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Effects of Paternal Heat Exposure and Parental Care on the Development of Offspring in *Gasterosteus aculeatus*

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Honors Thesis
Emma Borgert
Department: Biology
Advisor: Karolyn Hansen, Ph.D.
April 2024

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Abstract

Humans are profoundly altering the abundance and distribution of organisms via climate change. Warming temperatures are affecting marine and freshwater ecosystems by increasing physiological stress, limiting growth, and decreasing dissolved oxygen. With all this change occurring, an understanding of how organisms are going to cope is crucial. Transgenerational plasticity – when parental experiences alter offspring traits - can allow organisms to rapidly adapt to environmental change. Previous literature has focused on the role of maternal experiences on offspring traits, but paternal experiences are just as important. Fathers can influence their offspring in multiple ways, including changes via both sperm and paternal care behaviors. Three-spined sticklebacks, *Gasterosteus aculeatus,* are a small fish found in freshwater and marine ecosystems with paternal-only care. Many populations are facing the threats of drought and wildfire, making it important to understand how they will adapt to climate change. To understand how paternal heat exposure alters offspring development, I exposed parents to cool (~17°C) or warm (~20°C) water temperature and then manipulated whether fathers provided paternal care or offspring were artificially aerated; this generated offspring that received cues of heat exposure from gametes alone versus from gametes and paternal care. I found that offspring survival was not affected by parental heat exposure but was lower when offspring themselves were exposed to warm temperatures. Additionally, parental care influenced the development of offspring traits and boldness in a novel environment. Overall, these results suggest that heat exposure alters both paternal and offspring traits, and that the effects on offspring depend on the mechanism of paternal effects (care versus sperm).

Disclaimer

To my knowledge, all this data has been collected and analyzed without any bias.

Acknowledgements

Thank you to members in the Hellmann Lab for aiding in the completion of this research. Thank you to Dr. Jennifer Hellmann for advising this research and mentoring me along the way; you've taught me fascinating things and I'm very grateful to have had this opportunity to expand my knowledge of the area that I am passionate about.

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Introduction

Humans are altering ecosystems in several ways (e.g., habitat fragmentation, climate change, agricultural runoff), which increasingly exposes organisms to conditions that they have never encountered in their evolutionary history. This includes elevated temperatures, which are having detrimental consequences for marine and freshwater ecosystems. This is having detrimental consequences on marine and freshwater ecosystems by increasing physiological stress, restricting growth, and constraining the availability of oxygen for organisms to consume (Doney et al. 2012). The response to and consequences of this increased temperature exposure can vary across different habitats and organisms. In aquatic environments, increases in the size of a species causes a greater restriction of body growth than those of the same dry mass in terrestrial ecosystems due to the major role of oxygen within temperature-size responses in aquatic organisms (Forster et al. 2012). This suggests that exposure to increased temperatures within aquatic environments has a greater effect compared to terrestrial environments.

The effect of increasing temperatures on organisms in aquatic freshwater ecosystems might also be more detrimental than those in marine ecosystems. For instance, the peak magnitudes of plankton were not affected by temperature in freshwater systems, but higher water temperatures increased the blooming time of plankton within freshwater and marine ecosystems (Winder et al. 2012). In marine ecosystems, organisms may have the ability to swim further down to cooler temperatures, escaping the warming. Organisms in freshwater ecosystems, especially streams, may not be able to do this if there is less variation in the temperature or the depth of their habitat. Therefore, they must biologically adapt to the increased temperature. With all this change happening to the climate of organisms' habitats, there is increasing interest in understanding how freshwater organisms are going to cope.

One of the main mechanisms' organisms use to cope with rapid change is phenotypic plasticity (Seebacher et al. 2014), which is how environmental conditions experienced within a lifetime affect the development of traits. Plasticity is important for organisms facing climate change because it acts much faster than evolution via genetic changes, allowing organisms to quickly adapt to the changed climate (Donelan et al 2019, Donelson et al 2018). Long-term exposure to increased temperatures resulted in the increase of the critical thermal maximum and the temperature at which behavioral signs of stress occurred in the pugnose shiner (Potts et al. 2021). Individuals can also cope with changing environments via transgenerational plasticity, where the conditions experienced by one generation can influence the development of traits in future generations (Donelson et al. 2018). For example, exposing parents and grandparents to heat had a positive effect on the aerobic capacity of offspring regardless of the thermal conditions that were experienced by the offspring in later life stages (Bernal et al. 2022). Compared to within-generational plasticity, transgenerational plasticity is more beneficial when the offspring cannot test out their future environments, whether it be due to physical constraint or risk, and selective pressures are high within the early stages of life (Donelan et al. 2019).

Previous literature in transgenerational plasticity has primarily focused on maternal experiences, in part because the mother is the primary provider of care in most species. Increasingly, we are recognizing that paternal experiences have consequences for offspring (Guillaume et al. 2015). The role of paternal experiences might be particularly important in species where fathers provide paternal care, either with the mother (e.g., humans) or by themselves (e.g., many fish, amphibians).

The three-spined stickleback (*Gasterosteus aculeatus*) is a small species of fish in the middle of the trophic web that is found through marine and freshwater ecosystems in the Northern Hemisphere. Stickleback fathers are the ones who care and rear the offspring (Kraak et al. 1999). These fish, especially populations in California, are subject to increasing temperatures, drought, and wildfire. This is altering reproductive behavior: populations of sticklebacks in high-latitude lakes in Alaska are altering their breeding time to be earlier due to early ice breaking that is being caused by increased temperatures (Hovel et al. 2016). Previous studies have found that maternal exposure to warming temperatures produces smaller egg size, lower hatching success rate, and offspring with smaller body sizes; these characteristics observed are not adaptive (Shama 2015). Examining the ways in which paternal heat exposure alters offspring is important because the quality of parental care directly impacts the development of the offspring (Hellmann et al. 2021) and changes in parental care from the fathers in warmer conditions could change how offspring adapt to their environment. Previous studies have shown that fathers rearing offspring in temperatures increased 2-6°C above ambient levels exhibited more paternal care behaviors, specifically fanning, but had overall lower levels of successful reproduction (Hopkins et al. 2010). Changes in physiological pathways have been shown to yield smaller eggs from mothers and reduce sperm counts in fathers. Additionally, in relation to parental care caused by elevated temperatures, increased cortisol within produced eggs and a lower probability of offspring survival have been observed (Burt et al. 2010).

