

Effects of Temperature and Food Availability on Growth and Development of *Pisaster ochraceus* Larvae

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1 Abstract

Climate change is expected to increase temperatures and shift the algal regime and biomass in the Salish Sea. Previous studies suggest that growth and survival of echinoderm larvae are dependent on temperature and access to food. However, no study we know of has tested the combined effects of these factors on larvae of the sea star *Pisaster ochraceus*. We tested the effects of sustained low and high temperatures and feeding regimes on *Pisaster ochraceus* bipinnariae and brachiolariae larvae. Thirty-three and 40 days after fertilization, we measured larval length and width, and survival. In line with expectations, size and development of larvae was heavily affected by the level of food available to them. High water temperatures of 18 °C had a small negative effect on length and width of larvae, much less than the effect of feeding. While sample size was low for survival, our study suggests that 18°C temperatures may have increased survival of *Pisaster ochraceus* larvae, while feeding treatment had no effect. These results suggest that *Pisaster* larvae are resilient to direct effects of sustained ocean warming, but may be heavily affected by secondary ecological effects like shifting algal ecology.

2 Introduction

Climate change will have profound impacts on the marine ecology of the U.S. Pacific Northwest. Models predict that sea surface temperatures will rise by an average of 1.51°C in the Salish Sea and by 4°C along the outer coast of Washington (Khangaonkar et al. 2019). Warming leads to decreased survival, (Lang et al. 2022) foraging, (Arribas et al. 2021, Bonaviri et al. 2017) and size (Petes et al. 2008) in many adult asteroids and increases the prevalence of disease (Bates et al. 2009, Eisenlord et al. 2016, Menge et al. 2016). Climate change also depletes dissolved oxygen which may further exacerbates disease (Aquino et al. 2021) and other negative health effects. Experimental data suggests that the larvae of many echinoderms are negatively impacted by warming. Echinoid larvae raised in high temperature water developed slower and experienced higher mortality, which was exacerbated by food shortage (Freehan et al. 2018). Crown-of-Thorns sea star larvae from the tropical Pacific, did not experience higher mortality when temperatures were raised, but developed slowly, and achieved a smaller size (Hue et al. 2022). Climate change will also affect the concentration and species composition of algae within the Salish Sea (Khangaonkar et al. 2019). Total algal biomass may increase, but models predict an increased proportion of diatoms seasonally over the next hundred years (Khangaonkar et al. 2019).

The effects of temperature on planktotrophic larvae are complicated by the effects of adequate food and nutrition. Larvae exposed to warmer than average temperatures may be able to development faster, but only if there is adequate food supply (Hoegh-Guldberg and Pearse, 1995). Larvae are highly plastic in their response to food shortage. They may grow longer ciliated bands to facilitate increased clearance of algae (Hart and Strathmann

1994). The speed of development is variable and is largely regulated by access to protein (Ellison et al. 2021). Nevertheless, larvae that eat more are clearly advantaged as they grow to larger juvenile sizes, pass through the vulnerable larval stage more quickly, and have a higher rate of survival (Hart and Strathmann 1994).

Optimistically, as algal biomass increases, the total concentration of species on which *Pisaster* larvae feed could increase. Models predict that total dinoflagellate abundance will increase with warming (Khangaonkar et al. 2019). While we don't know how larvae feed in the wild, many dinoflagellate taxa, such as *Dunaliella spp.*, are considered optimal, nutritious food sources for culturing larvae (Hinegardner 1969, Lucas 1982). However, these theoretical benefits may be cancelled out by an increase in the proportion of inedible species during certain times of the year. No species of diatom are considered a good food source for larval echinoderms (Hinegardner 1969), and because they are difficult to consume, they can harm the feeding ability of small larvae. During active feeding larvae cannot choose between algal particles, so they frequently must expel inedible or overly large cells (Strathmann 1971). Dealing with these cells slows the clearance rate of all particles, including edible ones (Strathmann 1971). Thus, increasing relative abundance of diatoms could effectively decrease the access of larvae to preferred food sources. There are also more subtle aspects of larval nutrition that may be affected by climate change. Edible species are cleared at different rates from each other, perhaps related to their digestibility, swimming speed and size, (Lucas 1982) meaning that even changes in the concentration of different edible species could affect food access. Lastly, the nutritional content of different algal species is of critical importance to larvae. Particularly in food shortage conditions, larvae grow largest when receiving a mixed diet

of preferable species (Basch 1996). Climate change will shift the nutritional content of such familiar algal species in ways we are only beginning to understand (Holm et al. 2022). Unfortunately, little is known about the feeding habits of wild larvae, but some observational data suggests that food availability regulates larval abundance. In the Tropical Pacific, irregularly high concentrations of planktonic algae are thought to drive crown-of-thorns sea star blooms (Fabricius et al. 2010, Lucas 1982). There are significant annual variations in the concentration of algae in the Salish Sea (Del Bel Beluz et al. 2021), but no research to date has discerned how this affects juvenile recruitment in local invertebrates.

Pisaster ochraceus, the common purple sea star, is a keystone predator in the Salish Sea that regulates local intertidal diversity by consuming mussels and creating open space for an array of taxa (Paine 1966). In 2013 sea star wasting disease caused an unprecedented die-off of the species throughout the West Coast of North America (Miner et al. 2018), which allowed for increased mussel abundance (Traiger et al. 2022), and probably caused long-term changes to the local intertidal community (Menge et al. 2016). *Pisaster* larvae are planktonic and feed primarily on algae. Natural mortality is estimated to be greater than 99%, but because females lay over 40 million eggs each (George, personal observations) it is believed that only 0.00005% to 0.0001% larval survival is needed to maintain a stable population of adults (Jablonski and Lutz, 1983). A healthy population of *Pisaster ochraceus* is crucial for the maintenance of the intertidal zone throughout its range, and we are concerned with how the effects of climate change on food availability and temperature will impact *Pisaster bipinnaria* and brachiolaria larvae.

