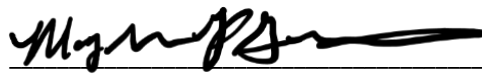Maternal Lineage Affects Metabolism and Growth Throughout *Petrolisthes cinctipes* (Porcelain crab) Embryonic Development

A Senior Thesis Presented to

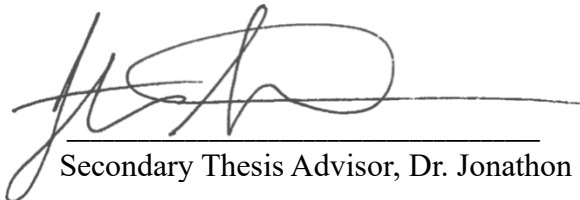The Faculty of the Department of Organismal Biology & Ecology,

Colorado College

By

Abigail Rivas

Bachelor of Arts Degree in Organismal Biology & Ecology

25th day of April, 2025

Approved by:

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Primary Thesis Advisor, Dr. Maybellene Gamboa

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Abstract

Atmospheric heat waves and marine heat waves continue to increase in temperature, frequency, and duration. Intertidal organisms are hypothesized to be particularly vulnerable to acute heat stress, or heat shock, events given their susceptibility to both atmospheric and marine heat waves. Indeed, evidence suggests heat shock can adversely affect intertidal adult metabolism, reproduction and survival. Yet, little is known about how heat shock affects critical developmental stages in intertidal organisms. Previous research on porcelain crabs (*Petrolisthes cinctipes*), a midintertidal organism, across life stages found significantly higher thermal tolerance in embryos. However, it is unclear to what extent heat shock during different embryonic stages affects physiology. Here, we test the hypothesis that *P. cinctipes* heat shocked during different embryonic stages affects metabolism, growth, and survival. We applied a 32°C heat shock for one hour on 12 related embryos and five maternal lineages across seven developmental weeks ($n = 420$) from June to August 2024. We imaged embryos and compared embryo area, yolk consumption, and hatch success of heat shocked individuals to 12 control embryos within each family ($n = 60$). We expected higher mortality, lower growth, and increased yolk consumption in individuals heat shocked later in embryonic development given the stressful transition from the embryonic to the larval stage. Contrary to our predictions, we found that heat shock does not significantly affect hatch success across families. Additionally, we found heat shock did not affect yolk consumption and growth compared to within family controls. However, when we observe maternal lineage, we find significant differences in yolk consumption and growth among families. Our results suggest that maternal effects act as the primary factor in embryonic yolk consumption and growth variability, and heat shock week does not play a role. Further research into genetic differences and transgenerational plasticity is necessary to fully understand how *P. cinctipes* embryos respond to acute thermal stress.

Introduction

Marine heatwaves (MHW) and atmospheric heatwaves (AHW) are increasing in frequency, duration, and intensity (Domeisen et al., 2023; Stillman et al., 2025) and affect animal metabolism, growth, and mortality (Brown et al., 2004; Liu et al., 2024). For example, MHW can lead to community shifts in kelp forests, reproductive failure of megafauna, and global mortality of corals (Smith et al., 2023). Similarly, AHW affect migratory patterns , thermoregulatory behaviors, and energetic allocation for heat shock protein synthesis (Stillman, 2019). While the effects of acute thermal stress events have been well-documented in organisms that experience either MHW (Guppy & Withers, 1999; Lemoine & Burkepile, 2012; Shanks et al., 2020; Somero, 2020) or AHW (Dahlhoff & Rank, 2000; Langham et al., 2015; Mitchell et al., 2018; Parmesan et al., 1999; Soren et al., 2018; Sunday et al., 2014), less certainty surrounds adaptive physiological flexibility in organisms that experience both MHW and AHW, such as intertidal organisms.

