Effects of Thermal Stress on Ascidian Larval Development

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Abstract:
Rising ocean temperatures caused by climate change have the potential to cause detrimental effects to the development of many marine organisms. Numerous species of ascidians have been shown to undergo faster rates of larval development when subject to heat stress. In this study, we investigated the effects of increased temperature on the development and survival of embryos in the species *Boltenia villosa*. *B. villosa* embryos were placed under three different temperature conditions, ambient (11-12°C), mid (14-15.5°C) or high (16.5-18°C), and rates of development to important stage markers were observed. Survival rate to larval stage was also recorded. Our results show that embryos subjected to higher temperatures develop faster than those at ambient temperatures but have lower survival rates. This suggests that while higher temperatures speed up development, they also have detrimental effects on the quality of development.

Introduction:
As climate change driven by human activity worsens, ocean temperatures continue to rise with the average annual temperature from 2001-2020 being 0.99°C higher than in pre-industrial times (IPCC 6, 2021). On top of the steady increase in average sea surface temperature, there has also been an increased occurrence of marine heatwaves that are projected to intensify in the coming years (Athanase et al., 2023). This increase in temperature can have devastating ecological consequences, including decreasing biodiversity, increasing extinction risk, especially in endemic species, and disrupting typical developmental and physiological processes (IPCC 6, 2021). In order to better understand the scope of these consequences and how to respond effectively to them it is important to study the effects of temperature on both entire ecosystems and on individual species. This paper aims to investigate the effects of temperature on the development *Boltenia villosa*, a native ascidian species.

Adult ascidians are sessile, filter feeding tunicates often found on docks and hulls of ships. They play an important role in the ecosystem as they are a food source for both larger invertebrates such as molluscs and crabs and some vertebrates such as fish and birds. They also serve as a reliable place for smaller organisms including algae and sponges to settle. Ascidians reproduce via spawning, releasing hundreds of eggs and sperm at a time to be fertilized in the open ocean. Once fertilized, the embryos develop into motile, tadpole-like larvae that swim through the currents before finding a place to settle and metamorphose (Fodor et al, 2021).

Embryonic development has been well documented in many species of ascidians, both solitary and colonial. (Strathmann, 1987 and Swalla, 2004). In solitary ascidians, fertilization is marked by the movement of myoplasm to the vegetal pole of the egg, followed by six rounds of bilateral cleavage (Swalla, 2004). This results in a 64-cell embryo that will then go through gastrulation, neurulation, and finally notochord convergence and extension to form a tadpole. Ascidians develop remarkably quickly, with this process all occurring in under 24 hours. Previous studies have shown that abiotic factors such as temperature and salinity affect development and survival

of both the solitary ascidians *Styela plicata* and *Ciona savignyi* and the colonial ascidians *Botryllus schlosseri* and *Botrylloides violaceus* (Thiyagarajan and Qian, 2003, Nomaguchi et al., 1997, & Epelbaum et al., 2009).

I am investigating how increases in sea water temperature to levels that are projected to become more common in the next fifty years affect the developmental success and rates of development in ascidians native to the Salish Sea (IPCC 6, 2021). I hypothesize that increased temperatures will disrupt development in the ascidian *Boltenia villosa* compared to ambient temperatures consistent with the disruptions seen in previously studied species (Thiyagarajan and Qian, 2003, Nomaguchi et al., 1997, & Epelbaum et al., 2009). I predict that when subjected to increased temperatures, *B. villosa* larvae will on average take less time to reach key developmental stages including first cleavage and gastrulation, and a larger percentage will not reach the tadpole-like larva stage.

Methods:
This study was conducted from May 11th to May 27th, 2023, at Friday Harbor Laboratories (FHL) in Lab 10. Adult *B. villosa* (Fig 1) were collected from tires on the FHL dock and kept from one day to three weeks in lab sea tables under a lamp at all times to prevent spawning. The *B. villosa* were then fertilized following the methods outlined in Swalla (2004) at room temperature.

Figure 1: Adult *Boltenia villosa*.

Thirty minutes post fertilization controls were moved to temperature-controlled sea tables with bag-filtered seawater which were used like water baths to keep cultures at specific temperatures. Thermometers were used to track the temperature. Treatment groups were moved to three separate sea tables, one ambient table at 11-12°C and two treated tables warmed by aquarium heaters to 14.5-15.5°C and 16.5-18°C respectively. Multiple fertilizations were done, resulting in three replicate jars per treatment. Data was collected by taking samples of each jar at regular intervals and calculating the percent of each sample at important developmental stages: 2 cell, 4 cell, 8 cell, 16 cell, 32 cell, gastrula, neurula, tailbud (Fig 2). Time until each developmental stage was recorded when over 50% of developing embryos in a sample had reached that stage.

