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Infrequent Fluctuations in Temperature and Salinity May Enhance Feeding in *Pisaster ochraceus* (Asteroidea) but Not in *Dendraster excentricus* (Echinoidea) Larvae

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Abstract. In recent years, low-salinity events characterized by high temperatures (18–23 °C) and low-salinity waters (20‰–22‰) have increased during late spring and summer, when many marine invertebrate larvae are developing. The present study examines the effects of low-salinity events on particle ingestion for larvae of two echinoderm species, the sea star *Pisaster ochraceus* and the sand dollar *Dendraster excentricus*. Larvae were exposed to high temperatures and low salinities for 24 hours, followed by feeding on the alga *Isochrysis galbana* in high or low salinity for another 10 minutes. Exposing *Pisaster* larvae to high temperatures and low salinities, followed by feeding in low salinity, did not impair ingestion rates. In fact, these larvae ingested particles at similar and sometimes higher rates than those in the controls. In sharp contrast, a 24-hour exposure to a high temperature and low salinity, followed by continued exposure to low salinity to feed, led to a decrease in the number of particles ingested by 8-arm *Dendraster* larvae. Larvae of both species captured very few particles when returned to 30‰ after a low-salinity event, indicating that continuous interruption of larval feeding by low-salinity events during development could be deleterious. Sand dollar larvae may have responded negatively to low-salinity events in our experiments because they are found in protected bays, where they may seldom experience these events.

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Abbreviations: AAL, anterolateral arm length; CBL, ciliated band length; FSW, filtered seawater; HSD, honest significant difference; PAL, preoral arm length; TLL, total larval length; TLW, total larval width; T↓S↑, low temperature-high salinity control treatment; T↓S↓, low temperature-low salinity treatment; T↑S↓, high temperature-low salinity treatment.

Introduction

Over the past few decades, the uptake of more than 90% of excess heat from the earth's atmosphere has led to an increase in ocean temperatures. Climate models predict that the oceans will continue to take up heat, with temperatures in the top 200 m projected to increase by between 1.5 °C and 2.0 °C by 2100 (Bindoff *et al.*, 2019). Coastal ecosystems are particularly sensitive to ocean warming, with temperatures expected to rise even higher (2.9–3.4 °C on rocky shores and 2.3–3.0 °C on sandy shores; Bindoff *et al.*, 2019).

The consequences of rising temperatures on marine invertebrates, the most abundant and diverse group of animals living in coastal ecosystems, are enormous. Rising temperatures are decreasing adult foraging time, growth, survival, and reproduction and are increasing susceptibility to disease (Harley, 2011; Hewson *et al.*, 2014; Jurgens *et al.*, 2015; Eisenlord *et al.*, 2016; Menge *et al. et al.*, 2016; Montecino-Latorre *et al.*, 2016; Miner *et al.*, 2018; Schiebelhut *et al.*, 2018; Harvell *et al.*, 2019). Thorson (1950) estimated that 70% of these marine invertebrates have planktotrophic larval development. Planktotrophic larvae need to feed in the plankton for a certain length of time (a few days to months, depending on species) to grow and develop to metamorphosis. High temperatures can boost developmental rates only if larvae acquire enough food to sustain the increased rate of growth (Hoegh-Guldberg and Pearse, 1995). Food scarcity and predation already take a tremendous toll on marine invertebrate larvae, with mortality exceeding 99% (Thorson, 1950). Because females of some marine invertebrates produce up to 70 million offspring annually (Jablonksi and Lutz, 1983; SBG, pers. obs.), it is believed that only 0.0001% to 0.00005% is needed to maintain adult populations. However, adult populations are already under considerable stress, the number of offspring produced varies

seasonally and annually (George, 1996; George and Walker 2007), and not all marine invertebrates with planktotrophic larvae produce millions of offspring. Further environmental stress during offspring development, such as rising temperatures, could have a negative effect on larval feeding, leading to increases in the time spent in the plankton, increases in larval mortality, shifts in population distribution, a decline in population abundance, and, ultimately, food web instability (Prather *et al.*, 2013).

Another global concern is that rising temperatures are accelerating the rate at which ice is melting. The effects of ice melt are becoming quite noticeable in many parts of the world. For example, an increase in arctic ice melt is translating into an increase in the amplitude and frequency of freshwater discharge from rivers into estuaries and other coastal habitats (Khangaonkar *et al.*, 2011, 2017, 2019). In the Pacific Northwest, sea surface salinities can drop suddenly to 20‰, with values less than 9‰ recorded near the mouths of rivers (Held and Harley, 2009; Bashevkin *et al.*, 2016). These values are recorded in a region historically characterized by cold waters with high sea surface salinities of between 26‰ and 32‰ (Strathmann, 1971; Sutherland *et al.*, 2011). Even the timing of maximum river discharge has shifted to spring (Masson and Pena, 2009; Riche *et al.*, 2014), the start of the reproductive season for many marine invertebrates (Strathmann, 1987).

The effect of low salinity is a major threat for coastal marine invertebrates, especially those with poor abilities to osmoregulate their extracellular fluids (Kinne, 1971; Stickle and Diehl, 1987). In a recent review by Russell (2013), 43 echinoderm species were found in hyposaline environments. Despite species being found in low-salinity environments, low salinity induces metabolic depression (Rivera-Ingraham and Lignot, 2017) and decreases adult foraging activity (Stickle and Diehl, 1987; Held and Harley, 2009; Garza and Robles, 2010), mobility (Held and Harley, 2009; Garza and Robles, 2010), and reproductive output (Stickle and Diehl, 1987; Held and Harley, 2009).

Echinoderm larvae are even more sensitive to low salinity. Though they can tolerate salinities of 20‰ or less (Russell, 2013), there have been reports of empty larval stomachs (Roller and Stickle, 1993), abnormal larval development, high larval mortality (Roller and Stickle, 1985, 1994; Stickle and Diehl, 1987; see Russell, 2013; Bashevkin *et al.*, 2016), low juvenile production (George and Walker, 2007), and poor larval swimming (Bashevkin *et al.*, 2016). For instance, when early larval stages of *Pisaster ochraceus* were exposed to low salinity, advanced larval stages had difficulty maintaining their position in haloclines with a food patch (Bashevkin *et al.*, 2016). Salinity-induced morphological changes have also been observed for echinoderm larvae exposed to low-salinity waters. Pia *et al.* (2012) noted that sea star gastrulae of *P. ochraceus* exposed for 3 days and bipinnaria larvae exposed for 14 days at 20‰ developed into shorter and wider larvae, while brachiolaria larvae exposed for 3 days at 20‰ and those reared at 30‰ throughout development were longer and more slender. Similar salinity-induced morphological changes were observed by George and Walker (2007) and Bashevkin *et al.* (2016). In George and Walker's (2007) study, 15- and 18-day-old sand dollar larvae exposed to 22‰ for 7 days, starting from the 4-arm stage, were bigger, with longer posterodorsal and anterolateral arms than those exposed to low salinity, starting from the 6-arm larval stage. However, the consequences of these changes on the ability to feed in the water column were not addressed.

Several studies (Strathmann, 1971; Hart, 1991; Hart and Strathmann, 1995) have documented feeding by planktotrophic echinoderm larvae, with those having longer ciliated bands capturing more particles (Hart and Strathmann, 1994; Rendleman *et al.*, 2018). Although feeding by planktotrophic echinoderm larvae has been very well documented, to our knowledge the combined effects of temperature and salinity on the ingestion of particles have not. A recent meta-analysis study of the effects of temperature and salinity on marine invertebrate larvae showed that interactions between these two variables were synergistic (Przeslawski *et al.*, 2015). Thus, the present study investigated the combined effects of temperature and salinity on particle ingestion by *P. ochraceus* and *Dendraster excentricus* larvae—the former a keystone sea star in the rocky intertidal zone (Paine, 1966) and the latter a sand dollar among the most abundant macroinvertebrates of the northeastern Pacific Ocean (Mooi, 1997). To predict the future of marine invertebrate populations, it is important to document the effects of a changing climate, specifically, increasing temperatures and decreasing salinities on larval feeding.

