

Temperature Accelerates Growth of *Pisaster ochraceus* Larvae

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Abstract

Ocean temperatures are slowly increasing as a result of climate change. The purpose of this study was to determine the effects of rising temperatures on growth and development of *Pisaster ochraceus* larvae. Larvae were kept at 22°C for 13 days then transferred to another tank where they were kept between 15°C to 18°C for a further 15 days. For both temperature treatments, samples were collected for 23-, 31-, and 37-day-old larvae and used ImageJ to measure total larval length, total larval width, stomach width and length and ciliated band length. All larvae developed successfully, which may imply that larval development is not harmed by temperature increases. Generally, larvae were significantly larger at high temperatures. Larger larvae developed longer ciliated bands, which may have allowed for more efficient feeding and faster growth and development at high water temperatures. These results suggest that if food is abundant in the water column, *P. ochraceus* larvae may be able to survive marine heat waves posed by climate change.

Introduction

Climate change has had an unquestionable effect on marine ecosystems and is an issue that seems to be worsening. The temperature of the ocean is slowly rising due to a combination of anthropogenic and natural forcings (Yuan et al., 2023). One example of this is the build-up of greenhouse gases in the atmosphere (Sarkwa et al., 2023). The global average surface temperature is predicted to increase between 1.8°C and 4.0°C by 2100 (Sarkwa et al., 2023). Previous research has shown that growth of *P. ochraceus* is influenced by temperature changes (Sanford, 2002). The decline of *Pisaster ochraceus*, a keystone species in the Pacific Northwest,

resulted in major ecological impacts on the biodiversity of marine communities (Menge et al., 2016).

During the spring and summer months, temperatures rise to between 18 and 24°C, which happens to be when echinoderm larvae are developing in the water column (George et al. 2021). This study indicated that temperature fluctuations may amplify *Pisaster* feeding. However, the effect of prolong exposure to high temperatures on larval growth and development of this species is unknown.

The current study determined whether *P. ochraceus* larvae can withstand prolong exposure to high temperatures when food is abundant. It is predicted that growth of *P. ochraceus* larvae in the high temperature treatment will be amplified. Like other ectotherms, *P. ochraceus* experience increased metabolic rates as a result of increased water temperatures (Fly, et al., 2012). As a result, this species may be more inclined to ingest particles from the water, at a higher rate and grow a lot faster at higher temperatures.

Methods

This experiment began on May 2, 2023, and was conducted in Lab 1 at Friday Harbor Laboratories in Friday Harbor, WA (48°32'45" N, 123°0'47" W). Five adult *Pisaster ochraceus* were collected on April 15th from Friday Harbor, WA. Four of the five adults were injected with 4 mL of 100 micromolar 1-Methyladenine to induce spawning on April 17th, 2023. Three males and 1 female spawned. Eggs were fertilized with spawn from one of the males. Fertilization was 99%. The adults were kept in the lab and fed mussels and clams on a regular basis. Larvae were stored in stock jars until the experiment began. Preliminary work began for this project prior to May 2nd.

The experimental design for this project utilized two large (104.5 cm x 104.5 cm x 14.5 cm) saltwater tanks: one to mimic current ocean temperatures (control) and another to simulate rising water temperatures due to climate change (treatment group). These temperatures were established and manipulated by utilizing two Hygger saltwater tank titanium tube submersible pinpoint aquarium heaters and two Inkbird ITC-308 digital temperature controllers. Three of these probes were used for the treatment, and one was used for the control. The design includes eight 1.8 Liter jars: four for the high temperature treatment and four for low temperature. Five hundred larvae were kept in each jar, with a total sample size of 4000 (1 larva per 2 mL of water).

All jars were monitored daily to ensure that temperatures were consistent and that paddles were functioning without issues. A system of swinging paddles was used to maintain water flow at all times. Paddles were rinsed with tap water and reverse osmosis (RO) water every two days to reduce risks of contamination. Initially, *P. ochraceus* larvae were fed 2300 cells/mL *Rhodomonas lens* and 2500 cells/ml of *Dunaliella tertiolecta* every other day, gradually increasing the concentration of cells to between 3500 cells/mL and 4000 cells/mL for both algae towards the end of the experiment to accommodate for growth. Larval cultures were drained and replaced with fresh 0.45 μ m filtered seawater once a week (Figure 1). Unfortunately, some of the larval cultures were contaminated, so a second experiment was started, using the same experimental design (four jars in the high temperature treatments and four in the controls). The second experiment began on May 2, 2023, when larvae were 15 days old.

Tank temperature was the independent variable and the dependent variables were growth measurements (total larval length, total larval width stomach length, stomach width and ciliated band length). Initially, the two established temperatures were 12°C and 22°C. However, the high

temperature (22°C) was lowered to 15°C on May 5th, 2023, to reduce the risks of heat stress (Figure 2a and 2b). To minimize pseudo-replication, jars and paddles from the high temperature tank were switched with those from the low temperature tanks on May 5th, 2023, and on May 19th, 2023. Also, both tanks were set to new temperatures, essentially swapping the role of each tank. A temperature data logger was placed in each tank (Figure 2a and 2b).

On May 10th, 2023, the first set of photographs for 23-day old larvae were captured using a HAYEAR 34MP microscope camera attached to a Nikon Eclipse Ci upright trinocular compound microscope. All pictures were taken using the 4x objective on the microscope. HAYEAR software was used to capture and save all images, and ImageJ was used to measure the various larval dimensions. Pixels in the photographs were converted to microns by setting a scale on ImageJ (103.1 pixels = 100 microns). Twelve to 17 larvae were randomly selected and measured per jar for a total of approximately 136 larvae for each age group.

Data Analysis

A mixed model nested ANOVA was used to process recordings in RStudio. Temperature was a fixed factor, and jars were a random factor nested within temperature treatments. A Type III analysis of variance using Satterthwaite's method was utilized to determine correlation between dependent variables, displayed in Tables 1-3. Scatterplot matrices were made using ggally and ggplot packages in RStudio 4.3. Due to time constraints and gaps in the data, the scatterplot matrix for 37-day old larvae did not include measurements of the ciliated band length. The Pearson correlation coefficients were included in all figures.