Spatial zonation of intertidal organisms correlates with thermal tolerances of those organisms—more thermally tolerant species are found in the mid-high intertidal zones with frequent emersion, whereas less thermally tolerant species are found in the low-sub intertidal zones with frequent immersion (Stillman, 2002). For example, *Petrolisthes armatus* (green porcelain crab), a tropical high-intertidal zone crab exhibits higher thermal tolerance due to frequent immersion in a warm climate. On the contrary, *Petrolisthes eriomerus* (flattop crab) and *Petrolisthes manimaculis* (chocolate porcelain crab) show lower thermal tolerance as subtidal crabs with frequent inundation in a temperate climate (Somero, 2002). Co-occuring with *P. eriomerus* and *P. manimaculis*, *Petrolisthes cinctipes* (flat porcelain crab) exhibits moderate thermal tolerance compared to its congeners. While *P. cinctipes* and *P. manimaculis* have a similar critical thermal maximum, or the temperature at which an organism experiences loss of function, *P. cinctipes* exhibits a lower thermal safety margin, or the difference between the organism's thermal tolerance and the temperature the organism regularly experiences, compared to *P. manimaculus* (Stillman, 2002). The thermal tolerance found in P. cinctipes may be attributed to the more variable thermal environment that *P. cinctipes* occupies and may suggest higher adaptive capacity in response to increasing heatwaves. Thus, ecology and population history will inevitably affect the breadth of adaptive responses to combined AHW and MHW. Environmental heterogeneity and pre-existing adaptive capacity interact to shape complex acute

heat stress responses. Highly variable environments select for greater plasticity to increase the likelihood of survival and reproduction (Collier et al., 2018). Adaptive phenotypic plasticity may buffer genetically-fixed physiological and behavioral thermal adaptation in response to thermal extremes events (Blewett et al., 2022; Collins et al., 2023). Even so, sudden, intense, and short-term exposure to thermal maxima and minima, or acute thermal stress events can shift communities, alter population responses and evolutionary trajectory, and increase mortality (Mieszkowska et al., 2021). For example, *Mytulis edulis* (blue mussel) experienced mass mortality during heatwave events where the thermal maximum is met in the intertidal zone and is more susceptible to subsequent acute thermal events (Seuront et al., 2019). On the contrary, many organisms are able to cope with extreme temperatures through the synthesis of heat shock proteins (*hsp*) (Berger & Emlet, 2007; Gracey et al., 2008; Miller et al., 2009). The threat of increasing acute heat stress events is exacerbated among intertidal populations, which currently experience temperatures exceeding their thermal maxima during warm days with a midday low tide (Helmuth et al., 2002; Scrosati et al., 2020). Understanding the extent of physiological responses to thermal stress in organisms that are susceptible to thermal extremes and variations will allow us to predict how populations and community dynamics will shift in the face of climate change.

Vertical stratification dictates organisms' varying thermal tolerances, but there are also differing thermal tolerances associated with life stages (Collin et al., 2021; Rebolledo & Collin, 2018; Truebano et al., 2018). For example, in *Petrolisthes*, the larval stage is significantly less thermally tolerant than the embryonic and adult life stages, likely given the comparatively stable environmental conditions within the pelagic zone where larvae develop (Leiva et al., 2018; Miller et al., 2013). Previous research has delved into metabolic changes and mortality due to thermal stress, in addition to how the embryonic, larval, and adult life stages differ in thermal stress responses (Garland et al., 2015; Paganini et al., 2014). *Petrolisthes* embryos are affixed to the abdomens of adults and experience similar thermal stresses, yet are not fully physiologically developed and are unable to exhibit escape behaviors (Aguila & Stillman, 2024). Consequently, *Petrolisthes* embryos have the highest thermal tolerance compared to other developmental stages (Miller et al., 2013). High thermal tolerance in the embryonic stage ensures development of vital structures and primes individuals for successful larval and adult stages (Xu et al., 2023), but the

extent of phenotypic plasticity it can display in response to heat shock throughout embryonic development is unknown.

