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Assessing the Rate and Extent of Transgenerational Acclimation and Adaptation to Ocean Warming

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"ASSESSING THE RATE AND EXTENT OF TRANSGENERATIONAL ACCLIMATION AND ADAPTATION TO OCEAN WARMING"

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Abstract

A primary goal of climate change research is to determine if species will be able to persist in a warmer environment. Most studies predict climate change will cause many species to become extinct. However, these predictions are based on experiments where only a single life stage or generation of a species was exposed to predicted future conditions (i.e. shock treatments), and thus overlook the possibility of species adapting or acclimatizing to new environmental conditions over multiple generations. As a result, current projections of species persistence through climate change are likely to overestimate species extinction. In this study, the rate and extent to which adaptation and transgenerational acclimation may allow species to persist through climate change was measured. Marine rotifers, *Brachionus plicatilis*, were reared for ~75 generations at: i) Optimal temperature (25°C), ii) Optimal temperature (25°C) with weekly sub-lethal shocks (35°C), iii) Maximum temperature (33°C), and iv) Maximum temperature (33°C) with weekly sub-lethal shocks (35°C). Changes in population growth rates and fitness were assessed weekly through rotifer density, adult size and aerobic performance (respiration rate). There was no adaptation observed, but there was evidence of transgenerational acclimation. However, populations were unable to acclimate when exposed to high temperature shocks. This study shows that acclimation through the selection of thermally tolerant individuals can occur over multiple generations in a thermally stable environment, as seen by a reversible increase in aerobic performance, and thus species with short life cycles may be better able to keep up with the pace of climate change. This multi-generational study can enhance our understanding of the rate and extent in which transgenerational acclimation may allow species to persist through climate change. These estimates can then be incorporated into models to improve projections of survival through climate change of species with longer lifespans.

Keywords: Rotifer, acclimation, adaptation, transgenerational, reproduction, population, respiration

1. Introduction

1.1 *Increasing Sea Surface Temperatures*

Rising carbon dioxide levels in the atmosphere have caused an increase in global temperatures since the industrial revolution (IPCC 2014). With no reduction of greenhouse gas emissions (particularly carbon dioxide, CO_2) in sight, temperatures are expected to be 0.3°C to 0.7°C higher than present by 2035, and 3°C to 8°C warmer by the end of the century (IPCC 2014). Sea surface temperatures are also predicted to rise within this century (Friedlingstein et al. 2006). Since 1971, the upper 75 m of the ocean has warmed by an average of 0.11°C per decade (IPCC 2014). Temperatures down to 3,000m have also increased 0.037°C between 1961 and 2003 (Solomon 2007). These data do not only undeniably show that the Earth's surface and its oceans are warming, but that this trend of increasing temperatures has no evidence of decelerating.

1.2 *Effect of Increased Temperature on Species Persistence*

Changing environmental conditions are projected to lead to the extinction of a great number of species (Thomas et al. 2004, Dawson et al. 2011, Bellard et al. 2012). High temperatures directly reduce the survival, growth and reproduction of plants and animals (Thomas et al. 2004, O'Connor et al. 2007). For example, a study conducted in Texas projects that climate change would lead to the loss of suitable habitats for a number of species of rodents, ultimately leading to their extinction (Cameron 2001). Montane Australian Queensland forests are predicted to have a 7-13% and 43-58% extinction risk due to climate change (for positive and business as usual climate scenarios, respectively) (Thomas et al. 2004). Elevated temperatures also cause an increase in the frequency and intensity of severe storms, as well as the melting of sea ice platforms and sea level rise, which results in the loss of suitable feeding, resting and breeding habitats for many animals (Burek et al. 2008). A rise in ocean temperature is predicted to have negative impacts on marine species' abilities to persist in future environmental conditions (Johansen and Jones 2011, Donelson and Munday 2012).

However, most projections of species persistence through climate change are based on the results of short-term experiments and/or models, which assume that species response to environmental stress will remain unchanged.

Current projections of species persistence through climate change use baseline experimental studies that test the effect of the future environmental conditions on a single life stage or generation of a species (Munday et al. 2013). Short-term experiments overlook evolutionary processes that could lead to a higher tolerance for the future climate, such as acclimatization and adaptation. Adaptation differs from acclimatization in that it is a genetic change that helps an organism survive and reproduce better in its environment, and therefore tends to be passed down from one generation to the next (i.e. naturally selected). Acclimatization is a process in which an individual organism adjusts to a gradual change in its environment through gene expression, allowing it to maintain performance across a range of environmental conditions, without genetic change (Kinnison et al. 2007, Munday et al. 2013, Sunday et al. 2014). Furthermore, animals are often exposed to environmental conditions predicted for the end of the century, which are outside of their current environmental temperature range, and therefore only reflect shock responses to stress. As a result, these experimental designs and resulting current model projections of species persistence through climate change are likely to overestimate species extinction. Multi-generational studies are needed to determine the extent of the impacts of global climate change on species persistence (Sunday et al. 2014). To accurately assess an organisms' ability to cope, they should be exposed to stressors incrementally rather than exclusively with "shock" treatments. The rate and extent in which acclimatization and adaptation (individually or in combination) may contribute to species persistence under climate change remains unknown.

1.3 *Adaptation and Acclimation*

To accurately predict future species abundance and distribution, it is imperative to understand how species respond to climate change and the mechanisms that may underlie such responses. Adaptation, a process that involves genetic change within a population or species, has been shown to contribute to the survival of a species under new

environmental conditions. If some individuals within a population survive exposure to a new environmental condition, it can be concluded that there is enough variation within the population for natural selection, and therefore adaptation, to occur (Lohbeck et al. 2012). In other words, individuals that have a higher tolerance to higher temperatures are more likely to survive and reproduce in an environment with increasing temperatures. As the genes of these individuals are passed on to their offspring, a greater proportion of individuals in the new generation will also be better able to cope with the increasing temperatures (Munday et al. 2013). Mutations that confer higher resistance to the new environmental conditions can also accumulate over multiple generations. Individuals possessing favorable mutations will likely be positively selected, i.e. exhibit disproportionally higher survival and reproduction, and the overall population should become better adapted **(**Munday et al. 2013). For example, a study on the wild rabbit, *Oryctolagus cuniculus*, showed a genetic difference in body core shape and ear and foot size among regional populations when reared under different temperatures (Williams and Moore 1989). In another study performed to assess the response of coccolithophores to increasing CO_2 levels through 500 generations, a direct positive adaptation via genotypic selection enhanced calcification by 50% relative to a non-adapted culture (Lohbeck at el. 2012). However, adaptation is a slow process which requires multiple generations, leading some researchers to suggest that the rate of climate change will outpace the rate of adaptation (Lynch and Lande 1993).

Natural populations can also enhance their chances of survival in a new environment through acclimation (here meaning both natural acclimatization and laboratory-based acclimation), which does not require genetic change, but rather the expression (up-regulation or down-regulation) of already existing genes caused by new environmental conditions (Sunday et al. 2014). There are three types of acclimation: reversible, developmental and transgenerational. Reversible acclimation can occur over very short periods of time within a life stage, e.g. seasonal physiological or behavioral adjustment, as opposed to developmental acclimation, which occurs when exposure to a novel environment during ontogeny (embryonic and/or larval development) enhances performance in that environment later in life. For example, the juvenile and adults of the spiny chromis damselfish, *Acanthochromis polyacanthus*, which have been exposed to

higher temperatures during early ontogeny, are better able to cope with higher temperatures than individuals reared through ontogeny at current conditions (Donelson et al. 2011). In particular, transgenerational acclimation consists of developing a phenotype (physiological, morphological, or behavioral) that is better suited to the environment to which the previous generations were exposed (Marshal 2008, Donelson et al. 2012, Miller et al. 2012). To maximize the chances of survival of the next generation, parents can include certain proteins in the eggs that can shutoff/deactivate certain parts of the DNA, and turn on/activate other parts (Rossiter 1996). Examples of transgenerational acclimation range from plants to humans. For example, the increase in atmospheric CO_2 levels since the Industrial Revolution has altered the density of the stomata (minute openings on the leaves of plants through which CO_2 enters and water leaves the plant) in some deciduous trees; experimental results indicate that this response is reversible and thus a result of phenotypic plasticity (Wagner et al. 1996). Transgenerational acclimation has also been recorded in juvenile damselfish whose parents were exposed to high temperatures and were able to compensate for the negative effects of elevated seawater temperature on metabolic rate and aerobic scope (Donelson et al. 2014). Identifying and understanding the capacity for acclimation will aid in the prediction of species persistence in response to global climate change.