Sea star larvae were subjected to a 24-hour low-salinity event, consisting of high temperature and low salinity, conditions they experience in the field almost every summer (Bashevkin *et al.*, 2016; SBG, pers. obs.), and to a low-temperature and low-salinity treatment, conditions they may also experience as they develop in the water column. The number of particles ingested was assessed, ciliated band length (CBL) was measured for all bipinnaria larvae, and CBL and total larval length (TLL) and total larval width (TLW) were measured for 50- and 54-day-old brachiolaria larvae. The number of particles ingested was also assessed for sand dollar larvae acclimated to a low-salinity event. The TLL, anterolateral arm length (AAL), preoral arm length (PAL), and CBL of sand dollar larvae were measured. Because both species are stenohaline osmocomformers and generally develop at temperatures between 12 °C and 15 °C, we expect that sudden changes in salinity (from 30‰ to 20‰ or from 20‰ to 30‰) and high temperatures (18–23 °C) will decrease the number of particles ingested. We also expect that the number of cells ingested will increase with larval size and CBL. We further hypothesized that *Pisaster* larvae may be less susceptible and *Dendraster* larvae more susceptible to low-salinity events, because adult populations of these two species do not experience similar environmental conditions in nature. *Pisaster* populations are found in the rocky intertidal and subtidal zone, with some

populations close to areas where salinity can be as low as 9‰, while *Dendraster* populations do not generally experience salinities below 28‰ and are found in sandy exposed and protected coastal areas (Emlet, 1986; Mooi, 1997).

Materials and Methods

Pisaster ochraceus

Adult collection, spawning, fertilization, and larval rearing. Spawning, fertilization, and rearing, from the embryo through the early (bipinnaria) and advanced (brachiolaria) larval stages, were accomplished using established methods (Strathmann, 1987, 2014; George, 1999; Bashevkin *et al.*, 2016; Hodin *et al.*, 2019). In May and June 2017, 5 and 7 adult sea stars, respectively, were collected from the rocky intertidal at Point Caution, San Juan Island, Washington (48.563240, −123.028537), and transported to Friday Harbor Laboratories in buckets containing a few rocks and a small amount of seawater to provide attachment and to keep them moist. They were held in a lab outfitted with running seawater tables pumped in directly from the harbor and fed an abundant supply of mussels daily; tanks were cleaned often to maintain a high-quality environment for the sea stars and to prevent clogging of the sea tables. All sea stars were injected with between 2.5 and 4 mL of 10^{-4} 1-methyladenine (depending on their size) to induce spawning. In May, all individuals spawned. In June, only one male and one female spawned.

Eggs and sperm were collected from the May and June spawning events and fertilized with 8–10 drops of dilute sperm. Fertilization success was 99%–100%. Fertilized eggs were split into 4 or 5 stock jars containing 30‰ filtered seawater (FSW) and left to develop to the gastrula stage. Two days later, swimming sea star embryos were distributed into 15, 3.78-L glass jars (1 larva per 10 mL) and placed in the sea table. For May cultures, all embryos were kept at 30‰ throughout development; for June cultures, 4 jars were kept at a salinity of 20‰, and the rest were kept at 30‰. Temperatures in the sea table varied between 12 °C and 14 °C. As the embryos progressed through the various larval stages, they were fed *Dunaliella tertiolecta* and *Rhodomonas* sp. (1000–5000 cells mL^{-1}), known to promote optimal growth conditions (George, 1999; Schiopu *et al.*, 2006; Pia *et al.*, 2012), and kept in suspension by using a system of swinging paddles (Strathmann, 1987). Both May and June sea star embryos and larvae were kept in clearly labeled jars in the sea table and allowed to develop for 16 days before the first set of feeding experiments. Larvae were starved for several days before all feeding experiments. All algae, including *Isochrysis galbana*, were grown in f/2 medium under a 12h:12h light:dark cycle and harvested at the exponential phase.

Experiment 1: effect of low-salinity events (T↑S↓) on the number of particles ingested and the ciliated band length for Pisaster *larvae*

Bipinnariae. To determine the effect of low-salinity events on the number of particles ingested per minute by *Pisaster* larvae, 16-day-old bipinnariae were selected from several 3.78-L jars containing 30‰ 0.45-μm FSW, from the June spawning event, and distributed into 6 1000-mL beakers: 2 replicate beakers served as the controls, low temperature (12–14° C) and high salinity (30‰–32‰) (T↓S↑); 2 beakers were held at low temperature and low salinity (20‰) (T↓S↓); and 2 beakers simulated high-temperature (18–23 °C) and low-salinity (T↑S↓)—conditions they may encounter as they develop in the plankton (see Bashevkin *et al.*, 2016). A high-temperature and high-salinity treatment was not used because these environmental conditions have not been reported in this region (see Khangaonkar *et al.*, 2011, 2019; Sutherland *et al.*, 2011). Each beaker contained 500 mL of 0.45-μm FSW and 20–30 bipinnariae. For each treatment, an attempt was made to select bipinnariae that were visibly similar in size.

After 24 hours, 10–15 bipinnariae per beaker were transferred into each of 10 150-mL bowls filled with 100 mL of 0.45-μm FSW water and 1000 cells mL^{-1} of the small alga *I. galbana* (algal cell diameter: 5–6 μL): 2 bowls for the controls, 4 bowls for the T↓S↓ treatments, and 4 bowls for the T↑S↓ treatments. For the T↓S↓ and T↑S↓ treatments, 2 of the bowls contained 30‰ FSW and algal cells (referred to as T↓S↓ fed in 30‰ and T↑S↓ fed in 30‰, respectively), and 2 contained 20‰ FSW and algal cells (referred to as T↓S↓ fed in 20‰ and T↑S↓ fed in 20‰ treatments, respectively). *Isochrysis galbana* was chosen for these experiments because it can be cultured in 20‰ and 30‰ and at variable temperatures (Kaplan *et al.*, 1986). Furthermore, these algal particles can be easily seen in larval stomachs. An estimate of the total numbers of algal cells ingested per minute was made using methods similar to those described in Strathmann (1971).

After 10 minutes, 10 bipinnariae were gently removed from each bowl with a 2-mL plastic pipette and placed on a slide, and the number of cells ingested, clearly seen in their stomachs, was counted under a compound microscope. Although ingestion rates obtained using this technique can be low and quite variable, Strathmann (1971) noted that it is useful for comparative studies, as described here. Feeding experiments were repeated for 26-, 39-, and 41-day-old bipinnariae (from the June spawning, reared in 30‰ FSW throughout their development) and for 28-day-old bipinnariae (from the June spawning, reared in 20‰ FSW throughout their development; Fig. 1A).

Pictures and videos of 10 larvae per treatment were taken to measure CBL and TLL (Fig. 1A, B). The CBL was obtained by summing the traced perimeter measurements of the median dorsal and median ventral processes of bipinnariae in ImageJ (https://imagej.nih.gov/ij/; Fig. 1A, B). A total of 5 larval ages, corresponding to 750 early and late bipinnariae, were used in these experiments. All digital images of larvae were taken with a video camera attached to an Olympus compound microscope. Images captured using BTV Pro (Ben Software, London) were analyzed in ImageJ.

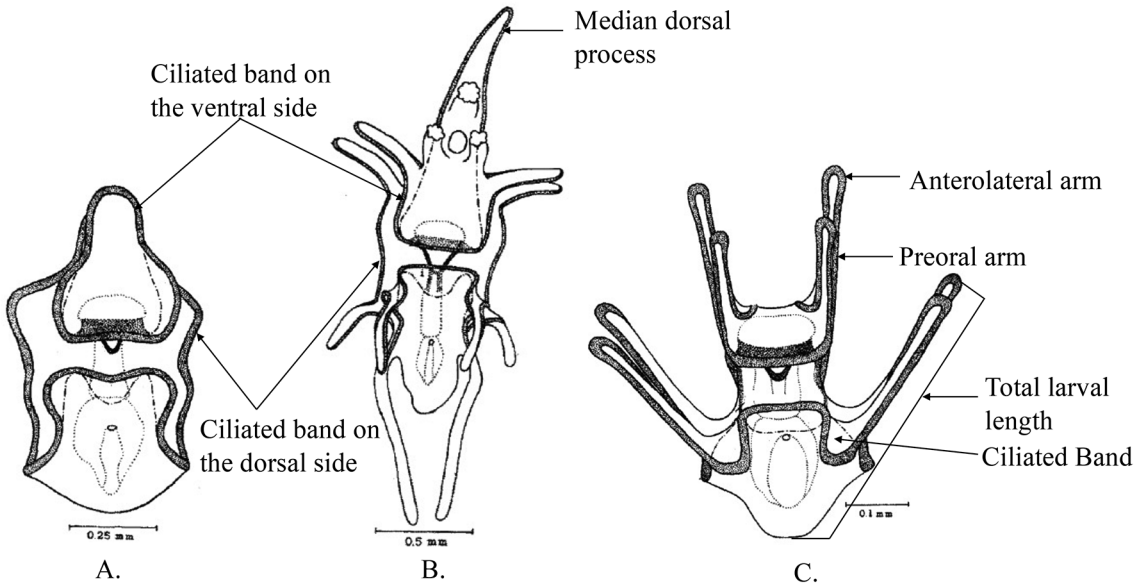

Figure 1. Echinoderm larvae showing measurements made. Ciliated band, median dorsal, and ventral processes of bipinnaria larvae (A) and brachiolaria larvae (B) for the sea star *Pisaster ochraceus* and the ciliated band, total larval length (where total larval length refers to the post-oral arm rod plus the body rod), anterolateral arm length, and preoral arm length for larvae (C) of the sand dollar *Dendraster excentricus*. Adapted and modified with permission from Elsevier (from Strathmann, 1971).

