effects on larvae.

The only exceptions to this pattern of daily maintenance came in the Feb 2019 experiment, during which rain events lowered the salinity of raw seawater to 25 at 9 dpf, and 28 at 13 dpf. On both of these days, we did not carry out water changes to avoid exposing larvae to low salinity water. Instead, on those two days we simply supplemented the contents of all treatment beakers with sufficient stock suspension of *R. lens* to provide an additional 1000 cells•mL^{-1}. This concentration of *R. lens* was chosen with the intent of bringing initial chl *a* concentrations on those two days back within the range of initial chl *a* concentrations in the NS treatment in prior days of that experiment. At 10 and 14 dpf, salinity had returned to normal or nearly so (33 and 30, respectively), and we resumed normal water changes.

2.4. Larval form

Prior work suggests that larvae of *D. excentricus* can display food-level induced phenotypic plasticity at ages of at least 3-12 dpf (Hart & Strathmann 1994; Miner 2007; Rendleman et al. 2018), with well-fed larvae having shorter postoral arms relative to midline body length than starved or poorly fed larvae. At 7 dpf, during normal maintenance and after thoroughly stirring to resuspend larvae, we removed ~8-10 larvae from each experimental beaker by pipet and placed them in a drop of seawater on a glass slide. Larvae were briefly relaxed in a 1:1 solution of seawater and 7.5% $MgCl_2$, then killed by addition of dilute formalin. An eyelash probe was used to orient each larva ventral side up, and a coverslip supported by Plasticene modelling material was placed over them. Larvae were observed using an Olympus BX-51 compound microscope (Olympus Scientific Solutions Americas Corporation, Waltham, Massachusetts, USA). The first

five correctly oriented larvae encountered on the slide were imaged using a QIClick monochrome camera (Teledyne Photometrics, Tucson, Arizona, USA). A stage micrometer was also imaged. We later opened these images in ImageJ 2.0.0 (Rueden et al. 2017) to estimate the length of the right postoral arm (from the ventral transverse rod to its tip) and midline body length (Fig. S1). Only the right postoral arm was measured to avoid between-arm variation due to directional asymmetry (Hodin et al. 2016).

2.5. Time to metamorphic competence

Beginning at 10 dpf, during daily water changes (and after thoroughly stirring to resuspend larvae) we removed ~10-15 larvae from each experimental beaker by pipet and placed them in 2 mL of FSW in a randomly assigned well of one of two six-well tissue culture plates. After all experimental beakers had been processed, we added 2 mL of a solution of FSW with 80 mM excess KCl to each well, yielding a final concentration of 40 mM excess KCl (the amount recommended for inducing metamorphosis in *D. excentricus*: Hodin et al. 2019). The plates were incubated at 16 °C for 1 hour, and then individuals in each well were scored for metamorphosis. Individuals were scored as metamorphosed when the tissue on each larval arm had pulled back from the tips of the arms and coalesced in a ball at the posterior end of the larva. Larvae that had not metamorphosed had not changed in form at all. The number of metamorphs and unmetamorphosed larvae were recorded. This process was repeated daily for each of the experimental beakers. When the samples of larvae from all beakers in a given treatment reached ≥50% metamorphosis for two days in a row, the remaining larvae in each of the beakers of that treatment were concentrated on to a mesh, rinsed into a 50-mL centrifuge tube, preserved with dilute formalin, and later counted in a Bogorov tray.

2.6. Larval mortality

For four of the six experiments we had independent estimates of the initial number of larvae in each beaker. We had also recorded the number of larvae removed from each beaker for imaging of larval form and estimating time to metamorphic competence, and the number of larvae remaining in each beaker when the treatment was terminated. These values allowed us to estimate instantaneous mortality rates in each beaker. Mortality rates were estimated by solving for m in the equation $N_t = N_0 e^{-mt}$, where N_t is the total number of larvae accounted for in a beaker, N_0 is the initial number of larvae in a beaker, m is instantaneous mortality (d^{-1}), and t is the length of the experiment (d).

2.7. Analysis and data availability

Except where otherwise noted, statistical analyses were carried out using Prism 8 (GraphPad Software, San Diego, California, USA). Morphological responses to food concentration in echinoid larvae are often estimated by comparison of the ratio of postoral arm length to body length (McAlister & Miner 2018). We compared the average within-beaker ratio of right postoral arm length to body length among treatments for each experiment using one-way ANOVA. All larval form data met assumptions of normality and homoscedasticity, as assessed with Shapiro-Wilk and Bartlett's tests, respectively. Where ANOVA indicated significant differences among treatments, we used post-hoc Dunnett's tests to make the two comparisons of interest, between the NS+ and NS treatments, and the NS- and NS treatments. Median time to metamorphic competence (when 50% of an experimental group exhibits metamorphic competence: TC_{50}) for each beaker in each experiment was estimated by logistic regression using R 3.6.2 (R Core Team

2019). TC_{50} was compared among treatments for each experiment using one-way ANOVA, with post hoc tests as above. Assumptions of normality and homoscedasticity were met for all TC_{50} data except for Feb 2019, where a Shapiro-Wilk test indicated that data were not normally distributed. We did not transform data for that month, however, since visual examination of the quantile-quantile plot suggested that deviations from normality were minor, and because one-way ANOVA is typically robust to even considerable deviations from normality (Zar 1996).

The data collected and analyzed during this study and the R code used to estimate TC_{50} are available from the National Science Foundation Biological and Chemical Oceanography Data Management Office (https://www.bco-dmo.org/project/727167).

3. RESULTS

3.1. Treatment conditions

Immediately after collection, the mean temperature of raw seawater in the six experiments ranged from 14.5-21.4 °C, with an overall grand mean among experiments of 17.8 °C (Table 2). The mean salinity of raw seawater was fairly consistent, ranging from 33.2 to 35.0 (overall grand mean = 34.0). These averages, however, mask substantial variation in one experiment, that of Feb 2019. As noted in the methods, two rain events during that experiment lowered the salinity of collected seawater to 25 at 9 dpf and 28 at 13 dpf.

Initial chl *a* levels in the NS treatment (the treatment most similar to raw seawater) varied both among and within experiments (Table 2; Fig. 1). Among experiments, mean initial chl *a* in NS varied by about 2.5-fold, from 1.88 $\mu g \cdot L^{-1}$ in the Feb 2019 experiment to 4.74 $\mu g \cdot L^{-1}$ in the Jul 2017 experiment. Among days within experiments, initial chl *a* levels typically varied by no

more than about 2.5-fold, as well (Table 2; Fig. 1). The exceptions were two experiments – Apr 2018 and Nov 2018 – during which initial chl *a* levels varied by slightly more than 3-fold.