After 24 hours, survival rates were calculated as percent of eggs in a sample that developed completely into the hatched tadpole-like larva stage.

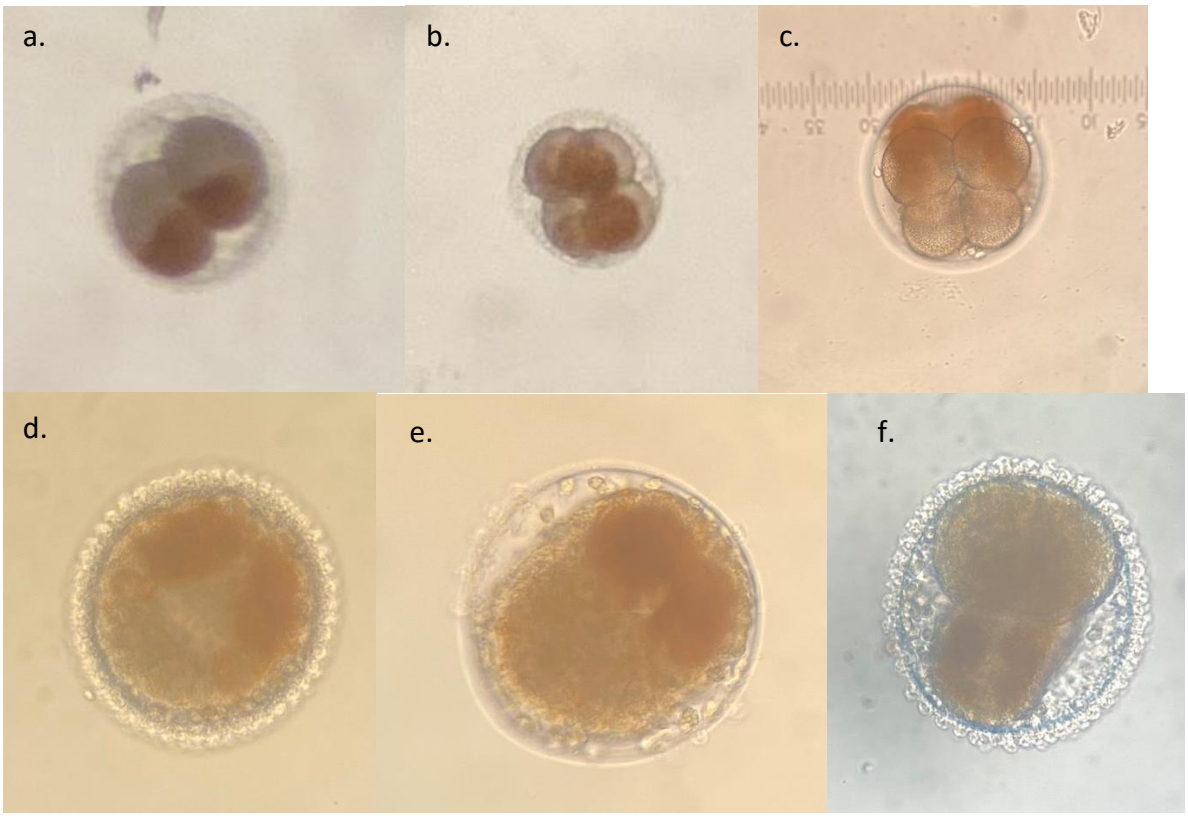

Figure 2: Developmental stages of *Boltenia villosa* embryo. (a) 2 cell (b) 4 cell (c) 8 cell (d) Gastrula (e) Neurula (f) Tail bud

Data was analyzed using statistical software in R. A one-way ANOVA was used to determine the statistical significance between time to development in treatment groups, followed by Tukey tests for each developmental stage. Another one-way ANOVA was used to analyze survival rate data.