In the present study, we tested the effects of sustained low (10-14°C) and high temperatures (18-20°C) and feeding regimes (mixed algal diet of *Dunaliella tertiolecta*, low food, 1000-1250 cells/ml, high food 2500 to 5000 cells/ml; and *Rhodomonas* sp. low food, 500 -1000 cells/ml, high food, 2000-4000 cells/ml) on *Pisaster ochraceus* bipinnariae and brachiolariae larvae. Thirty-three and 40 days after fertilization, we measured larval length, width, and survival. We predicted that *Pisaster ochraceus* larvae raised in warmer than average temperatures would grow to smaller overall sizes and experience higher mortality. We expected that high access to food might mitigate the detriments of warming, and even increase the speed of development, but the healthiest, largest larvae, with the lowest mortality rate would be those raised in low temperatures with high access to food.

3 Methods

Collection and Fertilization

On June 15th, adult *Pisaster ochraceus* were collected from The Friday Harbor labs pump house (N 48° 32' 46.065097" W 123° 0' 28.341992") and injected with 3 ml (1 mL in 3 different arms) of 100mM methyladenine to induce spawning. Eggs were collected from 4 females, and sperm was collected from 2 males. Gametes were washed through a 200µm Nitex mesh to remove debris. Eggs were fertilized with 8 drops of dilute sperm with fertilization success of 99%. The embryos were transferred into 15 3.5-liter glass jars with 2,600mL of 12°C, 33‰ filtered sea water pumped from San Juan Channel. Jars were placed into a sea table with continuously flowing sea water of 11-14 °C. A paddle stirrer, developed by Strathmann (1987), was used to facilitate oxygen and temperature diffusion, and keep larvae and food suspended in the jars. For the first 13

days, larvae were fed a mixed diet of *Dunaliella tertiolecta* and *Rhodomonas spp.* ad libitum (Table 1). To estimate algal cell concentration, cells were stained and fixed with Lugol's solution, then counted using a hemocytometer.

Temperature and Feeding Experiment

Thirteen-day old bipinnariae were used in experimental treatments. Bipinnariae from stock jars were combined and concentrated by siphoning off water. Using a small sample, the number of larvae per mL was estimated, and 1,500 larvae were added to each of 16 jars containing 2000mL of filtered sea water. To explore the combined effects of food availability and temperature on *P. ochraceus* larvae, four treatments were studied: low temperature with high food availability; low temperature with low food availability; high temperature with high food availability; and high temperature with low food availability. Table 1 shows the feeding treatments over time. *Rhodomonas* concentrations were raised over the course of the study to account for growth of the larvae. However, *Dunaliella* concentrations were capped at 5,000 cells/mL as it can be toxic if consumed in high quantities (Hinegardner 1969). Larvae were fed at the start of the experiment, and then every two days. When the larvae were sixteen days old (three days after the feeding treatment was implemented) the jars were divided into a high and low temperature treatments. For the high temperature treatment, four jars with high feeding and four jars with low feeding were slowly warmed from 12° to 18° C. In the low temperature (control) treatment, four jars from both feeding treatments were kept at around 12° C with flowing sea water. Due to variation in the temperature of the inflow water, water temperatures in the high temperature treatment regularly varied from 18-20°C, and in the low temperature varied from 11-14°C. A warming event in the Salish Sea towards the

end of July caused the temperature to temporarily increase to 21°C for the high temperature treatment and 15.5°C for the low temperature treatment.

Morphology:

Once per week, ten larvae from each jar were photographed under a light compound microscope (Nikon Eclipse Ci) with an Infinity 2 camera. The length and width of the larvae were measured using image J (Figure 1).

Data analysis

In R (R version 4.2.0, CAR package) we performed two-way multivariate ANOVAs to test the effect of temperature and food level on the length and width when larvae were 33 days old and 40 days. We also tested for interactions between temperature and food level factors. We removed a jar with abnormal larval size and survival in the low food, low temperature treatment from our analysis. The effect of individual jars was tested as a nested variable within temperature and food level. Significance of effects of temperature and food availability on length and width was found with a Tukey HSD test. An ANOVA was also performed to compare the survival from day 13-40 of larvae in the different temperature and feeding treatments.