Brachiolariae. The above experiment was repeated for similar-sized 50- and 54-day-old brachiolariae (from the May spawning reared in 30‰ FSW throughout their development); 50- and 54-day-old brachiolariae (Fig. 1B) from the May spawning event fed at extremely high rates and, thus, were allowed to feed for only 30 seconds (see video on feeding by brachiolariae larvae in George and Strathmann, 2019). Strathmann (1971) also observed very high clearance rates for advanced stages of this species. After 30 seconds, brachiolariae were removed individually from each bowl, and the total number of cells in the stomachs of each were counted under the compound microscope for 30 seconds. Pictures and videos of 10 larvae per treatment were taken to measure CBL and TLL and TLW (Fig. 1A, B). A total of 300 brachiolariae were used in these experiments. Images captured using BTV Pro were analyzed in ImageJ.

To summarize, bipinnaria and brachiolaria larvae were reared under T↓S↑ or T↓S↓ until the day of the experiment. These larvae were exposed for 24 hours to one of the following treatments: T↓S↑ (control), T↓S↓, or T↑S↓. The 10-minute (bipinnariae) and 30-second (brachiolariae) feeding trials took place the next day at 30‰ for all 3 treatments and at 20‰ for T↓S↓ and T↑S↓.

Data analysis. To determine the effects of low-salinity events on larval morphology, differences in CBL for all seven larval ages (bipinnariae and brachiolariae), and total length and width measurements for brachiolaria larvae from all five treatments, were assessed with a one-way or two-way ANOVA with interaction (larval age × temperature-salinity treatment). Larval age and temperature-salinity treatment were fixed factors. The effect of low-salinity events on the number of particles ingested per minute was also analyzed using a two-way ANOVA with interaction (larval age and temperature-salinity treatment as fixed factors). The effect of CBL on particle ingestion was also assessed with an ANCOVA; CBL was the covariate, and CBL × temperature-salinity treatment was used to determine equality of slopes. The data did not satisfy the assumptions of an ANOVA and were Box-Cox transformed before analysis. When differences were significant, pairwise comparison of means was made using Tukey honest significant difference (HSD) (Sokal and Rohlf, 2014; JMP ver. 13, SAS Institute, Cary, NC).

Dendraster excentricus

Spawning, fertilization, and larval rearing. Adult sand dollars were collected in May from Crescent Beach, East Sound, on Orcas Island, Washington (48.695359, −122.894712). To induce spawning, 8 individuals were injected with 2 mL of 0.5 mol L^{-1} KCL in June 2017. All individuals spawned. Eggs from five females were pooled and fertilized with sperm from three males. Fertilization success was 100%. After 24 hours, an estimate of the total number of swimming embryos was made by counting 5, 1-mL subsamples from stock jars (for more details, see George and Walker, 2007). Embryos were distributed into 10 jars, each having an initial density of 0.67 embryos mL^{-1} (2000 embryos in 3000 mL of fresh FSW). They were

fed a similar diet to *Pisaster* larvae and kept in suspension in a sea table with a system of swinging paddles (Strathmann, 1987) at temperatures of between 12 °C and 14 °C. Prior to each feeding experiment, larvae were starved for several days to clear their stomachs of algal particles.

Experiment 2: effect of low-salinity events (T↑S↓) on the number of particles ingested and the ciliated band length for Dendraster *larvae*

We wished to determine whether sand dollar larvae that were acclimated to a T↑S↓ treatment (temperatures between 20 °C and 23 °C and salinity 20‰) would ingest particles at a higher rate than those acclimated to a T↓S↓ treatment (temperatures between 12 °C and 14 °C and salinity of 20‰). Thus, 2-day-old sand dollar larvae were distributed into 3 treatments: 4 replicate 3.78-L jars for the controls (temperatures between 12 °C and 14 °C and salinity of 30‰, referred to as T↓S↑ in figures), 4 replicate jars for the T↓S↓ treatment (referred to as T↓S↓ in figures), and 2 replicate jars for the T↑S↓ treatment (referred to as T↑S↓ in figures). A 10-day acclimation period was used based on observations made by George and Walker (2007).

After 10 days, 20–30, 12-day-old 8-arm sand dollar larvae from the control acclimated treatment were distributed into 3 treatments for a further 24 hours: 2 replicate beakers for the control (T↓S↑), 2 for the T↓S↓ treatment, and 2 for the T↑S↓ treatment. Each replicate beaker contained 500 mL of 0.45-μm FSW. This was repeated for the other two acclimation treatments, with six beakers per acclimation treatment. A total of 18 beakers were set up for the 3 acclimation treatments.

After 24 hours, 15–20, 8-arm sand dollar larvae per beaker were transferred into each of 10 150-mL bowls filled with 100 mL of 0.45-μm FSW and 1000 cells mL^{-1} of the small alga *Isochrysis galbana*: 2 bowls for the controls (T↓S↑), 4 bowls for the T↓S↓ treatment, and 4 bowls for the T↑S↓ treatment. For the T↓S↓ and T↑S↓ treatments, 2 bowls contained 30‰ FSW and 2 contained 20‰ FSW. After 10 minutes, 10 sand dollar larvae were removed from each bowl, and the total number of algal cells in the stomachs of each larva was counted under a compound microscope and recorded. This experiment was repeated for larvae acclimated to the other two treatments (T↑S↓ and T↓S↓). Images captured using BTV pro software were analyzed using ImageJ. The CBL was obtained by tracing the ciliated band along all eight larval arms and the oral hood of sand dollar larvae in ImageJ (Fig. 1C). The TLL (where TLL refers to the post-oral arm rod plus the body rod), AAL, and PAL were also measured.

To summarize, 2-day-old sand dollar larvae were reared for 10 days under 3 treatments, T↓S↑, T↓S↓, or T↑S↓. Larvae from the T↓S↑ treatment were further exposed to T↓S↑ (control), T↓S↓, or T↑S↓ for 24 hours. This was repeated for T↓S↓ and T↑S↓. The 10-minute feeding trials took place the next day at 30‰ for all 3 treatments and at 20‰ for T↓S↓ and T↑S↓.

Data analysis. To determine the effects of low-salinity events on larval morphology, differences in TLL, AAL, PAL, and CBL were assessed with a one-way ANOVA. Temperature-salinity treatment was a fixed factor. The number of algal cells in larval stomachs (ingested per minute) was analyzed using a two-way ANOVA with interaction (temperature-salinity and acclimation treatment were main factors) after square-root or Box-Cox transformation. The effect of CBL on particle ingestion for sand dollar larvae from the five treatments was also assessed using ANCOVA, with CBL as a covariate and with the CBL × temperature-salinity treatment used to determine equality of slopes. JMP statistical software (ver. 13) was used to analyze all data.

Results

Ciliated band length and other morphological measurements for Pisaster *larvae*

Bipinnariae. Bipinnariae from the T↑S↓ treatment fed in 20‰ had ciliated bands that were just as long as those from the controls (T↓S↑) and the T↓S↓ treatment fed in 20‰ (Fig. 2A; 2-way ANOVA with interaction, Tukey HSD, $P < 0.05$). Larvae from these treatments had the longest CBLs. Those from the T↑S↓ and T↓S↓ treatments fed in 30‰ had the shortest CBLs (Fig. 2A). There was no interaction between larval age and salinity treatment (2-way ANOVA with interaction, n [total number of individuals] = 544, $P = 0.1222$).

As expected, CBL increased significantly with larval age for all 5 salinity treatments ($P < 0.0001$, $n = 544$): 16-day-old bipinnariae had the shortest CBL (3738.9 ± 165.4 μm), followed by 26- and 28-day-old bipinnariae (5339.9 ± 153.1 μm and 5523.8 ± 141.9 μm, respectively); 39- and 41-day-old bipinnariae had the longest CBL (8295.4 ± 144.9 μm and 8094.9 ± 146.2 μm, respectively. All values are means ± standard error; Fig. 2A).