Results:

Percent of successful fertilization ranged from 10 to 25%. The larvae in the high temperature treatment reached each developmental stage the fastest, followed by those at the middle temperature, and then those at ambient temperature (Fig 3). The time to each developmental stage was statistically significantly different ($p<0.05$) between the ambient and high temperature groups for all developmental stages except the 4-cell stage (Fig 4). There were no statistically significant differences in time to the 2-cell stage or time to the 4-cell stage between the ambient and mid temperature groups. There were statistically significant differences in the time to each stage between the ambient and mid temperature groups for all stages starting from the 8-cell stage. The mid and high temp groups followed the same pattern, with no statistically significant differences in time to the 2-cell stage or 4-cell stage. These two treatments were also statistically significant in difference in time to each developmental stage starting with the 8-cell stage. Overall, there were statistically significant differences between all three treatment groups from the 8-cell stage onward.

	Ambient (11-12 C)	Mid (14.5-15.5 C)	High (16.5-18 C)
2 Cell	75	63.3	51.7
4 Cell	106.7	96.7	85
8 Cell	151.7	131.7	118.3
16 Cell	206.7	158.3	136.7
32 Cell	266.7	206.7	176.7
Gastrula	356.7	296.7	236.7
Neurula	521.7	431.7	356.7
Tail Bud	656.7	576.7	516.7

Table 1: Time post fertilization (minutes) to reach each developmental stage under each temperature treatment.

	Ambient-Mid	Ambient-High	Mid-High
2 Cell	0.21	0.02	0.21
4 Cell	0.52	0.10	0.43
8 Cell	0.0023	0.0001	0.017
16 Cell	0.000016	0.0000018	0.0015
32 Cell	0.000035	0.000003	0.0017
Gastrula	3.55e-05	8.0e-07	3.55e-05
Neurula	3.0e-06	1.0e-07	9.2e-06
Tail Bud	0.0005	0.00002	0.0023

Table 2: P-values for differences between average time to each developmental stage.