Previous studies on Decapoda report conflicting effects of thermal stress across ontogeny. For example, elevated temperatures accelerate embryonic development but decrease survival in *Leptuca thayeri* (mangrove fiddler crab) and *Thenus orientalis* (flathead lobster) (Pardo & Costa, 2021; Wang et al., 1998). In contrast, thermal stress appears to have little effect on embryonic development or survival in *Limulus polyphemus* (American horseshoe crab) (Botton et al., 2006). To better understand thermal tolerance during this critical developmental period in intertidal organisms vulnerable to both AHW and MHW, we investigated how embryonic responses to acute thermal stress vary across developmental stages in *Petrolisthes cinctipes*. Embryos undergo key morphological transitions throughout development—including yolk reduction, organogenesis, and eye development—rendering later-stage embryos more physiologically complex and energetically active. Additionally, inherited maternal or transgenerational traits may influence embryonic physiology (Collin & Salazar, 2010; Yockachonis et al., 2020), potentially modifying responses to acute stress. Therefore, we predicted that embryos experiencing heat shock at later developmental stages would show increased metabolic and growth rates, but lower hatch success.
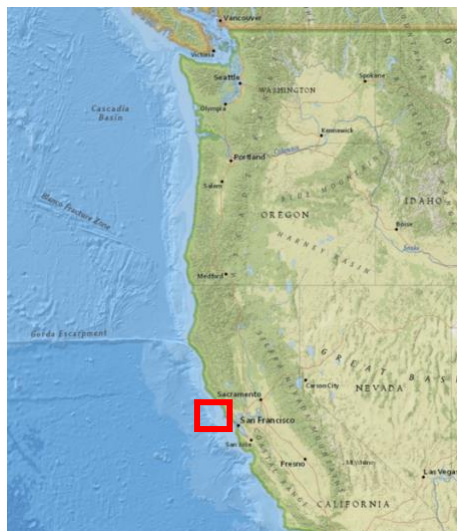
Methods

Figure 1: Map of *Petrolisthes cinctipes* distribution from Point Conception, California, United States to British Columbia, Canada with sampling location, Fort Ross, California, United State, boxed in red (U.S. West Coast Map | U.S. Geological Survey).

Specimen collection

　　We collected specimens at Reef Campground in the mid to high intertidal zone at a boulder intertidal zone in Fort Ross State Park, California (38°30′13.3"N 123°13′52.6"W) (Fig. 1; Aguila & Stillman, 2024). We collected five females with oviposited embryos between 1-3 weeks old via California Fish and Wildlife Scientific Collecting Permit (No. GM-232760002-23289-001) in June 2024. We weighed and measured females prior to extracting their embryos in the. We extracted approximately 100 embryos by opening the female's abdomen and gently removing them, then placed the embryos in a test tube with seawater until returning to San Francisco State university. Once we returned to lab, we rinsed embryos with deionized water to remove parasites. We counted and weighed each brood to determine biomass, then randomly placed embryos in a 96-well plate with 0.45μm filtered sea water. 96-well plates were placed atop a damp sponge in an enclosed plastic container and incubated at 14-16° C.

Heat shock treatment

　　We designated 12 embryos in the first row of all 96-well plates as the control group for their brood, and designated all remaining rows as treatment, where the first treatment row experienced heat shock in week 1 and each row subsequently until week 7. During the acute heat stress week for each treatment group, we placed individual embryos into centrifuge tubes and labeled them based on brood number and plate position. The embryos then received a one-hour 32°C acute heat shock (LT_{50}) during its designated week in a temperature-controlled water circulator. Once heat shock was completed, we placed embryos in their original wells and returned embryos to the incubator.

Estimating yolk consumption and growth rates

　　Once a week, we imaged each individual embryo using a Nikon SMZ-18 with 1x objective at 13.5x magnification with occlusion set at the midpoint. We placed six embryos on a microscope slide at a time, imaged the plate, and saved the image as a .nd2 file. We rinsed embryos in DI water then returned embryos to a new 96-well plate in their original position with freshly filtered seawater. We rinsed microscope slides with deionized water between uses to remove parasites. Once all embryos either hatched or expired, we converted all .nd2 files to .tif

files for FIJI: ImageJ (Schindelin et al., 2012) to analyze. Embryo area and yolk area were measured in pixels, converted to mm^2, and analyzed over time for each embryo to determine rate of growth and yolk consumption, a proxy for metabolism (Yockachonis et al., 2020).

Identifying hatch success

During weekly imaging, we inferred the wellness of individuals observationally. We placed embryos that appeared to be developing with dark red yolk decreasing over time were placed back into their respective wells after imaging, whereas we recorded dead embryos as such and disposed of. We determined deceased embryos via white discoloration, a swelled look, or parasitism. We determined a successful hatch when an embryo had normal development for the duration of the imaging process and hatched into an expectedly healthy larvae with appropriate eye spot, streamlined body, and swimming ability.