To determine the rate and extent in which transgenerational acclimation and adaptation will facilitate species persistence, the two mechanisms need to be studied separately and concurrently (Munday et al. 2013). Adaptation is expected to eventually confer a greater resistance to the new environmental conditions, although it occurs slowly. In contrast, acclimation can quickly help species survive smaller environmental changes. It remains unclear if acclimation will hinder or help species to adapt to future environmental conditions. Interpretation of results in studies on multiple generations often assume that the changes in populations' fitness are solely due to genetic adaptation, and ignore the chance for transgenerational acclimation (Sunday et al. 2014). Acclimation may allow populations to cope with initial environmental changes and thus "buy time" for adaptation to eventually occur. In other words, the process of acclimation may provide time for heat-resistant individuals to be selected (survive and reproduce better over multiple generations) (Chevin et al. 2010). However, acclimation may also

impede adaptation. Acclimated individuals may survive regardless of having good or mal-adapted genes, meaning that genes conferring higher survival (or fitness) will not be preferentially transferred to the subsequent generations.

To fully understand the impacts of global climate change on Earth's organisms, it is imperative to determine the potential for acclimation and/or adaptation to elevated temperature in a variety of species (Logan et al. 2014). Efforts to determine the effects of climate change need to concentrate on key functional groups (Sunday et al. 2014), such as organisms at lower trophic levels. Changes in abundance, distribution and composition of organisms that occupy the lower trophic levels will inevitably impact upper trophic levels (Sunday 2014). It has been theorized that any significant change in plankton biomass will ultimately ascend up the food chain, ultimately leading to worldwide extinctions **(**Lynch and Land 1993). To better estimate persistence in a less extensive time period, species from a key functional group with a relatively short life cycle should be used as model organisms.

2. Objectives

The overall aim of this study was to increase our understanding of what role adaptation and acclimation have in allowing species to persist through climate change. Specifically, I used a species that occupies the lower trophic level of the food web, the marine rotifer *Brachionus plicatilis*, as a model organism to*:*

(1) Quantitatively assess the rate and extent of the contribution of transgenerational acclimation to the size, growth and fitness (aerobic performance) of a population at elevated temperatures;
(2) Determine the rate and extent of the contribution of adaptation to the size, growth and fitness (aerobic performance) of a population at elevated temperatures;
(3) Evaluate the interaction between acclimation and adaptation processes in conferring enhanced size, growth and fitness to a population at elevated temperatures.

3. Methods

3.1 *Species Description*

Rotifers of the species *Brachionus plicatilis* are microscopic aquatic animals (Figure 1) found in a variety of saltwater environments and play an important role in the distribution of energy and dynamics of the ocean food web (Wallace and Smith 2013). They are primarily omnivorous and their lifespan is estimated to be between 4 and 7 days, generally reaching adulthood between 0.5-1.5 days (Lavens and Sorgeloos 1996). Adult females usually lay eggs every four hours and can produce either mictic or amictic eggs, depending on environmental conditions (Figure 2). Amictic eggs do not need to be fertilized and develop into females. Mictic eggs will develop into males if left unfertilized, or females if fertilized by the mictic male (Lavens and Sorgeloos 1996).

Figure 1 (above): Female *B. plicatilis*. Female on top is carrying an egg.

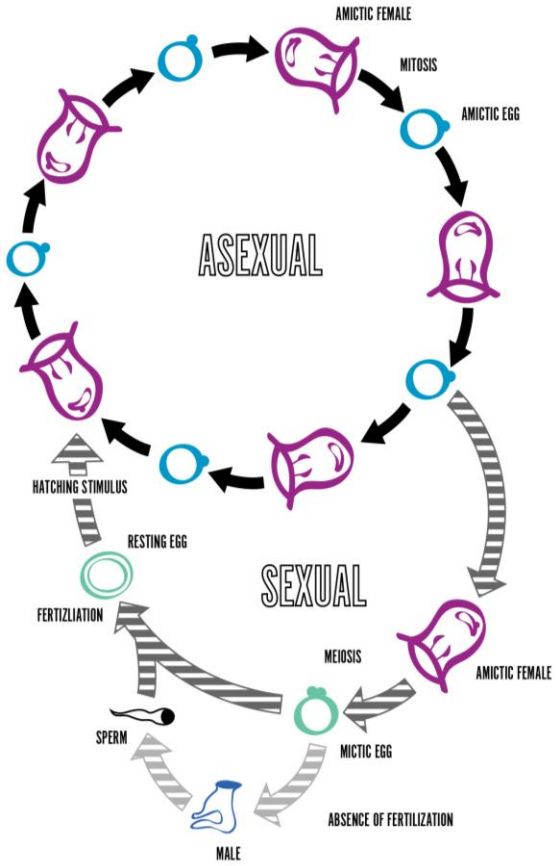

Figure 2 (right): Lifecycle of marine rotifer, *B. plicatilis*, depicting sexual and asexual reproduction (adapted from Hoff and Snell, 1987).

This species was selected for three main reasons, the first of which is their ecological relevance. Marine rotifers are planktonic animals at the base of the marine food web (Wallace and Smith 2013), i.e. constitute the food for marine larvae, small invertebrates and smaller fish, which are in turn consumed by larger organisms. Changes in abundance, distribution and composition of planktonic organisms will ultimately affect organisms at higher trophic levels, potentially leading to the collapse of food webs and worldwide extinctions (Lynch and Land 1993). A better understanding of this key functional group's ability to cope with climate change is essential to model the ability of marine ecosystems to persist in projected future conditions. Secondly, marine rotifers have a well-established culture methodology. They have been cultured in captivity for more than fifty years to be used as feed for larval fish in aquaculture farms. The optimal conditions to raise them in captivity have been extensively described (Hoff and Snell 1987, Arnold and Holt 1991). And lastly, because marine rotifers have a relatively short lifespan (less than a week, Arnold and Holt 1991), this allows for studying multiple generations in a laboratory setting under controlled environmental conditions.

3.2 *Population Development and Culture Maintenance*

The original culture batch of *B. plicatilis* was purchased through Pentair Aquatic Eco-systems®. Upon arrival, the batch was transferred to a 10L plastic cylindrical tank kept at 25°C (ambient) under a 16h light: 8h dark light cycle with constant moderate aeration (to prevent anoxic conditions and allow equal mixing of rotifers and food). The rotifers were fed daily with a commercial algae paste of Instant Algae® (*Nannochloropsis sp.*). Food quantities were based on population densities (which were assessed every day). The population remained in these conditions until the start of the 16-week experiment. To avoid water quality issues, each tank received a 50% water change three times a week, and a 100% water change once a week. All water changes were performed after all measurements (*see below*) had been completed. During water changes, the rotifer cultures were filtered through a 56 μm sieve, which allowed (broken down) dead rotifers, detritus and all other small organic matter to filter out while the

larger rotifers of all ages/sizes remained in the sieve. The tanks were cleaned and the rotifers were returned to their tanks with adequately cleaned and heated seawater, and subsequently fed. Rearing rotifers at high concentrations have direct repercussions for reproduction, and a concentration at or above 500 rotifers/mL is considered high density. It was determine that keeping the population density between 200-300 rotifers/mL would allow for best growing conditions. For the temperature tolerance experiments (*section 3.3*), rotifer populations were reared at a starting population density of 300 rotifers/mL. However, because of the larger size of the tanks used for the 16-week experiment (*section 3.4*) and the exponential population growth rate of the rotifers, it was determined that the best starting population density for the 16-week experiment was 200 rotifers/mL. The population density for the 16-week experiment was checked before every 100% water change (*section 3.5.1*). If the population density was found to be above 200 rotifers/mL, then excess individuals were removed before beginning the water change to bring the culture back to 200 rotifers/mL. If the population density was at or below 200 rotifers/mL, then the culture population was left alone and the water change continued per usual. These conditions remained the same throughout the entirety of the experiment.

3.3 *Temperature Tolerance*

Prior to conducting the experiments to assess the rate and extent to which rotifer *B. plicatilis* can acclimate and/or adapt to ocean warming, two preliminary experiments were conducted. One experiment was performed in order to identify the optimal temperature (T_{opt}, temperature at which the population growth rate is the highest) and the maximum survivable temperature (T_{max}, temperature higher than the optimal in which population density is similar to the initial density, i.e. a temperature in which populations survive but do not increase). Using this information, a second experiment was performed to determine the shock temperature (T_{shock}, temperature at which 50% of the population dies within 24 hours).