Results

Larval development:

When the larvae were 23 days old, bipinnaria larvae were the only stages of *Pisaster* present in both high and low temperatures (Figures 3a-c). Posterolateral arms were observed, albeit they were hardly defined or developed at this stage. Brachiolaria larvae were not encountered among 23-day old larvae. After 31 days, bipinnariae were still seen, and brachiolariae within the larval cultures were observed (Figure 4a-b). Spicule formation was witnessed during this stage (Figure 4c). At 37 days, brachiolaria larvae became the majority of larvae observed. At this point, posterolateral arms were conspicuously elongated (Figure 5a). Bipinnaria larvae were not observed among 37-day old larvae from both temperature treatments. Spicule development was more advanced in several specimens in the high temperature treatment (Figure 5b). By the end of the experiment, high survival was observed in all jars irrespective of temperature treatment.

Larval growth:

Total larval length and larval width did not vary significantly among 23 day old *Pisaster* larvae from the low and high temperature treatments (Figure 6a, Table 1, Nested Analysis of Variance (ANOVA), $F = 2.66$, $p = 0.2019$, $F = 2.66$, $p = 0.2007$ respectively, $n = 117$; where n is the total number of larvae measured) but stomach length and width, and ciliated band length varied significantly (Table 1, nested ANOVA, $F = 27.55$, $p < 0.0001$, $F = 58.59$, $p < 0.0001$ and $F = 12.10$, $p = 0.0008$ respectively, $n = 120$). Larvae in the high temperature treatment had larger stomachs and longer ciliated bands (Figure 6a). All dimensions measured varied significantly

among 31-day-old *Pisaster* larvae from the low and high temperature treatments (Table 2). Larvae from the high temperature treatment were significantly longer and wider with longer ciliated bands than those in the low temperature treatment (Figure 6b). Most of these differences disappeared among 37-day old larvae from the two temperature treatments but remained significant for total larval width and ciliated band length (Table 3, nested ANOVA, $F = 24.19$, $p < 0.0001$, and $F = 63.59$, $p < 0.0001$ respectively, $n = 95$). Larvae in the high temperature treatment were significantly wider (Figure 6c) with longer ciliated bands than those in the low temperature treatment.

Scatterplot matrix and Pearson's correlation coefficients further highlight the above differences. For 23-day old larvae the correlation coefficient between all pairs of variables measured indicated that they were highly correlated. Correlation among pairs of variables was positive in most cases (Figure 7a) with the exception of a couple of cases when a very weak negative correlation was observed for stomach width and ciliated band length and stomach width and total larval length for 31-day old *Pisaster* larvae from the low temperature treatment (Figure 7b). The strongest positive correlations were between total larval length and total larval width ($r = 0.963$), stomach length and stomach width ($r = 0.954$) and between total larval length and ciliated band length (0.884 , Figure 7a) for larvae from both temperature treatments. The correlation coefficient was highest for the relationship between ciliated band length and total larval length and width for larvae at high temperatures. The next scatterplot matrix (Figure 7b) also shows significant positive correlation between variables but with the exception of a strong positive correlation between stomach length and stomach width ($r = 0.958$) for larvae from the low temperature treatment, the correlation between most of the other variables was weak compared to observations made for 23-day old larvae (Figure 7b). The weakest relationship was

between stomach width and ciliated band length ($r = 0.141$, $p > 0.05$) and between stomach width and total larval length ($r = 0.144$, $p > 0.05$, Figure 7b) for larvae from the low temperature treatment. However, the relationship between these latter two pairs of variables remained significantly correlated for larvae from the high temperature treatment ($r = 0.498$, and $r = 0.368$, $p < 0.05$ respectively, Figure 7b). The final scatter plot also indicates further weakening of the relationship between all variables measured with the weakest relationships between stomach width and total width (Figure 7c, $r = 0.292$, $p < 0.05$) and between stomach width and total length (Figure 7c, $r = 0.077$, $p > 0.05$) for larvae in the high temperature treatment.

An example of Simpson's paradox was observed for 31-day old larvae (Figure 7b). The total correlation coefficients were very low and, in some instances, slightly negative for relationships between stomach length and ciliated band length ($r = 0.131$); stomach width and ciliated band length ($r = -0.047$); total width and stomach width ($r = 0.188$), total length and stomach width ($r = -0.040$), stomach length and total width ($r = 0.294$) and between total length and stomach length ($r = 0.105$). These relationships reversed when the correlation coefficient for larvae in the two temperature treatments were viewed separately. Correlation coefficients between stomach length and total width and between total length and stomach length for larvae from the low food treatment were positive and significantly higher ($r = 0.804$ and $r = 0.721$ respectively, $p < 0.05$). Correlation coefficients for the other 4 pairs of variables were also significantly higher for *Pisaster* larvae in the high temperature treatments (Figure 7b).

Discussion

We hypothesized that increased water temperatures would have a considerable effect on the growth rate of *Pisaster ochraceus* larvae. For all measurements made, larvae maintained in

higher temperatures tended to be significantly larger than those kept in the control temperature. Also, larvae kept in higher temperatures were more developed with brachiolar arms present and longer posterolateral arms. These results support our biological hypothesis. Temperature had a significant effect on the growth and development of *Pisaster* larvae. This is not surprising since larvae were fed a good mixed algal diet of *Dunaliella tertiolecta* and *Rhodomonas* sp. every two days and at high temperatures larvae may have cleared more algal particles from the water column than at low temperatures. George et al., (2021) noted that wavering temperatures may not only increase the feeding of *Pisaster* larvae, but may also influence their rate of growth and development. Titus & Hearther (2019) also found that increased temperatures resulted in faster larval growth rates and development for this species. Larval survival in the present study was high in both temperature treatments. The observed high larval survival in our study could not have been due to cloning; as cloning was rarely observed in either of our high or low temperature treatments. This is in agreement with the Titus & Hearther (2019) study, who noted very low cloning in their high (~22°C) and low (~9°C) temperature treatments, but more cloning in an intermediate temperature (~16°C) treatment. However, Titus & Hearther (2019) noted a higher risk of mortality at high temperatures. The differences between studies might have to do with larval age. In the current study 23-day old bipinnariae and 31 and 37-day old brachiolariae were used and larvae were kept at high temperatures of 22°C for only 3 days and between 15 and 20°C for the rest of the experimental period. In the Titus & Hearther (2019) study, much younger (14 and 17-day old) bipinnariae were used and larvae were kept in the high temperature treatment for the duration of their experiment. Another study by Utsch & George (2022), that looked at the combined effects of temperature and food on larval growth and development, found that temperature had less of an effect on larval growth and development while access to food had

a greater effect on growth of *Pisaster* larvae. The results of these studies imply that increased warming may have a positive effect on larval development of *P. ochraceus* as long as food is abundant in the water column. Our study also indicates that older larvae may be more resilient to extreme temperatures.

