The Effect of Temperature on the Vertical Distribution and Swimming Speeds of *Pycnopodia helianthoides* and *Pisaster ochraceus* Larvae in haloclines

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Abstract

We investigated the effect of high temperatures on the vertical distribution and swim speeds of *Pisaster ochraceus* and *Pycnopodia helianthoides* larvae in haloclines. The haloclines were kept at 12-13 °C for the low temperature treatment and 18 °C for the high temperature treatment. We saw that larvae kept in low temperatures showed higher proportions of larvae at the halocline or swimming just above the bottom of the chamber than larvae kept in high temperatures, who tended not to swim. Data analysis suggests that these differences were not significant, though we expect that they would be significant with increased sample sizes. We also saw no significant difference in swimming speeds between species and temperatures, likely due to low sample size. These results suggest that high ocean temperatures experienced during heatwaves in the Pacific Northwest would have a significant effect on the distribution of these species in the water column and therefore their ability to feed, as the phytoplankton they eat is mainly distributed within or at the base of haloclines.

Introduction

Nearshore ocean surface temperatures during Pacific Northwest heatwaves, such as the heatwave of June 2021, can get as high as 23.2 °C, causing physical stress for many coastal organisms (White *et al*., 2023). High temperatures are known to have an impact on echinoderm larvae, increasing their rates of feeding (George *et al*. 2021) and swim speeds (Civelek *et al*. 2013). Additionally, the Salish Sea receives freshwater input from several rivers, including the Fraser River, creating a stratification of salinity near the surface. Phytoplankton, which echinoderm larvae eat, tend to aggregate at the halocline (Erga *et al.* 2003), so remaining at the halocline is important to the survival of these larvae.

Pycnopodia helianthoides (Asteroidea) is an important predator in maintaining kelp forest ecosystems on the North American west coast (Galloway *et al.*, 2023) and its population levels have declined by over 97% since 2013 with few signs of recovery due to sea star wasting disease (Hamilton *et al*. 2021). *Pisaster ochraceus* (Asteroidea), also affected by the wasting disease, is a keystone species in the rocky intertidal habitat of the Pacific Northwest (Paine, 1966). While the adults of these species have been extensively studied, few studies have been done on their larval stages, especially for *Pycnopodia*.

We analyzed how *Pycnopodia* and *Pisaster* larvae vertical swim speeds and distribution within haloclines are affected by temperature. Temperature has been shown to have a positive

correlation with swimming speeds in the asteroid *Asterias rubens* (Civelek *et al*. 2013). So, it was hypothesized that for both *Pycnopodia* and *Pisaster* larvae, increases in temperature (from 12-13 °C to 18 °C) would result in increased swimming speeds. We also hypothesized that increased temperature would result in different larval distributions in the water column around a halocline.

Methods

Spawning, fertilization, and larval rearing:

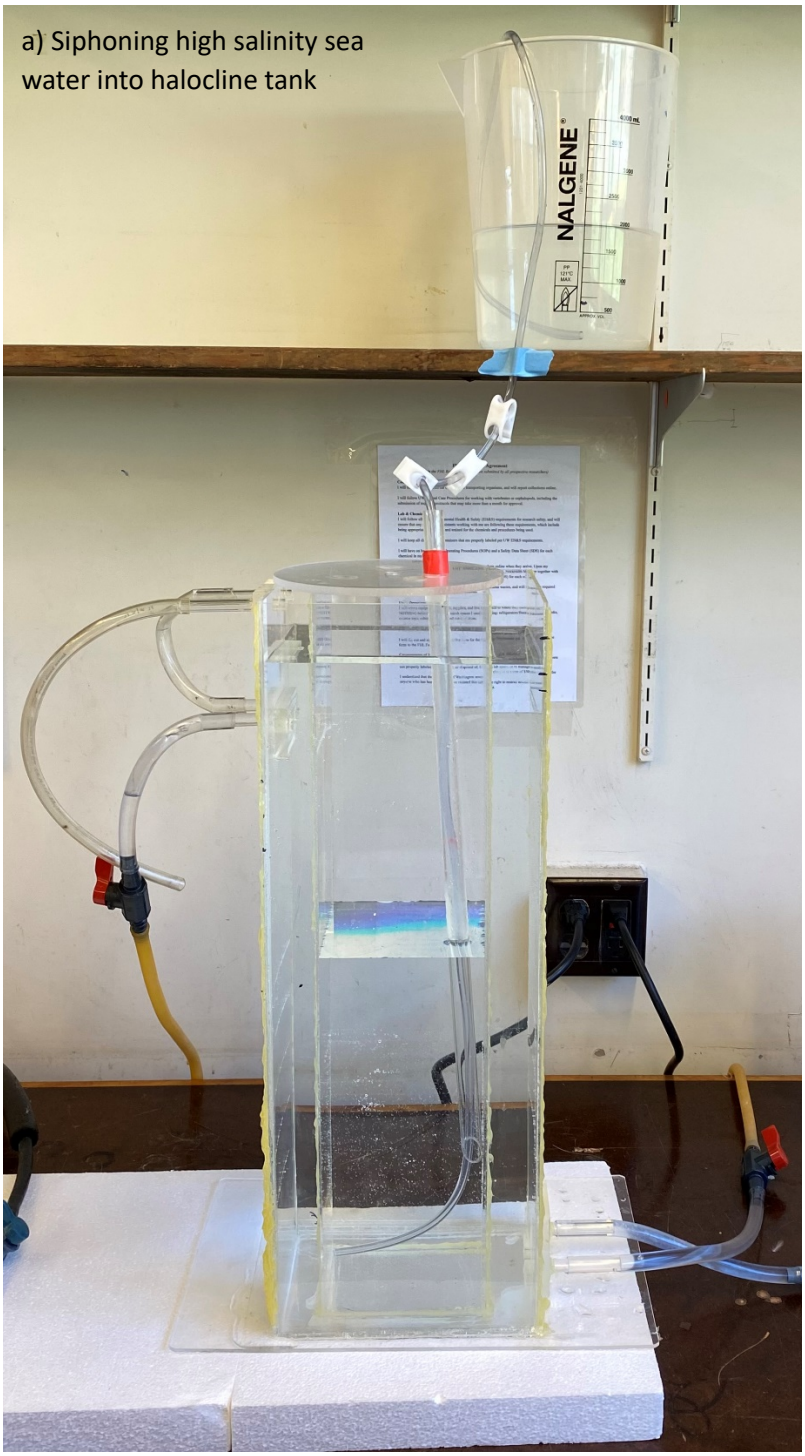
Five adults of *Pisaster* were collected near Friday Harbor Laboratories in Friday Harbor, WA (48°32'45''N, 123°0'47'' W) on April 15th, 2023. They were maintained in seawater tanks with constant flow-through at Friday Harbor Laboratories and fed mussels regularly. Spawning was induced in three males and one female on April 17th, 2023 by injecting 4 mL of 100 µmol 1-Methyladenine into each adult. Sperm and eggs were collected from the sea stars and fertilized the same day. 2 mL of dilute sperm was used to fertilize the eggs with a fertilization success of 99%.

Three wild caught *Pycnopodia* (one female and two males) spawned and fertilization occurred on March 21, 2023. Two captive reared (Hodin *et al*, 2021) *Pycnopodia* females were induced to spawn via 1- Methyladenine injection and fertilized on March 31, 2023 with sperm from a wild-caught male that spawned spontaneously on March 22, 2023.

Larvae of both species were kept in jars constantly stirred by a set of swinging paddles to keep the food and larvae in suspension and ensure aeration (Strathmann, 1987). These jars were kept at ambient ocean temperature, 10-14 ° C. Larvae in all jars were fed 2500 cells of *Rhodomonas* and 2500 cells of *Dunaliella* every two days. Once a week, 60-90% of the water was drained from each culture and replaced with fresh filtered seawater.