Statistical analyses

We compared yolk consumption, growth rate, and hatch success among control and heat shock weeks while controlling for within-family variation, or brood, using linear mixed effects models in R (v4.4.2; R Core Team 2024). Specifically, we used the *lmer* function to run linear mixed effects models in the package *lme4* (Bates et al., 2015). We used heat shock week as a fixed effect and yolk consumption and growth as continuous responses in separate models. We modeled hatch success as a binary distribution (0 = deceased, 1 = hatched) and used hatch success as a response in a linear mixed effects model with heat shock week as a fixed effect. To account for within family variation, all models used maternal lineage, or brood, as a random effect. Multi Model Inference was used to determine which variables in addition to heat shock weak led to variation in our three parameters using the function *r.squaredGLMM()* in the R package *MuMIn* (Bartoń, 2010). Finally, we ran the *anova* and *ranova* functions using the package *lmerTest* (Kuznetsova et al., 2017) to estimate pairwise differences among control and treatment weeks.

Results

Effect of acute thermal stress on yolk consumption rate

Treatment does not show any significant impact on rate of yolk consumption in comparison to control ($F_{(1,458)} = 0.0234$, $P > 0.05$) (Fig. 2). However, there were significant differences across heat shock weeks within treatment ($F_{(7,452)} = 4.1836$, $P < 0.001$) (Fig. 4). Importantly, heat shock week only explained 3% of the observed variation, while brood explained 52% of the observed variation at 52% in rate of yolk consumption ($F_{(4,455)} = 93.8377$, $P < 0.001$) (Fig. 3).

Effect of acute thermal stress on growth rate

Control and treatment were not significantly different for growth rate (ANOVA $F_{(1,458)} = 0.4761$, $P > 0.05$) (Fig. 4). There were significant differences across the heat stress weeks within the treatment category, but only contributed to 3% of the observed variation (ANOVA $F_{(7,452)} = 6.3985$, $P < 0.001$) (Fig. 5). Brood, however, led to 69% of the variation in growth rate (ANOVA $F_{(4,455)} = 199.9387$, $P < 0.001$) (Fig. 7).

Effect of acute thermal stress on hatch success

The application of treatment or time of heat shock did not have an affect on percent hatch success for embryos (ANOVA $F_{(1,6)} = 1.135$, $P > 0.05$) (Fig. 6).

Discussion

We investigated how the presence and developmental timing of acute heat stress on *P. cinctipes* embryos affects metabolism as inferred via yolk consumption, growth, and hatch success. Contrary to our prediction, we found no significant difference in rate of yolk consumption or growth between control and treatment embryos across all heat shock weeks (Figs. 3, 4, 6, 7). Our data suggest *P. cinctipes* embryonic thermal tolerance is innate and individuals maintain the same extent of thermal tolerance throughout the entirety of embryonic development. Our previous understandings of *P. cinctipes* adults shows thermal stress catalyzing metabolic changes but, adults also have limited plasticity to adapt to thermal stress events (Stillman, 2003; Stillman & Somero, 1996). Embryos may have different mechanisms for phenotypic plasticity that inhibit long-term metabolic changes following a heat shock. A study

performed on *L. polyphemus* embryos found heat shock did not play a role in metabolism because embryos had a baseline heightened concentration of *hsp70* so that synthesis would not be necessary during a thermally stressful event (Botton et al., 2006). *L. polyphemus* embryos, similarly to *P. cinctipes*, develop in the thermally variable intertidal zone, suggesting a possible mechanism by which *P. cinctipes* embryos could be physiologically preparing themselves for thermal maxima. The rates of yolk consumption and growth may be already primed to handle thermal fluctuations. Protein sequencing is necessary to further understand the role of heat shock proteins in embryonic development and thermal resilience.