The observed case of Simpson's paradox in this study, specifically for 31-day old larvae is interesting. But can be explained by differences in the rate of growth of the different larval dimensions for larvae at high and low temperature. For instance, stomach size for 31-day old larvae in the low temperature treatment continued to increase sharply with total larval width but did not for 31-day old larvae in the high temperature treatment. While ciliated band length continued to increase sharply with stomach size for 31-day old larvae in the high temperature treatment but not for larvae in the low temperature treatment. Larvae may be prioritizing the growth of these different dimensions based on needs. Keeping in mind that longer ciliated bands favor higher clearance rate of algal particles; larger stomach sizes may be due to the extension of the stomach to accommodate large amounts of food. This in turn will accelerate growth for larvae at high temperatures. The differences among these pairs of variables disappear for the aggregated data from both temperature treatments leading to a reversal and in a couple of cases a slight negative correlation coefficient among variables. Pearl (2014) goes into a lot of detail on this topic.

Future studies should focus on the relationship between the length of the ciliated band and stomach size for larvae kept under various temperature treatments. We also recommend that if replicate large tanks are impossible, future studies should swap tanks and paddles more frequently to further minimize pseudo-replication.

It is imperative that scientists understand the negative impacts of climate change, on these species, note what can be done to combat these effects, and how best to spread awareness to the general public, in order to conserve *Pisaster* populations and the marine intertidal ecosystem as a whole.

Table 1 Type III Nested Analysis of Variance Table with Satterthwaite's method (used to calculate the denominator degrees of freedom, DenDf) for 23-day-old larvae exposed to high and low temperatures. Jar nested within temperature. Highlighted p values indicate significant differences. Total sample size (n) = 117.

Growth	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Total Length (µm)	138914	138914	1	2.9823	2.6605	0.2019
Total Width (µm)	34134	34134	1	3.0158	2.6631	0.2007
Stomach Length (µm)	348961	348961	1	111.14	27.548	7.423e-07
Stomach Width (µm)	673040	673040	1	111.19	58.595	7.617e-12
Ciliated Band Length (µm)	11341274	11341274	1	71.572	12.103	0.0008607

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2 Type III Nested Analysis of Variance Table with Satterthwaite's method (used to calculate the denominator degrees of freedom, DenDf) for 31-day-old larvae exposed to high and low temperatures. Jar nested within temperature. Total sample size (n) = 120.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Total Length (µm)	804968	804968	1	3.0145	13.731	0.03387
Total Width (µm)	742845	742845	1	114.02	52.043	6.416e-11
Stomach Length (µm)	134195	134195	1	114.01	26.127	1.3e-06
Stomach Width (µm)	168602	168602	1	114.01	35.496	2.904e-08
Ciliated Band Length (µm)	50761883	50761883	1	114.01	31.494	1.424e-07

Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3 Type III Nested Analysis of Variance Table with Satterthwaite's method (used to calculate the denominator degrees of freedom, DenDf) for 37-day-old larvae exposed to high and low temperatures. Jar nested within temperature. Highlighted p values indicate significant differences. Total sample size (n) = 95.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Total Length (μm)	509579	509579	1	2.9848	9.2087	0.05648
Total Width (μm)	157668	157668	1	89.084	24.189	3.963e-06
Stomach Length (μm)	10447	10447	1	89.057	3.7919	0.05465
Stomach Width (μm)	6023.3	6023.3	1	89.031	2.5814	0.1117
Ciliated Band Length (μm)	141565978	141565978	1	81.027	63.595	8.411e-12

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



Figure 1. Larval cultures being drained and replaced with fresh 0.45 μ m filtered seawater.

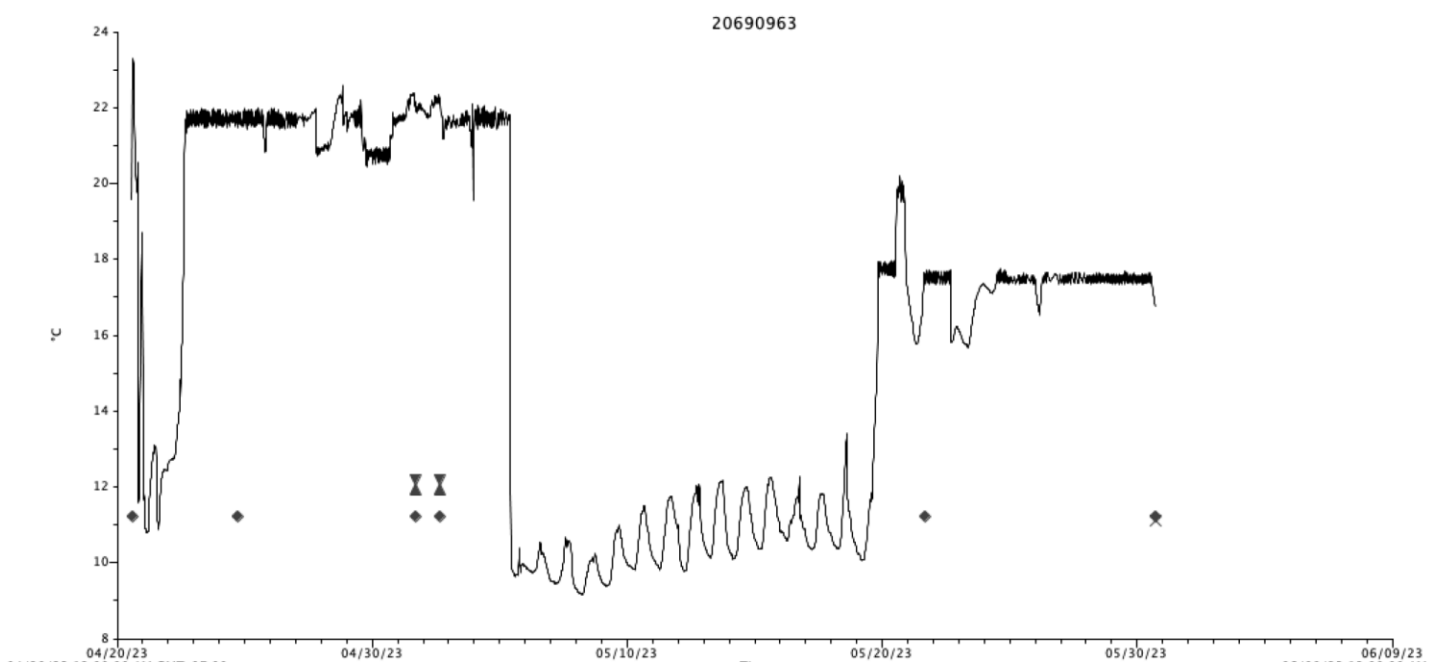


Figure 2a. Temperature logger recordings for Tank 1:

Initially, temperatures were maintained at 22°C with some fluctuations (lowest around 19°C) from April 23 through May 5). Temperatures were dropped to ~9°C with fluctuations between 9 and 13°C until May 20. Temperatures were increased to ~ 18°C for the rest of the experimental period. A temperature spike was observed on May 21 but returned to 18°C on May 22 with slight fluctuations thereafter.