Among experiments, mean initial chl *a* concentration in the NS- treatment averaged 55.4% of that in the corresponding NS treatment (range 49.9-59.4%; Table 2). Mean initial chl *a* concentration in the NS+ treatment averaged 0.9 $\mu g \cdot L^{-1}$ (range 0.78-1.11) higher than that in the corresponding NS treatment for the first five experiments, when 1000 $cells \cdot mL^{-1}$ of *R. lens* were added to generate the NS+ treatments. In the Aug 2019 experiment, 5000 $cells \cdot mL^{-1}$ were added, and in that experiment mean initial chl *a* concentration was 5.82 $\mu g \cdot L^{-1}$ higher than in the NS treatment (Table 2).

Chl *a* concentrations typically changed substantially over the 24 h between water changes. A fine-scale view of these changes (as well as within-experiment variation in initial chl *a* concentration) for the NS treatment of each of the six experiments is shown in Figure 1. Over almost all 24-h periods, chl *a* concentration declined sharply, presumably due primarily to larval feeding, as trophic competitors (primarily rotifers) were rare in cultures. Over a very few 24 h periods, chl *a* concentration increased for unknown reasons (see, e.g., the Jul 2017 and Aug 2019 experiments: Fig. 1). These patterns were very similar in the other two treatments (data not shown). Over each 24 h period, chl *a* concentrations declined an average 45.2% from initial concentrations in the NS- treatment, 43.3% in the NS treatment, and 46.3% in the NS+ treatment (Table 2).

Because larvae had such a strong effect on treatment conditions over such short timescales, the chl *a* level experienced by larvae in an experiment is best characterized as intermediate between the initial and final concentration. For simplicity, we used the mean of these two values

to describe overall mean chl *a* concentrations in an experiment. In the NS treatment, these values ranged from 1.39 to 4.23 among the six experiments (Table 2).

Our target initial density of larvae in each experiment was 250•L^{-1}. To determine how closely we approached this target, for the last four experiments we counted the number of larvae in two or three count control beakers established at the beginning of the experiment. For these four experiments the initial density of larvae averaged 219.5 (range 191-282; Fig. S2). In three of the four experiments, the initial density of larvae was ~20% lower than the target, and in the fourth (Aug 2019) it was ~13% higher than the target. There was little variation in the initial density of larvae within experiments (Fig. S2).

3.2. Larval form

The ratio of right postoral arm length to midline body length differed significantly among treatments in all experiments except for that of Jul 2017 (Fig. 2; Table 3). Post-hoc tests, however, showed that that ratio was significantly lower in the NS+ treatment relative to the NS treatment in only one experiment, that of Aug 2019 ($P = 0.043$). In four of six experiments, the ratio of right postoral arm length to midline body length was significantly lower in the NS relative to the NS- treatment (all $P < 0.039$ except for in the Aug 2019 experiment, where $P = 0.051$).

As described above, there was substantial variation among experiments in overall mean chl *a* levels in the NS treatments (Table 2). This variation permits two additional relevant comparisons. First, we predicted that among experiments, NS chl *a* level and the mean ratio of right postoral arm length to midline body length in that treatment should be inversely related – that is, the more chl *a* present, the smaller that ratio should be. There was a negative relationship

between these two variables (Fig. 3A; ordinary least squares regression, $P = 0.037$, $R^2 = 0.7027$). Second, we predicted that among experiments, food supplementation should have more of an effect on this ratio when NS chl *a* is low compared to when it is high. A scatterplot of these data hints at such a relationship, though a regression was not significant (Fig. 3B; $P = 0.182$, $R^2 = 0.3941$).

3.3. Time to 50% competence

Time to 50% competence differed significantly among treatments in all experiments (Fig. 2; Table 3). In two experiments, those of Feb 2019 and Nov 2018, TC_{50} was lower in the NS+ treatment relative to the NS treatment. In all six experiments, TC_{50} was lower in the NS treatment relative to the NS- treatment.

As above, we can make two more relevant comparisons using these data. We predicted that among experiments, NS chl *a* level and TC_{50} in that treatment should be inversely related – that is, the more chl *a* present, the less time larvae should require to reach metamorphic competence. A plot of these data faintly suggests such a relationship, though a regression was not significant (Fig. 3C; $P = 0.089$, $R^2 = 0.5558$). Second, we predicted that among experiments, food supplementation should have more of an effect on TC_{50} when NS chl *a* is low compared to when it is high. There was a strong negative relationship between overall mean chl *a* in the NS treatment and the difference in mean TC_{50} between NS+ and NS treatments (Fig. 3D; regression, $P = 0.005$, $R^2 = 0.8877$).

There was variation among experiments in the TC_{50} observed in NS+ treatments, from 11.0 days in Jul 2017 to 13.1 days in Nov 2018. This variation was not related to the concentration of chl *a* in the NS or NS+ treatments (data not shown).

3.4. Mortality

Average instantaneous mortality rates of larvae were generally near zero, with the exception of the Aug 2019 experiment where they were ~0.05 d^{-1} (Fig. S3). Within experiments, mortality rates did not differ significantly among treatments (ANOVA; results shown in Fig. S3).

4. DISCUSSION

Overall mean chl *a* levels in the NS treatment varied from 1.39 to 4.23 $\mu g \cdot L^{-1}$ among experiments, allowing us to explore a greater range of natural chl *a* levels than in previous experiments aimed at identifying food limitation in the larvae of temperate echinoderms (Table 1). We also expanded the range of chl *a* levels tested by supplementation and reduction of chl *a* levels. Unlike previous experiments, we examined the effects of these manipulations not only on larval form, but also on an estimate of average time to metamorphic competence. These efforts allow us to address two important questions: first, what is the chl *a* boundary above which larvae of the temperate sand dollar *Dendraster excentricus* are not food limited, and below which they are, and second, how strongly do food conditions experienced by larvae in nature affect time to competence?

4.1. What chl *a* conditions lead to limited vs. maximal rates of growth and development?

To address this question, the most critical comparisons in our experiments are of larval form and time to metamorphic competence between the NS+ and NS treatments. Larvae in NS+ treatments had phenotypes indicative of abundant food (lower postoral arm/midline body length ratios) while those in NS treatments had phenotypes indicative of scarce food (higher ratios) in

one experiment (Aug 2019), where the overall mean chl *a* level in the NS treatment was 2.39 $\mu g \cdot L^{-1}$. Larvae in NS+ treatments reached metamorphic competence more rapidly than did larvae in NS treatments in two experiments (Feb 2019 and Nov 2018), where the overall mean chl *a* levels were 1.39 and 3.01 $\mu g \cdot L^{-1}$, respectively. There was no indication of food limitation in either larval form or developmental rate in the remaining three experiments, where overall mean chl *a* levels were 2.83, 3.39, and 4.23 $\mu g \cdot L^{-1}$. These results suggest that the critical boundary below which larvae of *D. excentricus* experience food limitation, and above which they do not, is somewhere in the range of ~2.4-3 $\mu g \cdot L^{-1}$. Comparisons of larval form and time to metamorphic competence in the NS- and NS treatments are largely consistent with that result: four of six comparisons of larval form and all six comparisons of developmental rate indicate food limitation at chl *a* levels <2.1 $\mu g \cdot L^{-1}$ (the highest overall mean chl *a* level in the NS- treatment in any of our experiments, in Jul 2017).