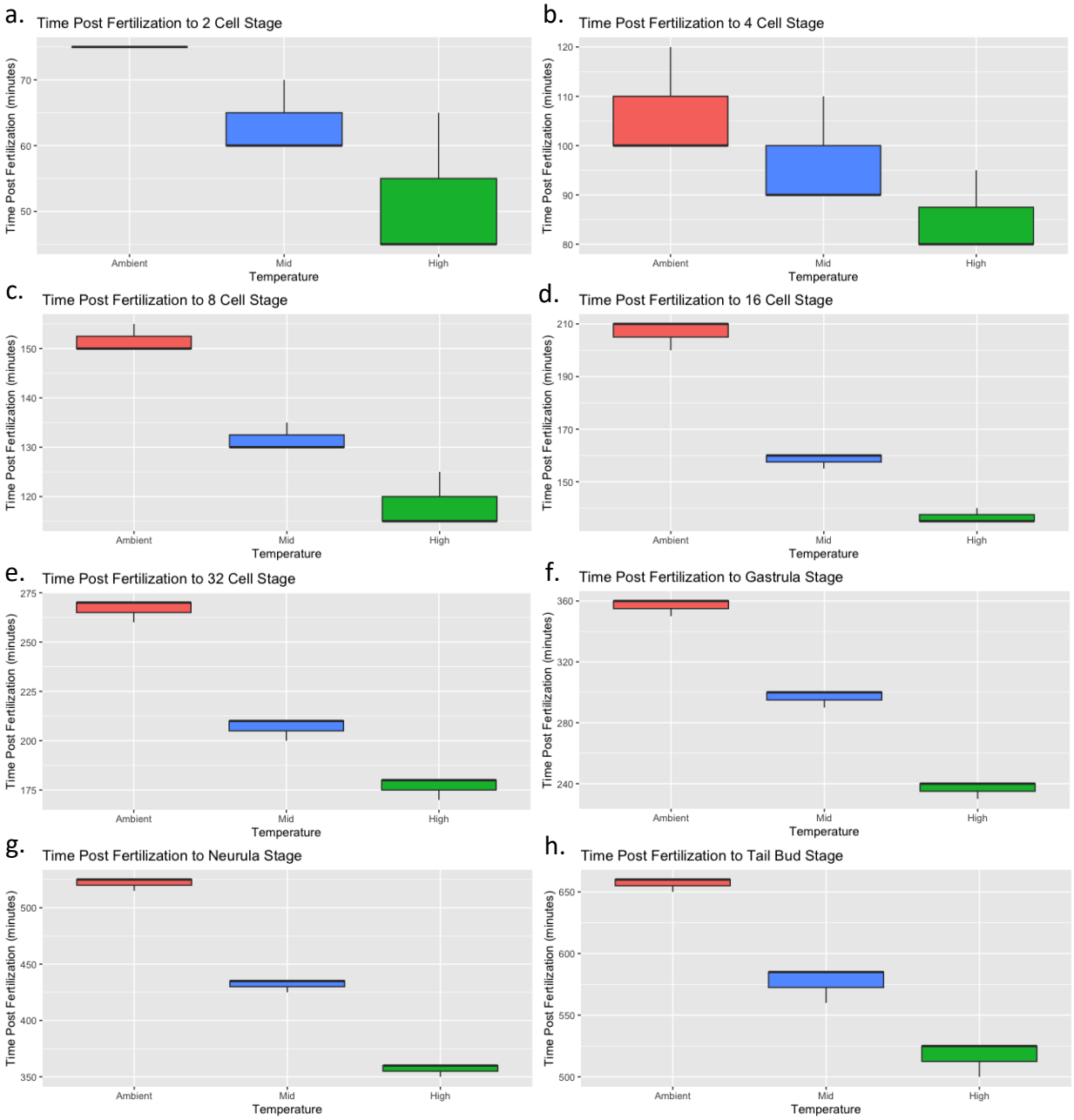

Figure 3: Average time post fertilization to reach each developmental stage for each treatment group. (a) Time to 2 cell (b) Time to 4 cell (c) Time to 8 cell (d) Time to 16 cell (e) Time to 32 cell (f) Time to gastrula (g) Time to neurula (h) Time to tail bud. See table 2 for p-values.

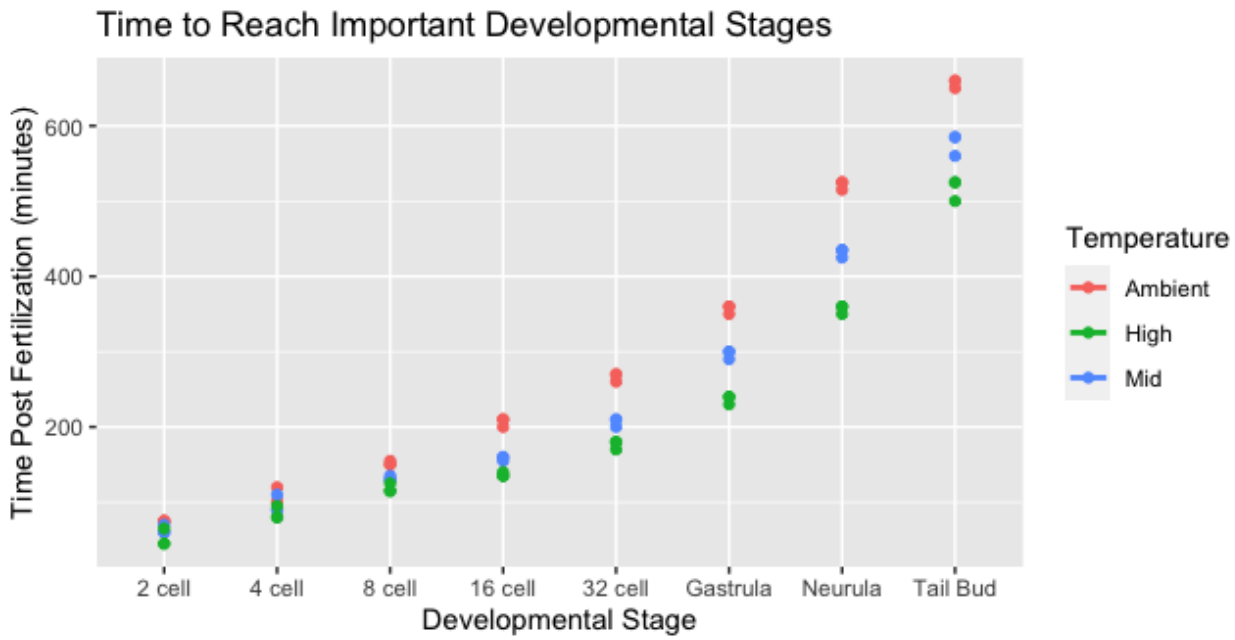

Figure 4: Time post fertilization to reach important developmental stages. Compiled comparison of time to each developmental between all temperature treatments. See table 2 for p-values.

The survival rate to the tadpole larva stage was highest in the ambient temperature group and lowest in the high temperature group (Fig 5). The ambient temperature group had an average survival rate of 1.91%, the mid temperature group had an average survival rate of 1.2% and the high temperature group had an average survival rate of 0.49%. The difference in survival rate was not statistically significant between the mid and high temperature groups (p=0.18) or between the ambient and mid temperature groups (p=0.20) but was statistically significant between the ambient and high temperature groups (p=0.02).