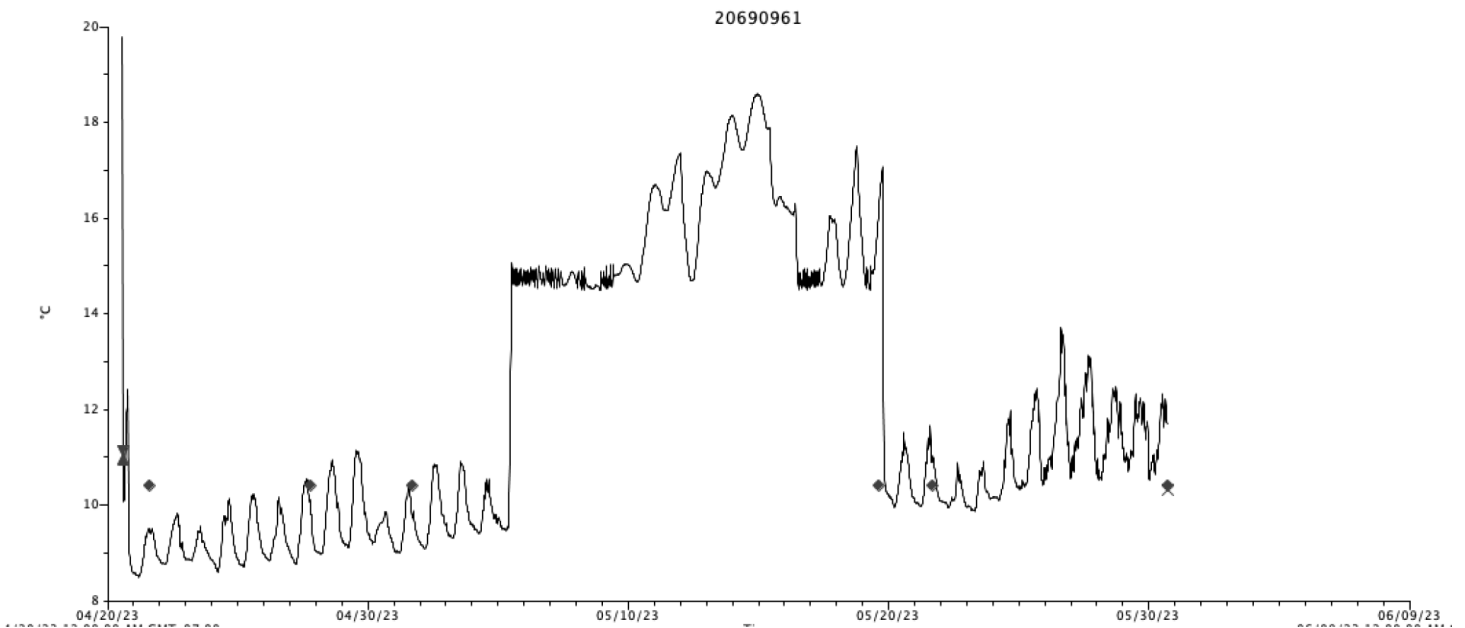


Figure 2b. Temperature logger recordings for Tank 2:

Initially, temperatures were maintained between 9 and 12°C with some fluctuations from April 23 through May 5). Temperatures were increased to 15°C on May 5. However, a heat wave caused temperatures to exceed 18 °C in this tank from May 12 to 16. On May 20, temperatures were decreased (temperatures ranged from 11°C to ~14°C for the rest of the experimental period).

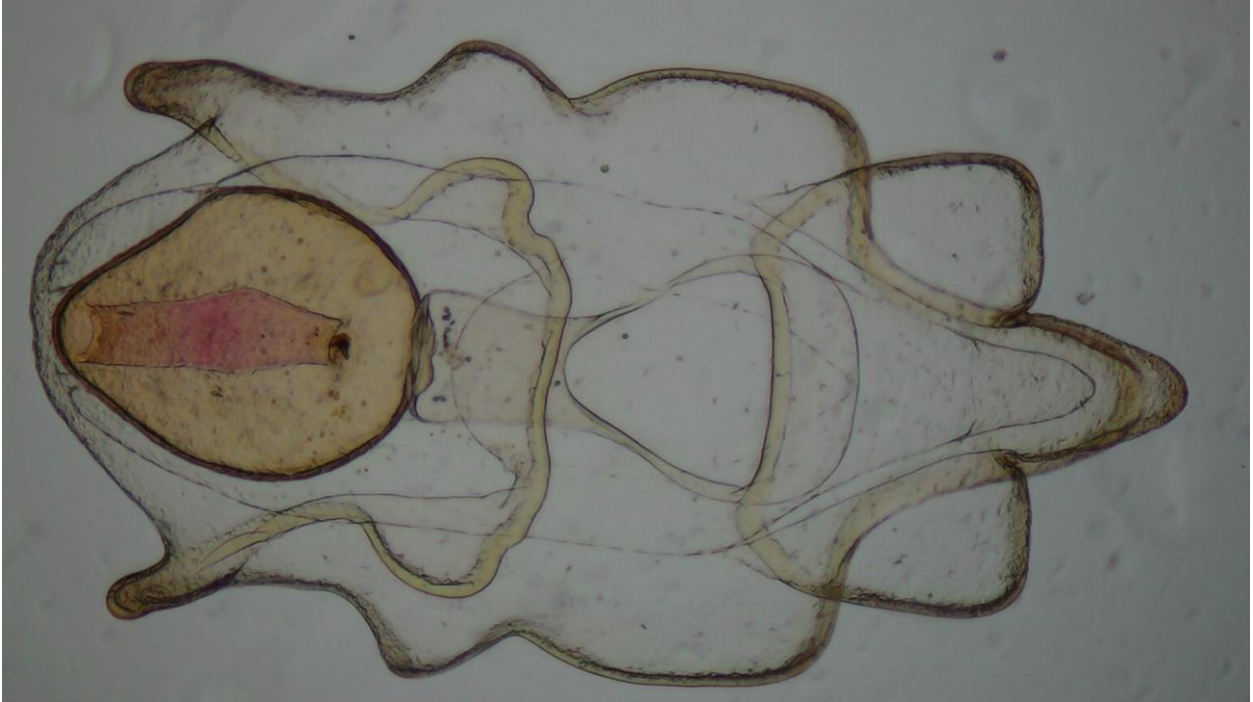


Figure 3a. 23-day-old Bipinnaria larva (total larval length 1777.9 μm , total larval width 560.7 μm) kept in high temperature treatment.