Our conclusion that larvae of *D. excentricus* experience food limitation at chl *a* levels below ~2.4-3 $\mu g \cdot L^{-1}$ is specific to our particular location. Results may differ elsewhere in this species' range (coastal northeastern Pacific from central Baja California to southeastern Alaska: Durham et al. 1980), where larvae may arise from genetically different source populations and experience different thermal regimes and food assemblages. However, both prior studies of larval food limitation in *D. excentricus*, which were carried out near the northern extent of its range, in Washington State, are consistent with our results: both demonstrated food limitation in rates of growth or development at natural chl *a* levels of ~1.3 and 1.8 $\mu g \cdot L^{-1}$, respectively (Paulay et al. 1985, Reitzel et al. 2004). Indeed, the results of all but one of the previous studies on food limitation in echinoderm larvae where natural chl *a* levels are reported are consistent with the tentative boundary that we have identified (Table 1). The exception is Olson's (1987) study of

Acanthaster planci, in which larvae of this tropical seastar did not appear to be food-limited even at the very low chl *a* level of 0.25 μg•L^{-1}. As described above, however, subsequent work suggests that this result may be incorrect and provided strong evidence that larvae of *A. planci* are indeed food limited at the low (≤0.5 μg•L^{-1}) chl *a* levels they typically experience (Okaji 1996, Fabricius et al. 2010). Further, Fabricius et al. (2010) report that the time for 50% of larvae to complete development decreased dramatically as food concentration increased up to chl *a* levels of at least 2 μg•L^{-1}, suggesting that the food limitation boundary for this tropical species may be similar to that of the temperate species we report on here.

There are, of course, caveats associated with our identification of this critical food level. One issue has to do with our choice of chl *a* concentration in the size fraction of edible particles as a metric of food abundance. As noted above, it is clear that chl *a* concentration is only imperfectly correlated with the abundance of larval food. However, it does appear to be sufficiently related to larval performance, in our study and many others (e.g., Table 1; Fig. 3), to serve as a reasonable proxy for food availability. Of course, we strongly encourage the development and evaluation of more sophisticated measures of food availability so that we can eventually better predict the quality of the larval feeding environment (Pernet 2018). As an aside, we note that some influential critiques of chl *a* as a metric of larval food supply merit reevaluation. For example, Olson et al. (1987) is sometimes cited as evidence that chl *a* is an inappropriate measure of food abundance for meroplankton (e.g., Hansen 1999), but Olson et al.'s conclusion is based on larval metabolic rate estimates that are very likely incorrect (Shilling & Manahan 1994).

There are also important limitations associated with our experimental design, which is similar to that used in several previous studies of larval food limitation (e.g., Paulay et al. 1985, Eckert 1995, Reitzel et al. 2004, Smith and Bolton 2007, Pedersen et al. 2010). Environmental

conditions in laboratory beakers are different from those in the field in a host of ways (Olson & Olson 1987, Fenaux et al. 1994). In particular, larvae in our laboratory beakers experienced two types of variation in food level that they are unlikely to experience in nature. First, we collected natural seawater, which was then modified into our treatment suspensions and used in complete water changes, every 24 hours. The progression of the tidal cycle, along with variation in other environmental drivers like winds, meant that each day we sampled water from a slightly or very different water mass. On some days, for example, we collected seawater on flood tides, and others on ebb tides. This meant that larvae were exposed to sometimes dramatic variation in initial chl *a* levels every 24 hours, variation that was most apparent in the experiments of April and Nov of 2018 (Fig. 1). In nature, larvae are probably drifting with water masses and thus unlikely to experience such dramatic changes in chl *a* levels at that short time scale.

In addition, larvae appeared to have a strong influence on treatment conditions, rapidly drawing down chl *a* levels in laboratory beakers (Fig. 1). Though we did not include no-larva control beakers in our experiments to assess other causes, this daily decline in chl *a* seems extremely likely to be due to grazing by larvae, which were reared at relatively high density in our beakers (0.25 larvae•mL^{-1}). In the field, larval density is probably at least an order of magnitude lower than this (Kacenas & Podolsky 2018), and the combination of larval swimming and turbulent mixing probably mean that larvae rarely deplete their immediate environs of food.

Researchers might use various strategies to minimize these problems during future laboratory experiments on food limitation, for example always collecting natural seawater during the same phase of the tidal cycle, rearing larvae at lower initial density in laboratory containers, or refreshing the food supply more frequently (or even continually, by dripping fresh treatment solution into experimental beakers). Beyond minimizing experimental artifacts or biases, an

additional strategy to increase confidence in studies of food limitation is to use as many different types of laboratory and field tests as possible, all aimed at the same question. Fenaux et al. (1994) did just this in what is certainly the most thorough study of food limitation in echinoderm larvae available. In our study we use two of the five methods that they describe: comparisons of larval form and developmental rate in the laboratory between larvae reared with natural food compared to those reared with an enhanced ration (Fig. 2), and relating larval form and developmental rate in the laboratory with the abundance of natural planktonic food (Fig. 3).

Our metric of time to metamorphic competence, TC_{50}, appeared to be a more sensitive indicator of food limitation than a morphological metric of phenotypic plasticity, the ratio of postoral arm length to body length. In eight of twelve comparisons (all six NS-/NS and two NS+/NS comparisons), TC_{50} was significantly different among treatments, but in the same experiments, the ratio of postoral arm length to body length differed only in five comparisons (four NS-/NS and one NS+/NS) (Table 3; Fig. 2). The apparent sensitivity of TC_{50} to food limitation is fortunate, since time to metamorphic competence is a parameter of direct ecological and evolutionary importance. To our knowledge, this is the first time that estimates of median time to metamorphic competence have been made in a study of food limitation. This method should work for any species in which reliable metamorphic cues have been identified. Among others, this includes all echinoids (see Hodin et al. 2019 for recommended concentrations of excess KCl for a diversity of species), as well as various annelids, gastropods, and bivalves (e.g., Yool et al. 1986, Pechenik & Heyman 1987, Beiras & Widdows 1995, Bryan et al. 1997).

4.2. How intensely does food limitation affect larval developmental rate?

It is important not only to identify when conditions are food limiting, but also to quantify the magnitude of their effects on parameters of interest. In our experiments, food limitation had a relatively small effect on the central parameter of interest, time to metamorphic competence. The magnitude of differences in TC_{50} between NS and NS+ treatments was negatively related to overall mean chl *a* concentration in NS treatments; the maximum difference we observed in our experiments was ~1.25 days, in the experiment with the lowest overall mean chl *a* concentration in the NS treatment (Fig. 3D). This might be an underestimate if the amount of supplemental food added to NS+ treatments (usually 1000 cells•mL^{-1} of *Rhodomonas lens*) was not sufficient to maximize development rate. That is possible, though in the Aug 2019 experiment the supplement was 5000 cells•mL^{-1}, and there was no significant difference between TC_{50} in NS+ and NS treatments in that experiment.

