EFFECTS OF RISING TEMPERATURES ON THE METABOLIC RATE AND GROWTH OF PLANKTIVOROUS FISH SPECIES IN NARRAGANSETT BAY

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Summary

Increasing ocean temperatures have been a problem for marine ecosystems and this issue is expected to get worse throughout the next century, with temperatures projected to increase by 2-3.5°C. Warmer waters can have various negative effects on marine ecosystems, such as loss of breeding areas, loss of biodiversity, or impacts on marine organisms' biological functions (e.g. decreased growth, increased metabolic rates, and altered reproduction). One particular area of concern is how these increasing temperatures can affect fish growth and physiology. Even though past studies have looked at the interaction between metabolic rate and increasing temperatures, there has been little research done on the species commonly found in Narragansett Bay. Scup (*Stenotomus chrysops*) and Atlantic silversides (*Menidia menidia*) are two abundant planktivorous fish species found in the Bay that serve as good model organisms to examine the effects of increasing water temperatures. Growth and condition indices were investigated in the *Menidia menidia* and respirometry experiments were used in both species to determine how these fish respond to increasing water temperatures. The Fulton index of condition for animals shortly after being wild caught was significantly higher than the two treatment groups that mimicked environment and elevated temperature by 2 ºC and there were no statistical differences between RNA:DNA ratios in these three treatments. Basal and maximum metabolic rates of both species increased with increasing testing temperatures, while the aerobic scope was unchanged with different held or testing temperatures. Maximum metabolic rate of *Menidia menidia* was also significantly affected by held temperature. By collecting this information, the impact of rising seawater temperatures in Narragansett Bay can be better assessed and communicated to decision-makers and community partners in an effort to protect these keystone species and maintain biodiversity and ecosystem health.

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Preface

The current Master of Arts thesis is presented in the form of two separate manuscripts that are being prepared for publication. Chapter 1 focuses on growth and condition of the planktivorous fish *Menidia menidia*, the Atlantic silverside as was completed as a collaboration with the Suckling lab at the University of Rhode Island. Chapter 2 focuses on metabolic rate as a proxy for physiological performance in both Atlantic silversides and scup, *Stenotomus chrysops*. These two fishes are keystone species of Narragansett Bay, which have received very little attention in past studies. This thesis also includes a general Introduction unifying the two chapters and a Conclusions section with future directions and management recommendations.

Introduction

The effects of climate change can be detrimental to ecosystems and the organisms found within them. Impacts of global warming include, but are not limited to, increased frequency and strength of storms, increases in air and water temperatures, rising sea levels, and increased droughts (Venkataramanan & Smitha, 2011). In recent years, increasing oceanic water temperatures have been a cause for concern among biologists, as these can have widespread effects on marine organisms and on their ecosystems. For instance, it can catalyze coral bleaching (Abdo *et al.*, 2012), increase the energetic costs needed for migration and spawning (Plumb, 2018), cause hypoxia (Roman *et al.* 2019), decrease growth rates (Collier & Waycott, 2014; Sun & Chen, 2014), increase various aquatic diseases such as *Flavobacterium columnare* (Karvonen *et al.*, 2010), and increase harmful algae blooms (Trombetta *et al.*, 2019).

Planktivorous fish are important parts of marine ecosystems and are being affected by global warming (Bernes *et al*., 2015). These species of fish feed on plankton, which are organisms found in the water column that move passively with the currents. The plankton helps maintain the nutrient-rich composition of their environment. Planktivorous fish species include, but are not limited to, scup, herrings, menhaden, and silversides. Moreover, some of the species found in Narragansett Bay may be considered keystone species. Keystone species are organisms that an ecosystem heavily depends on, and if removed from their native range, would cause extreme perturbations (Valls *et al.*, 2015). Species such as silversides feed heavily on the plankton found in the water column. If these fish were removed from the ecosystem, there may be shifts in the abundance of plankton found in Narragansett Bay. However, these fish are being disproportionally affected by global warming, such as temperature changes due to smaller home ranges compared to larger fish at higher trophic levels. While individual stressors, such as increased temperatures or decreased food sources, can affect the survival and well-being of fish, interactions between numerous stressors can also result in different impacts that would not have otherwise been seen. A study conducted by Gobler and colleagues (2018) using *Cyprinodon variegatus* and *Menidia beryllina* found that interactions between temperature, acidification, and food supply all had different outcomes than when each stressor was looked at individually (Gobler *et al.*, 2018).