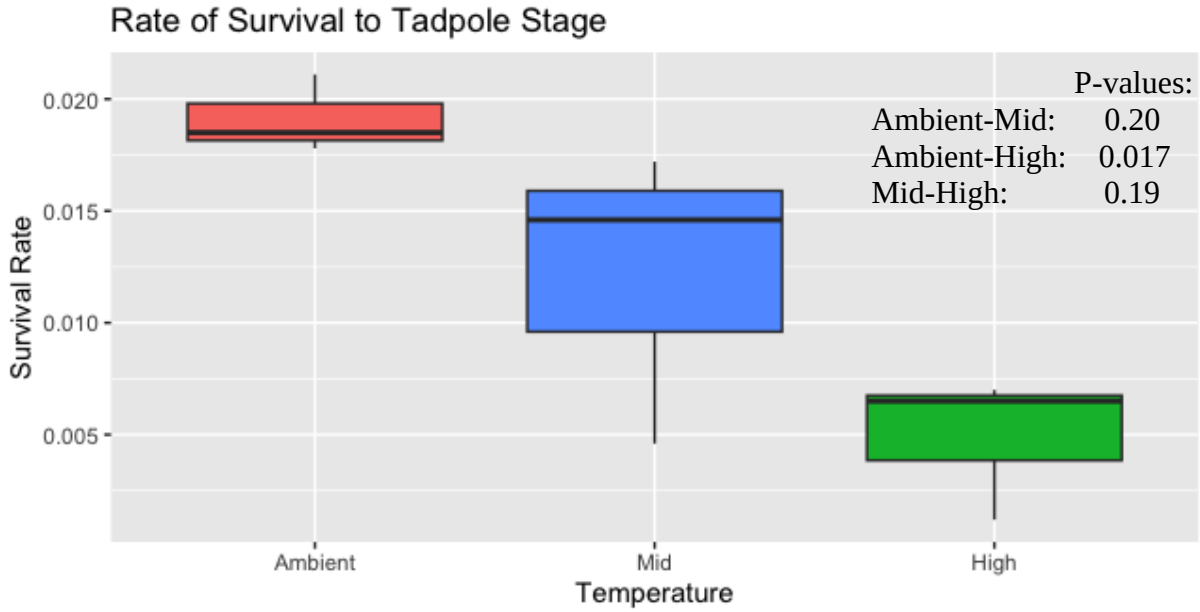

Figure 5: Rate of Survival to Tadpole Stage. Average survival rate for larvae kept at each temperature.

Discussion

This data supports our hypothesis that increases in sea water temperature lead to increases in rate of development in *B. villosa*, with embryos taking less time to reach each stage at high temperatures than at current ambient sea water temperatures. This data also showed that increased temperatures decreased rates of survival consistent with our proposed hypothesis. More replicates should be done to verify the pattern of lower survival rates. The Swalla lab has previously observed morphological differences in *B. villosa* that have developed at higher temperatures including large numbers of eye spots throughout the larvae. These observations taken together suggest that if ocean temperatures surge to levels that are too high the *B. villosa* population may decrease dramatically due to low survival rates and improper development. Due to the ecological role of ascidians, a decrease in the population size has the potential to disrupt the ecosystem and leave many organisms with fewer options for feeding or settlement.

It will be important to continue temperature trials such as these with more local species of tunicates as well as other local invertebrates to determine how many species will be affected by ocean temperatures rising and what those developmental and physiological effects may be. It will also become of vital importance to study how interspecies interactions change when subject to heat stress to determine how ecosystem dynamics as a whole will be affected. Within this it will be especially important to study the differences between how native and non-native species are affected by increased temperature. This will help to determine if nonnative species have an adaptive advantage in high temperature conditions over native species and which native species will need to be specifically targeted in conservation efforts.

Research on individual species development and physiology can be done in the lab following similar experimental procedures, placing multiple replicates under varying levels of heat stress, and observing the developmental timelines and morphological phenotypes. Research on ecological interactions may best be done in the field during or after marine heatwave events, observing and recording the percent cover of species, survival rate and competitive ability of species, and overall ecological productivity during and after a marine heating event. Research such as this will help to determine how ecosystems as a whole will change and inform decisions about how to best protect marine biodiversity.

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