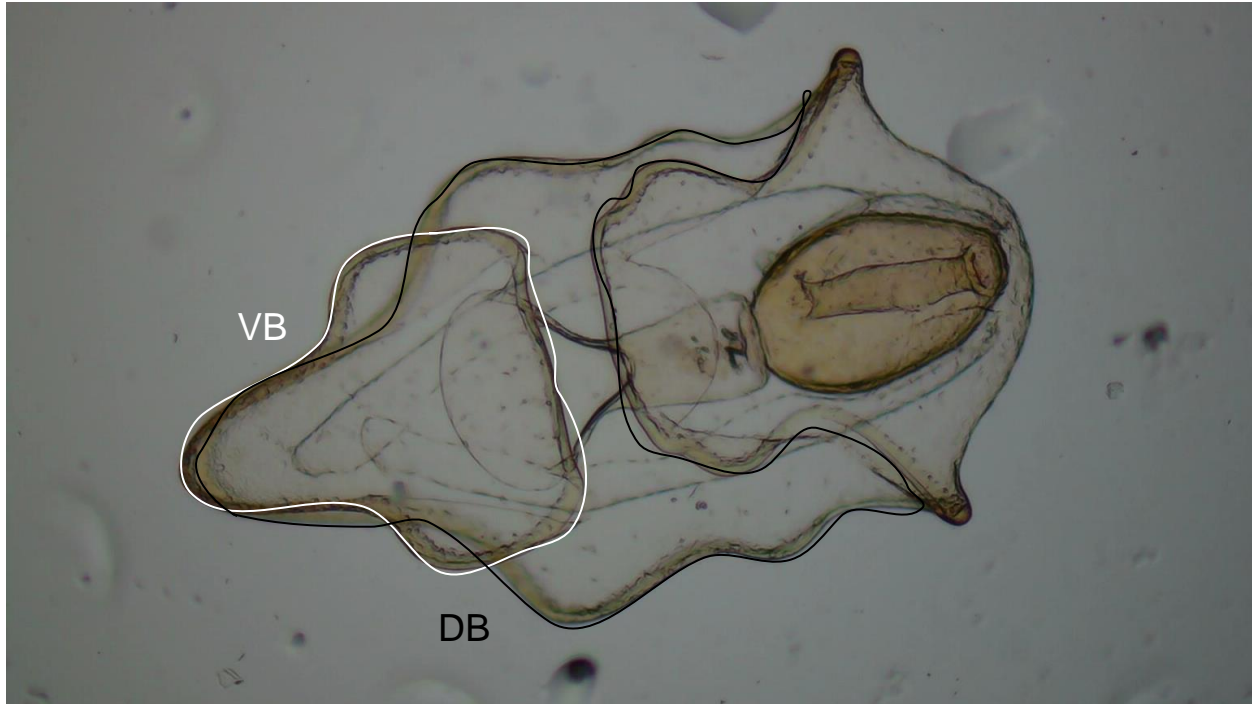


Figure 3b. 23-day-old Bipinnaria larva (total larval length 1346.5 μm , total larval width 702.4 μm) kept in low temperature treatment showing an outline of the ciliated band measured. The Ciliated Band Length on the ventral side (VB) is highlighted in white + Ciliated band on the dorsal side (DB) highlighted in black. All measurements were in microns.

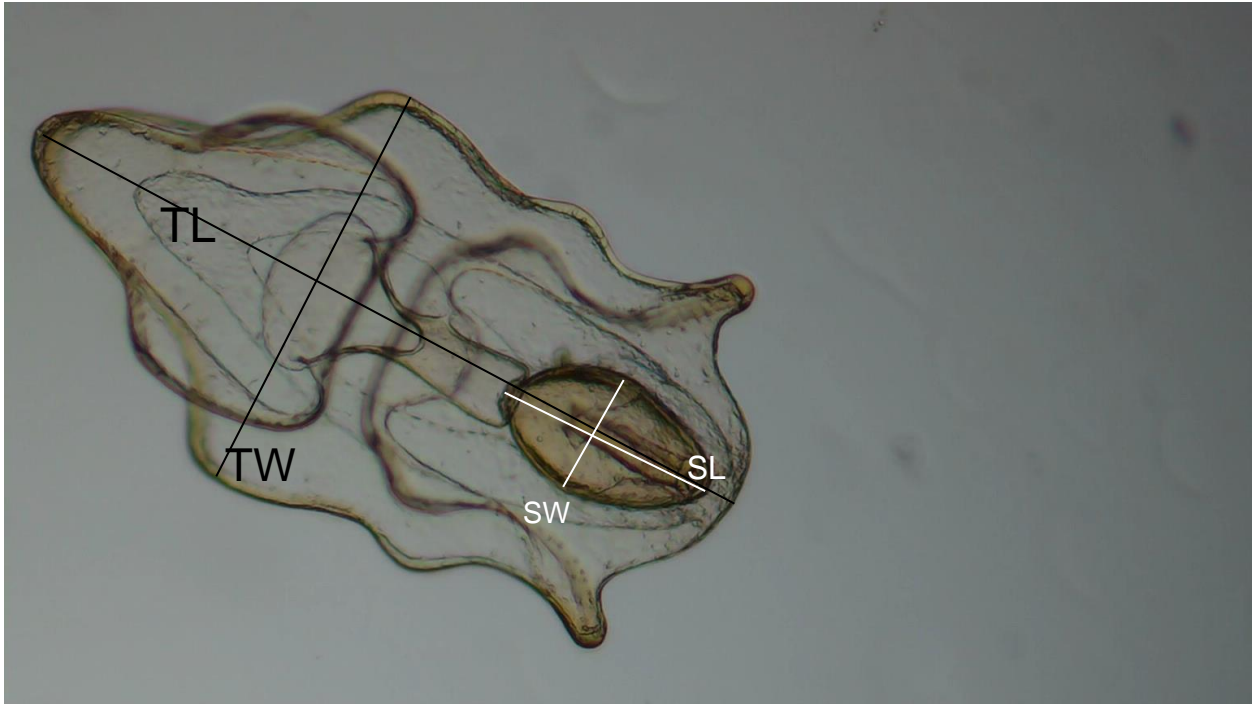


Figure 3c. 23-day-old Bipinnaria larva (total larval length 1208.9 μm , total larval width 630.2 μm) kept in low temperature treatment showing the posterolateral arms beginning to elongate and four of the measurements made. Total Length = TL, Total Width = TW, Stomach Length = SL, Stomach Width = SW. All measurements were in microns.