I seek to understand the effects of warming on paternal care and offspring trait development in three-spined sticklebacks. To do so, I exposed parents to cool (~17°C) or warm (~20°C) water temperature and then manipulated whether fathers provided paternal care or offspring were artificially aerated; this generated offspring that received cues of heat exposure from gametes alone versus from gametes and paternal care. Based on current literature, I hypothesize that the amount of parental care exhibited by stickleback fathers will increase under conditions of warming (Hopkins et al. 2010). With this increase in parental care, the offspring will develop traits, such as better endurance and faster growth, that will make them better adapted to the warming conditions. Offspring who received care would have greater adaptive benefits compared to those who did not.

Materials and Methods

Sticklebacks were collected from the Navarro River in California in July 2021 and were reared in the lab on recirculating racks under a common temperature (17.2-17.7°C). We maintained groups of 8-12 fish (mix of males and females) in an 18.9L (38L x 25W x 21H) tank containing gravel, two fake plants, and half of a PVC pipe. We fed the F0 generation ad libitum daily with a mix of frozen bloodworms (*Chironomus* spp.), brine shrimp (*Artemia* spp.) Mysis shrimp, and calanus. During the summer months, fish had a 16hr day: 8hr dark light schedule; for the winter months, the light schedule switched to 8 hr day: 16hrs dark.

To stimulate the natural conditions of exposure to increased temperatures, we exposed both males and females to either a cooler (17.2-17.7°C) or a warmer treatment (19.4-20°C) using recirculating racks with a heater or a chiller to control temperature for a period of 3 weeks starting in May 2022. Each treatment tank housed between 6-8 individuals. For the exposures to the warmer treatment, we increased the temperature of the heater by about 1°C for a period of three days so that the fish within the tanks on the rack had an adequate amount of time to adjust without causing heat shock. We recorded the temperatures of each tank on the recirculating racks once a day and recorded the dissolved oxygen of each tank once a week (average DO elevated treatment: 8.14 ppm, average DO control treatment: 8.72 ppm). We measured dissolved oxygen because temperature can alter dissolved oxygen (Mahaffey et al. 2020) and we wanted to control the dissolved oxygen while changing the temperature. Once the three-week exposure period was over, we slowly lowered the temperature of the heaters by 1°C per day till the temperature of the tanks were brought back down to control temperatures (17.2-17.7°C). We allowed males to nest at cool temperatures to ensure the eggs and newly hatched larvae all encountered the same developmental temperatures.

After the end of the heat exposure, we move males to individual tanks with nesting supplies, including a tray of sand and some algae. Once the male constructed his nest, we introduced a gravid female, which received the same treatment as the male, into the tank. We observed courtship behaviors, including zigzag dancing, female following male to the nest, and female poking at the nest (Lehto and Tinghitella 2020), using the program BORIS. We conducted a courtship trial with recorded observations for each male for up to 3 mating attempts. We recorded behaviors for 10 minutes after the introduction of the female to the male's tank. Courtship trials lasted for up to 1 hour if mating was not successful within the first 10 minutes of the female being introduced. Before each courtship trial, we weighed the selected gravid female, and measured her length. After the mating was successful, we removed the female from the male's tank, reweighed, marked her individually with elastomer dye (Northwest Technologies) for identification purposes, and placed her back into her original tank. If the female was in the male's tank for the maximum time of an hour and mating was not successful, we still reweighed the female and placed her back in her original tank. For heat-exposed males, there was an average of 15.36 days (range: 4-27 days) between when the treatment for the male ended and the male successfully mated. For each male with a nest that had fertilized eggs in it, we conducted parental care observations for 10-minute periods for 10 days after fertilization; the first observation was conducted an hour after successful mating. We conducted observations around the same time every day (if the male mated in the morning, observations were conducted between 0900-1300; if the male mated in the afternoon, observations were conducted between 1300-1800). We observed parental care behaviors, including egg fanning, spitting, and poking the nest, live and recorded them using the program BORIS. Fanning, in which the father aerates the eggs and recently hatched fry (eggs hatch about six days post-fertilization), is the most common parenting behavior shown by sticklebacks. On Day 10 post-fertilization, we removed the male from his nesting tank and ranked the male's body coloration and throat coloration and area on a number scale of 0-5 with 0.5 increments. We then marked him using elastomer dye (Northwest Technologies) and returned him to a stock tank.

For this study, we were interested in seeing how the presence or lack of parental care affects the growth and development of the offspring. To examine this, we simultaneously reared clutches that did not receive any parental care using in-vitro fertilization. We placed males into an identical nesting tank as described above. Once the male constructed his nest, he was removed, weighed, and measured before being euthanized via an overdose of MS-222. After the male was euthanized, he was dissected, and his testes were removed. The males' testes were crushed up in a solution of deionized water and pipetted over eggs extracted from a gravid female that received the same treatment as the male. The fertilized eggs were transferred into a plastic cup with mesh on the bottom and clipped to the side of the nesting tank that the male was removed from using a plastic clip and positioned above a bubbling stone that provided oxygen to the fertilized eggs. We created a total of 37 clutches: n =11 clutches parented by a male exposed to the elevated treatment, n =9 clutches parented by a male exposed to the control treatment, 10 not receiving parental care but their father exposed to the elevated treatment, and n= 7 clutches receiving no parental care, but their father exposed to the control treatment (in-vitro fertilization). Once hatched, we fed offspring newly hatched brine shrimp for two months and then gradually transitioned to frozen food (as above).