What might a delay of 1.25 days in time to competence mean for larval mortality and dispersal? If one assumes that larvae of *D. excentricus* growing at maximal rates reach competence at 11 dpf (Fig. 2), and that they experience an instantaneous mortality rate of 0.16•d^{-1} (the mean of estimates for three echinoids in Table 4 of Rumrill 1990), in the absence of food limitation 17.2% of the original larvae survive in the plankton and achieve competence. A delay of 1.25 days leads to additional mortality such that only 14.1% of the original larvae survive to competence. Larvae forced to delay development by the small increment of 1.25 days are thus exposed to substantial additional risk (~18%) of mortality over that time period. It is possible that the mortality caused by such delays might affect the dynamics of populations of adults. In addition, the increased mortality risk incurred by even slight food-limitation induced delays in time to competence seems likely to be a source of selection for maximal net rates of energy gain by larvae, potentially affecting the evolution of both larval physiology and larval form.

This argument assumes that larvae leave the plankton immediately once achieving metamorphic competence. However, laboratory experiments have shown that the planktotrophic larvae of many benthic marine invertebrates -- including *D. excentricus* – are capable of delaying settlement and metamorphosis for days or weeks in the absence of suitable settlement cues (Highsmith & Emlet 1986, Pechenik 1990). The frequency and duration of delayed settlement and metamorphosis due to the absence of cues in nature is very poorly understood, but if such delays are common and substantial, they likely have a stronger effect on larval mortality than the slight increases in time to competence due to food limitation that we have identified here.

We note that there was also substantial among-experiment variation in TC_{50} (from ~11-13 days) in the NS+ treatment. We suspect three potential causes of these differences. First, each experiment was carried out using the offspring of a unique pair of parents, so experimental differences may represent genetic differences among families. A second possibility is that there is seasonal variation in gamete quality (e.g., in egg energy content). We did not characterize egg energy content in this study. Finally, it is possible that the quality of the pool of potential food particles in the plankton varies seasonally. Chl *a* is not a sufficiently high-resolution metric of food conditions to capture such variation.

4.3. Temporal patterns of food limitation in coastal Southern California

The utility of identifying a tentative chl *a* boundary between food-limited and food-unlimited conditions is that existing data can now be explored to make inferences about spatial and temporal patterns of risk of food limitation for feeding larvae. We are particularly interested in how the risk of food limitation changes over time in nearshore habitats in southern California. We explored this using a record of chl *a* concentration in nearshore water from a sampling

station on the Newport Pier, ~23 km southeast of our study site. Chl *a* has been measured from just below the surface (2 m depth) at that site weekly from mid-2008 until the present, and data are publicly available (http://habs.sccoos.org/newport-pier). We obtained all complete years (2009-2019) of weekly chl *a* data from that location and used it to begin to explore the frequency and temporal patterning of risk of food limitation for larvae of *D. excentricus*.

Raw chl *a* data are plotted for all 51-52 sampled weeks of each of the 11 years in Figure 4A, where the dashed line indicates a chl *a* level of 2.4 $\mu g \cdot L^{-1}$, and the solid line indicates a chl *a* level of 3.0 $\mu g \cdot L^{-1}$, the minimum and maximum of our estimates of the threshold for food limitation, respectively. Chl *a* levels were $\leq$2.4 $\mu g \cdot L^{-1}$ in 305 of the 560 sampled weeks (54%), and $\leq$3.0 $\mu g \cdot L^{-1}$ in 372 of the 560 sampled weeks (66%). Inspection of Figure 4A also suggests a temporal pattern in chl *a* abundance – during the early part of each year, chl *a* abundance is often well above 3.0 $\mu g \cdot L^{-1}$. To visualize this more clearly, we averaged chl *a* concentrations for each of the 52 weeks of the year across all 11 sampled years (Fig 4B). For a portion of the average year – weeks 5-26, early Feb to late Jun – mean chl *a* concentration is usually above 3.0 $\mu g \cdot L^{-1}$ (solid line). During the late summer, fall, and early winter, mean chl *a* concentration is always below this boundary, and very often below 2.4 $\mu g \cdot L^{-1}$ (dashed line).

This pattern of chl *a* abundance is likely associated with the intensity of upwelling off of the southern California coast. Upwelling occurs through much of the year in southern California, but over a 30-year period (1967-2007), its intensity was maximal on average at about week 21 (with standard error limits ranging from weeks 8-34) of the year, similar but slightly delayed relative to the chl *a* pattern shown in Figure 4 (Bograd et al. 2009).

What does this pattern mean for larvae? If adult sand dollars release gametes throughout the year randomly with respect to chl *a* concentration, and if their larvae remain in nearshore coastal

waters, their larvae may often be at risk of food limitation. However, a simple adjustment of adult behavior – spawning only during the late winter, spring, and early summer – would mean that larvae experience food-limiting conditions much more rarely. If sand dollars have sufficient resources to reproduce only once a year, selection on adults to spawn only during the period when larvae are more likely to survive may thus be strong. This basic hypothesis – that marine invertebrates with feeding larvae time their reproduction to coincide with periods of high food availability for larvae, presumably maximizing larval success – has been suggested by many authors (e.g., Lacalli 1981, Olive 1992, Starr et al. 1993). Typically, however, the suggested linkages are qualitative, with authors suggesting that selection should favor adults spawning during periods of "high" planktonic food availability (but see Bos et al. 2006). An advantage of making quantitative estimates of the amount of food required to fuel maximal rates of larval growth and development is that we can be more precise in our predictions.

Do adults of *D. excentricus* spawn during high chl *a* periods in southern California? Niesen (1977) measured gonad index (which he defined as gonad volume divided by adult weight) in samples of *D. excentricus* from two populations in San Diego County over one year. Gonad index was highest from February to April or May (depending on the population); after the April or May peak, gonad index dropped sharply in both populations, suggesting that most individuals in the population had spawned. If patterns of chl *a* abundance in that area are similar to those at Newport Pier (~110 shoreline km to the north), this means that larvae would be in the plankton during the high chl *a* part of the year. It would be helpful to have independent corroboration of these inferred spawning events in the form of data on the abundance of larvae of *D. excentricus* in the plankton over time, but we know of no such data. Larvae of *D. excentricus* are easy to

distinguish from co-occurring echinoid larvae by skeletal morphology (Strathmann 1979), so such corroboration is possible.