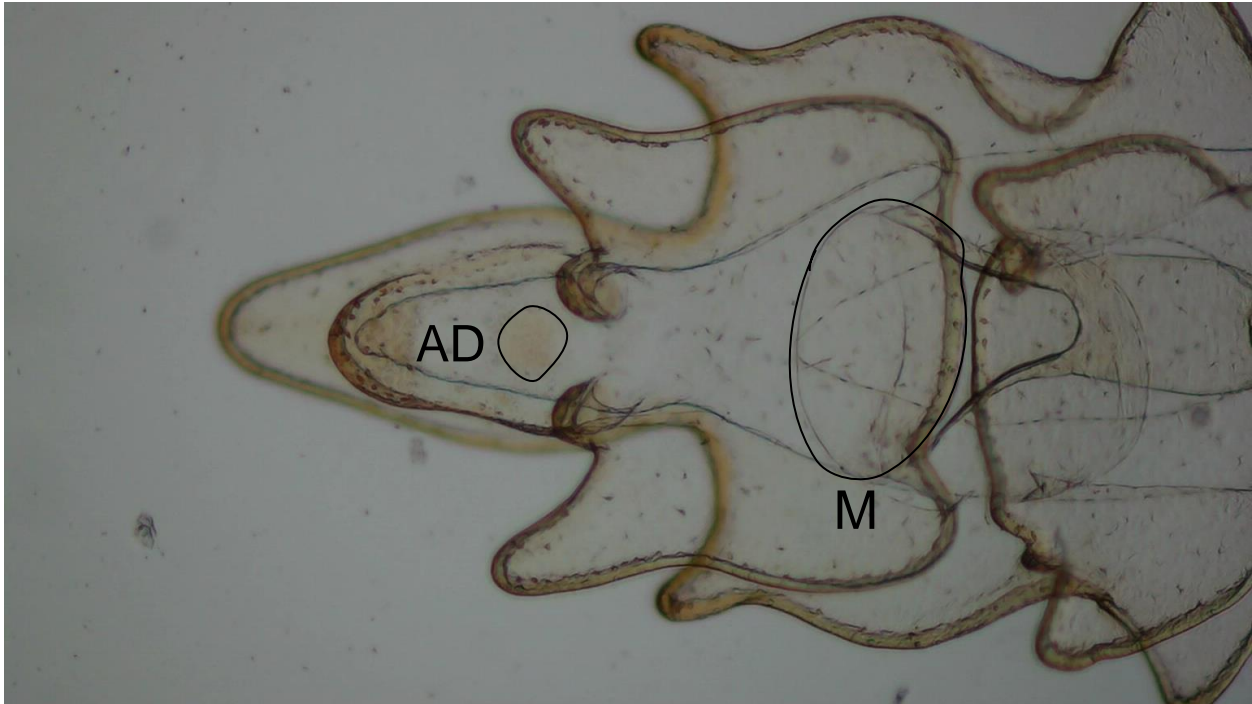


Figure 4a. 31-day-old Brachiolaria larva (total larval length 2210.6 μm , total larval width 1159.5 μm) kept in the high temperature treatment showing the development of the adhesive disk and appearance of brachiolar arms. Adhesive disk = AD, and Mouth = M are highlighted.



Figure 4b. 31-day-old Brachiolaria larva (total larval length 1941.9 μm , total larval width 811.3 μm) kept in low temperature treatment.

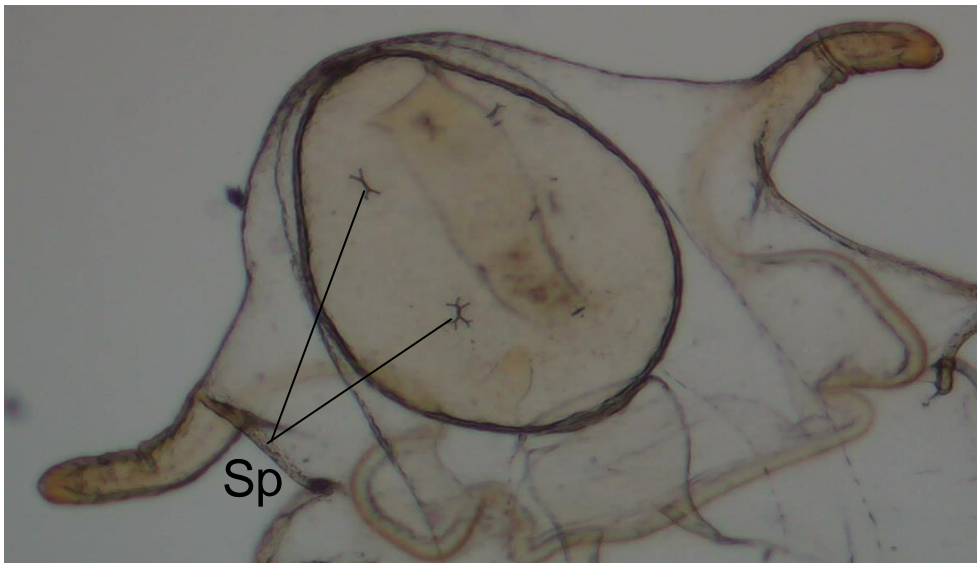


Figure 4c. Spicule formation for 31-day-old Brachiolaria larva (total larval length 625.5 μm , total larval width 1002.7 μm) kept in the low temperature treatment. Spicules = Sp.



Figure 5a. Developing posterolateral arms, in 37-day-old brachiolaria larva (total larval length 1850.4 μm , total larval width 987.1 μm) kept in the high temperature treatment.