One month after that the clutch had hatched, we counted and recorded the number of fry. At approximately 8 weeks (average: 56.05 days, range: 41-77 days), we split each clutch to expose offspring to either control (17.2-17.7°C) or elevated temperatures (19.4-20°C). Depending on the size of the clutch, we moved 4-11 fish into two new tanks (average: 6.84 fish for the control treatment and 6.5 fish for the elevated treatment). We began splitting clutches August 2022 and finished in late September 2022. Only 2 clutches reared without parental care had partial failure to mature; Clutch 22 reared from a warm exposed male did not have any offspring survive when re-exposed to warm temperatures and Clutch 26 reared from a cold exposed male did not have any offspring survive when re-exposed to cold temperatures. We left the fry in their exposure tanks until we conducted the assays (average: 35.1 days, range: 26-48 days), and we measured survival at the end of this period.

Offspring assays. To assess the offspring's swimming and behavior at different temperatures, we conducted a fast startle assay that measured the period of startle after exposure to a stimulus, as well as activity after the stimulus. and the period spent in different sections of a tank. This assay consists of a 5-gallon tank with 3 sides covered in black contact paper and an arena constructed out of polyglass taking up about ¾ of the tank. The tank was then positioned over a light board so that the arena was lit up while the holding area was dark. There was a small opening cut into the polyglass of the front of the arena which was where the fish had the ability to access the arena upon lifting of a barrier that restricted the fish to just the holding area. For the assay, the fish was placed into the holding area of the tank to acclimate for 5 minutes. After this acclimation period, we lifted the barrier that separated the holding area and the arena. We recorded how long the fish took to enter the arena. Once the fish’s body was at least halfway in the arena, we dropped a pebble into the holding area to elicit a startle response. When the pebble was dropped, we placed the barrier to block the fish from reentering the holding area and we recorded the behaviors of the fish after the startle, such as time spent swimming and time spent near the walls or the middle of the arena, for 3 minutes. After these 3 minutes, we removed the fish from the assay tank and placed it back into its designated section in one of the holding tanks. The maximum amount of time for the fish to enter the arena was 15 minutes, and if the fish did not enter the arena at the 15-minute mark then we ended the assay. Both assays were video recorded and analyzed using BORIS.

We assayed up to 11 fish per clutch, with an average of 6.94 and a range of 2-11. Some variation existed in how many fish from each clutch were selected to be assayed as some clutches experienced a lot of die-off. We assayed each fish in the control temperature (17.2-17.7°C) and the elevated temperature (19.4-20°C), in a random order with 48-hours between the two assays. Prior to the first assay, we captured the fish from their home tanks using plastic cups and placed them into one of three sections in holding tanks whose temperatures were in between that of the two treatments (18.0-18.4°C) and allowed them to adjust to these holding tanks for a period of 48 hours. After completion of the second assay, we removed the fish and its mass and length were recorded before being euthanized using a solution of MS-222. Upon euthanasia, we preserved the body in ethanol for genetic sexing. We extracted DNA from the tail of the fish using the QIAGEN Blood and Tissue Kit and then we used PCR methods outlined in Peichel et al. 2001 to amplify the DNA and visualize sex on an agarose gel (two bands for males or one band for females). A total of 248 fish were assayed, n= 56 offspring parented by control males, n= 75 offspring reared from control males with no parental care, n= 68 offspring parented by heat-exposed males, and n= 49 offspring reared from heat-exposed males with no parental care.

***Statistical analysis*.**

Paternal traits. We used Wilcox rank sum test to test for differences between control and heat-exposed males in the number of courtship attempts until successful mating and coloration. To analyze fanning behavior, we used linear mixed models (R package lme4 (Bates, Mächler, Bolker, & Walker, 2015)). Because fanning increases until Days 4-5 post-fertilization and then decreases, we included both a linear effect of day and a quadratic effect of day as fixed effects. We also included a fixed effect of paternal heat exposure, and interactions between paternal treatment with both the linear and quadratic effect of day (to understand if the shape of the fanning curve varied with paternal treatment). To account for repeated measures, we included paternal ID as a random effect.

Offspring traits. We used general linear mixed models to analyze variation in length and clutch survival. Both models included fixed effects of parental care, parental heat exposure, and offspring early life heat exposure, with a random effect of clutch identity. We used generalized linear mixed models to analyze variation in behavior. All models included fixed effects of parental care, parental heat exposure, offspring early life heat exposure, and standard length, with a random effect of clutch identity.

Results

Male traits. We found no difference in throat coloration (Wilcox rank sum test: W =107, p=0.11) or body coloration (W =52, p=0.16) between males exposed to heat compared to the control. Males exposed to heat did not take any more time to mate than control males (Wilcox rank sum test W = 51, p=0.12).

Parental care. We found no effect of temperature on parental care effort (Table 1). We saw no difference in fry survival to one month between clutches parented by males exposed to cold versus warm temperatures (Wilcox rank sum test W = 43, p=0.42).

Offspring survival. Offspring survival was significantly lower when they were exposed to heat compared to the control but did not vary with paternal care or paternal heat exposure (Table 2) (Figure 1). We found no evidence that paternal heat exposure primed offspring to survive heat exposure themselves, as there was no significant interaction between paternal heat exposure and offspring heat exposure.

Offspring size. We found an interaction between parental care and offspring early life heat exposure on offspring size (Table 2). Early life heat exposure tended to make orphaned offspring shorter ($t_{43.42}$=-1.70, p=0.10), while early life heat exposure tended to make parented offspring longer ($t_{50.48}$=1.73, p=0.09). We found no effect of parental heat exposure on offspring length (Table 2).