Heat shock does not affect hatch success

Similarly to metabolism and growth, heat shock did not affect hatch success of *P. cinctipes* embryos for all treatment weeks. The mechanism of phenotypic plasticity in our embryos is unknown, but consistent hatch success across all control and treatment groups suggest there are processes in place to ensure survival (Fig. 9). Previous studies on intertidal and marine organisms' embryonic responses to thermal stress highlight the importance of heat shock protein synthesis for survival. Research on sea urchin embryos found that *hsp70* synthesis only occurred post-blastula formation—embryos heat shocked between fertilization and blastula formation experienced mortality post heat shock, whereas embryos that received heat shock post-blastula formation survived and reached successful hatch (Giudice et al., 1999). A study on Atlantic Cod embryos found hsp production occurred immediately post-blastula formation as well, and was maintained throughout embryonic development (Skjærven et al., 2011). Further investigation into the stages of embryonic development in *P. cinctipes* is required to understand how blastula formation plays a role in thermal tolerance and hsp synthesis, but the aforementioned studies suggest that heat shock proteins are vital for embryonic success in the face of short-term thermal stress. Our results indicate that if heat shock proteins are responsible for embryonic thermal tolerance, that cellular defense mechanism is catalyzed at the earliest stages of development, allowing for phenotypic plasticity throughout the entire ontogenetic period.

Heat shock week affects yolk consumption and growth

While our analyses suggest that heat shock week affects yolk consumption and growth via pairwise comparison, the variation heat shock week introduces is not necessarily biologically relevant. Treatment and control groups are significantly similar for yolk consumption and growth, but the collapsed variation that comes with averaging rates for treatment groups limited the statistical power to detect differences (n=5). The statistical analyses used to detect differences between heat shock weeks did so by each individual embryo, therefore introducing greater statistical power to detect the 3% variation (n=60).

Maternal Lineage Affects Yolk Consumption and Growth

Little variation was observed from heat shock week, but maternal lineage introduced a significant amount of interbrood variation. There is inherent variation in embryo size between broods, so it is unsurprising that maternal lineage contributes significant variation to growth rate. Our main objective was to consider the variation from heat shock week, but there was such high variation from maternal lineage that it warranted further investigation (Collin & Salazar, 2010).Variation in brood response to heat shock has been observed before when studying single paternity vs. multiple paternity, where broods with multiple paternity exhibited higher survival than those with single paternity. Multiple paternity is hypothesized to be an evolutionary strategy—adding genetic variation within broods can increase survivability in particularly stressful and extreme environments (Yockachonis et al., 2020). We observe this variation within broods particularly when comparing growth rates across treatment groups, as illustrated by the substantial error bars (Fig. 6). Within brood variation also explains why we observed across brood variation—five unique maternal lineages with an undetermined amount of paternal lineages evidently leads to variable responses from embryos to acute thermal stress. Because we collected our specimens from the field, it is reasonable to assume they employ multiple paternity to maximize survival. To further test the role of single or multiple paternity in embryonic thermal stress response, embryos would need to be collected from adults raised in aquaria to control for paternal influence. Furthermore, we did not have the environmental history of the adults we collected, so we cannot make any claims about adult tolerance influencing embryonic tolerance. However, transgenerational plasticity has been observed in *P. cinctipes* and may play a role in the brood variation we observed (Hooper, 2024). The female crabs we collected had all exhibited

some thermal resilience, as they were able to reproduce and survive through the unusually warm early summer. If transgenerational plasticity is at play, mothers that experienced thermal stress will produce more thermally resilient broods, which we observed. *P. cinctipes*' ability to tolerate increasing thermal maxima demonstrates its plasticity and provides insight into how intertidal organisms will respond in the face of climate change.

Our results suggest that *P. cinctipes* embryos possess intrinsic thermal tolerance that buffers them against acute stress events, and that maternal lineage may be a stronger determinant of metabolic and developmental trajectories than heat shock timing. This highlights the potential for cryptic genetic or epigenetic factors to shape organismal responses to climate variability.