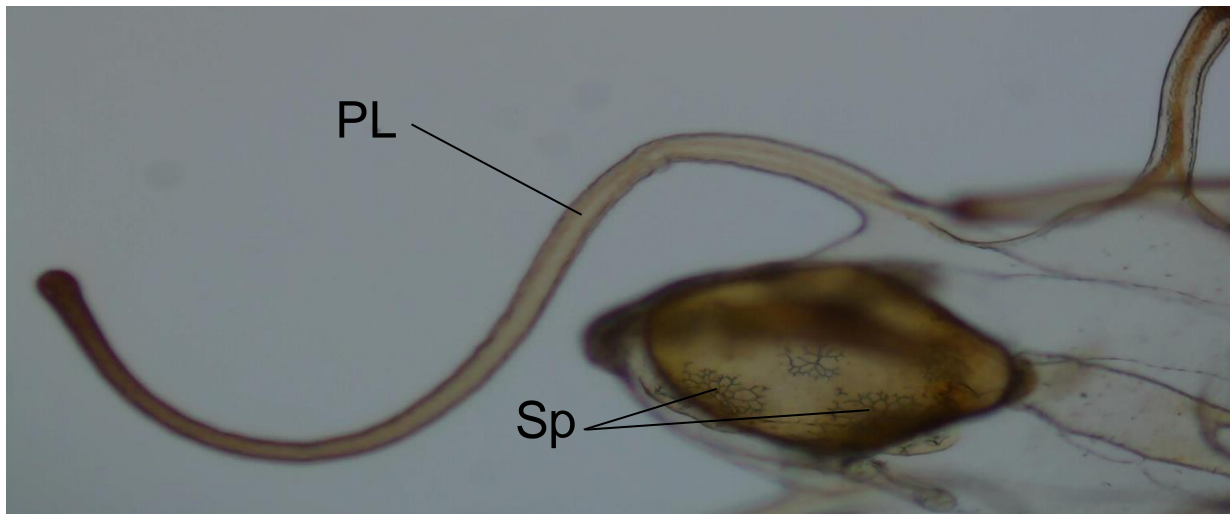
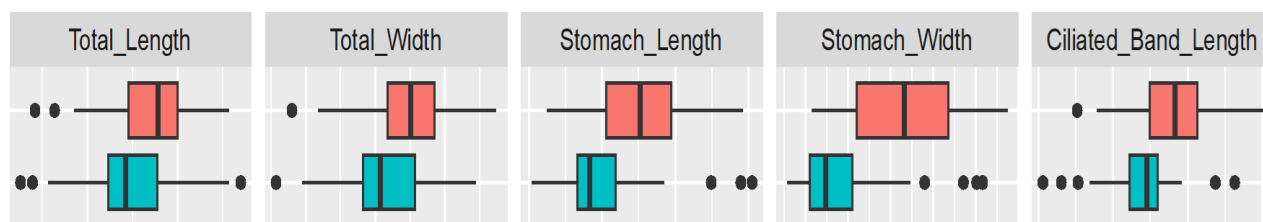
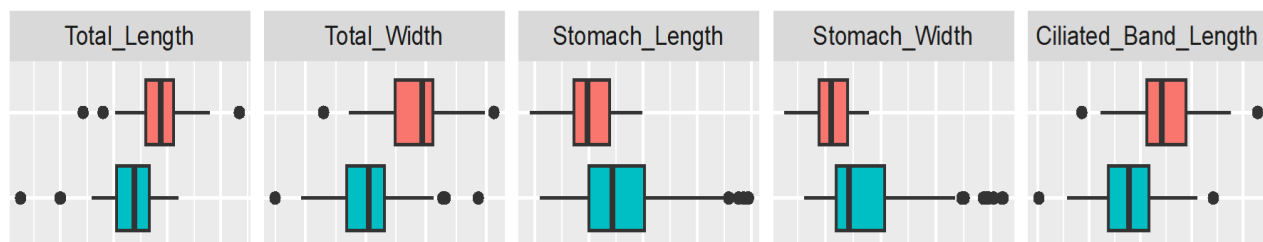


Figure 5b. Spicule formation and posterolateral arm of 37-day-old Brachiolaria larva (total larval length 2711.9 μm , total larval width 837.5 μm) kept in the high temperature treatment.

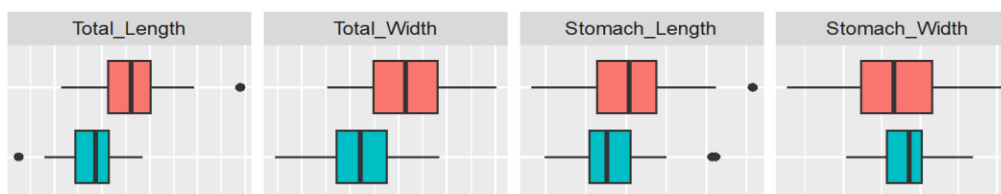
Posterolateral arm = PL, Spicules = Sp



a) 23-day old larvae.



b) 31-day old larvae.



c) 37-day old larvae.

Figure 6. Boxplots show total length, total width, stomach length, stomach width and the ciliated band length for a) 23-day-old, b) 31-day old, and c) 37-day old larvae (respectively) at high temperatures (15°C-22°C, shown in red) and low temperatures (9-12 °C, shown in blue). The vertical line in the middle of the box represents the median. Whiskers represent the maximum and minimum values. Dots represent outlier measurements that are outside the interquartile range.