Halocline setup:

Haloclines were created in rectangular plastic tanks consisting of an inner and outer chamber. Water was constantly flowing through the outer chamber and passing through either an Isotemp 1016 water heater or Isotemp 4100 water chiller to maintain the temperature of the inner chamber at either 18 °C or 12 °C, respectively. The larvae were observed in the inner chamber. Two rigid plastic tubes were inserted into the inner chamber through a lid at the top. To generate the halocline, 2700 mL of 20 ppt seawater was added to the inner chamber. All seawater used in this experiment was 0.45 µm filtered seawater. A flexible plastic tubing with clamps to control water flow was inserted into the inner chamber. 3500 mL of 30 ppt seawater was slowly siphoned through this tube into the bottom of the chamber, generating a halocline with 20 ppt water at the top and 30 ppt water at the bottom. Haloclines were left to stabilize for at least 30 minutes before adding larvae (Fig. 1). A clear grid of dots 1-inch apart from each other was placed behind the inner chamber to serve as a reference for distance swam by larvae.



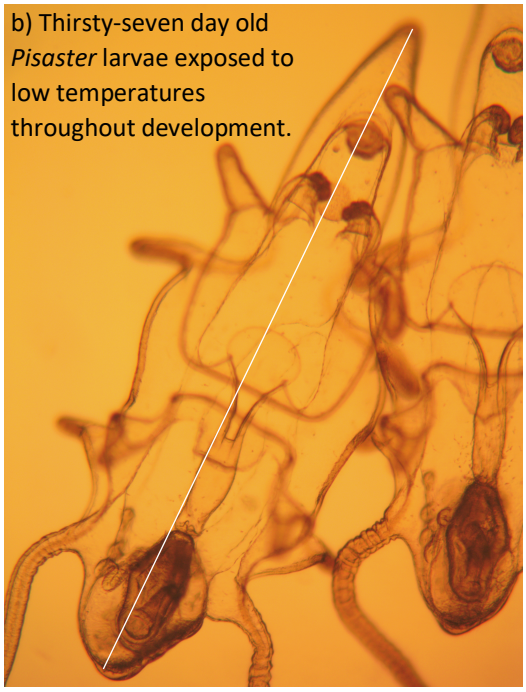


Fig. 1. a) Photo of the halocline apparatus. The contraption consists of an outer tank with water flowing inward from the bottom and outward from the top, cycling through a water heater or chiller. 30 ppt filtered seawater is being siphoned into the inner chamber beneath 20 ppt filtered seawater to fill the tank and generate a salinity gradient. Clamps are put on the water tube to slow water flow. b) *Pisaster ochraceus* larvae used in halocline experiments. The white line is the total larval length, 1961.96 µm.

Table 1: Temperature treatment, replicate number, date of trial, age of sea star larvae of *Pycnopodia helianthoides* and *Pisaster ochraceus*, and length of exposure to high temperatures before each halocline trial. All dates are in the year 2023.

Species	Temperature (°C)	Replicate	Date of trial	Age of larvae (days)	Exposure to 18 °C (hours)
Pycnopodia	12	1	May 19	49	0
Pycnopodia	12	2	May 19	49	0
Pycnopodia	18	1	May 21	51	40
Pycnopodia	18	2	May 22	52	40
Pisaster	12	1	May 23	36	0
Pisaster	12	2	May 23	36	0
Pisaster	18	1	May 24	37	45
Pisaster	18	2	May 24	37	50

For the 18-degree treatment, larvae were kept in a sea table at 18 °C for 40-50 hours (Table 1) before being introduced into the halocline. Larvae in the 12-degree treatment remained in the sea tables at ambient ocean temperature (10-14 °C). Two hundred to three hundred ml of seawater containing 130-160 larvae were slowly introduced to the bottom of the inner chamber using a flexible plastic tubing with clamps to control water flow. A video camera with a 55 mm lens and BTV Pro were used to capture videos of swimming larvae. 60 and 90 minutes after larvae were initially introduced to the halocline, larval distributions were recorded by counting the number of larvae at each depth in 0.5-inch increments (later converted to centimeters). The transparency of these larvae made it difficult to accurately count the larvae resting at the bottom of the chamber, so the exact number of larvae at the bottom was not recorded. After each trial, salinity and temperature readings were taken in 1-inch depth intervals throughout the halocline with an EcoSense EC300A probe. Data was gathered from a total of thirteen trials. Eight of these trials were analyzed, with two replicates of each temperature treatment per species (Table 1). The five omitted trials are reviewed in the discussion section.