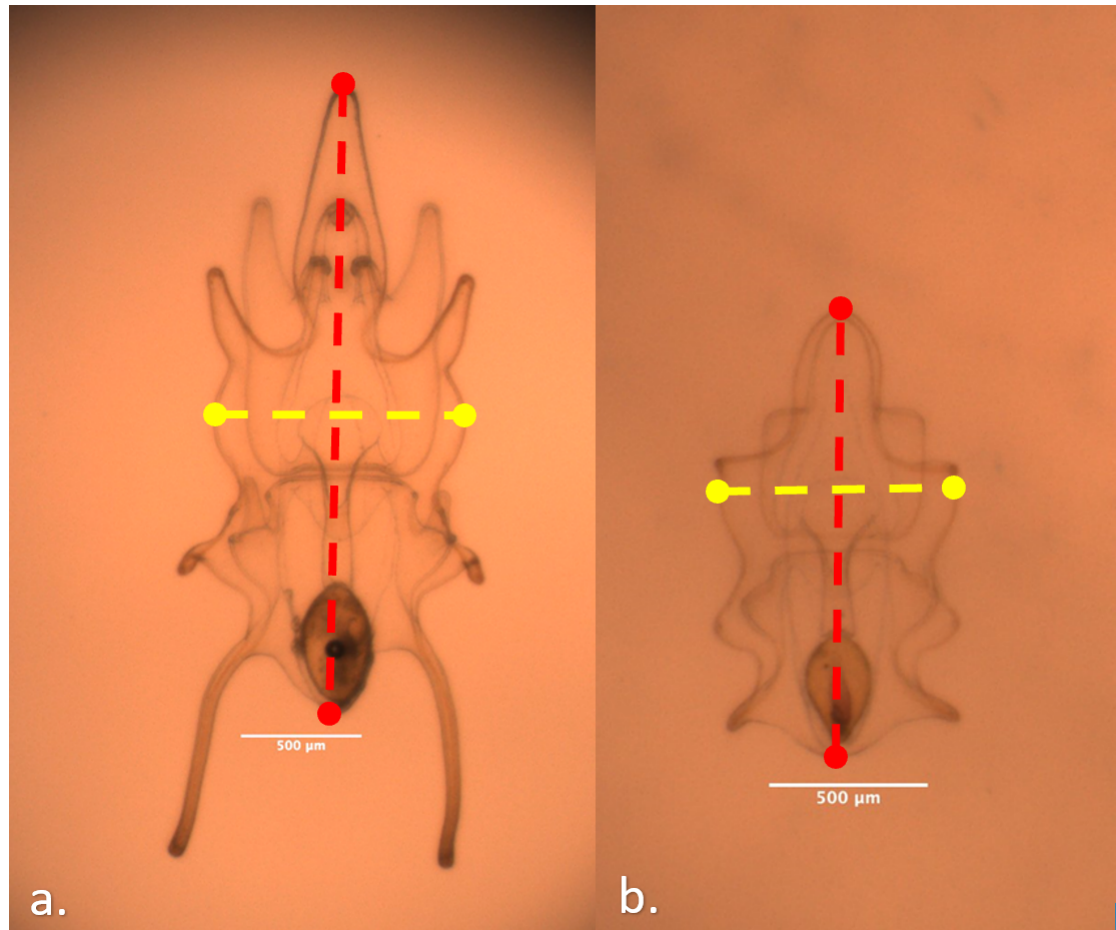


Figure 1 Brachiolaria (a.) and bipinnariae (b.) *Pisaster ochraceus* larvae shown with performed length (red dashed line) and width (yellow dashed line) measurements.

Table 1 Algal feeding concentration. Larvae were fed based on age, treatment, and the volume of sea water in each jar. The number of algal cells were added to each jar with an automatic pipet. Food was introduced every two days after the larvae were 13 days old.

Larval age	Treatment	<i>Dunaliella</i> cells/mL	<i>Rhodomonas</i> cells/mL
4 days	All Larvae	500	500
6-10 days	All Larvae	1000-2000	1000
13-23 days	High	2500-5000	2000-3000
	Low	1000-1250	500-750
24-34 days	High	5000	4000
	Low	1250	1000
35-45 days	High	6000	5000
	Low	1500	1250

4 Results

When larvae were 33 days old, the length ($n=148$, $F=940.9$, $P<0.0001$) and width ($n=148$, $F=458.3$, $P<0.0001$) of larvae in high feeding treatments were significantly greater than low feeding treatments (Figure 2, Table 2). Larvae fed more were 1.56 times longer and 1.25 times wider, on average. Larvae raised in cooler temperatures were also significantly longer ($n=148$, $F=1.776$, $p<0.0006$) and wider ($n=148$, $F=3.995$, $P<0.0006$), than those raised in warm temperatures at 33 days, but the extent of this effect was smaller than that of food, as they averaged only 1.05 times longer and 1.03 times wider. There was no significant interaction between temperature and food at 33 days.

At 40 days, the length ($n=150$, $F=926.7$, $p<0.0001$) and width ($n=150$, $F=713.0$, $p<0.0001$) of larvae in high feeding treatments were significantly greater than for the low feeding treatments (Figure 3, Table 2). Larvae fed a greater amount were 1.30 times wider than those in the low food regime. Both the length ($n=150$, $F=11.7$, $p<0.0001$), and width ($n=150$, $F=19.4$, $p<0.0001$) of larvae varied significantly between temperature treatments by 40 days. Larvae raised in low temperature treatments were 1.04 times wider than those in the high temperature treatments, but temperature had opposite effects on length at different feeding levels due to interactive effects ($n=150$, $F=4.45$, $p=0.0368$). Specifically, when feeding level was low, high temperature was associated with greater lengths, and when feeding levels were high, high temperatures were associated with shorter average length. There was no interactive effect of temperature and feeding on width ($n=150$, $F=1.1 \times 10^{-3}$, $p=0.9733$). There were significant differences among jars within treatments in terms of length and width of larvae at both larval ages (Table 2).

According to a two-way ANOVA, survival did not differ significantly among treatments, based on either food level ($n=15$, $F=0.596$, $p=0.4563$) or temperature ($n=15$, $F=4.41$, $p=0.0596$) (Figure 4).

Larvae in high food treatments showed evidence of faster development. They showed evidence of swimming arm growth, and brachiolaria arm development before larvae in the low food treatments (Figure 5).

Table 2 Results of two-way ANOVA tests for *Pisaster ochraceus* reared at four treatments: low temperature with high food availability; low temperature with low food availability; high temperature with high food availability; and high temperature with low food availability. P values for temperature and feeding differences are Tukey HSD adjusted. df, degrees of freedom, SS sum of squares.

Source of Variation	df	SS	F	P
33-day-old-larvae				
Temperature				
Length	1	36689	1.776	0.0006
Width	1	10321	3.995	0.0006
Feeding				
Length	1	19435752	940.9	<0.0001
Width	1	1183891	458.3	<0.0001
Feeding x Temperature				
Length	1	6656	0.322	0.5712
Width	1	4977	1.927	0.1675
Jar effects				
Length	11	2747180	7.688	<0.0001
Width	11	205954	7.248	<0.0001
40-day-old larvae				
Temperature				
Length	1	47604	1.516	0.0008
Width	1	17299	6.201	<0.0001
Feeding				
Length	1	29092858	926.7	<0.0001
Width	1	1989181	713.0	<0.0001
Feeding x Temperature				
Length	1	139558	4.445	0.0368
Width	1	3	0.001	0.9733
Jar effects				
Length	11	1200104	3.475	0.0003
Width	11	73954	2.410	0.0091
40-day Survival				
Temperature	1	0.0599	4.411	0.0596
Feeding	1	0.0081	0.596	0.4563
Feeding x Temperature	1	0.0001	0.012	0.9194