Offspring behavior. Offspring who received paternal care spent less time on the edge of the area compared to orphaned offspring, but time spent on the edge was not altered by parental or offspring heat exposure (Table 3) (Figure 2). Offspring of heat-exposed parents tended to spend more time swimming than offspring of control parents (Table 3). We found no effect of parental care, parental heat exposure, or offspring early life heat exposure on how long it took fish to emerge from the dark area or thigmotaxis behavior (Table 3). Larger size spent less time swimming and performed less thigmotaxis behavior.

Table 1: ***Paternal care***. Results of linear mixed models testing how total time fanning (seconds) varied with paternal heat exposure. We tested fixed effects of paternal heat exposure (control or heat-exposed), a linear effect day of paternal care (Days 0-9 post-fertilization) and quadratic effect of day (to account for non-linear curve of fanning behavior). To understand if the shape of the fanning curve varies with paternal treatment, we included interactions of paternal treatment with both the linear and quadratic effect of day. The model included a random effect of paternal identity.

	Fanning duration (sec)				
	Estimate	S.E.	t	df	P
Paternal heat	68.17	61.71	1.11	180.93	0.27
Day	48.62	17.18	2.81	196.38	0.005
Day^2	-2.81	1.68	-1.67	196.38	0.10
Predation exposure * Day	-28.51	25.46	-1.12	197.26	0.26
Predation exposure * Day^2	2.57	2.49	1.03	197.02	0.30

Table 2: ***Offspring traits*****.** Results of linear mixed models testing how paternal heat exposure, offspring heat exposure, and parental care influenced the survival of the clutches and the size of the produced offspring. Fixed effects of parental care, parental heat exposure, offspring early life heat exposure, with a random effect of clutch identity were utilized within this model.

	Clutch survival				
	Estimate	S.E.	t	df	P
Paternal heat exposure	0.25	0.77	0.32	34.00	0.75
Offspring heat exposure	-1.38	0.32	-4.27	36.00	<0.001
Paternal care	-0.20	0.76	-0.26	34.00	0.80
	Offspring size				
	Estimate	S.E.	t	df	P
Paternal heat exposure	0.15	0.60	0.26	32.31	0.80
Offspring heat exposure	-0.52	0.30	-1.75	214.83	0.08
Paternal care	-1.87	0.62	-3.02	214.83	0.004
Paternal care * offspring heat	0.83	0.41	2.04	214.30	0.04

Table 3: ***Offspring behavior*.** Results of linear mixed models testing how paternal heat exposure, offspring heat exposure, parental care, and standard length influenced certain traits (time to emerge, time swimming, time near dark edges, and thigmotaxis) that were measured during the conducted offspring assays. Fixed traits of parental care, parental heat exposure, offspring early life heat exposure, and standard length, with a random effect of clutch identity were utilized within this model.

	Time to emerge				
	Estimate	S.E.	Z	df	P
Paternal heat exposure	-0.02	0.08	-0.26	402	0.80
Offspring heat exposure	-0.03	0.08	-0.39	402	0.70
Paternal care	-0.01	0.08	-0.16	402	0.87
Standard length	0.005	0.02	0.28	402	0.78
	Time swimming				
	Estimate	S.E.	Z	df	P
Paternal heat exposure	0.20	0.12	1.71	250	0.09
Offspring heat exposure	0.08	0.11	0.69	250	0.49
Paternal care	0.08	0.13	0.64	250	0.52
Standard length	-0.06	0.03	-2.20	250	0.03
	Time near dark edges				
	Estimate	S.E.	Z	df	P
Paternal heat exposure	-0.20	0.22	-0.90	250	0.37
Offspring heat exposure	0.10	0.22	0.43	250	0.67
Paternal care	-0.53	0.24	-2.19	250	0.03
Standard length	0.05	0.06	0.84	250	0.40
	Thigmotaxis				
	Estimate	S.E.	Z	df	P
Paternal heat exposure	0.09	0.16	0.54	250	0.58

Offspring heat exposure	-0.03	0.16	-0.16	250	0.87
Paternal care	-0.06	0.17	-0.35	250	0.73
Standard length	-0.10	0.04	-2.50	250	0.01