Though natural spawnings of *D. excentricus* may be concentrated in the spring or early summer (Niesen 1977), our observations over the past five years indicate that most field-collected adults from our study site contain abundant fertilizable gametes throughout almost the whole year. The only months in which we sometimes fail to find ripe adults are December and January. Why would sand dollars invest energy in producing viable gametes during times when food conditions might prolong larval duration and reduce larval survivorship? One possibility, of course, is that sand dollar larvae actually do not experience food limitation in nature frequently. Chl *a* concentration in nature is likely patchy on very small scales, and if larvae are capable of identifying and orienting to high chl *a* concentration patches, then they may not experience the lower "averaged" levels of chl *a* that we measured in our experiments. Inferences on the frequency of food-limitation in *D. excentricus* based on field-collected larvae (like those reported by Fenaux et al. 1994 for another species) would be helpful in cross-checking our laboratory results. Two additional possibilities have to do with the hypothesis that because of their flattened shape, adult sand dollars simply do not have extra body volume in which to store many energy-rich compounds (Moss & Lawrence 1972). If this is true, then perhaps adult sand dollars use their gonads as energy storage depots (Moss & Lawrence 1972). In this case, the presence of fertilizable gametes in gonads outside of the spawning season does not necessarily indicate reproduction, just storage. Alternatively, once sand dollars approach maximum size they presumably no longer need to invest substantial energy into growth. If they continue to accumulate energy by feeding but do not have sufficient internal space in which to store it while awaiting spawning during the optimal period for larval success, then perhaps they spend that

excess energy by reproducing through much of the year, including at times that are suboptimal for larval success. Distinguishing between these hypotheses for *D. excentricus* will require better data on gonad indices over time (especially as related to adult body size), the timing of spawning events, and the presence of larvae in the plankton.

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Table 1. Results of food supplementation experiments and surveys aimed at assessing whether the availability of natural food limits growth or development of ciliated larvae. In supplementation experiments, rates of larval growth or development are compared between larvae reared on a natural diet and those reared on the natural diet supplemented with additional food; surveys use the characteristics of field-collected larvae to make inferences about food levels those larvae have experienced in nature. Chl *a* values are those of the least manipulated feeding treatment at the start of the experiment (for supplementation experiments) or the natural feeding environment (for surveys of larval form or condition); "--" indicates that chl *a* values were not reported in the study. Hansen's (1999) study was not replicated sufficiently to demonstrate food limitation in larval growth statistically, but the patterns observed are suggestive of limitation. Instead of growth rate measurements, Ginsburg (2007) estimated protein synthesis rates. Fenaux et al. (1994) used both experiments and surveys in their study.

	Method	Chl *a* ($\mu g \cdot L^{-1}$)	Evidence of food limitation?	Reference
Annelida				
Galeolaria caespitosa	expt	--	yes	Smith & Bolton 2007
Polydora ciliata	expt	~ 2-14	yes	Pedersen et al. 2010
Spionidae	expt	--	yes?	Hansen 1999
Mollusca: Bivalvia				
Mytilus edulis	expt	2.8	yes	Fotel et al. 1999
Echinodermata: Asteroidea				
Acanthaster planci	expt	0.25	no	Olson 1987
	expt	0.28-0.52	yes	Okaji 1996, Fabricius et al. 2010
Patiria miniata	expt	--	yes	Basch 1996
Echinodermata: Echinoidea				
Dendraster excentricus	expt	1.3	yes	Paulay et al. 1985
	expt	1.8	yes	Reitzel et al. 2004
Encope michelini	expt	1.3	yes	Eckert 1995
Lytechinus pictus	expt	0.49	yes	Ginsburg 2007
Paracentrotus lividus	both	0.1-0.5	yes	Fenaux et al. 1994
Strongylocentrotus purpuratus	survey	--	no	Miller & Emlet 1999
Echinodermata: Ophiuroidea				
Ophiopholis aculeata	expt	2.3	yes	Paulay et al. 1985

Table 2. Summary of seawater temperature and salinity at collection and chl *a* levels in each experiment. Most columns show means of daily measures (ranges). All treatments were maintained at 16°C after collection. NS was a natural seawater treatment, NS- a reduced food treatment (1:1 dilution of NS with filtered seawater), and NS+ a supplemented food treatment (NS with the addition of 1000 cells•mL^{-1} of *Rhodomonas lens*, except for Aug 2019, where 5000 cells•mL^{-1} of *R. lens* were added). NS- and NS+ treatments had identical or very similar salinities to NS treatments. Mean initial chl *a* was calculated as the mean of starting chl *a* levels across days of the experiment (n=12-14); mean final chl *a* was calculated as the mean of ending (~24 h after starting) chl *a* levels (n=12-14). Overall mean chl *a* is the grand mean of mean initial and final chl *a* (n=2).

Trial	Treatment	Temperature (°C)	Salinity	Mean initial chl *a* (µg•L^{-1})	Absolute (and %) difference in initial chl *a* relative to NS	Mean final chl *a* (µg•L^{-1})	Mean daily change in chl *a* (%)	Overall mean chl *a* (µg•L^{-1})
Feb 2019	NS-	--	--	1.02 (0.66-1.50)	0.86 (54.3)	0.53 (0.0-1.29)	-48	0.78
	NS	14.5 (13.3-15.4)	33.2 (25-35)	1.88 (1.36-2.26)	--	0.89 (0.32-1.48)	-53	1.39
	NS+	--	--	2.89 (1.65-4.01)	1.01 (153.7)	1.26 (0.29-2.09)	-56	2.08
Mar 2018	NS-	--	--	2.57 (1.45-4.50)	1.76 (59.4)	1.15 (0.59-2.19)	-55	1.86
	NS	15.9 (14.8-17.4)	34.0 (34.0)	4.33 (2.42-5.74)	--	2.44 (1.08-3.86)	-44	3.39
	NS+	--	--	5.21 (3.29-6.68)	0.88 (120.3)	2.65 (1.78-4.34)	-49	3.93
Apr 2018	NS-	--	--	2.23 (1.42-3.49)	1.74 (56.2)	0.83 (0.30-2.17)	-63	1.53
	NS	15.4 (15.0-16.0)	34.0 (34.0)	3.97 (2.27-7.29)	--	1.69 (0.55-2.78)	-57	2.83
	NS+	--	--	5.08 (2.82-7.92)	1.11 (130.0)	2.07 (0.96-3.30)	-59	3.58
Jul 2017	NS-	--	--	2.43 (1.53-3.51)	2.31 (51.3)	1.74 (0.91-3.36)	-28	2.09
	NS	21.4 (19.3-23.9)	33.8 (33-34)	4.74 (2.98-6.30)	--	3.72 (1.95-6.93)	-22	4.23
	NS+	--	--	5.54 (3.71-7.07)	0.80 (116.9)	4.49 (2.58-7.92)	-19	5.02
Aug 2019	NS-	--	--	1.58 (0.89-2.22)	1.41 (52.8)	0.93 (0.48-1.64)	-41	1.26
	NS	21.3 (20.0-22.3)	34.1 (34-35)	2.99 (1.75- 4.50)	--	1.79 (1.09-3.46)	-40	2.39
	NS+ (5000•mL^{-1})	--	--	8.81 (7.75- 10.35)	5.82 (294.6)	4.58 (2.38-7.33)	-48	6.70
Nov 2018	NS-	--	--	1.92 (0.88-2.96)	1.93 (49.9)	1.22 (0.35-2.33)	-36	1.57
	NS	18.4 (17.5-19.6)	35.0 (35.0)	3.85 (1.97-6.55)	--	2.17 (1.00-4.45)	-44	3.01
	NS+	--	--	4.63 (2.56-7.01)	0.78 (120.3)	2.44 (1.06-4.46)	-47	3.54

Table 3. Results of one-way ANOVA analyses on larval form and time to 50% competence for the six experiments. The results of Tukey's post-hoc comparisons are shown in Figure 2.