3.3.1 *Optimal and Maximum Temperature*

Twenty-four 400mL batches of rotifers, at an average density of 300 rotifers/mL, were removed from the initial culture and randomly assigned to a water bath at one of the following temperature treatments: 25, 27, 29, 31, 33, 35, 37 and 39°C (three replicates per temperature. Mass culture of marine rotifers are maintained best at temperatures between 20-30°C, with optimal growth between 18-25°C (Hirayama and Kusano 1972, Hoff and Snell, 1987). Therefore, these experimental temperatures range from temperatures at which rotifers are best maintained, to temperatures above this optimal range. The culture density within each replicate was measured (*section 3.5.1*) every day for a total of 14 days (ca. ~10 generations). The water was moderately aerated and the cultures were fed every day. After 14 days, population growth rate was calculated using the population density at the beginning and end of the trial for each temperature and these data were then used to determine the temperatures for T_{Opt} and T_{Max}.

3.3.2 *Shock Temperature*

Sixteen 400mL batches of rotifers, at an average density of 300 rotifers/mL, were removed from the initial culture and randomly assigned to a water bath at one of the following temperature treatments: 35, 36, 37 and 38°C (two replicates per temperature). Based on the results in the previous experiment (*see Results*), this temperature range was chosen to determine which temperature would kill 50% of the initial population in 24 hours, i.e. what temperature (T_{shock}) would lead to a population growth rate of -0.5/day. This 24h trial was repeated three times (three separate runs). After each 24h period, total populations were counted for all treatments. All population totals were compared and analyzed to determine the T_{Shock}.

3.4 *Experimental Design*

To assess the rate and extent at which rotifers can acclimate and/or adapt to ocean warming, the rotifers were reared for 16 weeks (approximately 75 generations) under four temperature treatments (Figure 3). In the first treatment (Optimal), rotifers were kept at 25°C (T_{opt}), and this represented the control. This temperature was determined during the preliminary experiment to give the highest population growth rate and therefore serves as the ambient (optimal) temperature. In the second treatment (Max), rotifers were kept at 33°C (T_{max}). This temperature was found in the preliminary experiment to be the maximum temperature within their natural thermal tolerance range and guarantees that the population is not shocked (i.e. individuals with heat-sensitive genes are not excluded from the population). Additionally, this temperature has the potential to alter gene expression over multiple generations to produce heat-resistant individuals through transgenerational acclimation. In the third treatment (Optimal+Shock), rotifers were kept at T_{opt} (25°C) temperature with weekly 24-hour shocks at 37°C (T_{shock}). The temperature shocks were determined from preliminary experiments and intended to eliminate individuals from the population whose genes are less adapted to higher temperatures. In other words, individuals with warm-resistant genes have the potential to become more prevalent in subsequent generations through adaptation. In the fourth treatment (Max+Shock), rotifers were kept at T_{max} (33°C) with weekly 24-hour shocks at 37°C (T_{shock}). This purpose of this treatment was to assess the interaction between the process of transgenerational acclimation and adaptation.

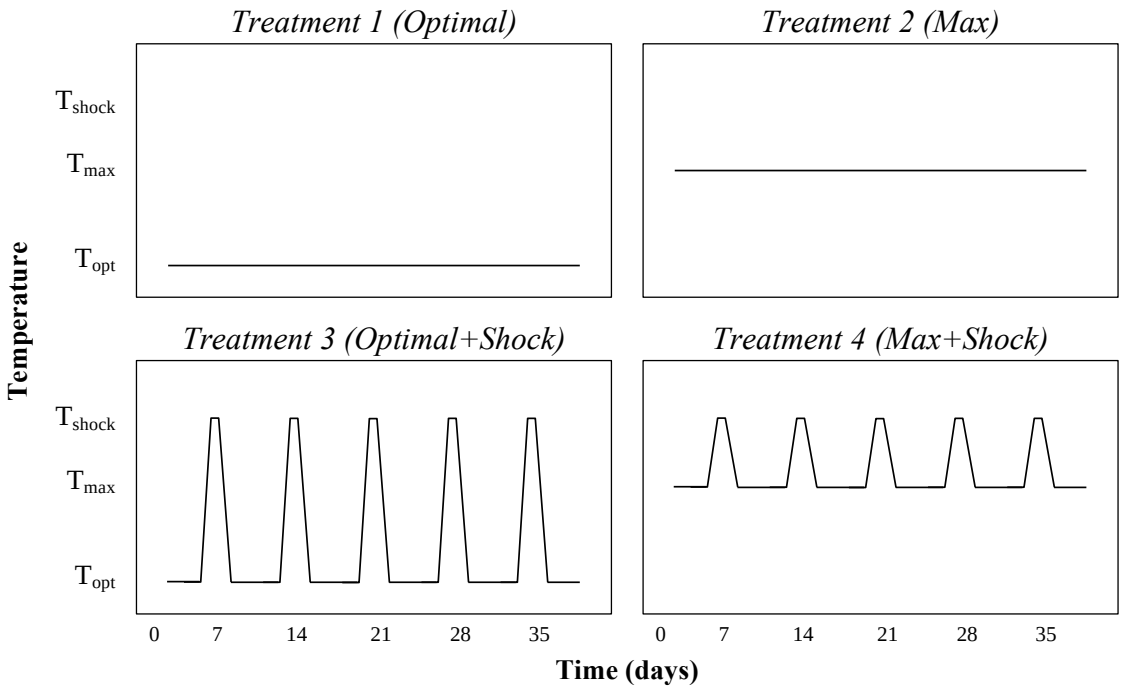

Figure 3: Schematic of all four treatments displaying their corresponding temperatures.

Each treatment had four replicates. Sixteen 10L tanks, with a stocking density of 200 rotifers/mL, were assigned to one of the four temperature treatments and each tank's location was randomly placed so that the replicates of each treatment were never in the same row or column (Figure 4). Each tank was supplied with constant, moderate aeration (to prevent anoxic conditions and allow equal mixing of rotifers and food) and a light cycle of 16h light: 8h dark. The tanks were heated with a 250watt titanium heater (one per tank) regulated by an Aqua Logic® 115V temperature controller (one per heater). Tanks were cleaned and fed as described previously. In the treatments for Max and Max+Shock, rotifers were acclimated to their treatment temperature at a rate of 1°C per day, until reaching their respective temperature of 33°C (T_{max}). The remaining tanks (Optimal and Optimal+Shock) stayed at 25°C (T_{opt}). For the two treatments requiring weekly shocks (Optimal+Shock and Max+Shock), once a week (on the same day every week) the temperature was increased to 37°C (T_{shock}) instantly by resetting the heater, and remained at that temperature for 24 hours, after which the heater was returned to the original temperature prior to the shock. The weekly 100% water change occurred on the day after the weekly shock treatment. To differentiate between acclimation from

adaptation, at the conclusion of the 16-week experiment, Max and Max+Shock were returned to T_{opt}, so that all treatments were at 25°C, and remained at that temperature for four more weeks. During the 16-week experiment and the four weeks at which all populations were at T_{opt}, measurements were taken weekly.