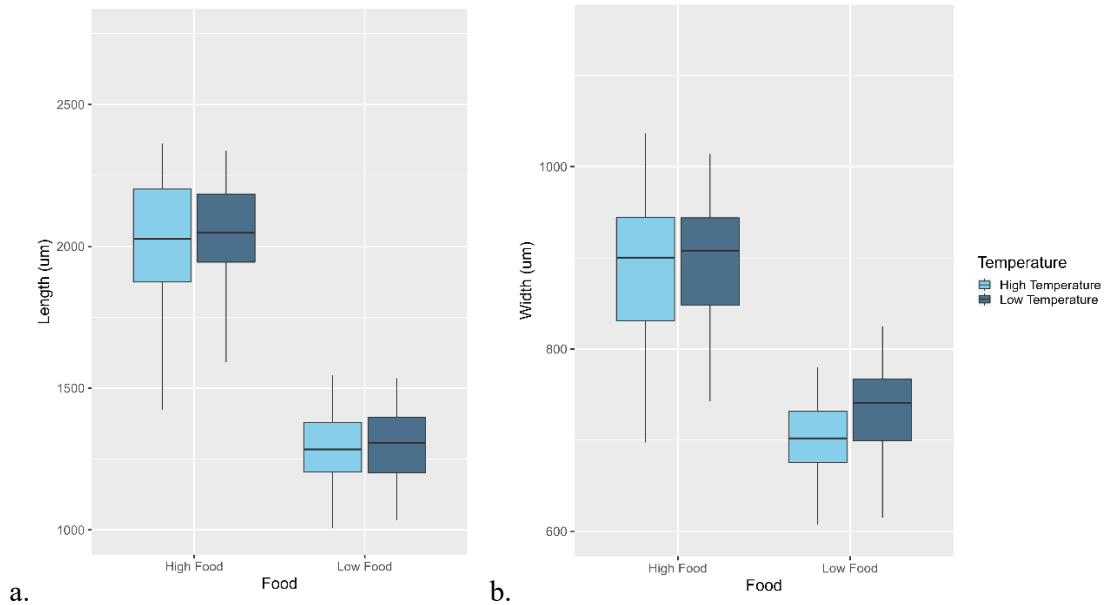


Figure 2 Length (a.) and width (b.) of larvae by food availability and temperature treatment at 33 days. Light shaded boxplots represent higher temperature treatments (18-20 °C), and dark shaded boxplots represent lower temperature treatments (11-14 °C). Boxes include interquartile range (IQR) from 25th percentile to 75th, divided by the median. Whiskers encompass data points within 1.5 times the IQR. Points outside this range are outliers.

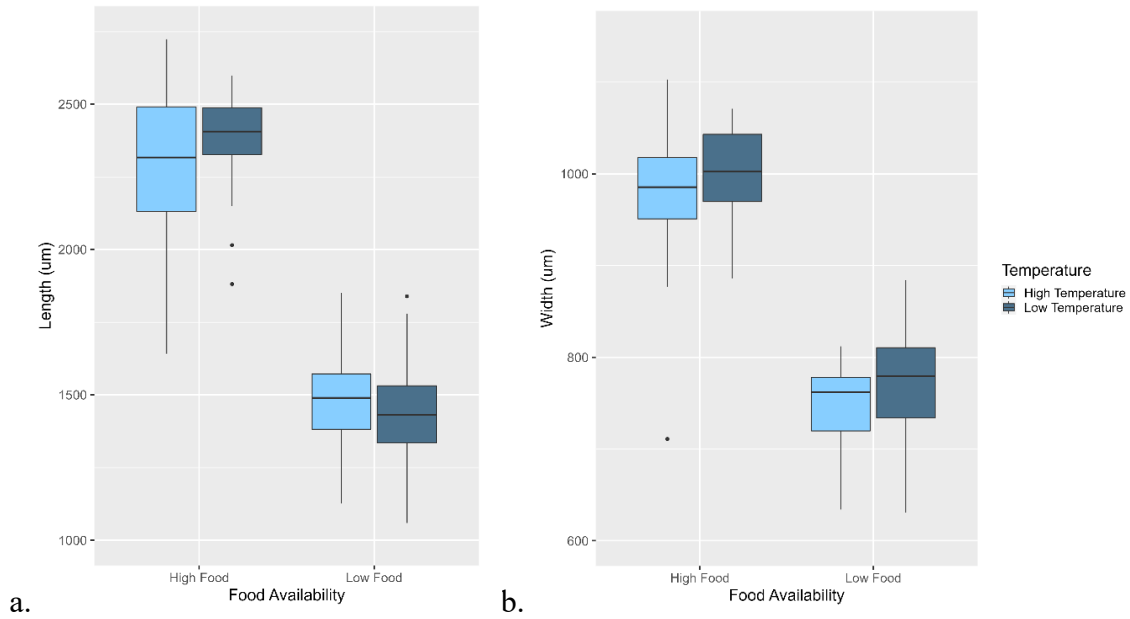


Figure 3 Length (a.) and width (b.) of larvae by food availability and temperature treatment at 40 days. Light shaded boxplots represent higher temperature treatments (18-20 °C), and dark shaded boxplots represent lower temperature treatments (11-14 °C). Boxes include interquartile range (IQR) from 25th percentile to 75th, divided by the median. Whiskers encompass data points within 1.5 times the IQR. Points outside this range are outliers.