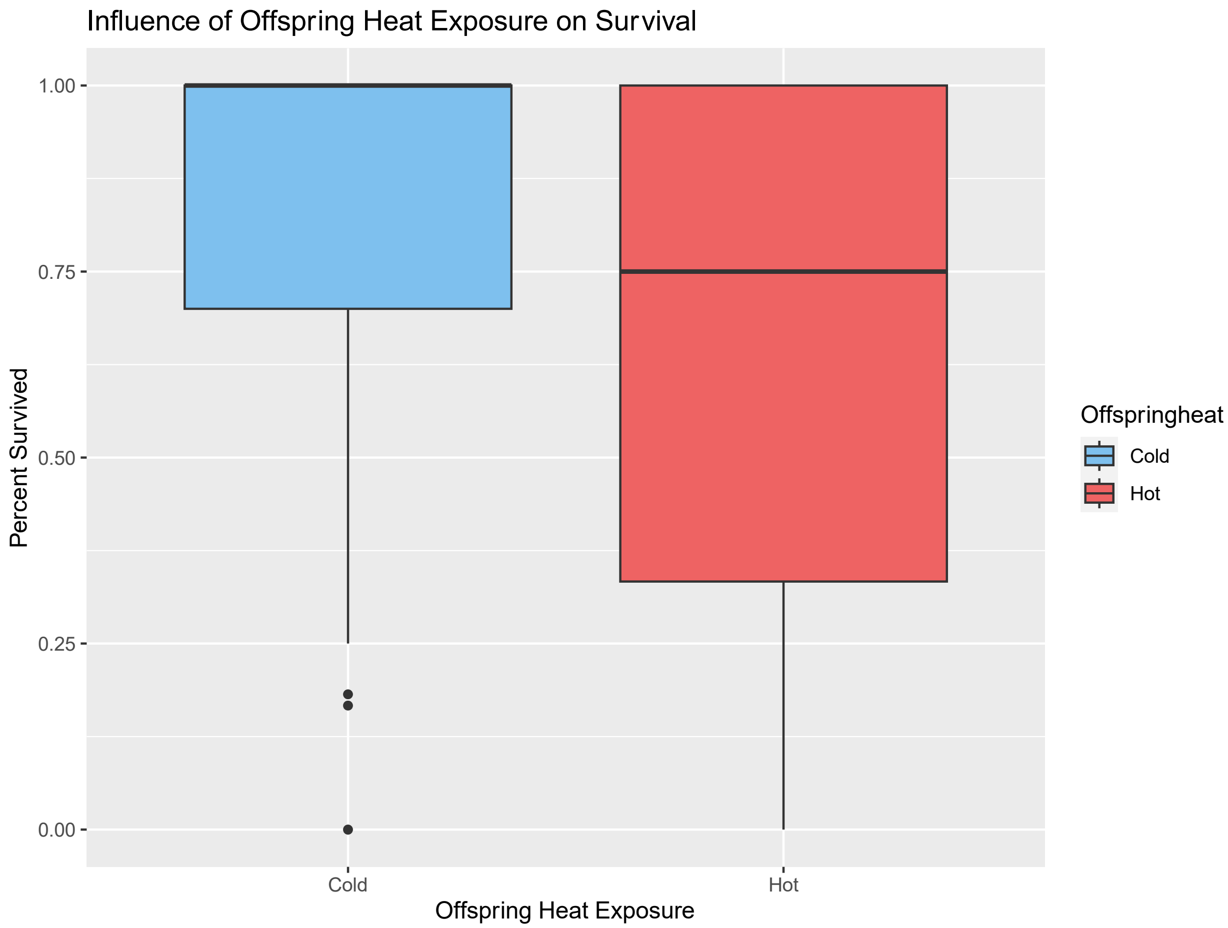

Figure 1: *Offspring heat exposure*. Boxplot of offspring heat exposure and the percentage of offspring survival. Offspring that were exposed to the control treatment (17.2-17.7 degrees Celsius) had a higher percentage of survival compared to those that were exposed to the heat treatment (19.4-20.0 degrees Celsius). This illustrates that heat exposure influences the percent of offspring that survive past their early life stages (~3 months after hatching).

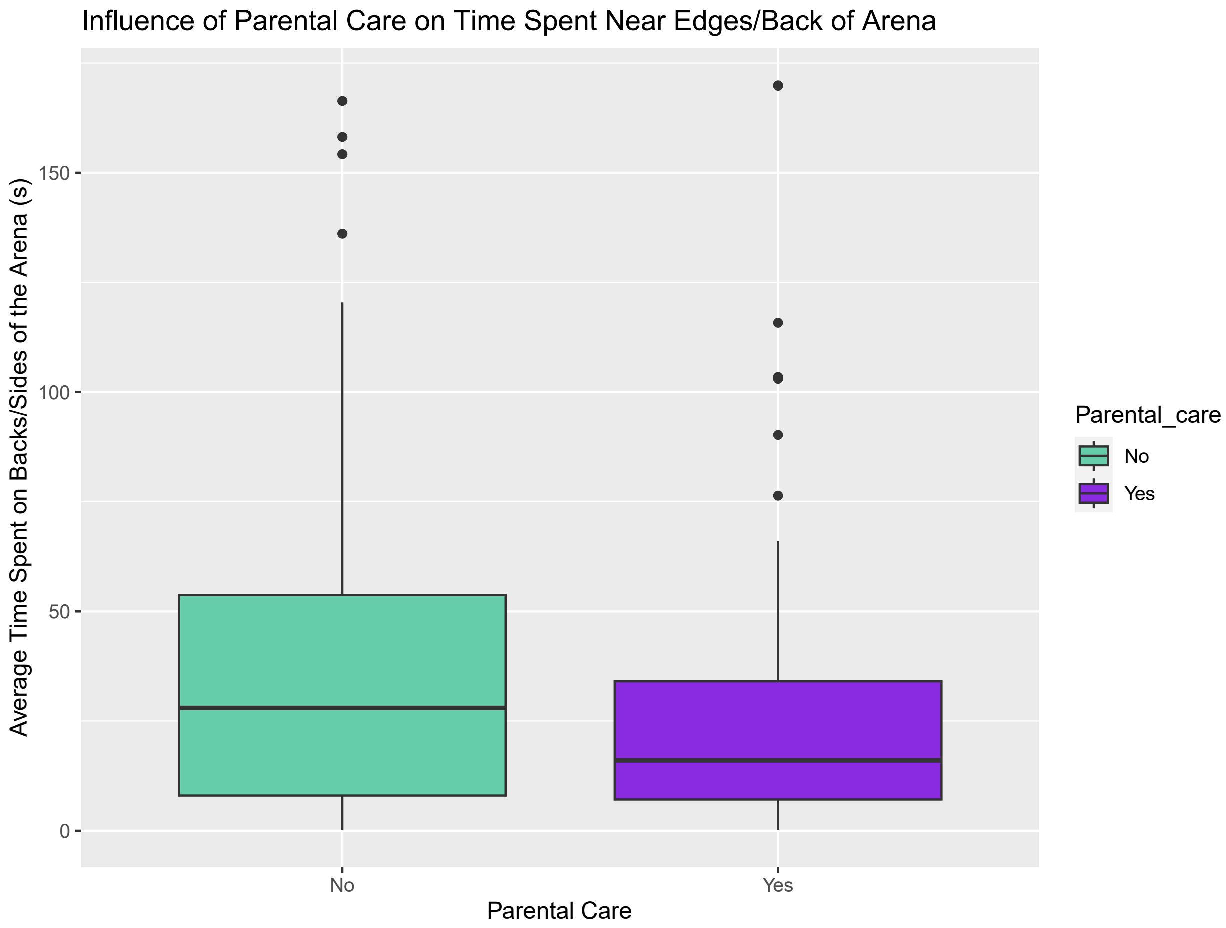

Figure 2*: Parental care and boldness.* Boxplot of parental care and the average time, in seconds, that the assayed offspring spent on the back/sides of the arena. Offspring who didn't receive parental care, regardless of their early life heat and the parental heat, spent more time on the back/sides of the arena while those that did receive parental care spent less time. This suggests that parental care is influencing the boldness of the produced offspring.