Brachiolariae. The CBL did not differ significantly between the 5 treatments for 50-day-old brachiolariae (range: 9394.4 ± 959.7 μm to 13,078.3 ± 910.4 μm) but did for 54-day-old brachiolaria larvae (2-way ANOVA with interaction, $n = 138$, $P = 0.0378$; Fig. 3A). Among 54-day-old brachiolariae, those from the T↑S↓ treatment fed in 20‰ had long CBLs that did not differ in length from those in the controls (8765.2 ± 643.8 μm and 6882.9 ± 627.5 μm, respectively). Those in the T↑S↓ treatment fed in 30‰ had the shortest CBLs (6123.6 ± 935.5 μm; Fig. 3A). The CBLs for larvae from the T↓S↓ treatment fed in 20‰ or 30‰ were 11,607.1 ± 627.5 μm and 10,746.4 ± 643.8 μm, respectively. A similar pattern was observed for TLL and TLW, with no significant differences among 50-day-old larvae (Fig. 4A). However, 54-day-old brachiolariae from the T↑S↓ treatment fed in 30‰ were shorter and narrower, while those in the other treatments were longer and wider (Fig. 4B). Basically, larval size decreased by 21% for brachiolariae from the T↑S↓ treatment fed in 30‰.

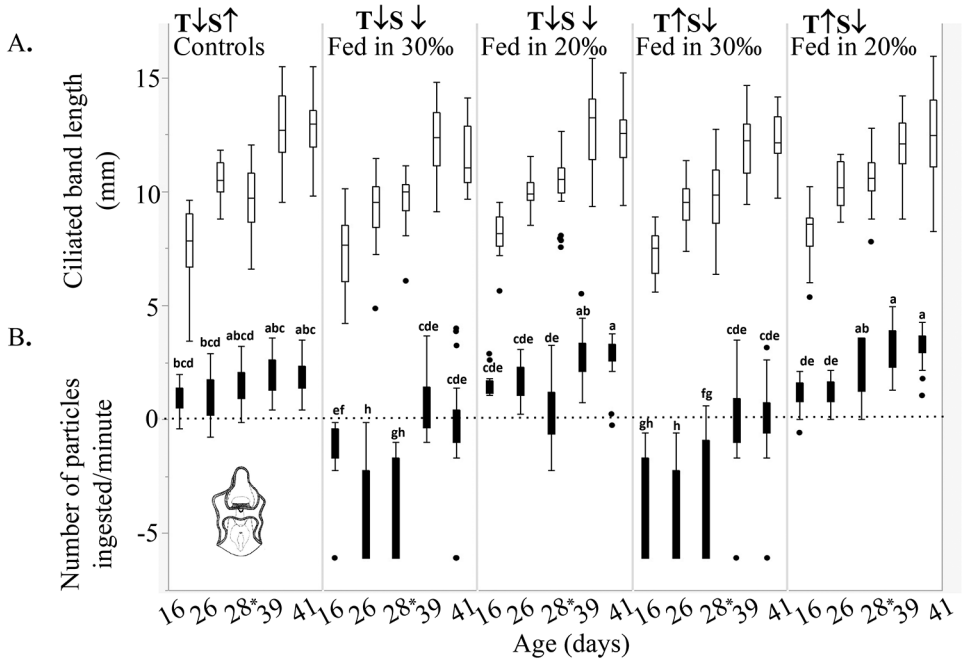

Figure 2. Transformed ciliated band length and particles ingested by bipinnariae exposed to 5 temperature-salinity treatments for 24 hours. Boxplots of ciliated band length (mm; A) and number of particles ingested per minute (B) by 16-, 26-, 28-, 39-, and 41-day-old bipinnariae of the sea star *Pisaster ochraceus* exposed to 5 treatments: control (T↓S↑), low temperature-low salinity (T↓S↓) fed in 30‰, low temperature-low salinity (T↓S↓) fed in 20‰, high temperature-low salinity (T↑S↓) fed in 30‰, and high temperature-low salinity (T↑S↓) fed in 20‰. The asterisk at 28 indicates that 28-day-old larvae were kept at low salinity for 26 days. All data were transformed before statistical analysis. The values on the *y*-axis are Box-Cox-transformed data. The following equations were used to transform the data: (ciliated band length in mm$^{-0.008}$ − 1)/−0.001334303 and (number of algal cells ingested per minute$^{0.2}$ − 1)/0.1642544745. The horizontal dotted line at 0 in the figure represents 1 algal cell ingested per minute; values below 0 represent no feeding by larvae, and values above 0 indicate that greater than 1 algal cell was ingested per minute by larvae from the 5 treatments. Different lowercase letters indicate significant differences in the number of particles ingested (two-way ANOVA with interaction, Tukey honest significant difference [HSD], $P < 0.05$) by larvae from the five treatments. The length of the box is the interquartile range; the horizontal line in the box is the median (when this line is in the middle of the box the data are symmetrical); the bottom and top of the box are the 25th and 75th quartiles, respectively; the upper and lower whiskers extend to the maximum and minimum values; and the dots are outliers.

Particles ingested per minute by Pisaster *larvae*

Bipinnariae. The number of algal cells ingested per minute by bipinnariae differed significantly between the five treatments within larval age groups (2-way ANOVA with interaction, $P < 0.0001$; Figs. A1, 2B; Table 1). The number of particles ingested was significantly higher for those in the control (T↓S↑), T↓S↓, and T↑S↓ treatments fed in 20‰ than for those from the T↑S↓ and T↓S↓ treatments fed in 30‰ (Fig. 2B). Low particle ingestion was especially noticeable between 16-, 26-, and 28-day-old larvae that were transferred into bowls with 30‰ to feed. Although the number of particles ingested increased for 39- and 41-day-old larvae kept in T↓S↓ and T↑S↓ treatments for 24 hours, then fed in 30‰ FSW, the number of particles remained significantly lower than for bipinnariae in the control (T↓S↑), T↓S↓ treatment fed in 20‰, and T↑S↓ treatment fed in 20‰ (Fig. 2B). Results remained the same when data were analyzed with CBL as a covariate (ANCOVA, $P < 0.0001$; Table 2). Data for 28-day-old larvae reared at 20‰ for 26 days should be viewed with caution because CBL varied significantly between temperature-salinity treatments (ANCOVA, $P = 0.0306$; Table 2).

Brachiolariae. A similar pattern in the number of particles ingested per minute was observed for 50- and 54-day-old brachiolariae from the 5 treatments, with significant differences in the number of cells ingested between treatments (2-way ANOVA with interaction, $P < 0.0001$; Fig. 3B). The number of particles ingested was significantly higher for those in the T↑S↓ treatment fed in 20‰, those in the control (T↓S↑), and those in the T↓S↓ treatment fed in 20‰ than for those from treatments fed in 30‰ (Fig. 3B; Tukey HSD, $P < 0.05$). The interaction between larval age and treatment was significant ($P = 0.0013$). This might be due to a significant drop in the number of particles ingested for 54-day-old brachiolariae in