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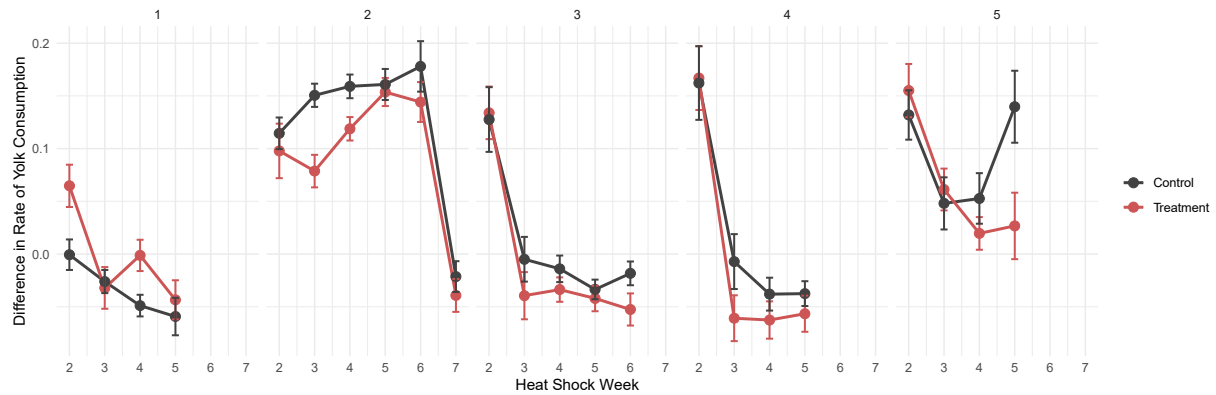
Figures

Figure 2: The mean difference in rate of yolk consumption for *P. cinctipes* before and after heat shock. Each data point represents the difference in rate for a treatment group in its respective brood and heat shock week with its accompanying standard error. Treatment groups are in red and control groups are in black.
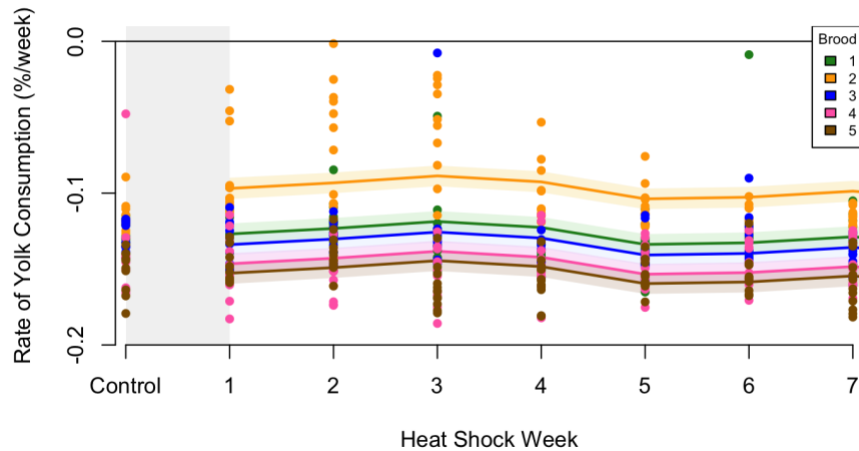