LARVAL FORM						
	Source	***SS***	***DF***	***MS***	***F* (*DFn, DFd*)**	***P***
Feb 2019	Treatment	0.0860	2	0.0432	15.15 (2, 9)	0.0013
	Residual	0.0256	9	0.0028		
	Total	0.1116	11			
Mar 2018	Treatment	0.0886	2	0.0443	4.581 (2, 9)	0.0424
	Residual	0.0871	9	0.0097		
	Total	0.1757	11			
Apr 2018	Treatment	0.0625	2	0.0312	21.53 (2, 9)	0.0004
	Residual	0.0131	9	0.0015		
	Total	0.0755	11			
Jul 2017	Treatment	0.0067	2	0.0033	1.119 (2, 6)	0.3864
	Residual	0.0179	6	0.0030		
	Total	0.0246	8			
Aug 2019	Treatment	0.0481	2	0.0240	13.54 (2 ,9)	0.0019
	Residual	0.0160	9	0.0018		
	Total	0.0640	11			
Nov 2018	Treatment	0.0171	2	0.0086	7.232 (2, 9)	0.0134
	Residual	0.0107	9	0.0012		
	Total	0.0278	11			
TIME TO 50% COMPETENCE						
	Source	***SS***	***DF***	***MS***	***F* (*DFn, DFd*)**	***P***
Feb 2019	Treatment	17.2600	2	8.6300	173.8 (2, 9)	< 0.0001
	Residual	0.4470	9	0.0497		
	Total	17.7100	11			
Mar2018	Treatment	2.8580	2	1.4290	26.88 (2, 9)	0.0002
	Residual	0.4784	9	0.0532		
	Total	3.3360	11			
Apr 2018	Treatment	4.5269	2	2.2630	27.75 (2, 9)	0.0001
	Residual	0.7339	9	0.0816		
	Total	5.2600	11			
Jul 2017	Treatment	2.4050	2	1.2020	226.4 (2, 6)	< 0.0001
	Residual	0.0319	6	0.0053		
	Total	2.4370	8			
Aug 2019	Treatment	25.1500	2	12.5700	99.01 (2, 8)	< 0.0001
	Residual	1.0160	8	0.1270		
	Total	26.1600	10			
Nov 2018	Treatment	7.7210	2	3.8600	76.94 (2, 8)	< 0.0001
	Residual	0.4014	8	0.0502		
	Total	8.1220	10			

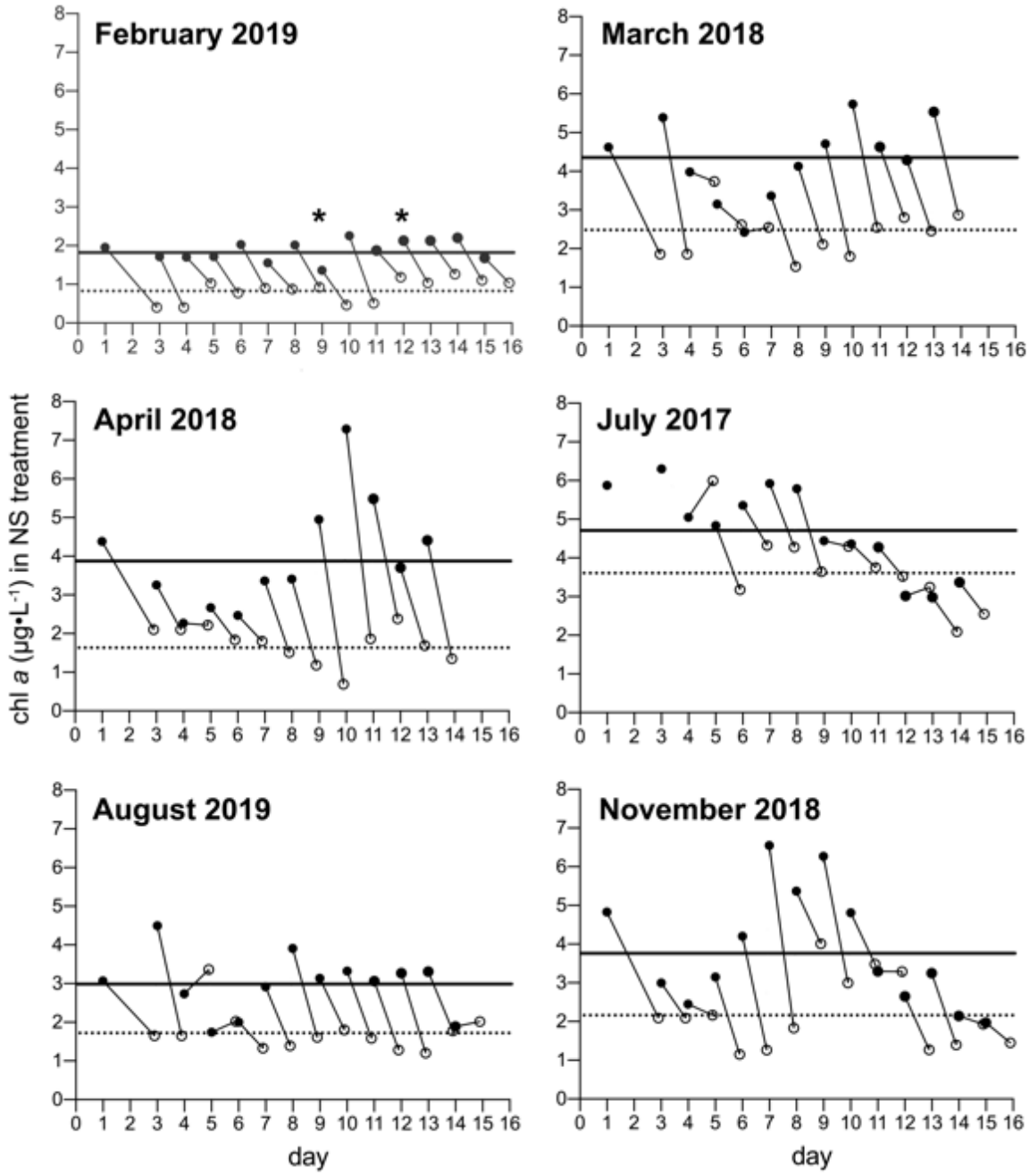

Fig. 1. Initial (filled points) and final (open points) chl *a* concentrations for each 24 h period in the NS (natural seawater) treatment for each day of each experiment. Lines connect initial chl *a* concentrations with those 24 h later. In all six experiments, water was not changed on the second day post-fertilization. The solid horizontal line indicates the mean initial chl *a* concentration in that experiment, and the dashed line the mean final chl *a* concentration in that experiment. Overall chl *a* concentration in each experiment (not shown in this figure) was calculated as the mean of initial and final concentration. Asterisks (*) in the February 2019 panel indicate days where water was not changed due to low salinity resulting from high rainfall; instead, a ration of 1000 cells•mL^{-1} of *Rhodomonas lens* was added to all treatment beakers on those day