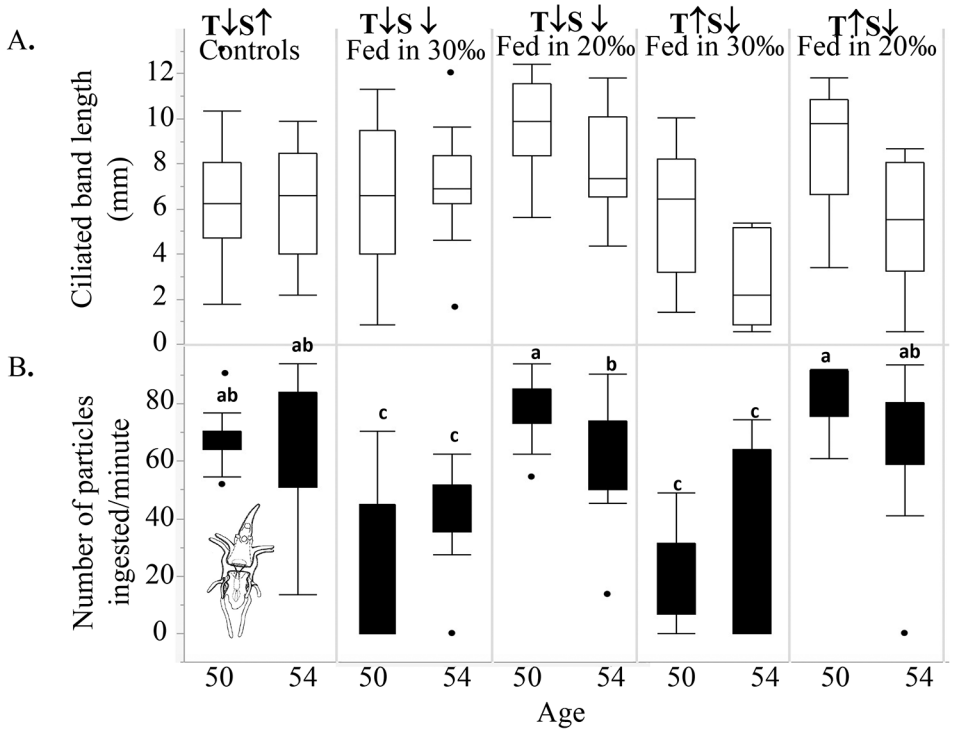

Figure 3. Transformed ciliated band length and particles ingested by brachiolariae exposed to 5 temperature-salinity treatments for 24 hours. Boxplots of ciliated band length (mm; A) and number of particles ingested per minute (B) by 50- and 54-day-old brachiolariae of the sea star *Pisaster ochraceus* exposed to 5 treatments: control (T↓S↑), low temperature-low salinity (T↓S↓) fed in 30‰, low temperature-low salinity (T↓S↓) fed in 20‰, high temperature-low salinity (T↑S↓) fed in 30‰, and high temperature-low salinity (T↑S↓) fed in 20‰. All data were transformed before statistical analysis. The values on the *y*-axis are Box-Cox-transformed data. The following equation was used to transform the data: (ciliated band length in $mm^{1.613} - 1$)/6.4538106967. Different lowercase letters indicate significant differences in the number of particles ingested (two-way ANOVA with interaction, Tukey honest significant difference [HSD], $P < 0.05$) by larvae from the five treatments. The length of the box is the interquartile range; the horizontal line in the box is the median (when this line is in the middle of the box the data are symmetrical); the bottom and top of the box are the 25th and 75th quartiles, respectively; the upper and lower whiskers extend to the maximum and minimum values; and the dots are outliers.

the T↓S↓ treatment fed in 20‰ compared to 50-day-old larvae. The 54-day-old brachiolariae in the T↑S↓ treatment fed in 20‰ ingested significantly higher numbers of algal cells than those from the T↓S↓ and T↑S↓ treatments fed in 30‰ (Fig. 3B). No effect of larval age was observed (2-way ANOVA with interaction, $P = 0.8935$; Table 1); CBL as a covariate did not affect particle ingestion (ANCOVA; Table 2). Maximum ingestion rates for 50-day-old brachiolariae were 75 cells min^{-1} for the controls, 182 cells min^{-1} for T↑S↓ fed in 20‰, 28 cells min^{-1} for T↑S↓ fed in 30‰, 86 cells min^{-1} for T↓S↓ fed in 20‰, and 11 cells min^{-1} for T↓S↓ fed in 30‰. For 54-day-old brachiolariae, maximum cells ingested were 121 cells min^{-1} for the controls, 108 cells min^{-1} for T↑S↓ fed in 20‰, 30 cells min^{-1} for T↑S↓ fed in 30‰, 72 cells min^{-1} for T↓S↓ fed in 20‰, and 19 cells min^{-1} for T↓S↓ fed in 30‰.

In summary, the T↑S↓ treatment had a positive effect on the number of particles ingested for sea star larvae of all ages, as long as they fed in the salinity they had been acclimated to for 24 hours. Particle ingestion by these larvae was four- to sevenfold higher than for the other salinity treatments. A return to 30‰ to feed after 24 hours at 20‰ led to a significant decrease in the number of particles ingested by all bipinnariae and brachiolariae. In some cases, larval stomachs were completely empty.

Ciliated band length and other morphological measurements for 8-arm Dendraster *larvae*

The CBL differed significantly among larvae from the three acclimation treatments (control T↓S↑, T↓S↓, and T↑S↓). Eight-arm sand dollar larvae in the controls had significantly longer CBLs than those acclimated to T↑S↓ and T↓S↓ treatments (2-way ANOVA with interaction, $P < 0.0001$; Fig. 5A). The CBL decreased by approximately 42% for sand larvae in the T↑S↓ and T↓S↓ treatments. Similar observations were made for TLL, AAL, and PAL (Fig. 6). Larval size decreased by 26% in TLL, by >50% in PAL, and by 33% in AAL for those in the T↑S↓ and T↓S↓ treatments. The CBL and all other

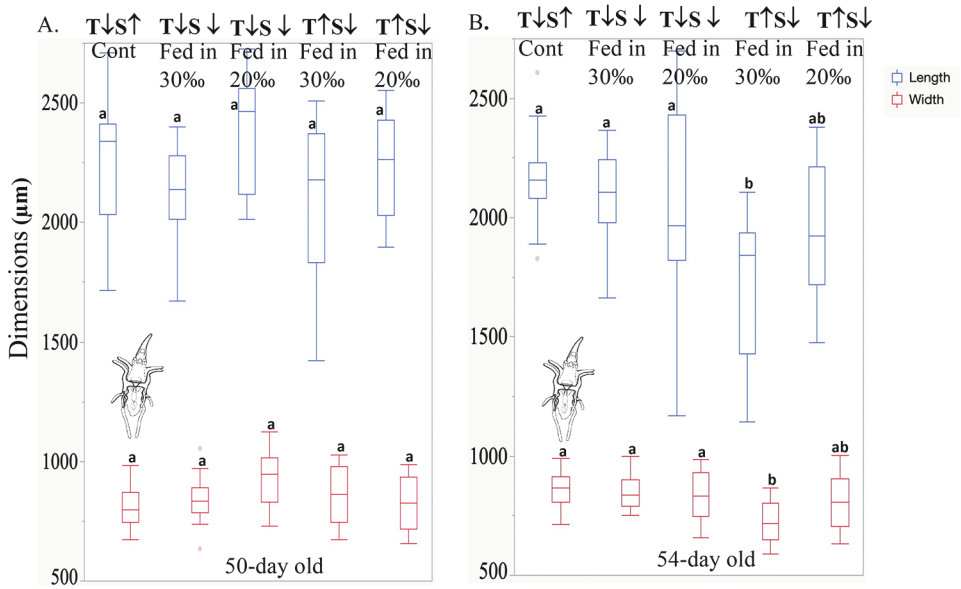

Figure 4. Total larval length and width for brachiolaria larvae exposed to 5 temperature-salinity treatments for 24 hours. Boxplots of total larval length and larval width (μm) for 50-day-old (A) and 54-day-old (B) brachiolariae of the sea star *Pisaster ochraceus* exposed to 5 salinity treatments: control (T↓S↑), low temperature-low salinity (T↓S↓) fed in 30‰, low temperature-low salinity (T↓S↓) fed in 20‰, high temperature-low salinity (T↑S↓) fed in 30‰, and high temperature-low salinity (T↑S↓) fed in 20‰. Different lowercase letters indicate significant differences in the total larval length and larval width (one-way ANOVA, Tukey honest significant difference [HSD], $P < 0.05$) by larvae from the five treatments. The length of the box is the interquartile range; the horizontal line in the box is the median (when this line is in the middle of the box the data are symmetrical); the bottom and top of the box are the 25th and 75th quartiles, respectively; the upper and lower whiskers extend to the maximum and minimum values; and the dots are outliers.

morphological measurements made did not differ significantly among sand dollar larvae from the five temperature-salinity treatments within acclimation treatments ($P > 0.05$; Fig. 6).

Particles ingested per minute by 8-arm Dendraster *larvae*

Surprisingly, a 10-day acclimation period to the 3 acclimation treatments—control (T↓S↑), T↓S↓, and T↑S↓—had no effect on the number of particles ingested by 8-arm sand dollar larvae (2-way ANOVA with interaction, $P = 0.1969$, $n = 310$; Fig. 5B; Table 1). However, the number of particles ingested varied significantly between the five treatments ($F = 51.8$, $P < 0.0001$, $n = 310$; Table 1; Fig. 5B). Eight-arm larvae from the T↑S↓ treatment fed in 20‰ and 30‰, and those from the T↓S↓ treatment fed in 30‰, ingested significantly fewer algal cells than the controls and those in the T↓S↓ treatment fed in 20‰ (Fig. 5B). The lowest particle ingestion was observed for sand dollar larvae from the T↑S↓ treatment fed in 30‰. Similar results were obtained with CBL as a covariate (ANCOVA, $P < 0.0001$; Table 3). The CBL for larvae in the control acclimated treatment differed between temperature-salinity treatments ($P = 0.0018$), and the slopes were not equal for this treatment (CBL × treatment, $P = 0.0015$; Table 3). The conclusions drawn from the ANCOVA for this acclimation treatment should, thus, be viewed with caution.

In summary, the T↑S↓ treatment (fed in either 20‰ or 30‰) led to a significant drop in the number of particles ingested by sand dollar larvae, regardless of acclimation treatment. A sudden return to 30‰ after a 24-hour exposure to 20‰ led to a significant drop in the number of particles ingested by all 8-arm *Dendraster* larvae.