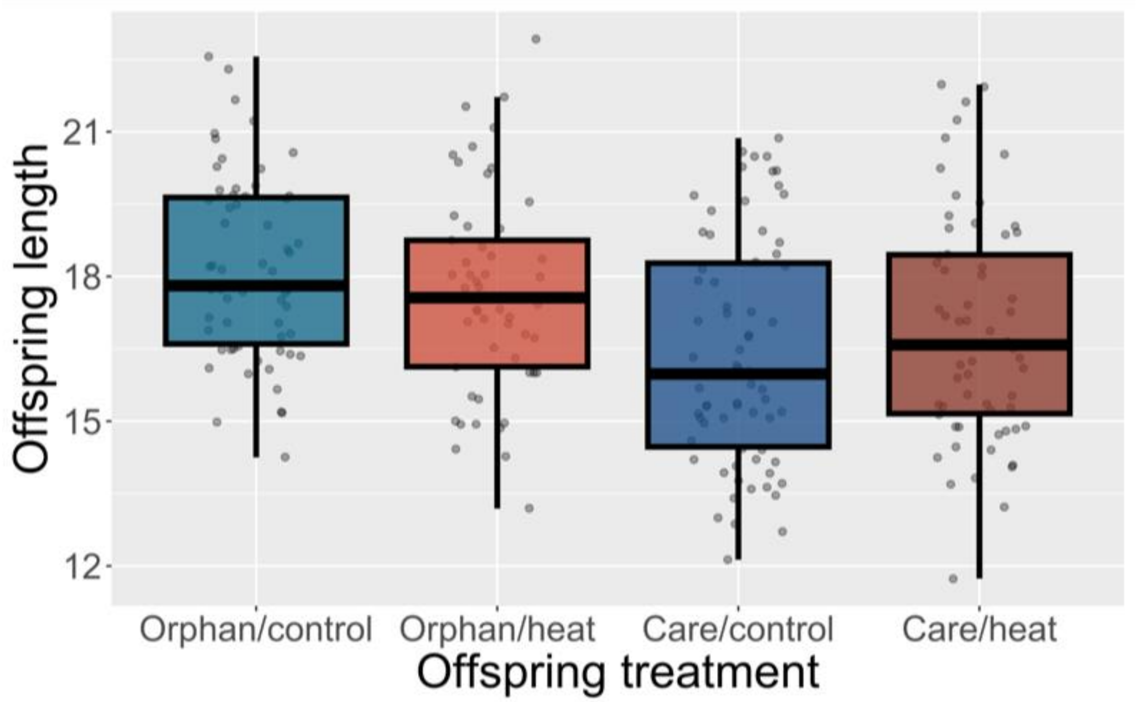

Figure 3: ***Offspring treatment and offspring length.*** Boxplot of the interaction of offspring treatment, early life heat exposure and parental care, and offspring length. Early life heat exposure made offspring shorter in comparison to the control treatment when they received no parental care. While, those that received parental care and were exposed to early life heat were longer than those who were parented and received the control treatment.

Discussion

For this research, we exposed the F0 generation of three-spined sticklebacks to either increased temperatures (19.4-20 degrees Celsius) or control temperatures (17.2-17.7 degrees Celsius). After this, we decided to allow some of the clutches to be parented by their father while others were generated via invitro fertilization to determine the influence of parental care in addition to elevated temperature exposure on the development of the offspring, specifically their length, weight, and willingness to explore a novel environment. To test the influence of parental heat exposure on the offspring, we exposed the offspring to the same treatments as the parents then conducted fast startle assays where each offspring was assayed in the heat and control treatment. This was done to determine if the parents were priming the offspring to be able to cope with increased temperature. Overall, we found that parental care influences the boldness of offspring and the development of offspring traits, and that early life heat exposure determines the rate of offspring survival.

We found that temperature influences the success of the offspring but seems to not affect the breeding behaviors nor the parental care exhibited by the males. This is interesting as within other aquatic organisms, such as the intertidal barnacle, exposure to heat stress resulted in a reduction of mating attempts and ultimately a lower number of offspring produced (Fraser and Chan, 2019). A reduction in mating attempts is commonly seen after heat exposure as organisms, especially fish, rely on certain environmental cues to initiate the start of the spawning season (Breedveld et al. 2023). When these cues aren’t present then there are decreased mating attempts as the parents feel as if the environmental conditions are not proper to be able to successfully rear offspring.

Additionally, a lack of increase in parental care behavior for the heat-exposed males is not what was expected to be seen. It was originally thought that heat-exposed males would fan their eggs more since there is typically a negative correlation between temperature and dissolved oxygen. This would mean that the males would have to accommodate by fanning more to oxygenate their eggs. However, for this study, all the males parented and mated in the control temperatures (17.4-17.7 degrees Celsius) which explains why there was no difference observed in the amount of time spent fanning and the time it took to mate between heat exposed males and control males. If we chose to have the males' mate and parent at elevated temperatures, our results might display similar outcomes to those previously found in literature and this is an area worth exploring in future studies. Furthermore, a difference between heat exposed and control males might have not been observed as the DO for each treatment was relatively the same (between 17-20 ppm). Dissolved oxygen often decreases with increased temperatures. With a decrease in dissolved oxygen, there are constraints on the availability of oxygen for organisms to consume which results in physiological stress and alterations in the development of traits (Crispo and Chapman 2010). Additionally, parents alter their care to better support the development of offspring in a changing environment; such as an increase in fanning by male sticklebacks under conditions of warming (Hopkins et al. 2010). Since the DO was consistent between treatments, this might mean that the males did not need to increase care to compensate in warmer temperatures, as what was observed in previous literature (Hopkins et al. 2010). Future research should analyze the combined effect of dissolved oxygen and increased temperature on parental care behaviors as organisms are soon going to be forced to cope with both of those stressors.