Figure 3: Rates of yolk consumption plotted atop a best-fit linear model with 95% confidence intervals. Each color is representative of one brood/maternal lineage and the columns of data points are rates at respective heat shock weeks.
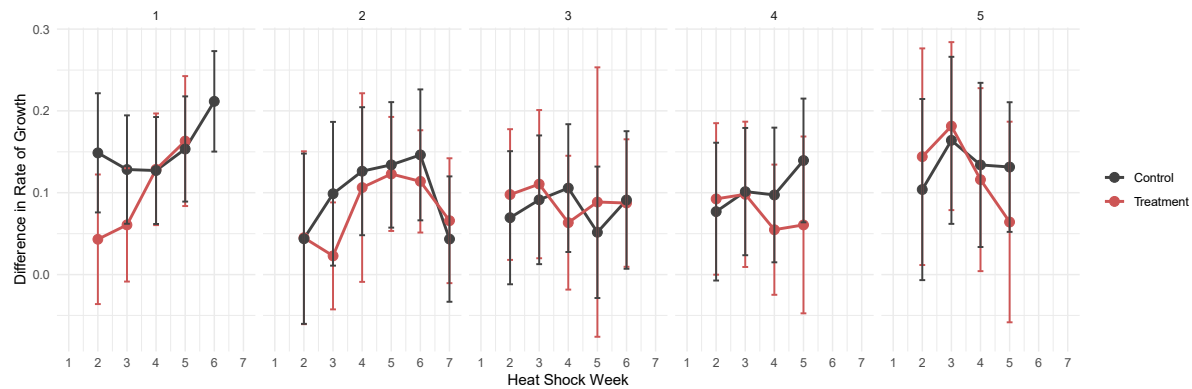
Figure 4: The mean difference in growth rate before and after heat shock. Each data point represents the difference in rate for a treatment group in its respective brood and heat shock week. Treatment groups are in red and control groups are in black.
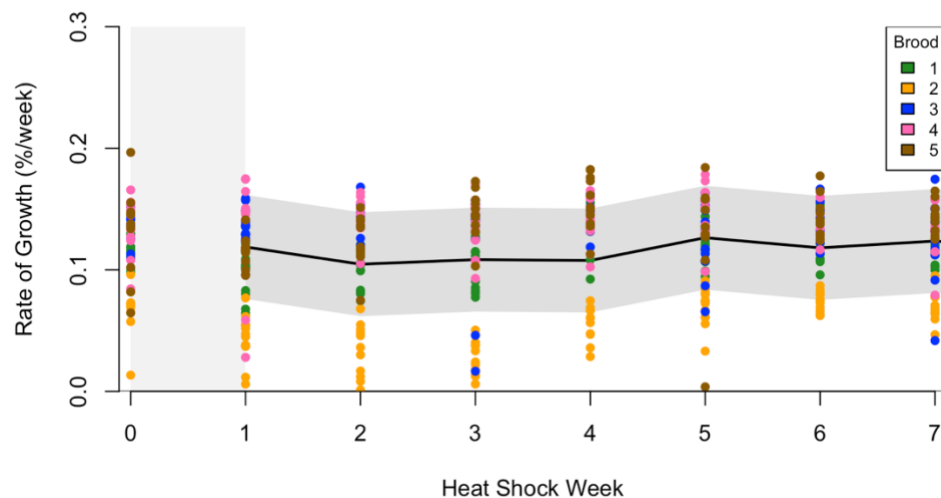

Figure 5: Growth rates for individual embryos plotted atop a linear mixed model with 95% confidence intervals. Each color is representative of one brood/maternal lineage and the columns of data points are rates at respective heat shock weeks.
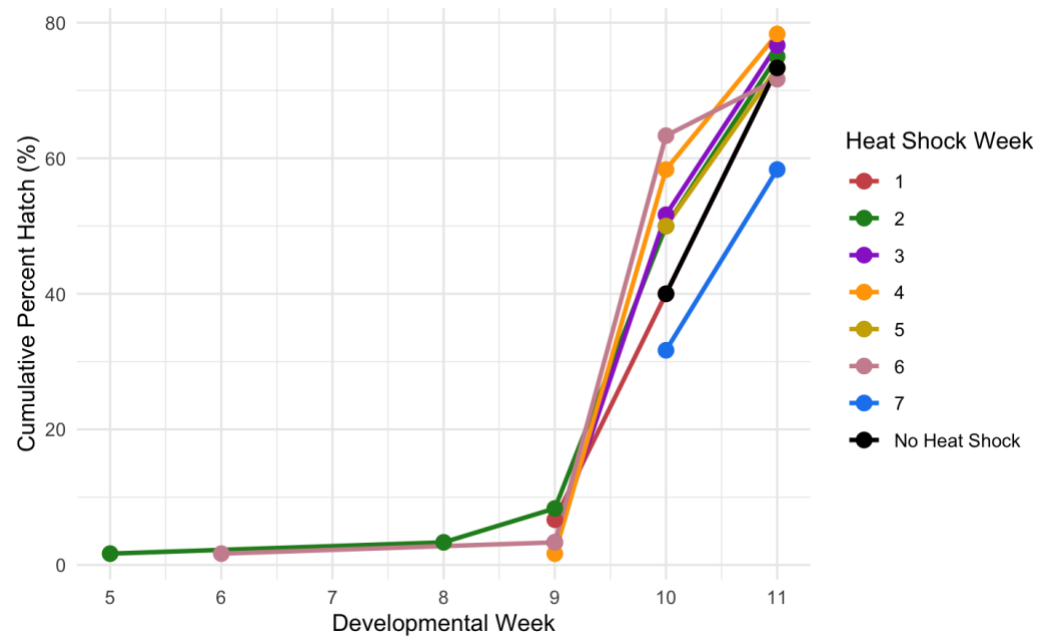
Figure 6: Cumulative percent hatch for all embryos. Colors indicate when heat shock was applied to the embryo.