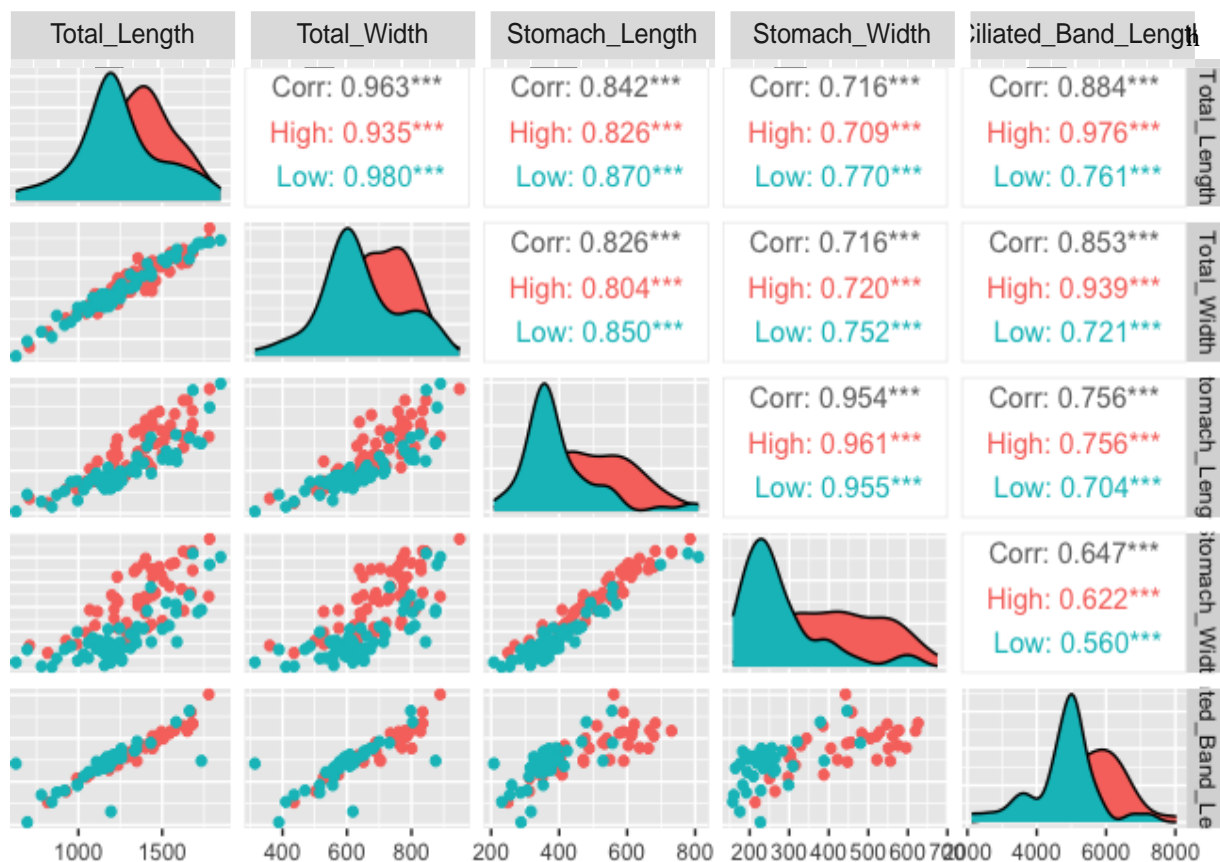


Figure 7a. Scatterplot matrix showing relationships between total length, total width, stomach length, stomach width, and the ciliated band length for 23-day-old larvae at high temperatures (22°C) and low temperatures (9-12 °C). Distribution of larval dimensions, density plots, and correlation coefficients for *Pisaster ochraceus* larvae at high and low temperatures are shown. All measurements are in microns.

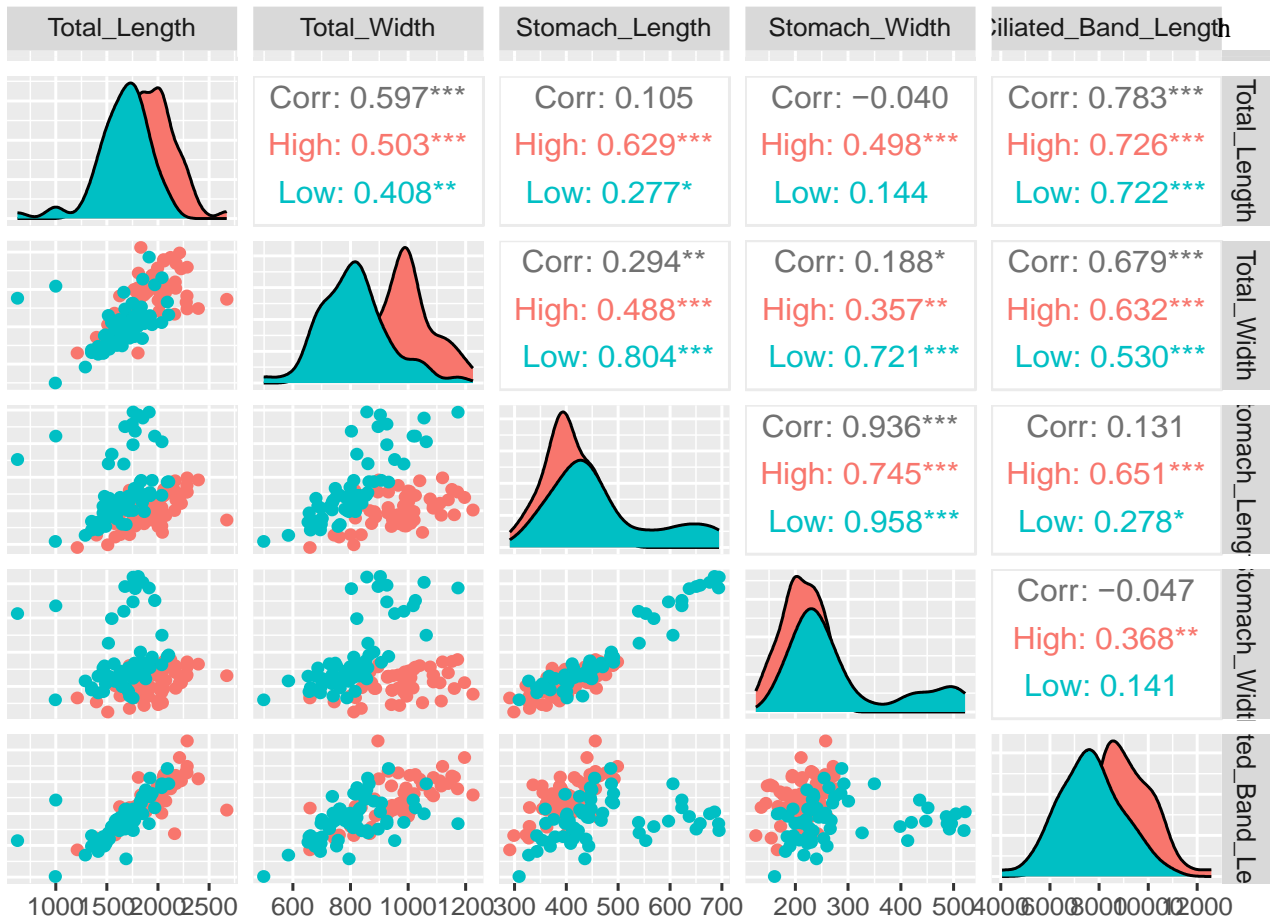


Figure 7b. Scatterplot matrix showing relationships between total length, total width, stomach length, stomach width, and the ciliated band length for 31-day-old larvae at high temperatures (22°C) and low temperatures (9-12 °C). Distribution, density plots, and correlation coefficients for *ochraceus* larvae at high and low temperatures are shown. All measurements are in microns.



Figure 7c. Scatterplot matrix showing relationships between total length, total width, stomach length, and stomach width for 37-day-old larvae at high temperatures (22°C) and low temperatures (9-12 °C). Distribution, density plots, and correlation coefficients for *Pisaster ochraceus* larvae at high and low temperatures are shown. All measurements are in microns.

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