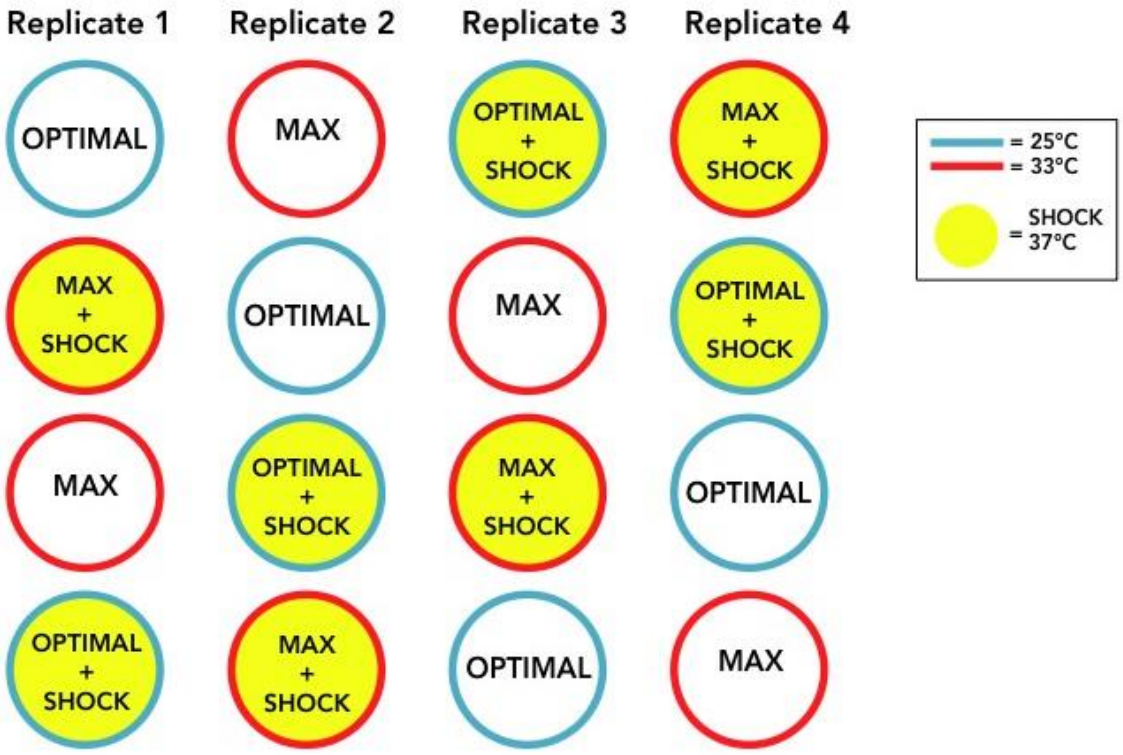

Figure 4: Experimental schematic showing the placement each treatment. Blue represents the treatments that were kept at T_{opt} (Optimal and Optimal+Shock). Red represents the treatments that were kept at T_{max} (Max and Max+Shock). The circles filled with yellow are the treatments at either T_{opt} or T_{max} that were shocked weekly (Optimal+Shock and Max+Shock) for 24 hours at 37°C (T_{shock}).

3

3.5 *Measurements*

The culture density (population growth rate) and adult size (area of rotifer, mm^2) in each replicate of each treatment were measured over time. Once a week, rotifers were randomly selected from each replicate of each treatment to measure aerobic performance as a representation of overall fitness. All measurements were recorded over 16 weeks. At the conclusion of the four weeks at T_{opt} (after the 16-week experiment), culture density, adult size and aerobic performance were also assessed for each treatment.

3.5.1 *Population Growth Rate*

To estimate population growth rate, the culture density of each treatment was measured by removing 1mL samples from each replicate of each treatment and placing them on a Sedgewick-Rafter slide (1mL). Each 1mL sample was fixed with iodine and the number of rotifers were counted under a compound microscope. The total population of rotifers for each replicate of each treatment was determined by multiplying the culture density (number of rotifers/mL) by the volume of the culture (10L). The population growth rate was then estimated using the following equation:

$$\mu = \frac{(N_t - N_0)}{N_0}\ x\ 100$$

Where μ is the population growth rate, N_0 is the initial density, and N_t is the final density at day (t) of culture period.

3.5.2 *Adult Size*

To determine changes in adult size between treatments at the conclusion of the 16-week experiment and after four weeks at T_{opt}, a minimum of 50 individuals per treatment were fixed in a solution of iodine and measured. Pictures of each individual were taken with an Olympus LC20 digital camera attached to an Olympus SZ61 dissecting microscope (4.5x magnification) and CellSens® was used to measure surface area (mm^2).

3.5.3 *Aerobic performance*

To evaluate aerobic performance, one 800 mL sample was removed from each replicate of each treatment and placed in a water bath at the respective treatment temperature. Each 800 mL sample was moderately aerated to create a homogenous sample and to saturate the samples with oxygen. From each 800 mL sample, nine 1 mL

samples were removed and each placed into a Wheaton vial. Eight of the vials were topped off with salt water (heated to the same temperature as the respective treatment and saturated with oxygen) and sealed with Parafilm^{TM}. The remaining vial was fixed with iodine and the number of rotifers in that vial were counted (see below) in order to calculate respiration rate. Vials from each replicate of each treatment were randomly assigned to a water bath at one of the following temperatures: 25, 27, 29, 31, 33, 35, 37 and 39°C (4 vials per temperature, one vial for each replicate). An oxygen sensor spot (SP-PSt3-NAU, Presens) was glued on the inside wall of each vial prior to measuring oxygen concentration. Each vial was given 30 minutes to acclimate to the temperature before any measurements were taken. A non-invasive polymer optical cable (POF, Presens) was then used to scan the oxygen sensor spot at 30 minutes and then again at 60 minutes. At each measurement, vials were removed from the water bath and gently inverted to ensure equal mixing. The O_2 saturation ($\%O_2$) was measured at the water bath temperature using the POF (Presens) which transfers a light from the cable to the sensor spot, back to the Fibox 4® meter (Presens). The number of rotifers in each vial was determined by placing the contents of the fixed vial on a Sedgewick-Rafter slide (1mL) and counting the number of rotifers under a dissecting microscope. Respiration rate was then calculated by taking the oxygen consumed (given in $\%O_2$) between minute 30 and minute 60 and dividing this number by the number of rotifers in the vial for each treatment to get the oxygen consumed per rotifer in 30 minutes. This number was then divided by time to get oxygen consumed per rotifer per minute. The formation of an anoxic environment in the vials placed in the water baths was prevented by *B. plicatilis'* natural tendency to swim continuously within the water column and rarely attach or settle, thus creating their own mixing within the vial and eliminating the need for a stir bar or any other similar mechanism. In order to determine rate of oxygen consumption by the oxygen probe, measurements of vials containing only salt water in each temperature were also made in order to determine rate of oxygen consumption by the oxygen probe. These measurements were then adjusted accordingly for each respiration trial in order to compensate for loss of oxygen by the probe or diffusion of oxygen into the vial. For each treatment, the respiration rate ($\%\ O_2.rotifer^{-1}.min^{-1}$) was considered to be the aerobic performance of the population for that treatment at that temperature. It is important to

note that oxygen consumption (respiration rate) is inversely related to aerobic performance in that an increase in oxygen consumption translates to a decrease in aerobic performance (fitness). Measurements were performed weekly, however, when the concentration of each vial reached below 100 rotifers/mL, the error was too high to properly assess aerobic performance. Therefore, only measurements from week 1, week 16 and week 20 were used.

3.6 *Data Analysis*

The software R was used to conduct all analysis.

3.6.1 *Optimal and Max Temperature*

A one-way ANOVA was used to compare population growth rates between temperatures followed by a post-hoc multiple comparisons test (Tukey) to determine differences between the treatments. Those results were then aligned with a graphical representation to determine T_{opt}. In order to establish T_{max}, a one sample T-test was used to determine which temperature had a population growth rate closest to 0.0/day.

3.6.2 *Shock Temperature*

A one-way ANOVA was used to compare population growth rates between temperatures after 24h of exposure. A post-hoc multiple comparisons test (Tukey) was then used to determine differences between the temperatures. In order to determine T_{shock}, a one sample T-test was used to determine which temperature had a population growth rate closest -0.5/day.

3.6.3 *Population Growth Rate*

A two-way repeated measures ANOVA was used to compare population growth rates between treatments over time. There was a significant effect of time and treatment,

so a one-way ANOVA was used to analyze how each treatment changed over time and also to analyze how treatments differed in each week. A one-way ANOVA was used to compare population growth rate between treatments after four weeks of T_{opt}.

3.6.4 *Rotifer Size*

A one-way ANOVA was used to compare rotifer size post 16 weeks between treatments and also to compare size after four weeks of T_{opt}.

3.6.5 *Aerobic Performance*

To evaluate aerobic performance, the respiration rate in each treatment was compared at each temperature (25, 27, 29, 31, 33, 35, 37, 39°C) for week 1 and week 16 using a factorial ANOVA. One-way ANOVA was performed for each treatment and each week to determine which factor was significant at each temperature. A one-way ANOVA was then used to compare each treatment after four weeks of T_{opt}.

4. Results

4.1 *Optimal and Max Temperature*

There was a significant difference in population growth rate between temperatures ($p=0.0006302$). The optimal temperature (T_{opt}), i.e. temperature at which the population growth rate significantly increased the most, was 25°C (Figure 5). The temperature at which populations survived but did not increase significantly in population growth rate (T_{max}) was 33°C (Figure 5). Population growth rates between 31°C and 33°C were similar, but because the temperature controllers vary by ±1°C, setting the temperature at 31°C puts the treatment at risk for fluctuating too close to a significantly better temperature in terms of population growth rate. Therefore 25°C (T_{opt}) was the temperature used for Optimal and Optimal+Shock treatments, and 33°C (T_{max}) was the temperature used for Max and Max+Shock treatments.