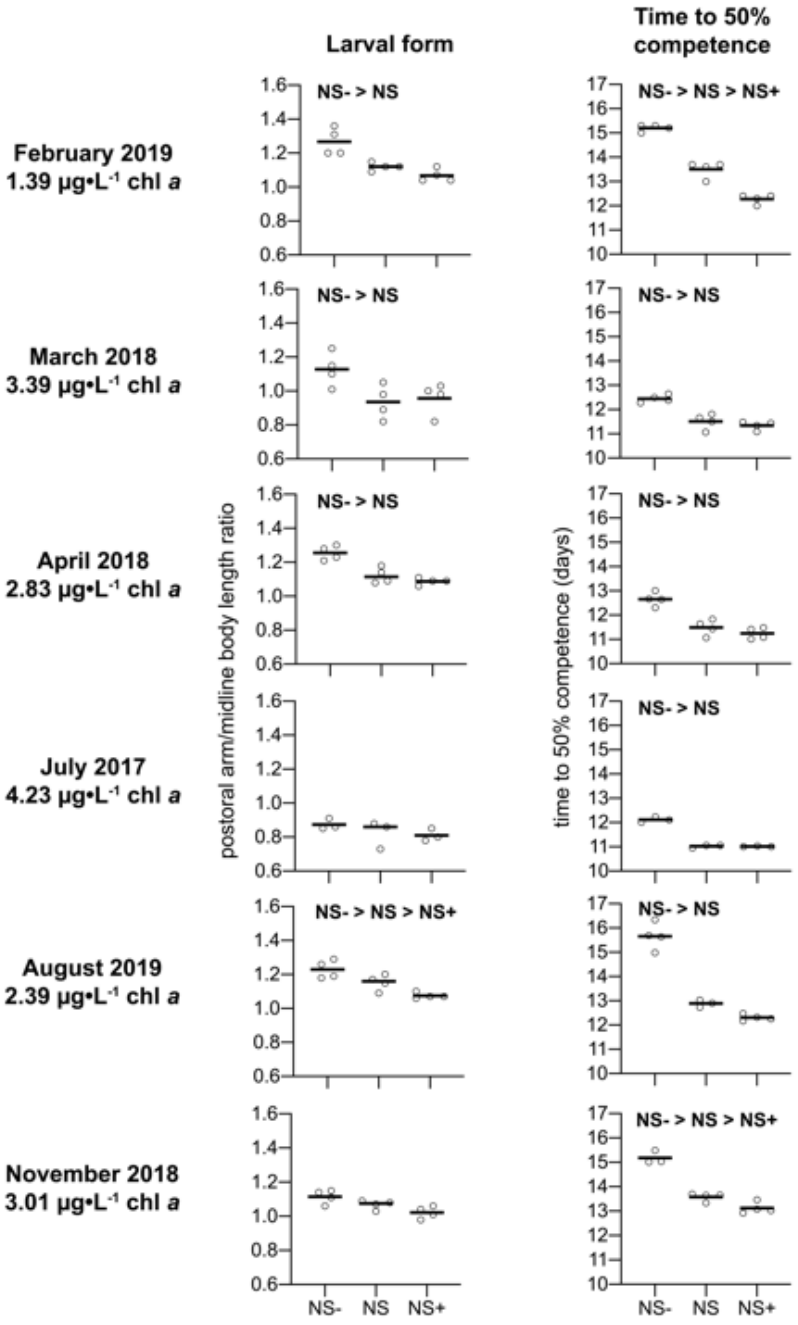

Fig. 2. Overall chl *a* concentration in the NS (natural seawater) treatment, ratio of right postoral arm length to midline body length, and time to 50% competence in each treatment of each of the six experiments. NS- was a reduced food treatment (1:1 dilution of NS with filtered seawater), and NS+ a supplemented food treatment (NS with the addition of *Rhodomonas lens*). Bars in each plot are means of the data points shown. Inequalities on each graph indicate the significant ($p < 0.05$) results of Dunnett's post-hoc tests comparing NS+ to NS and NS- to NS, with one exception – larval form differed between the NS- and NS treatments in the August 2019 experiment at $P = 0.051$.