Discussion

Only a few studies have examined the interactive effects of high temperature and low salinity on particle ingestion by echinoderm larvae. Variation in the number of particles ingested by sea star and sand dollar larvae within a treatment and between treatments was, in a few cases, extremely high. Despite high variation, several patterns emerged from this study. A sudden change in salinity led to a significant drop in the number of particles ingested for both species. However, their response to high temperatures (18–23 °C) and low salinities (20‰) differed significantly.

Exposure of Pisaster *larvae to a low-salinity event (T↑S↓) for 24 hours, followed by continued exposure to low salinity (20‰) to feed, did not impair ingestion rates*

Exposing young (bipinnariae) and advanced (brachiolariae) larvae of *Pisaster ochraceus* for 24 hours to high temperatures

Table 1

Two-way ANOVA with interaction of the number of particles ingested for 16- to 41-day-old bipinnaria larvae, 50- to 54-day-old brachiolariae of Pisaster ochraceus*, and 13- to 17-day-old plutei of* Dendraster excentricus *kept in 5 treatments*

Source of variation	df	SS	MS	*F*	*P*
16- to 41-day-old bipinnariae					
Temperature-salinity treatment	4	1643.5675	410.891875	178.9	<0.0001
Age	4	748.1208	187.0302	81.4	<0.0001
Age × temperature-salinity treatment	16	381.3932	23.837075	10.4	<0.0001
Model	24	2852.9677	118.873654	51.8	<0.0001
Error	517	1187.3187	2.29655455		
Total	541	4040.2863	7.4681817		
50- to 54-day-old brachiolariae					
Source of variation					
Temperature-salinity treatment	4	35,923.387	8980.84675	52.36337	<0.0001
Age	1	3.085	3.085	0.01798728	0.8935
Age × temperature-salinity treatment	4	4523.722	1130.9305	6.59395866	<0.0001
Model	9	43,382.764	4820.30711	28.1050921	<0.0001
Error	142	24,354.434	171.510099		
Total	151	67,737.198	448.590715		
13- to 17-day-old 8-arm plutei					
Source of variation					
Acclimation treatment	2	0.202322	0.101161	1.63413018	0.1969
Temperature-salinity treatment	4	12.826678	3.2066695	51.7997589	<0.0001
Acclimation × temperature-salinity treatment	8	3.338676	0.4173345	6.74151997	<0.0001
Model	14	16.469975	1.17642679	19.0037121	<0.0001
Error	296	18.323911	0.0619051		
Total	310	34.793887	0.11223835		

The 5 treatments were as follows: controls, low temperature-low salinity placed in bowls with 30‰ seawater to feed, low temperature-low salinity placed in bowls with 20‰ filtered seawater (FSW) to feed, high temperature-low salinity placed in bowls with 30‰ FSW to feed, and high temperature-low salinity placed in bowls with 20‰ FSW to feed. Data for *P. ochraceus* were Box-Cox transformed and for *D. excentricus* were square-root transformed. df, degrees of freedom; SS, sum of squares; MS, mean square.

(18–23 °C) and low salinities (20‰), environmental conditions that they probably encounter during development in late spring and summer in the Pacific Northwest, did not impair the number of algal cells ingested. In fact, *Pisaster* larvae from the above treatment ingested particles at similar and sometimes higher rates than those in the controls maintained at temperatures of 12–14 °C and salinities of 30‰, environmental conditions typical of early spring in the Pacific Northwest (Khangaonkar *et al.*, 2011; Sutherland *et al.*, 2011).

These results are intriguing but not entirely surprising. First, low-salinity events characterized by high temperatures and low salinities have become quite common in this region. They can be of short duration (24 hours; SBG, pers. obs.) or last for as long as nine days (Garza and Robles, 2010). The amplitude and frequency of low-salinity events during the spring and summer months have also increased over the past decade (Sutherland *et al.*, 2011; Bashevkin *et al.*, 2016). Environmental conditions, thus, can be quite unstable during the months when these sea star embryos and larvae are developing. We know that adults of this species can acclimate to salinities of between 15‰ and 30‰ (Held and Harley, 2009; Russell, 2013) and that young (bipinnariae) and older (brachiolariae) *Pisaster* larvae can acclimate to high temperatures and low salinities within 24 hours (this study). Thus, inherited physiological traits and physiological plasticity may enable *Pisaster* larvae to feed in both T↑S↓ and T↓S↑ waters, provided all other environmental conditions remain unchanged.

Second, a larva's shape and size may be due to the interaction between its genome, the environment it is exposed to during its development, its ontogenetic stage, and random variability that occurs during development, sometimes referred to as developmental noise (Fusco and Minelli, 2010; Oostra *et al.*, 2018; Klingenberg, 2019). For instance, brachiolariae were wider and shorter, or narrower and shorter, for those exposed to 20‰ and longer and slender for those in 30‰ seawater (Pia *et al.*, 2012; Bashevkin *et al.*, 2016). Based on these earlier studies, we expected different sizes and shapes for larvae from the T↑S↓ and T↓S↑ treatments (control) and for these differences to lead to differences in ingestion rates. However, the shape and size of larvae from these two treatments did not differ significantly. Larvae from both treatments were long and wide, with long ciliated bands. The differences observed may be because brachiolariae were exposed to low salinity for much longer (3, 7, and 14 days) in the Pia *et al.* (2012) and Bashevkin *et al.* (2016) studies and for 24 hours in the present study. As Pia *et al.* (2012) noted, subjecting brachiolariae to 3 days at 20‰ did not lead to salinity-induced morphological changes, or the differences may be partly due to variation in

Table 2

ANCOVA of ingestion rates, with ciliated band length (μm) as a covariate, for 16-, 26-, 28-, 39-, 41-, 50- and 54-day-old Pisaster ochraceus *larvae obtained from individuals collected from Point Caution*

Larval age		df	SS	*F*	*P*
16-day-old	CBL	1	0.09	0.05	0.8214
$r^2 = 0.74$	Temperature-salinity treatment	4	337.38	50.22	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	12.42	1.85	0.1276
	Model	9	373.48	24.71	<0.0001
	Total	89	507.85		
26-day-old	CBL	1	5.03	2.26	0.1363
$r^2 = 0.80$	Temperature-salinity treatment	4	648.04	72.79	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	2.66	0.30	0.8783
	Model	9	822.72	41.07	<0.0001
	Total	101	1027.50		
28-day-old	CBL	1	22.07	4.79	**0.0306**
$r^2 = 0.52$	Temperature-salinity treatment	4	522.41	28.36	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	12.02	0.65	0.6262
	Model	9	579.83	13.99	<0.0001
	Total	126	1118.70		
39-day-old	CBL	1	3.53	3.10	0.0813
$r^2 = 0.53$	Temperature-salinity treatment	4	112.04	24.59	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	5.24	1.15	0.3375
	Model	9	118.48	13.16	<0.0001
	Total	113	253.38		
41-day-old	CBL	1	0.00	0.00	0.9871
$r^2 = 0.60$	Temperature-salinity treatment	4	152.60	34.42	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	16.51	3.72	**0.0073**
	Model	9	164.40	16.48	<0.0001
	Total	107	273.02		
50-day-old	CBL	1	22.93	0.20	0.6606
$r^2 = 0.83$	Temperature-salinity treatment	4	14,826.78	31.66	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	1185.34	2.53	0.0562
	Model	9	21,562.43	20.46	<0.0001
	Total	47	26,011.37		
54-day-old	CBL	1	435.40	1.74	0.1917
$r^2 = 0.38$	Temperature-salinity treatment	4	7183.64	7.16	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	1966.82	1.96	0.1096
	Model	9	10,929.59	4.84	<0.0001
	Total	81	28,979.48		

All larvae were reared in 30‰ filtered seawater (FSW) before experiments, except 28-day-old larvae reared in 20‰ FSW for 26 days. The 5 treatments were controls, low temperature-low salinity placed in bowls with 30‰ FSW to feed, low temperature-low salinity placed in bowls with 30‰ FSW to feed, low temperature-low salinity placed in bowls with 20‰ FSW to feed, high temperature-low salinity placed in bowls with 30‰ FSW to feed, and high temperature-low salinity placed in bowls with 20‰ FSW to feed. Data were Box-Cox transformed before analysis. Values in bold indicate that one of the assumptions of the ANCOVA is violated. CBL, ciliated band length (μm); df, degrees of freedom; SS, sum of squares.

offspring produced among females in a population and among populations over time (George *et al.*, 1990; George, 1996, 1999). Regardless, if the number of particles ingested depends on the length of the ciliated band (McEdward, 1986; Hart, 1991), then the results obtained in the present study on ingestion rates for larvae from the T↑S↓ and T↓S↑ treatments are not too surprising. Larvae from both of these treatments may capitalize on high phytoplankton abundance that occurs during the spring when river discharge increases in the Pacific Northwest (Masson and Pena, 2009; Durham and Stocker, 2012; Benoit-Bird and McManus, 2014).