We observed no correlation between offspring survival and parental care or parental heat exposure. This suggests that parental heat did not enable offspring to better cope with exposure to increased temperature, and survival is ultimately not determined through transgenerational exposure, at least not within this research. Furthermore, we found that offspring that were exposed to heat in their early-life stage had a decreased offspring survival rate in comparison to those that weren't. This finding contradicts what we hypothesized as it was thought parental heat exposure and parental care would prime offspring to be better suited to survive in matching environmental conditions. However, it was observed that only offspring heat exposure has an effect on the survival rate and there was no priming effect from parental heat exposure and parental care. Previous literature has shown that the degree of exposure, whether once or multiple times within offspring development, has crucial influence on reproductive plasticity, as those exposed to thermal variability in early and later life stages exhibited beneficial acclimation in terms of hatching success and egg provisioning (Massey et al. 2022). This suggests that multiple exposures are more likely to initiate a transgenerational response as organisms become aware that certain conditions of the new environment are going to persist. This may select for changes within biological pathways to prevent physiological stress from becoming more permanent. Additionally, our findings contrast with previous literature where parental exposure to heat elicited adaptive offspring traits when the rates of environmental change were slower (Donelson et al. 2016). This would be beneficial as it would reduce the risk of heat stress or other forms of physiological stress as the organism would be gradually getting used to the changes instead of being thrown into an environment where the conditions are extremely different than previously experienced.

Our study didn’t observe the same results as previous studies likely do to the rapid exposure that the parents underwent. They were only exposed for a period of three weeks, compared to previous literature that exposed the parental generation to increased heat multiple times during their developmental stages until reproduction (Donelson et al. 2016). Additionally, there wasn’t a huge increase in temperature between the heat exposed and control treatments. A greater difference between the two treatment temperatures might be necessary in order to elicit stronger transgenerational effects. However, the lack of transgenerational responses observed within this research might illustrate that this is a good thing. Parents exposed to slightly elevated temperatures don’t produce offspring that have poor outcomes. This is in contrast to when offspring are directly exposed, which clearly is more detrimental. So, offspring might be more vulnerable to increased temperature exposure than parents.

There was an interaction found between parental care and offspring early life exposure. Offspring from control parents were shorter when they received care compared to when they didn’t. Additionally, early life heat exposure tended to make orphaned offspring shorter, but parented offspring longer. This agrees with previous literature as offspring of coral reef fishes were shorter when exposed to a combination of elevated temperatures compared to other treatments, but parental care was not measured (Donelson et al. 2016). A reduction in size in warming environments may prove to be beneficial as less nutrients are needed to be able to grow and reproduce as nutrients become scarce under conditions of warming; an example being the presence of carbon carbonate within the ocean for shelled organisms to be able to build their shell. However, parental care is also an important variable in the development of offspring traits, but only when the environment is the same as previously experienced within their evolutionary history. This might indicate the setbacks that are present in terms of the parents' ability to prime the offspring based on a minimal exposure to increased temperatures. Within previous studies there has been a positive effect on the development of the offspring in a novel environment when they received parental care and their parents were exposed to that environment (Massey and Dalziel 2023). Additionally, parental care influences the boldness of the offspring as those that received it were observed to spend less time at the edge of the assay arena. Parental care primes the offspring to be able to explore their environment more as they are learning through protection of their parents what exactly is a threat and what behaviors are necessary to be successful. This is important as the boldness to be able to explore environments is crucial under changing temperatures (Moran et al. 2016) as those that are within an environment that is intolerable must move to a new location or rapidly acclimate to their present one. Furthermore, these results suggest that parental care might play an important role in determining how offspring cope with heat. If parental care is influencing the boldness of offspring when exposed to a novel environment, then it is likely that parental care can prime the offspring to be able to cope with an increase in temperature of that environment. It has been found that exposure to increased temperatures alters the parental care of stickleback fathers by causing an increase in the effort of fanning (Hopkins et al. 2010). Further investigation into the effects of increased temperature exposure on parental care's ability to prime offspring is necessary as the results from this study suggest that but cannot prove it entirely.

Overall, if offspring are not being primed by their parents to be able to survive successfully in changing environments, then there is concern that the species will experience a decrease in its population size. Ultimately, impacts to the ecosystem would be observed as a decrease of the prevalence of a trophic level might cause a trophic cascade. Additionally, if offspring can’t acclimate to the environment that their parents are in then changes to the geographical ranges of species will be observed and there will be a greater migration of species to colder waters, but still possibly not an increased success rate of that species as those colder areas might lack resources that are necessary for growth and reproduction. Increasing temperatures are threatening the livelihood of species and it is crucial that a greater understanding of how severe these threats are is achieved.

Works Cited

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H., Singmann, H., ... & Grothendieck, G. (2015). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1–7. 2014.