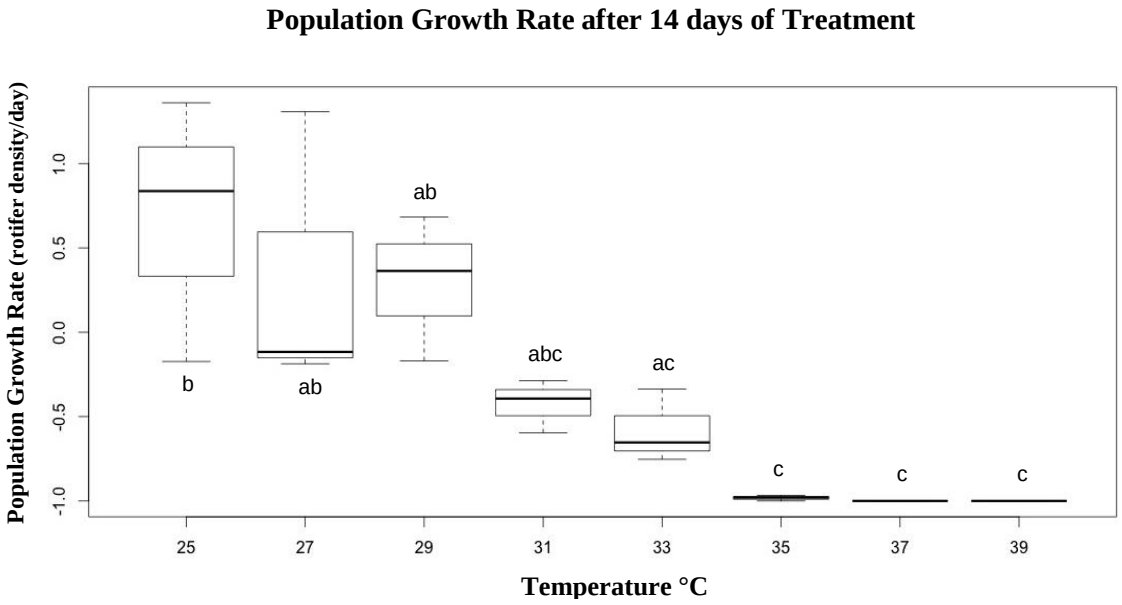

Figure 5: Population growth rate after 14 days of rearing populations at 25, 27, 29, 31, 33, 35, 37, 39°C.

4.2 *Shock Temperature*

The previous preliminary experiment established that 33°C was the maximum temperature (T_{max}) at which populations survived but did not increase. Since a temperature of 39°C would eventually kill the entire population, the temperature range of 35-38°C was used to determine T_{shock}. Population growth rate significantly decreased with increasing temperature ($p=0.04321$). The population growth rate reached -0.5/day (where population was reduced by 50%) between 36-38°C (Figure 6). The mean population growth rate was not significantly different between 36°C and 37°C (Figure 6). The heaters fluctuate by ±1°C, so any population shocked at 38°C or higher could potentially have experienced 39°C and more than 50% of the population would be killed. Conversely, shocking a population at a temperature of 36°C means the heater could fluctuate to 35°C and would have the potential to not kill enough of the population in one day. Therefore, T_{Shock} was determined to be 37°C and was used as the weekly shock temperature for the populations reared in Optimal+Shock and Max+Shock treatments.

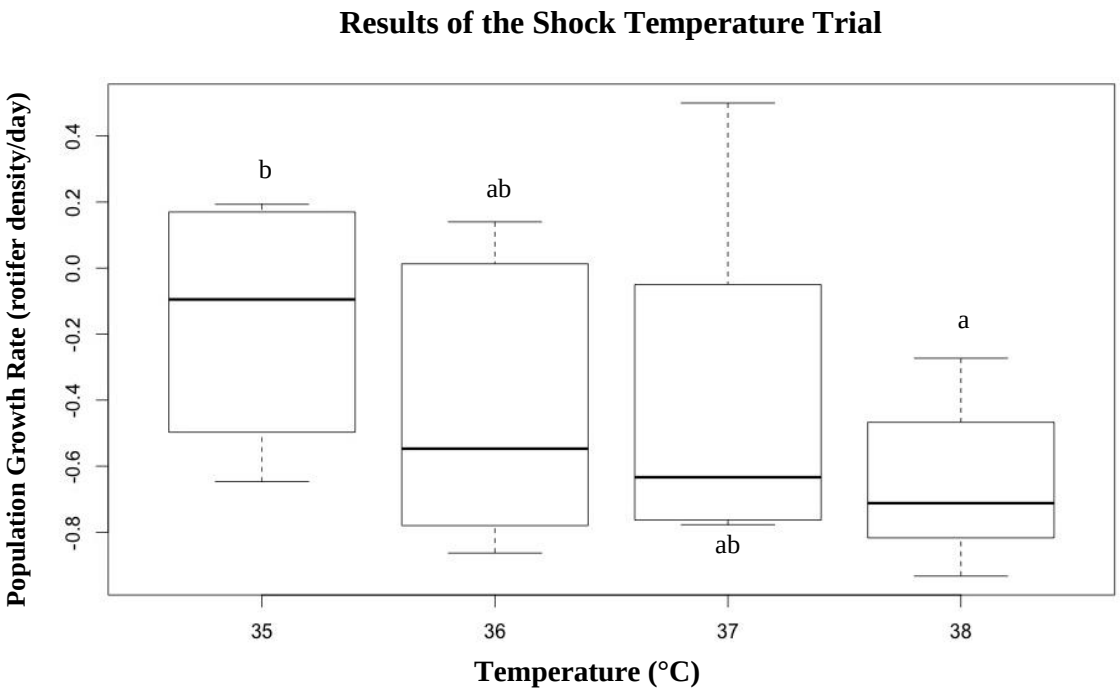

Figure 6: Population growth rates after 24h shock at 35, 36, 37, and 38°C.

4.3 *Population Growth Rate*

The population growth rate of the rotifers significantly differed between treatments ($p=0.0132$) and between weeks ($p=7.145x10^{-05}$). The population growth rate of rotifers in Optimal did not significantly change throughout the 16 weeks ($p=0.3792$). The population growth rate significantly changed over time within Optimal+Shock, Max, and Max+Shock treatments ($p=0.004452$, 0.002528, 0.000145, respectively). The rotifers in Optimal+Shock displayed an increased population growth rate from week 1 to week 2, then decreased into week 3, followed by an increase into week 8 and no change in population growth rate from week 8 to week 16 (Figure 7). The population growth rate for the rotifers in Max increased from week 1 to week 2, and remained the same through week 16. The rotifers in Max+Shock had an increase in population growth rate from week 1 to week 2, followed by a decrease to week 3 and then an increase through week 16 (Figure 7). Weeks 2, 3, and 8 had significantly similar population growth rates among treatments ($p=0.275$, 0.08056, 0.316, respectively). There were significantly different

population growth rates between treatments at week 1 and week 4 ($p=0.002738$ and 0.002582, respectively). However, at week 4 the population growth rates of the rotifers in Optimal+Shock, Max and Max+Shock were similar to each other, but significantly lower than Optimal (Figure 7). At the conclusion of the experiment (week 16), population growth rates for rotifers were significantly different in all treatments ($p=0.0055$), with Max and Max+Shock having higher population growth rates than Optimal and Optimal+Shock. After returning all populations to T_{opt} for four weeks, population growth rates were no longer significantly different between any treatments ($p=0.8463$) (Figure 8).