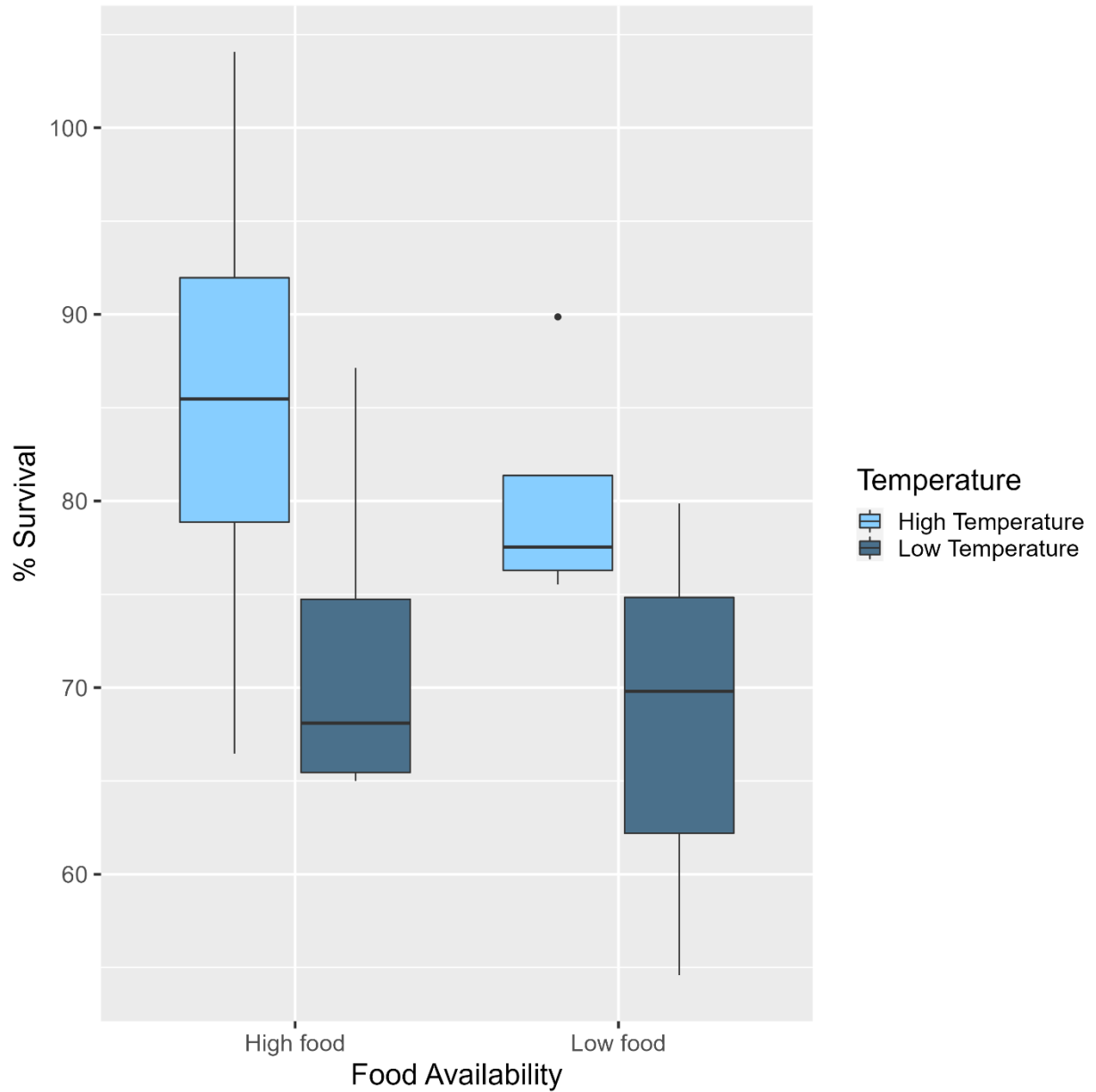


Figure 4 Percent survival from day 13 to day 40 by treatment. Light shaded boxplots represent higher temperature treatments (18-20 °C), and dark shaded boxplots represent lower temperature treatments (11-14 °C). Boxes include interquartile range (IQR) from 25th percentile to 75th, divided by the median. Whiskers encompass data points within 1.5 times the IQR. Points outside this range are outliers.