To calculate swimming speeds, videos were analyzed to measure the time it takes for a larva to swim vertically between two 1-inch apart dots. In cases where larvae did not swim the full span between two dots in a video, ImageJ was used to determine the distance swam. The vertical distance was divided by time and converted to cm/second.

Data analysis:

To analyze distribution data, data within treatments were pooled into three categories: at the halocline (12.7 cm depth to 20.32 cm depth), below the halocline (20.32 cm depth to 30.48 cm depth), and near the bottom (from 30.48 cm depth to the bottom of the chamber, excluding larvae resting at the bottom). Two-by-two contingency tables comparing temperature and number of larvae at each depth were used with the Pearson chi-squared test to determine temperature's effect on larval distribution around haloclines. Four contingency tables were made, comparing *Pycnopodia* or *Pisaster* distributions 60 minutes and 90 minutes after introduction. These tests were run on RStudio 2023.03.0. To determine temperature's effect on swimming speeds of *Pycnopodia* and *Pisaster* larvae, one-way ANOVAs were run on JMP. This program was also used to create Fig. 3. RStudio was used to run statistical tests on distribution data.

Results

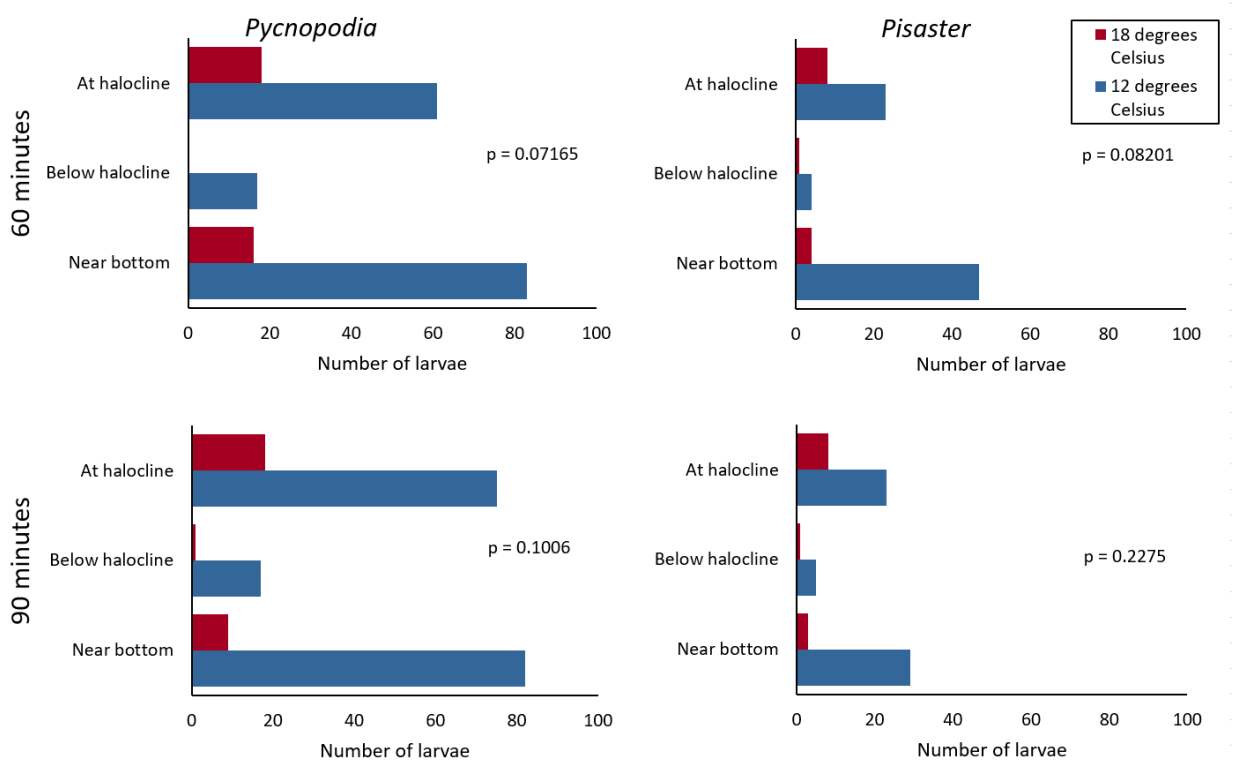


Fig. 2. Vertical distribution and pictures below of *Pycnopodia helianthoides* and *Pisaster ochraceus* larvae 60 and 90 minutes after introduction to haloclines. About 150 larvae were introduced to each halocline. Larvae in high temperature treatments were exposed to 18 °C for 40-50 hours (Table 1) before being introduced to an 18 °C halocline. Each temperature treatment was repeated twice for each species. Larval counts within each trial were pooled into three different depth categories discussed in the methods section: at the halocline, below the halocline, and near the bottom of the tank. Larvae resting at the bottom of the tank were not considered. n ~ 300 for each graph.

a) Fifty-two day old *Pycnopodia* larva exposed to high temperatures for 40 hours (Total larval length, 2095.6µm).

b) Fifty-two day old *Pycnopodia* larva at low temperatures throughout development (Total larval length, 1883.9 µm)

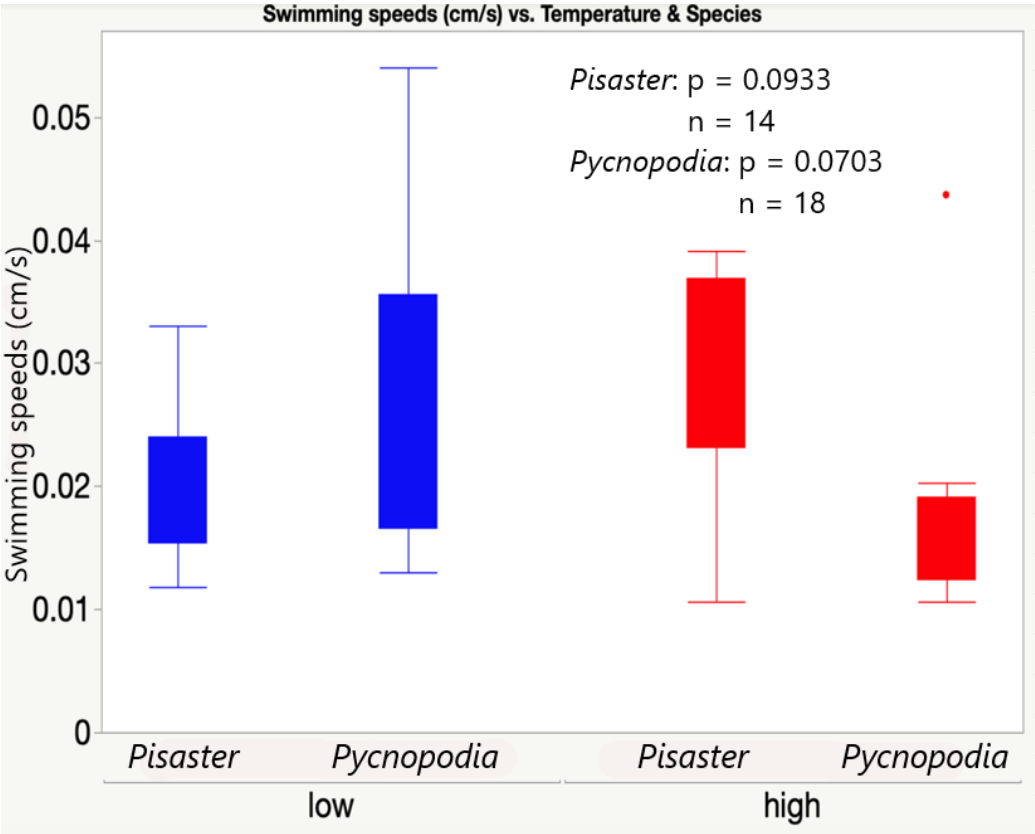


Fig. 3. Box plots showing swimming speeds (cm/s) for each species in either treatment. Swimming speeds were determined by measuring the time it takes for a larva to swim upward 1 inch or less. These measurements were taken from videos recorded with a Speco Technologies® video camera, a 55 mm lens, and BTV Pro.