Exposure of Pisaster *larvae to a low-salinity event (T↑S↓) for 24 hours, followed by a return to 30‰ to feed or prolonged exposure to low salinity, had a negative effect on particle ingestion*

Exposure to T↑S↓ or T↓S↓ conditions (followed by a return to 30‰ within 24 hours), simulating receding low-salinity waters, had an immediate negative effect on particle ingestion. High temperatures may increase ciliary beat frequency, as suggested by Civelek *et al.* (2013), but a sudden return from 20‰ to 30‰ salinity may reverse this response. Of note is that

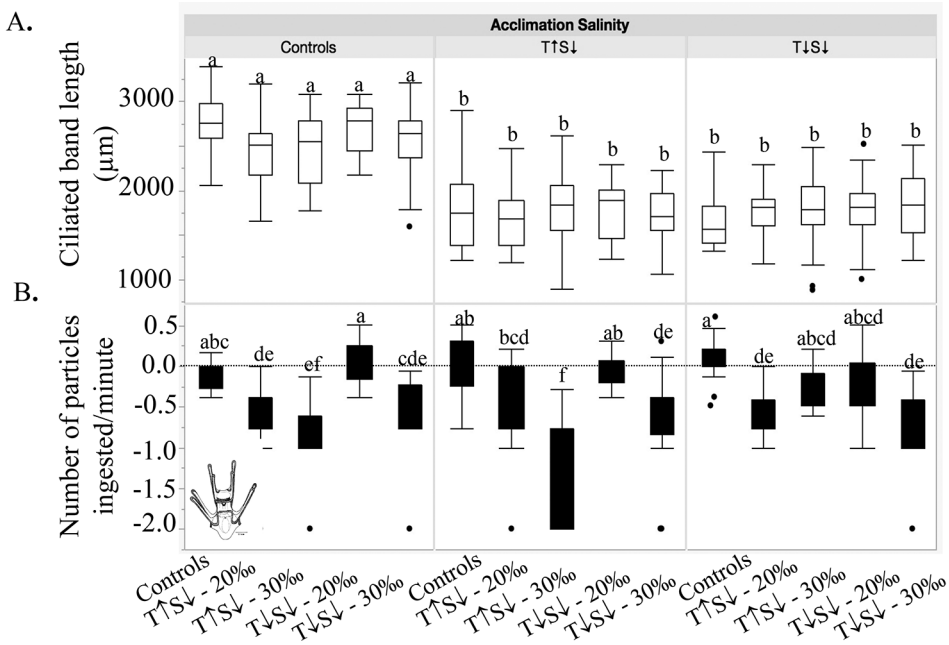

Figure 5. Transformed ciliated band length and particles ingested by plutei exposed to 5 temperature-salinity treatments for 24 hours. Boxplots of ciliated band length (μm; A) number of particles ingested per minute (B) by plutei of the sand dollar *Dendraster excentricus* acclimated to control (T↓S↑), high temperature-low salinity treatment (T↑S↓), and low temperature-low salinity (T↓S↓) treatments for 10 days, followed by exposure to 5 treatments: control (T↓S↑), T↑S↓ fed in 20‰, T↑S↓ fed in 30‰, T↓S↓ fed in 20‰, and T↓S↓ fed in 30‰. All data were transformed before statistical analysis. The values on the *y*-axis are Box-Cox-transformed data. The following equations were used to transform the data: (ciliated band length in $mm^{0.988} - 1)/0.9023000657$ and (number of algal cells ingested per $minute^{0.303} - 1)/0.5012077013$. The horizontal dotted line at 0 in the figure represents 1 algal cell ingested per minute; values below 0 represent no feeding by larvae, and values above indicate that greater than 1 algal cell was ingested per minute by larvae from the 5 treatments. Different lowercase letters indicate significant differences in the number of particles ingested (two-way ANOVA with interaction, Tukey honest significant difference [HSD], $P < 0.05$) by larvae from the five treatments. The length of the box is the interquartile range; the horizontal line in the box is the median (when this line is in the middle of the box the data are symmetrical); the bottom and top of the box are the 25th and 75th quartiles, respectively; the upper and lower whiskers extend to the maximum and minimum values; and the dots are outliers.

brachiolariae from the T↑S↓ treatment fed in 30‰ were short and narrow, with shorter ciliated bands. George and Walker (2007) and Pia *et al.* (2012) also noted that larvae reared in low-salinity waters were smaller. As mentioned in George and Walker (2007), energy is divided into the development of larval and adult structures and maintenance (*e.g.*, swimming, respiration, and feeding). The energetic costs associated with cell volume regulation and higher respiratory rates when exposed to the combined effects of temperature and salinity may have led to shrinking and arrested larval feeding. In several instances, larval stomachs were completely empty.

The 28-day-old bipinnariae exposed to 26 days in the T↓S↓ treatments fed in either 20‰ or 30‰ caught a similar number or fewer particles than young (16- and 26-day-old) bipinnariae. These results raise the question as to which is more harmful, sudden changes in salinity or prolonged exposure to low salinity, and whether there is a specific stage during development where one of these environmental conditions is more harmful than the other.

Exposure of Dendraster *larvae to a low-salinity event (T↑S↓) for 24 hours, followed by continued exposure to low salinity (20‰) to feed, had a negative effect on particle ingestion*

In sharp contrast to the response by *Pisaster* larvae, a 24-hour exposure to high temperature and low salinity, followed by continued exposure to low salinity (20‰) to feed, had a negative effect on the number of particles ingested by 8-arm *Dendraster* larvae. These results were not surprising, given the much more stable environmental conditions in East Sound, where adult sand dollar populations and developing larvae are commonly found (Emlet, 1986). Sand dollars from this population live in an extremely protected bay where waters are weakly stratified (Durham and Stocker, 2012), the effects of river discharge are negligible, and salinities remain between 28‰ and 29‰ throughout the year (Emlet, 1986). If precipitation increases, as predicted by the Intergovernmental Panel on Climate Change (Bindoff *et al.*, 2019), and if temperatures

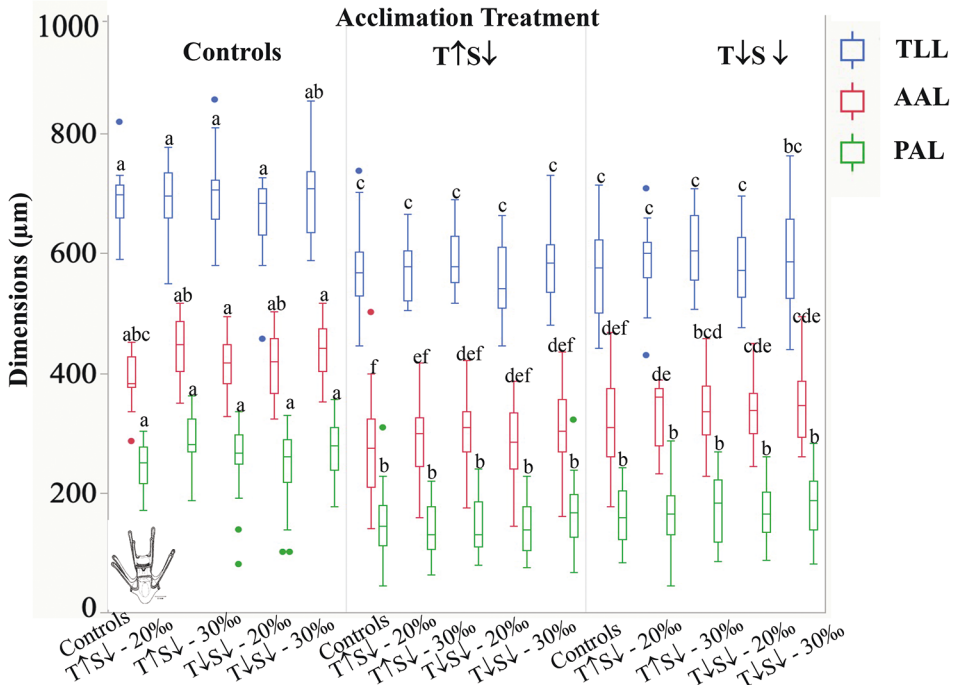

Figure 6. Morphological measurements made for sand dollar plutei acclimated to 3 treatments for 10 days and exposed to 5 temperature-salinity treatments for 24 hours. Morphological traits: total larval length (TLL), anterolateral arm length (AAL), and preoral arm length (PAL) measured for larvae of the sand dollar *Dendraster excentricus* from three acclimation treatments: control, low temperature (12–14 °C) and high salinity (30‰), and a low-salinity event (LSE) characterized by high temperature (18–21 °C) and low salinity (20‰); and 5 temperature-salinity treatments: control (T↓S↑), high temperature-low salinity (T↑S↓) fed in 20‰, high temperature-low salinity (T↑S↓) fed in 30‰, low temperature-low salinity (T↓S↓) fed in 20‰, and low temperature-low salinity (T↓S↓) fed in 30‰. Different lowercase letters indicate significant differences in the total larval length and larval width (two-way ANOVA with interaction, Tukey honest significant difference [HSD], $P < 0.05$) by larvae from the five treatments. The length of the box is the interquartile range; the horizontal line in the box is the median (when this line is in the middle of the box the data are symmetrical); the bottom and top of the box are the 25th and 75th quartiles, respectively; the upper and lower whiskers extend to the maximum and minimum values; and the dots are outliers.