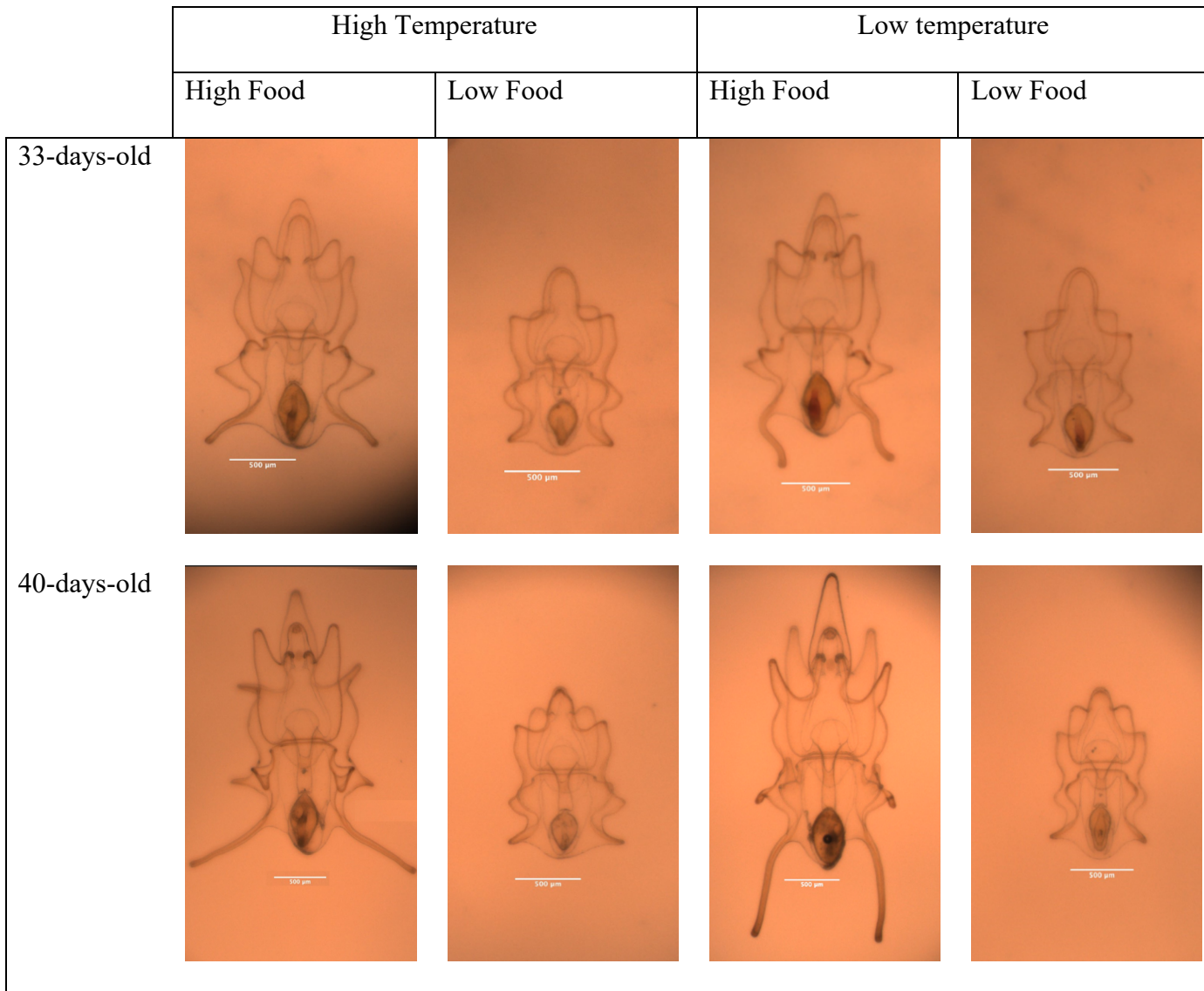


Figure 5 Representative sample of *Pisaster ochraceus* larvae from each treatment at 33 days and 40 days, with 500µm bar for scale.

5 Discussion

When larvae were 33-days old we found that both low temperature treatments and higher food availability were associated with greater average length and width of *Pisaster* larvae, however, the size of these effects were vastly different. Access to food had a pronounced positive effect on larval growth and development. Larvae fed a greater amount were not only physically larger, had more advanced swimming arms, showing their transition out of the bipinnariae started earlier than for food shorted larvae. In contrast, temperature affected the size of larvae to a much smaller extent than expected. Larvae kept in sustained temperatures of over 18°C, which is more than 6° hotter than average regional summer sea temperatures, were on average less than 5% shorter than larvae raised around historic temperature levels.

By 40-days-old, the effect of food was still largely associated with faster growth and development of *Pisaster* larvae. Larvae in high food treatments were almost all at the brachiolaria stage with well-developed brachiolaria arms, while those in lower food conditions were small, and frequently had not developed a single pair of swimming arms. Temperature was associated with smaller larval width, but to a small extent. Interestingly, at forty days, there was an interactive effect of feeding and temperature on the length of larvae. When food was abundant, larvae in cooler temperatures grew longer, but when there was less food, larvae in warmer temperatures grew longer. The effect was small, but significant, and counter to our expectations. We believed that when larvae were in higher temperature water their metabolism would be increased, and they might be able to grow large if food was plentiful, but would be synergistically negatively affected if their

metabolism was increased without access to nutrition. Previous studies of *Pisaster* larvae, with different temperature levels, found that growth and mortality increase with temperature (Titus and Hearther, 2019). In echinoids, both temperature and low food access appear to have large and negative effects on the growth and development of larvae (Freehan et al. 2018). Our study diverges slightly from these findings, as the size of larvae in different feeding treatments did not have a universally positive or negative responses to temperature.

It is noteworthy that, generally, adult animals show a greater response to their larval conditions after metamorphosis (Forster and Hirst 2012). Differences that appear minimal during *Pisaster*'s larval stage, could become pronounced after settlement. The temperature size rule describes a trend observed in over 83% of ectotherms where exposure to high temperatures during development is associated with smaller size attainment in adults (Atkinson 1994). Temperature size dependence in aquatic species may be driven in large part by access to oxygen, as larger species tend to be more affected (Forster et al. 2012). If temperature size dependence is driven largely by oxygen concentration, this could explain why the effect of prolonged heating alone on size were less than anticipated. Future research should question the extent to which dissolved oxygen concentration, which will decrease locally due to climate change, affects the survival and health of *Pisaster* larvae.

It is difficult to make conclusions as to how our treatments affected mortality due to the high variation and relatively low sample size of different jars among treatments. Survival levels for larvae were high in each sample, surpassing 50% in every case. Nevertheless, there appeared to be some degree of positive association between

temperature and survival. This conflicts with previous findings that high temperatures lower survival in *Pisaster* larvae (Titus and Hearther, 2019). There appeared to be no effect of food concentration on survival in the larvae. It is interesting that larvae with less access to food grew to significantly smaller sizes, but did not experience heightened mortality. This highlights *Pisaster* larvae's plasticity in their response to food shortage, where they are able to minimize health consequences at the expense of growth and development in the lab. However, such behavior would likely lead to significantly higher mortality in the wild. Larvae that develop more slowly may be susceptible to high predation for a longer period of time and may be more likely to be transported to a suboptimal habitat.

Pisaster larvae can asexually reproduce by cloning (Vickery and McClintock 2000). We noted the presence of some abnormally small larvae within some of our treatments that could have been produced by cloning. Cloning rate in *Pisaster* is affected by a variety of factors, including temperature (Titus and Hearther, 2019) and nutrition (Vickery and McClintock 2000). Cloning can decrease the average size of larvae within a sample, because clones are much smaller than other larvae. At the same time, it increases the total number of larvae in a jar affecting perceived levels of survival. High incidence of cloning may explain why the survival level was close to, or above, 100% in some of our jars. We do not know which temperature treatments may have produced higher incidence of cloning, but if a high rate of cloning occurred in the high temperature, high food treatment at 18°C it would explain why there were high rates of perceived survival, a broader distribution of larval sizes, and smaller larvae on average, compared to the low temperature, high food treatment.