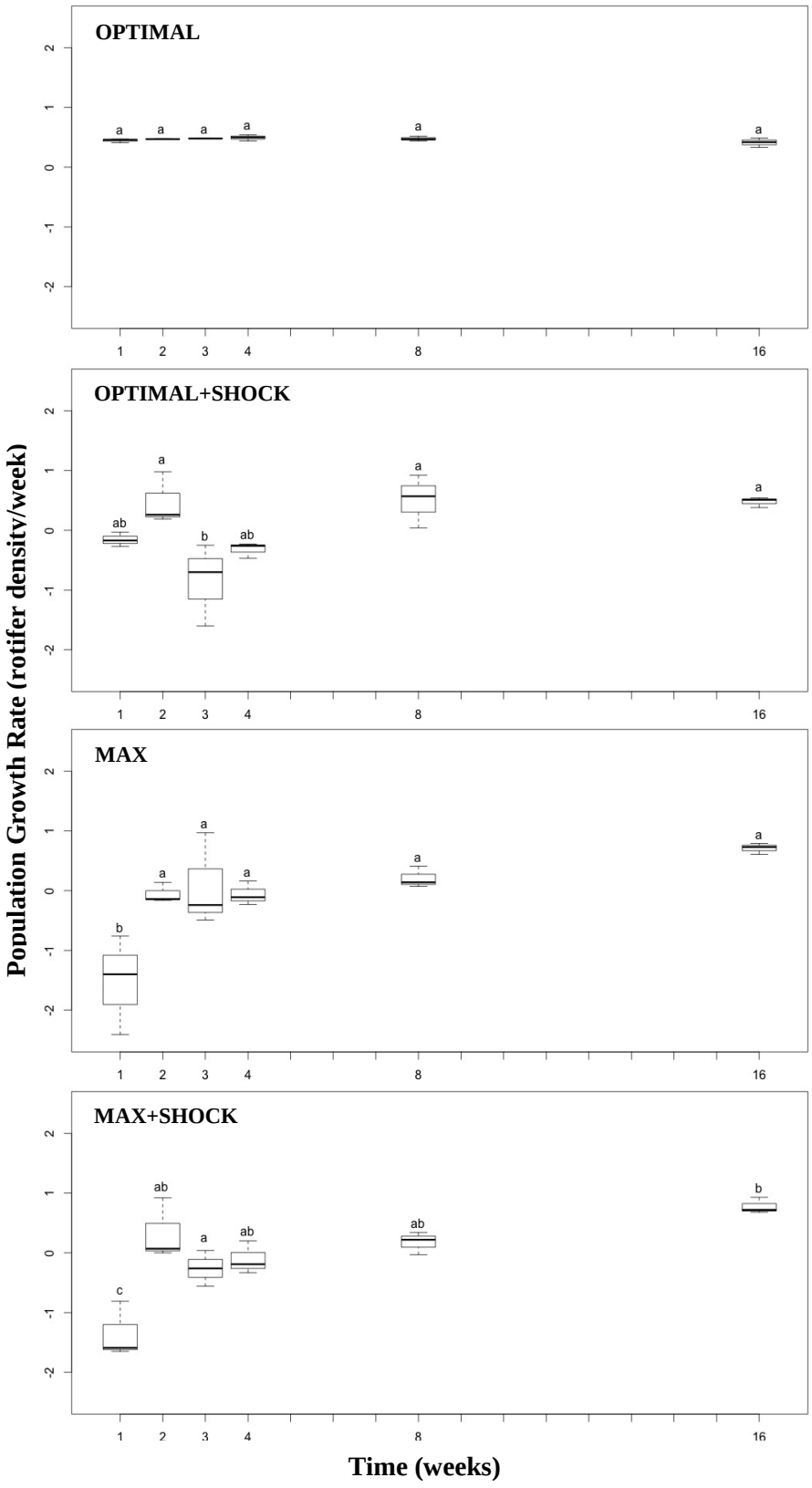

Figure 7: Population growth rates in each treatment over time (weeks).

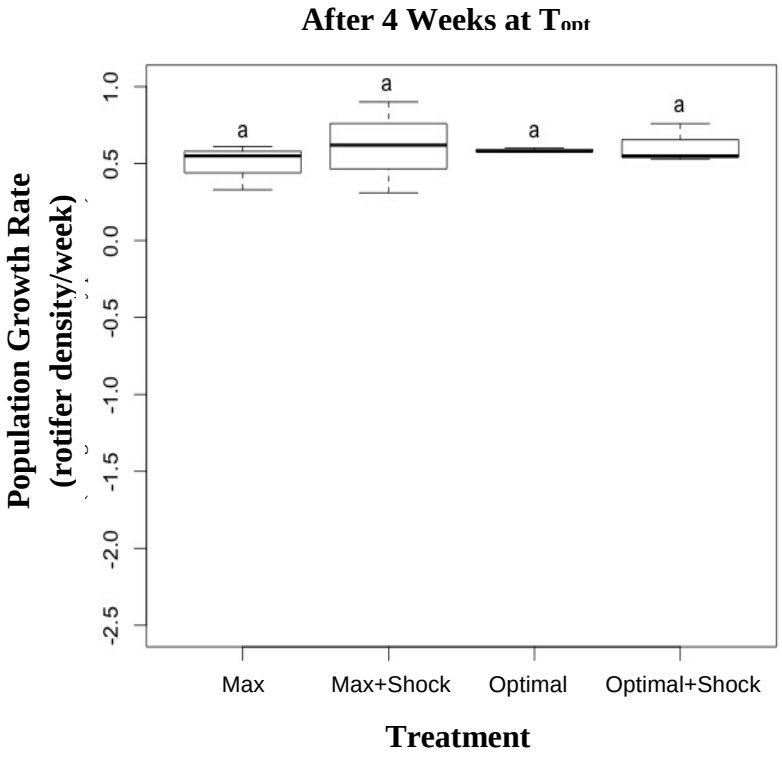

Figure 8: Population growth rate of each treatment after four weeks at T_{opt}.

4.4 *Rotifer Size*

After 16 weeks of treatments, Optimal and Optimal+Shock did not have significantly different sizes of rotifers from each other (Figure 9). Max and Max+Shock did not have significantly difference sizes of rotifers bu had significantly smaller rotifers then Optimal and Optimal+Shock (Figure 9). After four weeks at T_{opt}, the rotifer size in Max and Max+Shock had significantly increased from week 16 ($p=0.002892$ and 0.0001109, respectively) (Figure 9). In fact, Max+Shock had the largest increase in overall rotifer body size for all treatments after four weeks at T_{opt}.

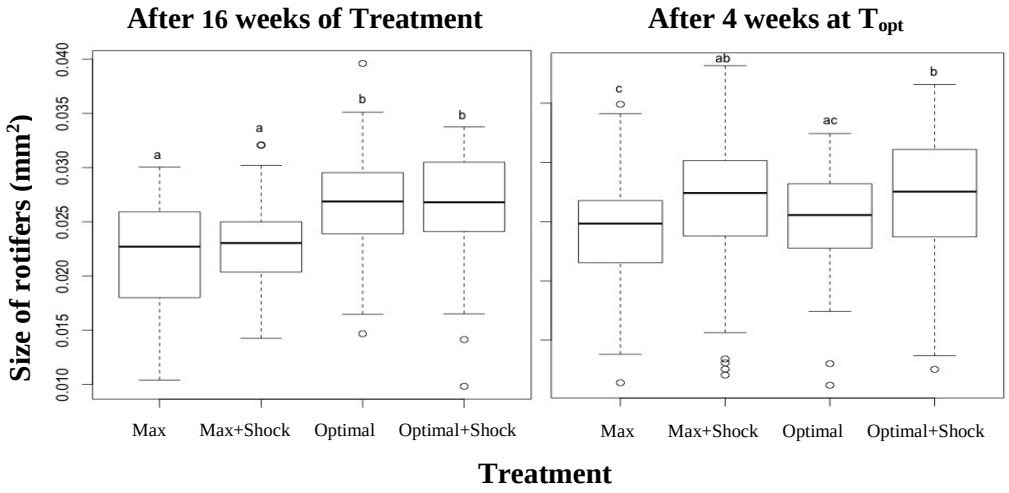

Figure 9: Comparison of rotifer sizes in each treatment. Left: Rotifer size for each treatment after the 16-week experiment. Right: Rotifer size for the same treatments after they were returned back to optimal temperature (25°C)