In each treatment, larvae tended to be at the halocline or near the bottom of the tank. Many larvae rested at the bottom of the tank instead of swimming upwards. The 18 °C haloclines showed significantly fewer swimming larvae than the 12 °C haloclines. In either temperature, swimming larvae were mostly distributed at the haloclines or near the bottom of the haloclines, with a few swimming below the haloclines. Additionally, fewer *Pisaster* were observed swimming than *Pycnopodia*. However, statistical analysis with Pearson's chi-squared tests showed that there was no significant difference between temperature treatments (p-values > 0.05) (Fig. 2). In one low-temperature *Pycnopodia* trial done on May 18 and not used in data analysis for reasons explained in the discussion section, 37 larvae (fertilized on March 31) swam past the halocline and remained at the surface of the water.

All swimming speeds were between 0.01 cm/s and 0.06 cm/s. For *Pisaster*, swimming speeds were observed to increase with temperature. However, a one-way Anova test comparing the two treatments had a $p > 0.05$, a power of 0.4384, and a least significant number of 16.60386. For *Pycnopodia* , swimming speeds were observed to decrease with temperature. A one-way Anova gave a $p > 0.05$, a power of 0.4173, and a least significant number of 22.50134 (Fig. 3).

Discussion

Our hypothesis that increased temperature would result in different vertical larval distributions around a halocline was not supported statistically by our results; the differences between temperature treatments were not statistically significant. However, we consistently observed that in either temperature treatment, swimming larvae were distributed near the halocline or above the bottom of the tank. Additionally, higher temperatures resulted in fewer larvae swimming than in the 12°C treatment. The p-values comparing larval distributions in 12 °C vs 18°C 60 minutes after introducing larvae are close to 0.05, and it is likely that with more trials and a higher sample size, these differences would be statistically significant. These findings are consistent with another study, where *Pisaster* larvae tended to aggregate within a 20 ppt/30 ppt halocline (Bashevkin *et al*., 2016).

Additionally, our hypothesis that increased temperature would result in increased vertical swimming speeds was not supported by our results. No conclusions could be drawn about temperature's effect on larval swim speeds due to small sample size. With more replicates and time to complete this experiment, sufficient data on larval swim speeds could be gathered. Power tests revealed that sample sizes of 16 for *P. ochraceous* and 22 for *Pycnopodia* were needed to obtain significant statistical differences. If the observed differences in swimming speeds are not due to random chance, it is likely that these differences are due to the stages of larvae, as *Pisaster* used were younger than *Pycnopodia*, and high temperature trials were completed 1-3 days after low temperature trials. Larval swimming speeds were within the same range of those recorded for *Asterias rubens* in another study (Civelek *et al.,* 2013). In future experiments, larvae used should be similar ages to remove effects caused by age. Additionally, differences in swimming speeds of different echinoderm larvae ages could be studied.

Thirteen total halocline trials were completed, but only eight were used in the data analysis. Two low-temperature *Pycnopodia* trials were omitted to account for differences in stages of development, as these larvae were one and two weeks older than the larvae used in other *Pycnopodia* trials. Another low-temperature *Pycnopodia* trial was omitted due to a low number of larvae being added to the halocline. One high-temperature *Pycnopodia* was omitted due to loss of larvae. One low-temperature *Pisaster* trial was omitted due to high temperatures in the halocline. In the trial omitted due to low numbers of larvae, about 35 larvae swam past the halocline to the surface of the water. This could be due to these larvae being selected from the top of a jar; however, no larvae swam to the surface in any other trial. Slight variations in age, halocline temperature, and speed at which larvae are added to the halocline could have affected larval behavior.

Heatwaves that have been occurring during the summers in the Pacific Northwest can bring surface ocean temperatures to 18 °C and higher (White *et al*., 2023). Under these conditions, *Pycnopodia* and *Pisaster* larvae are less able to swim to the halocline where phytoplankton is located (Erga *et al*., 2003), possibly resulting in mass starvation. The adults of these species are already under pressure of sea star wasting disease (Hamilton *et al*. 2021), and additional pressure caused by rising ocean temperatures could further hinder their survival. Marine heatwaves could therefore further hinder *Pycnopodia'*s ability to facilitate kelp forest ecosystems (Galloway *et al*., 2023) and put pressure on *Pisaster*, keystone predators in rocky intertidal habitats of the Pacific Northwest (Paine, 1966).

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