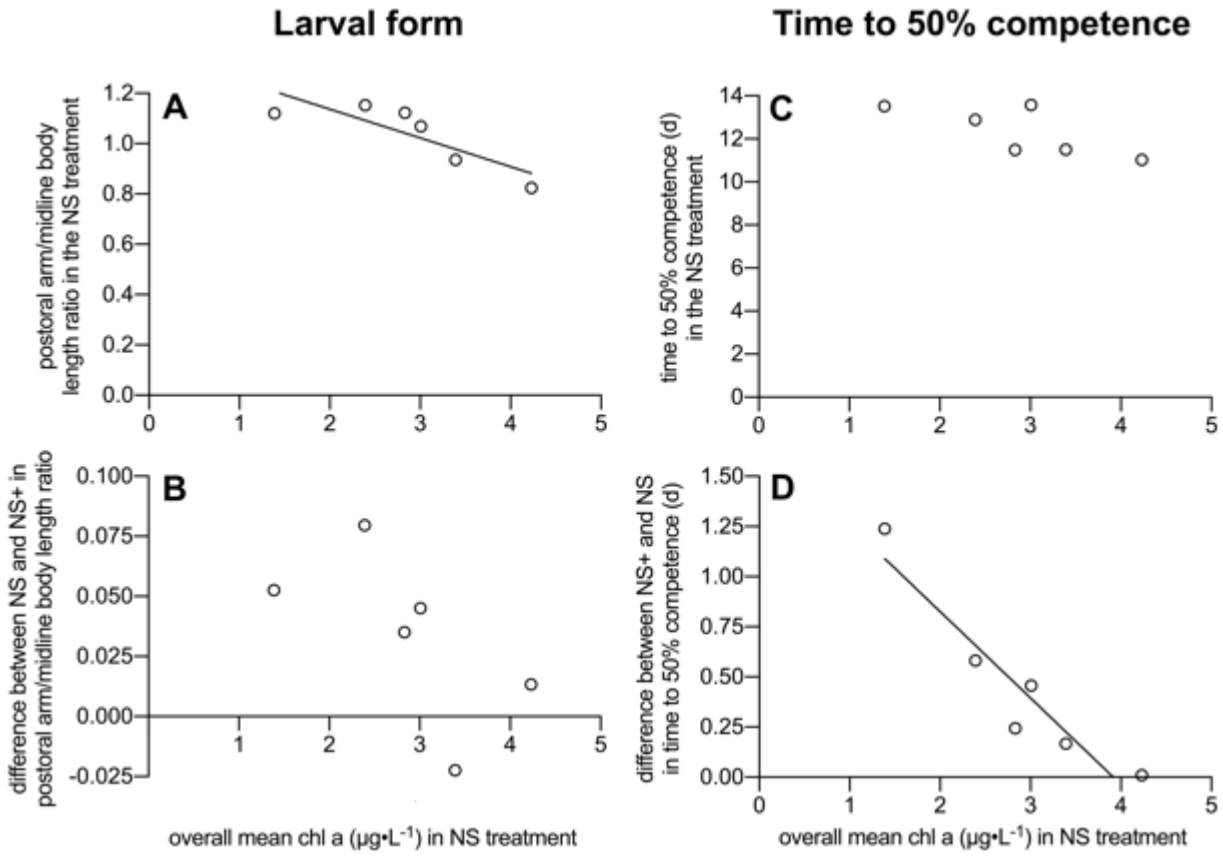

Fig. 3. Relationships between overall chl *a* concentration in the NS (natural seawater) treatment and metrics of larval form and time to 50% competence among the six experiments. NS+ was a supplemented food treatment (NS with the addition of *Rhodomonas lens*). (A) Mean postoral arm/midline body length ratio larvae in the NS treatment. The line is an ordinary least squares regression ($P = 0.037$, $R^2 = 0.7027$). (B) Difference between larvae in the NS and NS+ treatments in mean postoral arm/midline body length ratio. (C) Mean time to 50% competence of larvae in the NS treatment. (D) Difference in mean time to 50% competence between larvae in NS+ and NS treatments. The line is an ordinary least squares regression ($P = 0.005$, $R^2 = 0.8877$).

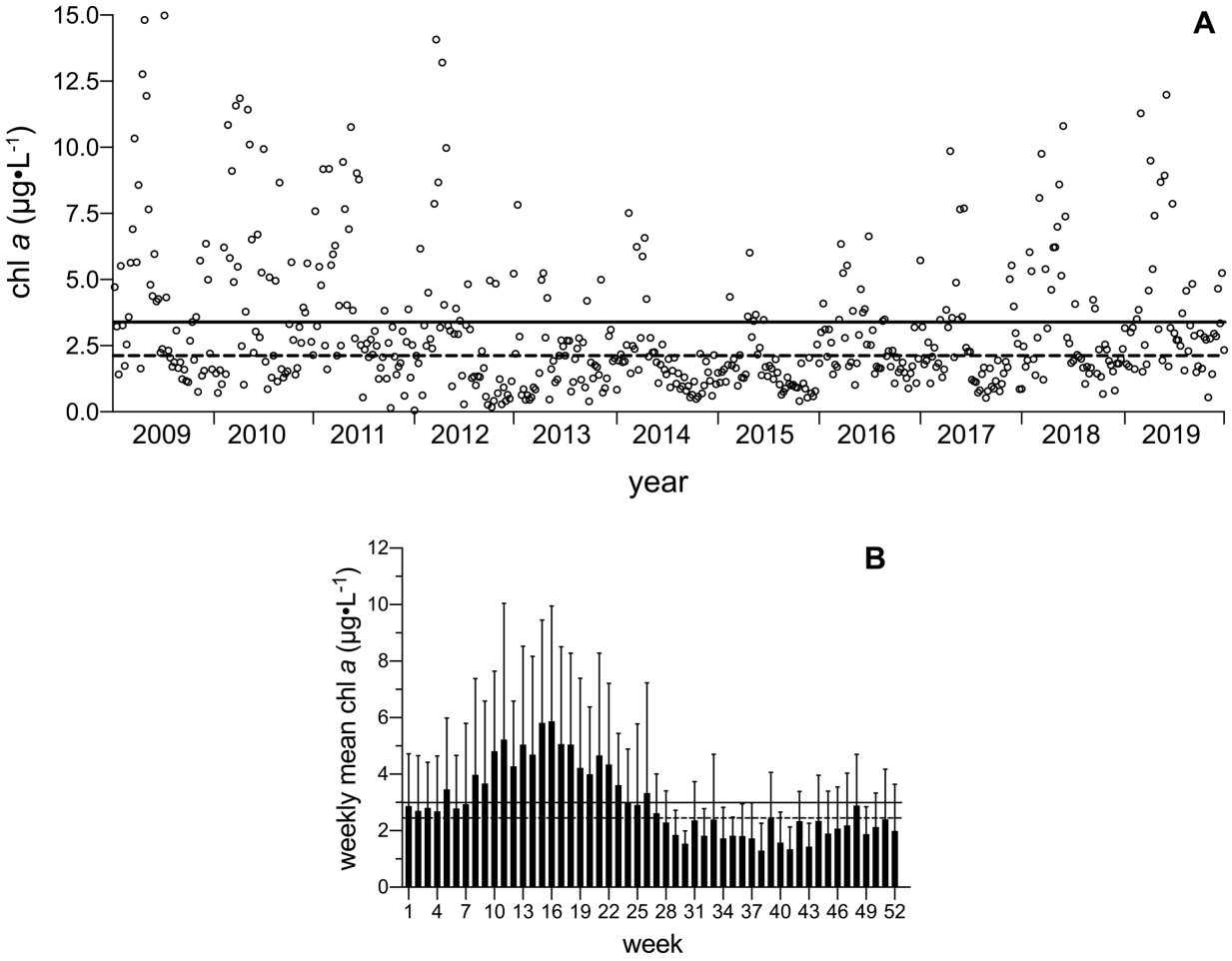

Fig. 4. An 11-year record (2009-2019) of weekly chl a levels at Newport Pier, Orange County, ~23 km shoreline distance southeast of the current study site. (A) Weekly chl *a* measurements at Newport Pier. Plotted values are averages of two independent measures of chl *a* concentration at each time point. Four chl *a* values >>15 µg•L^{-1} (of a total of 560 weekly measurements) are omitted from the plot. Data downloaded 5 April 2020 from California HABMAP website (http://habs.sccoos.org/newport-pier). Dashed horizontal line indicates 2.4 µg•L^{-1}, and solid horizontal line indicates 3.0 µg•L^{-1}. (B) Weekly mean chl *a* levels (and standard deviation) at Newport Pier averaged over 11 years (2009-2019). Dashed horizontal line indicates 2.4 µg•L^{-1}, and solid horizontal line indicates 3.0 µg•L^{-1}.