4.5 *Aerobic Performance*

Respiration rate (inversely related to aerobic performance in that an increase in respiration rate translates to a decrease in aerobic performance, i.e. fitness) was found to be significantly different between weeks, treatments and temperatures (p=0.01858, $3.704x10^{-06}$, $6.887x10^{-10}$, respectively). The respiration rates of the rotifers in the Optimal treatment were not significantly different between weeks (p=0.2351), but the respiration rates were significantly different between temperatures (p=0.0002963) with a gradual increase in respiration rate as the temperatures increased until reaching 31-33°C, where there was a slight decrease before the respiration rates increased up to the highest rate (poorest aerobic performance) at 39°C (Figure 10). The respiration rates of Optimal+Shock were significantly different between weeks ($p=8.329x10^{-08}$) and temperatures (p=0.02406). The respiration rates for Optimal+Shock significantly increased (which translates to a decrease in aerobic performance) from week 1 to week 16 ($p=1.081x10^{-07}$), and then significantly decreased after being kept at T_{opt} for four weeks (week 20) (p=0.01263). The respiration rates of the rotifers in Optimal+Shock between week 1 and the final week of being kept at T_{opt} (week 20) were not significantly

different (p=0.8941), which means the respiration rates of the rotifers in the Optimal+Shock returned back to the same respiration rates present at the beginning of the treatment (week 1) after being reared in optimal temperatures with no sub-lethal shocks. The respiration rates of rotifers in the Max treatment were significantly different between weeks (p=0.001017), and had the highest respiration rate (poorest aerobic performance) in week 1 for all treatments. The respiration rates in week 1 were significantly higher than in week 16 (p=0.0002789) and in the final week of being kept at T_{opt} (week 20) (p = 0.035). The respiration rates of the rotifers in week 16 were significantly lower (higher aerobic performance) than after the four weeks in optimal temperatures (T_{opt}) (p=0.8664). Max+Shock had significantly different respiration rates between weeks (p=0.0115) and temperatures (p=0.002555), with the highest respiration rate (poorest aerobic performance) present in week 16 in all treatments, but by week 20 (after four weeks of T_{opt}), the respiration rate significantly decreased (increased in aerobic performance) (p=0.2759) back to a rate that was not significantly different from week 1 (p=0.69) (Figure 10). When comparing weeks, respiration rates in all treatments were significantly different between week 1 ($p=2.226x10^{-06}$) and week 16 ($p=1.507x10^{-08}$). However, by the end of the week 20 (after four weeks of all populations being reared at T_{opt}), the respiration rates of all treatments were no longer significantly different (p=0.1162) (Figure 10).

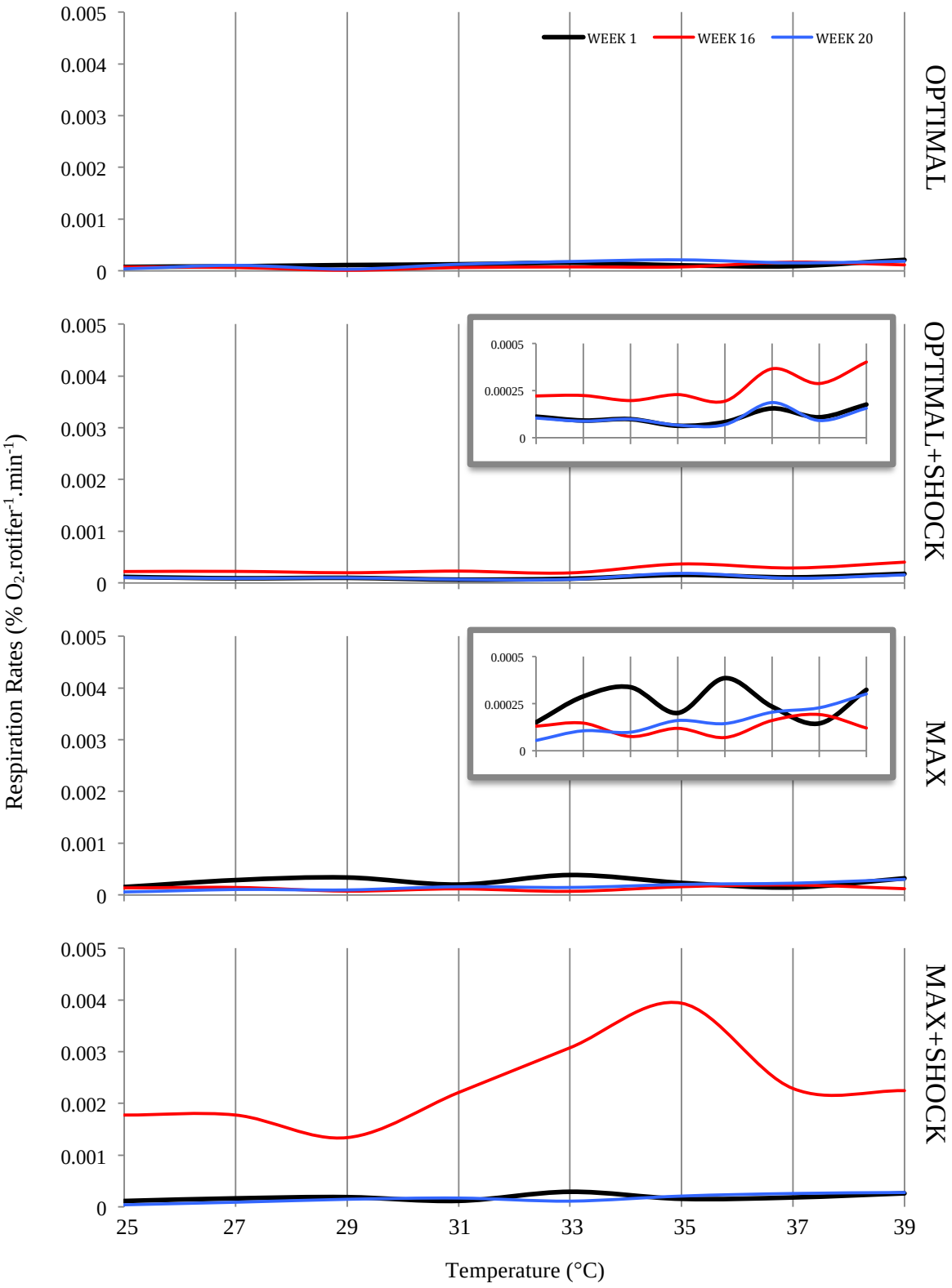

Figure 10: Respiration rates (inversely related to aerobic respiration) for each treatment at each water bath temperature. Week 20 refers to the final week of the all populations being reared at T_{opt}.

5. Discussion

Exposure to higher temperatures within the natural range of the marine rotifer, *Brachionus plicatilis*, was found to facilitate transgenerational acclimation. However, acclimation became hindered when the rotifers were frequently exposed to severe disturbances in temperature, i.e. weekly sub-lethal temperature shocks. There was no indication of adaptation and higher temperatures were found to have an overall negative effect. Rotifers reared in higher temperatures were smaller in size, but once they were returned to optimal conditions after the 16-week experiment, their sizes increased. Exposure to optimal conditions after the 16-week experiment had an overall positive effect in rotifer size, population growth rate and aerobic performance.

Transgenerational acclimation was observed when the population was exposed to a maximum temperature within their natural temperature range. In a matter of two weeks (ca. ~10 generations) there was a significant increase in population growth rates among rotifers were reared in a maximum temperature within their natural range (Max treatment) and among rotifers reared in the same maximum temperature but with weekly sub-lethal shocks (Max+Shock treatment), indicating transgenerational acclimation had occurred. Similarly, in a species of water flea, increasing temperatures were found to cause the water flea to mature at a younger age, leading to earlier reproduction, which ultimately increased overall population growth rates (Heugens at el. 2006). Comparably, it has also been observed that a decrease in temperature has a negative impact on the population growth rate of many species of insects (Frazier et al. 2017). Increased temperatures have been observed to accelerate physiological processes, such as metabolism, which cause population growth rates to accelerate (Cairns et al. 1975, Savage et al. 2004).

Rotifer populations kept at a maximum temperature that was still within their natural range (Max treatment) showed a decrease in respiration rates at all temperatures over the 16 week experiment as a result of transgenerational acclimation. A decrease in respiration rate (inversely related to aerobic performance) translates to an increase in fitness (Ikeda 1985, Ikeda et al. 2001, Lehette et al. 2016). The observed increase in fitness of the rotifers in the Max treatment could be explained by a mechanism known as

gene expression plasticity, as seen in some corals under variable temperature environments (Kenkel and Matz 2016). In other words, as the rotifers were exposed to a higher temperature over time, it was likely that the parents were able to up-regulate specific genes within their genome that better suited their new environmental conditions and pass those genes on to subsequent generations, in turn allowing the population to better survive in higher temperatures. After exposure to 16 weeks of high temperatures in the Max treatment, the population was returned to optimal conditions of 25°C, for four weeks. The rotifers that had become acclimated to the higher temperatures in the Max treatment had better aerobic performance (lower respiration rates) than they did at the start of the 16-week experiment. However, the population of rotifers in the Max treatment had better aerobic performance across all temperatures when they were being kept at their maximum temperature than when they were put back to optimal temperatures (Figure 10). This suggests that transgenerational acclimation occurred, and that the newest generations of rotifers were better able to cope with the higher temperatures than the cooler, optimal temperatures because they had acclimated to their higher temperature environment by up-regulating more heat-resistant genes that were within their genome.