Previous research suggested that heightened temperature increases the feeding rate of *Pisaster* larvae (George et al. 2021). When comparing images of high food availability treatments, larvae raised in low temperatures often had a colorful red stomach, while larvae raised in high temperatures had a dull colored stomach. *Rhodomonas spp.*, one of the two algae taxa used in this study, are a similar shade of red. This could indicate that larvae in higher temperature treatments cleared *Rhodomonas spp.* more quickly, and circumstantially points to higher levels of feeding in high temperature treatments.

Collectively, our findings show that temperature and food availability may regulate different aspects of *Pisaster* health. Food availability clearly has an immense influence on the growth and development of *Pisaster* larvae. Higher access to food allowed for faster transition into later larval stages, and would probably have led to faster settlement, and higher survival in the wild. Temperature had a lesser effect on growth and development, but may have positively regulated survival and feeding. This suggests warming within 6°C will have minimal direct negative effects, and perhaps even benefit *Pisaster* larvae, but that ecosystem level effects that shift their access to food, will cause major disruption.

This study also highlights how related taxa can have vastly different responses to climate effects. *Pisaster ochraceus* has frequently surprised researchers by reacting to climate stressors in opposite ways from other echinoderms. For most adult asteroids studied, high pH sea water limits growth and limb regeneration (Keppel et al. 2015, Randazzo 2021), but it increases both growth and feeding rate in *Pisaster ochraceus* (Gooding et al. 2009). Feeding rate decreases with temperature for many sea star species,

but *Pisaster ochraceus* adults feed more under most warm temperature conditions (Pincebourde et al. 2012, Gooding et al. 2009). Because *Pisaster* is an outlier in many respects, future research should be cautious extrapolating the findings of this study to presume that other sea star larvae will be as resilient to temperature as these larvae were. *Pisaster* is a crucial keystone species, but it may be a poor representative for asteroids.

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References

- Arribas, L. P., J. E. F. Alfaya, M.G. Palomo, S. Giulianelli, R. A. N. Vilela, and G. Bigatti. 2021. Ocean warming lead to heat shock protein expression and decrease in the feeding rate of the Patagonian sea star *Anasterias minuta*. *Journal of Experimental Marine Biology and Ecology*. 546:151661
- Aquino, C. A., R. M. Besemer, C. M. DeRito, J. Kocian, I. R. Porter, P. T. Raimondi, J. E. Rede, L. M. Schiebelhut, J. P. Sparks, J. P. Wares and I. Hewson. 2021. Evidence That Microorganisms at the Animal-Water Interface Drive Sea Star Wasting Disease. *Frontiers in Microbiology* <https://doi.org/10.3389/fmicb.2020.610009>
- Atkinson, D. 1994. Temperature and organism size – a biological law for ectotherms. *Advances in Ecological Research* 25: 1-58
- Basch, L. V. 1996. Effects of algal and larval densities on development and survival of asteroid larvae. *Marine Biology*. 126(4): 693-701.
- Bates A.E., B. J. Hilton, and C. D. Harley. 2009 Effects of temperature, season and locality on wasting disease in the keystone predatory sea star *Pisaster ochraceus*. *Dis Aquat Organ*. 9;86(3):245-51. doi: 10.3354/dao02125. PMID: 20066959.
- Bonaviri C, M. Graham , P. Gianguzza, and N.T. Shears. 2017. Warmer temperatures reduce the influence of an important keystone predator. *J Anim Ecol*. 86(3):490-500.
- Del Bel Beluz, J., M.A. Peña, J.M. Jackson, N. Nemcek. 2021. Phytoplankton Composition and Environmental Drivers in the Northern Strait of Georgia (Salish Sea), British Columbia, Canada. *Estuaries and Coasts* 44, 1419–1439. <https://doi.org/10.1007/s12237-020-00858-2>
- Eisenlord M. E., M. L. Groner, R. M. Yoshioka, J. Elliott, J. Maynard, S. Fradkin, M. Turner, K. Pyne, N. Rivlin, R. van Hooidek and D. C. Harvell. 2016. Ochre star mortality during the 2014 wasting disease epizootic: role of population size structure and temperature *Phil. Trans. R. Soc.* B371
- Ellison, A., A. Pouv and D. A. Pace. 2021. Different protein metabolic strategies for growth during food-induced physiological plasticity in echinoid larvae. *Journal of Experimental Biology* 224 10.1242/jeb.230748
- Fabricius, K. E., K. Okaji, and G. De'ath. 2010. Three lines of evidence to link outbreaks of the crown-of-thorns seastar *Acanthaster planci* to the release of larval food limitation. *Coral Reefs* 29:593–605
- Forster, J. and A. G. Hirst. 2012. The temperature-size rule emerges from ontogenetic differences between growth and development rates. *Functional Ecology*, 26: 483-492