Bernal, M. A., Ravasi, T., Rodgers, G. G., Munday, P. L., & Donelson, J. M. (2022). Plasticity to ocean warming is influenced by transgenerational, reproductive, and developmental exposure in a coral reef fish. *Evolutionary Applications*, *15*(2), 249–261. https://doi.org/10.1111/eva.13337

Breedveld, M. C., Devigili, A., Borgheresi, O., & Gasparini, C. (2023). Reproducing in hot water: Experimental heatwaves deteriorate multiple reproductive traits in a freshwater ectotherm. *Functional Ecology*, *37*(4), 989–1004. https://doi.org/10.1111/1365-2435.14279

Burt, J. M., Hinch, S. G., & Patterson, D. A. (2010). The importance of parentage in assessing temperature effects on fish early life history: A review of the experimental literature. *Reviews in Fish Biology and Fisheries*, *21*(3), 377–406. https://doi.org/10.1007/s11160-010-9179-1

CRISPO, E., & CHAPMAN, L. J. (2010). Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. *Journal of Evolutionary Biology*, *23*(10), 2091–2103. https://doi.org/10.1111/j.1420-9101.2010.02069.x

Donelan, S. C., Hellmann, J. K., Bell, A. M., Luttbeg, B., Orrock, J. L., Sheriff, M. J., & Sih, A. (2019). Transgenerational plasticity in human-altered environments. *Trends in Ecology & Evolution*, *35*(2), 115–124. https://doi.org/10.1016/j.tree.2019.09.003

Donelson, J. M., Salinas, S., Munday, P. L., & Shama, L. N. (2017). Transgenerational plasticity and climate change experiments: Where do we go from here? *Global Change Biology*, *24*(1), 13–34. https://doi.org/10.1111/gcb.13903

Donelson, J. M., Wong, M., Booth, D. J., & Munday, P. L. (2016). Transgenerational plasticity of reproduction depends on rate of warming across generations. *Evolutionary Applications*, *9*(9), 1072–1081. https://doi.org/10.1111/eva.12386

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N., Polovina, J., Rabalais, N. N., Sydeman, W. J., & Talley, L. D. (2012). Climate change impacts on marine ecosystems. *Annual review of marine science*, *4*, 11–37. https://doi.org/10.1146/annurev-marine-041911-111611

Fraser, C., & Chan, B. (2019). Too hot for sex: Mating behaviour and fitness in the intertidal barnacle fistulobalanus albicostatus under extreme heat stress. *Marine Ecology Progress Series*, *610*, 99–108. https://doi.org/10.3354/meps12848

Forster, J., Hirst, A. G., & Atkinson, D. (2012). Warming-induced reductions in body size are greater in aquatic than terrestrial species. *Proceedings of the National Academy of Sciences*, *109*(47), 19310–19314. https://doi.org/10.1073/pnas.1210460109

Guillaume, A. S., Monro, K., & Marshall, D. J. (2015). Transgenerational plasticity and environmental stress: Do paternal effects act as a conduit or a buffer? *Functional Ecology*, *30*(7), 1175–1184. https://doi.org/10.1111/1365-2435.12604

Hellmann, J. K., Carlson, E. R., & Bell, A. M. (2021). The interplay between sperm-mediated and care-mediated paternal effects in threespine sticklebacks. *Animal Behaviour*, *179*, 267–277. https://doi.org/10.1016/j.anbehav.2021.07.005

Hopkins, K., Moss, B. R., & Gill, A. B. (2010). Increased ambient temperature alters the parental care behaviour and reproductive success of the Three-spined stickleback (gasterosteus aculeatus). *Environmental Biology of Fishes*, *90*(2), 121–129. https://doi.org/10.1007/s10641-010-9724-8

Hovel, R. A., Carlson, S. M., & Quinn, T. P. (2016). Climate change alters the reproductive phenology and investment of a lacustrine fish, the three-Spine stickleback. *Global Change Biology*, *23*(6), 2308–2320. https://doi.org/10.1111/gcb.13531

Kraak, S. B., Bakker, T. C. M., & Mundwiler, B. (1999). Sexual selection in sticklebacks in the field: Correlates of reproductive, mating, and paternal success. *Behavioral Ecology*, *10*(6), 696–706. https://doi.org/10.1093/beheco/10.6.696

Lehto, W. R., & Tinghitella, R. M. (2020). Predator-induced maternal and paternal effects independently alter sexual selection. *Evolution*, *74*(2), 404–418. https://doi.org/10.1111/evo.13906

Mahaffey, C., Palmer, M., Greenwood, N., & Sharples, J. (2020). Impacts of climate change on dissolved oxygen concentration relevant to the coastal and marine environment around the UK. *MCCIP Science Review*, 31–53. https://doi.org/https://doi.org/10.14465/2020.arc02.oxy

Massey, M. D., & Dalziel, A. C. (2023). Parental early life environments drive transgenerational plasticity of offspring metabolism in a freshwater fish (*danio rerio*). *Biology Letters*, *19*(10), 1–6. https://doi.org/10.1098/rsbl.2023.0266

Moran, N. P., Mossop, K. D., Thompson, R. M., & Wong, B. B. M. (2016). Boldness in extreme environments: Temperament divergence in a desert-dwelling fish. *Animal Behaviour*, *122*, 125–133. https://doi.org/10.1016/j.anbehav.2016.09.024

Peichel, C. L., Nereng, K. S., Ohgi, K. A., Cole, B. L., Colosimo, P. F., Buerkle, C. A., Schluter, D., & Kingsley, D. M. (2001). The genetic architecture of divergence between threespine stickleback species. *Nature*, *414*(6866), 901–905. https://doi.org/10.1038/414901a

Potts, L. B., Mandrak, N. E., & Chapman, L. J. (2021). Coping with climate change: Phenotypic plasticity in an imperilled freshwater fish in response to elevated water temperature. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *31*(10), 2726–2736. https://doi.org/10.1002/aqc.3620

Seebacher, F., White, C. R., & Franklin, C. E. (2014). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, *5*(1), 61–66. https://doi.org/10.1038/nclimate2457

Shama, L. N. (2015). Bet hedging in a warming ocean: Predictability of maternal environment shapes offspring size variation in marine sticklebacks. *Global Change Biology*, *21*(12), 4387–4400. https://doi.org/10.1111/gcb.13041

Winder, M., & Sommer, U. (2012). Phytoplankton response to a changing climate. *Hydrobiologia*, *698*(1), 5–16. https://doi.org/10.1007/s10750-012-1149-2