Exposure to frequent temperature shocks did not allow transgenerational acclimation to occur. When the parents were in an environment that was at their thermal maximum and was frequently unstable or fluctuating (such as being exposed to temperature shocks, i.e. Max+Shock treatments), it is likely that the parents were unable to pass the specific genetic information to their offspring that would allow their offspring to cope with the higher temperatures. As a result, the genome expression of the offspring did not reflect the environment to which the parents were exposed, so the population was unable to acclimate to their environmental conditions, even after 16 weeks. This was apparent in the increased respiration rates of rotifer populations in the Max+Shock treatments, translating to a decrease in aerobic performance and fitness among the population over time. Similar results were found in copepod species, in which increased temperatures caused increased respiration rates, suggesting an inability to acclimate to short-term temperature stressors (Isla and Perissinotto 2004, Lehette et al. 2016). Any change in temperature outside of the natural thermal range can lead to an increase in metabolic costs when related to short-term temperature fluctuations (Gaudy et al. 2000).

It is possible that an increase in metabolic costs (caused by the temperature shocks) could have compromised the ability of the population to acclimate to the warmer environment. Therefore, the subsequent generations were unable to cope with their higher temperature environment because the specific heat-tolerant genes were never passed from parent to offspring. When populations from the Max+Shock treatments were returned to optimal conditions, their respiration rates decreased (aerobic performance and fitness increased) back to rates similar to the ones observed at the beginning of the 16-week experiment. No matter what the specific mechanism is that is responsible for the decrease in aerobic respiration at higher temperatures and increase at lower temperatures, the results of this experiment indicate transgenerational acclimation to the warmer temperatures never occurred in the presence of weekly temperature shocks.

Although there was evidence of transgenerational acclimation, adaptation was never observed. Rotifers exposed to their optimal temperature but stressed with fluctuating high temperatures over multiple generations (i.e. Optimal+Shock treatment) did not show any increase in population growth rate, size or aerobic performance. The purpose of the temperature shocks was to eliminate individuals from the population whose genes were less adapted to higher temperatures. In other words, the temperature shocks would reduce the population and the remaining individuals would potentially possess favorable warm-resistant genes and would likely exhibit higher survival, growth and reproduction, creating a population better adapted to higher, fluctuating temperatures **(**Munday et al. 2013). There was no indication adaptation because the populations that were reared in the Optimal+Shock treatment did not increase in aerobic performance or size. There was an apparent negative effect of temperature on individual size of rotifers (Max+Shock treatments). In fact, there was an overall decrease in body size in rotifer populations that were subjected to constant higher temperatures (Max and Max+Shock treatments). These findings are consistent with previous studies showing that an increase in environmental temperature can cause a decrease in growth of individuals (Atkinson 1995, Munday et al. 2008, Motson and Donelson 2017). A decrease in body size from increased temperatures is often explained by a decrease in individual growth (McLaren 1963). Elevated and fluctuating temperatures can create an increase in metabolic costs, resulting in less available energy for things such as growth (Heugens et al. 2006). For

example, zebrafish reared in a thermally variable environment incurred significant energetic costs causing the population to be significantly smaller than zebrafish reared in more thermally stable environments (Schaefer and Ryan 2006). However, due to the rotifer's short generation times and the difficulty in identifying adults versus juveniles in a given population, it cannot be determined what caused the decrease in rotifer sizes. Once the populations were returned to optimal conditions (25°C), the individual body sizes of rotifers in Max and Max+Shock increased to the sizes they were prior to their increased temperature treatments and the aerobic performance of rotifers in the Max+Shock treatment returned to their rates previously observed at the beginning of the 16-week experiment. This reverse of sizes and respiration rates suggests that adaptation did not occur.

Adaptation has often been assumed to be the ultimate factor determining a species ability to outpace a changing environment (Munday et al. 2008a), however, acclimation has been known to induce adaptive changes allowing species to cope with their changing environment (Egginton and Sidell 1989). Plasticity has been known to act as a "buffer" against evolutionary change by producing phenotypes that can lead to persistence in a population (Chevin and Lande 2010, Pavey et al. 2010). Some fish species have been found to acclimate to warming ocean temperatures through phenotypic plasticity (Munday et al. 2008b). Similarly, in this study the rotifer populations kept at their thermal maximum (Max treatments) were given enough time to induce genomic expression (plasticity) of their heat-tolerant genes which were inherited by subsequent generations, allowing for better fitness. As opposed to waiting for a genetic mutation or recombination to promote survival in a changing environment, plasticity can provide the first steps in moving a population closer to optimum phenotypic advantages in the new environment (Ghalambor et al. 2007).

The potential for species to acclimate, or even adapt, depends largely on their generation times and their potential for phenotypic plasticity (Munday et al. 2008a). Species with short generation times have the highest potential for adaptation because genetic selection can occur over a larger number of generations in a relatively short period of time. Genetic adaptation to climate change is already proceeding in a number of animal populations (Bradshaw and Holzapfel 2006, Skelly et al. 2007, Munday et al.

2008). Although adaptation was not evident in this study, it does not mean that it is not possible in this species. Sexual reproduction was never observed among any of the populations during this study, meaning all new generations were the result of asexual reproduction so no new genetic material was introduced in subsequent generations. Had there been sexual reproduction, adaptation through genetic recombination would have been more likely to occur. It is possible this study did not last for enough generations to allow for adaptation through selection to occur. This emphasizes the need to conduct long term (multi-generational) experimental studies to more accurately project species' persistence through climate change.

To increase the reliability of projections of species persistence through climate change, it is important to not only determine the potential for transgenerational acclimation and/or adaptation to projected future temperatures, but to also relate this temperature change to species' natural temperature range. Many species have been found to have different thermal tolerance limits depending on their latitude, with a predicted pattern of increasing impact of climate change as latitudes decrease (Coles et al. 1976, Deutsch et al. 2008). The Mediterranean bivalve, *Mytilus galloprovincialis,* lives close to its thermal acclimation limits and long-term exposure to these limits was found to provoke lethal stress responses (Anestis et al. 2007). Contrastingly, Antarctic copepods exhibited higher tolerances when exposed to elevated temperatures (Lahdes 1995). Species with wider thermal ranges are likely to have different adaptation and/or acclimation rates than species with more narrow thermal ranges. Geographically based environmental temperatures are thought to be very important in determining temperature tolerances in species (Coles et al. 1976). With increasing temperatures, species are predicted to move closer to the poles (Parmesan and Yohe 2003). Two–thirds of the North Sea fish species have shown climate-related shifts in latitude and depth (Perry et al. 2005). Therefore it is important to not only study the potential for acclimation and adaptation across generations, but also across latitudes (Logan et al. 2014). This study used a species that has a relatively large natural thermal range, so increasing the temperature in their environment by 1 or 2°C would have less of an effect that it would on a species with narrow thermal ranges, such as corals in the tropics. This emphasizes

the importance of incorporating other factors into future studies in hopes of accurately studying and predicting species' acclimation and/or adaptation potentials.

This study provided valid evidence that over time, transgenerational acclimation can occur, and also demonstrated that in the presence of frequent high temperature stressors, acclimation can be hindered. It required almost the entirety of the 75 generations before the rotifer populations in Max and Max+Shock were able to surpass the population growth rate of rotifers in optimal temperature treatments. This highlights the importance of incorporating multiple generations into ocean warming studies (Sunday et al. 2014). Additionally, this study suggests that if a population is forced to live in an environment at or near its thermal limits, there is the possibility that the population will survive and eventually become acclimatized to the new environment, if given adequate adjustment time. If sexual reproduction had occurred among the populations of rotifers, there would be a greater potential for adaptation to have occurred within just 75 generations, however, only asexual reproduction was present, therefore there was no mutation or genetic recombination. This study focuses specifically on temperature as the only stressor, and is a very simplified version of challenges in a very diverse and changing climate. For this reason, it is important for future studies to not only implement multiple generational studies, but also to incorporate multiple stressors with the aim of creating a more realistic experiment to represent real-life transgenerational acclimation and adaptation potentials. Data from this study provided valuable insight into mechanisms used by species to cope with climate change, and can be applied to other organisms to produce accurate projections of the potential outcome to increasing ocean temperatures.

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