continue to rise (Khangaonkar *et al.*, 2017, 2019), sand dollar populations could be at risk in shallow protected bays.

A 10-day acclimation period to a low-salinity event (T↑S↓) or low temperature-low salinity environment (T↓S↓) had no effect on particle ingestion by Dendraster *larvae*

Some very interesting patterns emerged when 2-day-old larvae were acclimated to low salinity for 10 days. All four morphological traits (CBL, TLL, PAL, and AAL) differed significantly among sand dollar larvae reared in the three acclimation treatments. Those from the control acclimated treatment had significantly longer larval arms with longer ciliated bands, while those from the other two acclimated (T↑S↓ and T↓S↓) treatments had shorter larval arms with shorter ciliated bands. Surprisingly, despite over a 20%–50% decrease in the size of the different larval arms, including the CBL among plutei from the three acclimation treatments, the number of particles ingested did not vary.

However, morphological traits did not differ significantly among sand dollar larvae from the five treatments within each acclimation treatment, but the number of particles ingested did. The number of particles ingested was always significantly higher for larvae that continued to feed in the salinity environment that they had been acclimated to (*e.g.*, control salinity T↓S↑ and the T↓S↓ treatment fed in 20‰), while the number of particles ingested dropped significantly for treatments that involved high temperatures or a sudden increase in salinity (*e.g.*, T↑S↓ fed in 20‰ or 30‰ and T↓S↓ fed in 30‰). This further highlights the energetic costs of osmotic stress, increased respiratory rates, and swimming. Changes in larval swimming behavior, such as attempting to escape when exposed to a sudden decrease in salinity, may add to total energetic costs.

To summarize, *Pisaster* larvae were less susceptible and *Dendraster* larvae more susceptible to low-salinity events. The difference in response may be partly due to differences in where they reside. *Pisaster* is found where environmental conditions can be quite unstable during larval development, while

Table 3

ANCOVA of the number of cells ingested, with ciliated band length (μm) as a covariate, for Dendraster excentricus *larvae*

Treatment		df	SS	*F*	*P*
Control acclimated	CBL	1	63.62	10.51	**0.0018**
$r^2 = 0.63$	Temperature-salinity treatment	4	689.03	28.46	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	118.16	4.88	**0.0015**
	Model	9	1108.34	20.34	<0.0001
	Total	86	1568.38		
Low salinity acclimated	CBL	1	1.84	0.16	0.6948
$r^2 = 0.48$	Temperature-salinity treatment	4	861.86	18.15	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	18.28	0.39	0.8187
	Model	9	961.73	16.65	<0.0001
	Total	97	2024.42		
LSE acclimated	CBL	1	0.28	0.02	0.8789
$r^2 = 0.47$	Temperature-salinity treatment	4	1237.94	18.44	<0.0001
Equality of slopes	CBL × temperature-salinity treatment	4	11.28	0.17	0.9541
	Model	9	1266.94	8.39	<0.0001
	Total	93	2676.85		

Larvae were acclimated to the following treatments for 10 days: control, low temperature (12–14 °C) and high salinity (30‰), and a low-salinity event (LSE) characterized by high temperature (18–21 °C) and low salinity (20‰). Larvae were then transferred to 5 treatments (controls, low temperature-low salinity placed in bowls with 30‰ filtered seawater (FSW) to feed, low temperature-low salinity placed in bowls with 30‰ FSW to feed, low temperature-low salinity placed in bowls with 20‰ FSW to feed, high temperature-low salinity placed in bowls with 30‰ FSW to feed, and high temperature-low salinity placed in bowls with 20‰ FSW to feed) for 24 hours, and the number of particles ingested per minute was recorded. Adults were collected from East Sound, Orcas Island, Washington. Values in bold indicate that one of the assumptions of the ANCOVA is violated. CBL, ciliated band length; df, degrees of freedom; SS, sum of squares.

Dendraster is found in much more stable environments. The present study also indicates that brachiolariae may be more resilient to some environmental changes than bipinnariae. These results suggest that continuous interruption of larval feeding by multiple low-salinity events during development will slow growth and development, while infrequent low-salinity waters may be advantageous for growth and development to metamorphosis. As temperatures continue to increase in the Pacific Northwest, populations of both species, as well as those of many other marine invertebrates, may become at risk. Similarly, as the oceans continue to take up heat and salinities decrease, marine organisms worldwide may be affected. Finally, particle concentration was kept constant at 1000 cells mL^{-1} of *Isochrysis galbana*. Future studies should determine whether the clearance rate of larvae subjected to the above temperature-salinity treatments differs across several algal food concentrations and types.

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Appendix

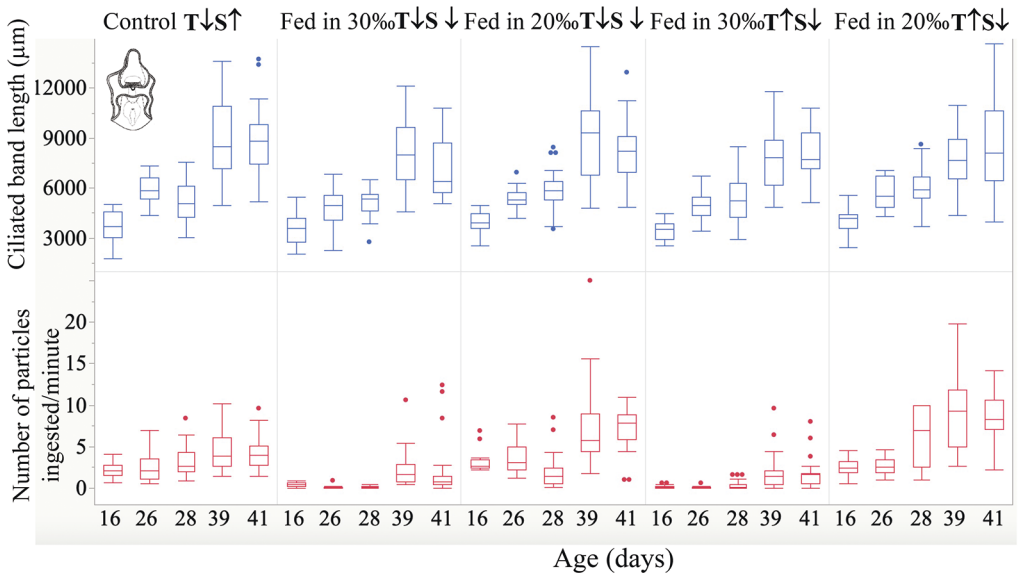

Figure A1. Untransformed ciliated band length and particles ingested by bipinnariae exposed to 5 temperature-salinity treatments for 24 hours. Boxplots of untransformed ciliated band length (μm) and untransformed number of particles ingested per minute by 16-, 26-, 28-, 39-, and 41-day-old bipinnariae of the sea star *Pisaster ochraceus* exposed to 5 salinity treatments: control (T↓S↑), low temperature-low salinity (T↓S↓) fed in 30‰, low temperature-low salinity (T↓S↓) fed in 20‰, high temperature-low salinity (T↑S↓) fed in 30‰, and high temperature-low salinity (T↑S↓) fed in 20‰. Note that 28-day-old larvae were kept at low salinity for 26 days. The length of the box is the interquartile range; the horizontal line in the box is the median (when this line is in the middle of the box the data are symmetrical); the bottom and top of the box are the 25th and 75th quartiles, respectively; the upper and lower whiskers extend to the maximum and minimum values; and the dots are outliers.