- Forster, J., A. G. Hirst, and D. Atkinson. 2012. Warming-induced reductions in body size are greater in aquatic than terrestrial species. *PNAS* 109(47): 19310-19314.
- Freehan, C., Z. Ludwig, S. Yu, D. K. Adams. 2018. Synergistic negative effects of thermal stress and altered food resources on echinoid larvae. *Nature Scientific Reports* 8, 12229 <https://doi.org/10.1038/s41598-018-30572-w>
- George S., E. Navarro, D. Kawano. 2021 Infrequent Fluctuations in Temperature and Salinity May Enhance Feeding in *Pisaster ochraceus* (Asteroidea) but Not in *Dendraster excentricus* (Echinoidea) Larvae. *Biol. Bull.* 241: 77-91
- Gooding, R. A., C. D. G. Harley, and E. Tang. 2009. Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. *PNAS* 106(23): 9316-9321.
- Hart, M. W. and R. R. Strathmann. 1994. Functional Consequences of Phenotypic Plasticity in Echinoid Larvae. *Biol. Bull.* 186(3): 291-299
- Hinegardner, R. T. 1969. Growth and Development of the Laboratory Cultured Sea Urchin. *Biol. Bull.*, 137: 465-475
- Hoegh-Guldberg, O.V.E., and J. S, Pearse. 1995. Temperature, Food Availability and The Development of Marine Invertebrate Larvae. *American Zoologist* 35(4): 415-425.
- Holm, H. C., H. F. Fredricks, S. M. Bent, D. P. Lowenstein, J. E. Ossolinski, K. W. Becker, W. M. Johnson, K. Schrage and B. A. S. Van Mooy. 2022. Global ocean lipidomes show a universal relationship between temperature and lipid unsaturation. *Science*. 376(6600): 1487
- Hue, T., O. Chateau, G. Lecellier, C. Marin, N. Coulombier, L. L. Dean, H. Gossuin, M. Adjeroud, and P. Dumas. 2022. Impact of near-future ocean warming and acidification on the larval development of coral-eating starfish *Acanthaster cf. solaris* after parental exposure. *Journal of Experimental Marine Biology and Ecology*. 548: 151685 <https://doi.org/10.1016/j.jembe.2021.151685>
- Jablonski, D., and R. A. Lutz. 1983. Larval Ecology of Marine Benthic Invertebrates: Palaeobiological Implications. *Cambridge Philosophical Society Biological Reviews*. 58(1): 21-89.
- Kacenas, S., and R. Podolsky. 2018. Density-dependent expression of plasticity in larval morphology: effects of actual and apparent competitors. *Marine Ecology Progress Series* 593: 1–13.
- Keppel, E. A., R. A. Scrosati and S. C. Courtenay. 2015. Interactive effects of ocean acidification and warming on subtidal mussels and sea stars from Atlantic Canada. *Marine Biology Research* 11(4):337-348.

Khangaonkar, T., A. Nugraha, W. Xu and K. Balaguru. 2019. Salish Sea Response to Global Climate Change, Sea Level Rise, and Future Nutrient Loads. *Journal of Geophysical Research: Oceans* 124, 3876– 3904. <https://doi.org/10.1029/2018JC014670>

Lang, B.J., J.M. Donelson, C.F. Caballes, S. Uthicke, P.C. Doll and M. S. Pratchett. 2022. Effects of elevated temperature on the performance and survival of pacific crown-of-thorns starfish (*Acanthaster cf. solaris*). *Mar Biol* 169, 43

Leveque-Eichhorn, L. 2020. The effect of temperature and food patch presence on two life stages of *Pisaster ochraceus* larval swimming behavior. Published online at the Friday Harbor laboratories, University of Washington library

Lucas, J. S. 1982. Quantitative Studies of Feeding and Nutrition During Larval Development of the Coral Reef Asteroid *Acanthaster Placi* (L.) *J. Exp. Mar. Biol. Ecol.* 65: 173-193

Menge, B. A., E. B. Cerny-Chipman, A. Johnson, J. Sullivan, S. Gravem, and F.Chan. 2016. Sea Star Wasting Disease in the Keystone Predator *Pisaster ochraceus* in Oregon: Insights into Differential Population Impacts, Recovery, Predation Rate, and Temperature Effects from Long-Term Research. *PLOS ONE* DOI:10.1371/journal.pone.0153994

Miner, C.M., J. L. Burnaford, R. F. Ambrose, L. Antrim, H. Bohlmann, et al. 2018. Large-scale impacts of sea star wasting disease (SSWD) on intertidal sea stars and implications for recovery. *PLOS ONE* 13(3): e0192870.

S. Pincebourde, E. Sanford, J. Casas, and B. Helmuth (2012), Temporal coincidence of environmental stress events modulates predation rates. *Ecology Letters*, 15: 680-688. <https://doi.org/10.1111/j.1461-0248.2012.01785.x>

Paine, R. T. 1966. Food Web Complexity and Species Diversity. *The American Naturalist*, 100(910), 65–75.

Petes, L. E., M. E. Mouchka, R. H. Milston-Clements, T. S. Momoda, and B. A. Menge. 2008. Effects of environmental stress on intertidal mussels and their sea star predators. *Oecologia* 156, 671–680

Randazzo, H. L. 2021. Down in arms: marine climate stress inhibits growth and calcification of regenerating *Asterias forbesi* (Echinodermata: Asteroidea) arms. Honors Projects, 222

Strathmann, R. R. 1971. The Feeding Behavior of Planktotrophic Echinoderm Larvae: Mechanisms, Regulation, and Rate of Suspension-Feeding. *J. exp. mar. Biol. Ecol.* 6:109-160;

Strathmann, R. R. and D. Grünbaum. 2006. Good eaters, poor swimmers: compromises in larval form. *Integrative and Comparative Biology*. 46(3): 312-322.

Titus, S. and K. Hearther. 2019. A disaster for the *Pisaster*? Temperature effects on Ochre sea star larval development. Undergraduate Thesis.

Torres, G., and L. Gimenez. 2020. Temperature modulates compensatory responses to food limitation at metamorphosis in a marine invertebrate. *Functional Ecology*. 1564–1576.

Traiger, S. B., J. L. Bodkin, H. A. Coletti, B. Ballachey, T. Dean, D. Esler, K. Iken, B. Konar, M. R. Lindeberg, D. Monson, B. Robinson, R. M. Suryan, and B. P. Weitzman. 2022. Evidence of increased mussel abundance related to the Pacific marine heatwave and sea star wasting. *Marine Ecology*, 43, e12715.
<https://doi.org/10.1111/maec.12715>

Vickery, M., and J. McClintock. 2000. Incidence of Cloning in Planktotrophic Larvae of the Sea Star *Pisaster ochraceus*. *Biol. Bull.* 199(3):298-304