Seawater temperature is incredibly important to maintaining various biological functions with increased water temperature harming many marine organisms. Studies found that temperature is a main predictor of metabolism in *Menidia menidia* embryos and larvae and that temperature plays a more important role than CO_2 concentrations (Schwemmer *et al.*, 2020; Murray & Baumann, 2018). A study conducted in Narragansett Bay found that a 1 °C increase in water temperature caused a reduction in phytoplankton bloom throughout the winter and spring months (Smith *et al.*, 2010). Phytoplankton is a major food source for many species of fish and is also large contributors to chlorophyll-a production. In addition, a temperature increase of 1 °C can cause the release of zooxanthellae from corals leading to bleaching (Brierley & Kingsford, 2009) as well as an increase in harmful algal blooms (HABs) (Wells *et al*., 2015). Temperature also influences dissolved oxygen (DO) solubility (Roman *et al*., 2019). As temperatures increase, so does hypoxia, which increases stress on the fish (Roman *et al.*, 2019) and other organisms.

It is important to understand how increasing water temperatures affect marine organisms as oceans have continued to warm over the past several decades. The National Oceanic and Atmospheric Administration (NOAA) reports that sea surface temperatures are rising approximately 0.13 °C each decade or approximately 1.3 °C since 1979 (Brierley & Kingsford, 2009; United States Environmental Protection Agency, 2023). In particular, the average temperature of Narragansett Bay has risen by 1.2 °C since 1950 (Smith *et al.*, 2010). It is also projected that oceanic water temperatures will increase by 2-3.5°C, while waters near the Arctic may increase by 8 °C by 2100 (Richardson, 2008).

One particular biological function that can be impacted by global warming is metabolic rate, which, in fish, indicates how much oxygen the fish is consuming in order to maintain bodily functions (Chabot, McKenzie, *et al*., 2016). These functions include maintaining homeostasis, growth, digestion, and reproduction (Metcalfe *et al.*, 2016; Farrell, 2016). Metabolic processes can increase by 2- or 3-fold when there is a 10 °C increase in temperature (Volkoff & Rønnestad, 2020). Moreover, Roman and colleagues (2019) reported that global warming can increase coastal hypoxia. While hypoxia can have slightly different definitions depending on the source, they define it as water with dissolved oxygen concentrations less than 2 mg/L. As water temperatures increase, oxygen solubility decreases, which means that there is less dissolved oxygen in the water at higher temperatures (Roman *et al*., 2019).

Fish have a particular respiration rate that they need to maintain in order to survive (Rubalcaba *et al.*, 2020). Basal metabolic rate (BMR) is the amount of oxygen an animal can consume after a period of rest. In addition to basal metabolic rate is standard metabolic rate (SMR), which accounts for minimal activity of the animals (Chabot, Steffensen, *et al.*, 2016). When the oxygen supply decreases below the basal (standard) rate, the fish would have a more difficult time extracting oxygen and the fish is unlikely to be able to survive (Mitra *et al*., 2014). Areas of low oxygen – hypoxic areas – can pose a challenge for fish. Planktivorous fish must either remain in the hypoxic water, which significantly increases their stress, or they must use behavioral strategies, such as avoidance, (Roman *et al*., 2019) As salt concentrations increase, there is a decrease in oxygen solubility (Debelius *et al*., 2009). This is even more important in an estuary like Narragansett Bay, where salinity varies widely with the ebb and flow of the tides. In addition, hypoxia is also seasonal and dependent on pollutants, biodiversity and water temperature (Roman *et al*., 2019). Phytoplankton, zooplankton and microbiota all impact the oxygen levels (concentration) in the water (Robinson, 2019). Increased primary productivity by itself can increase oxygen concentration, while zooplankton density decreases oxygen. When algae blooms sink or are grazed by zooplankton, microbes break down this material and consume much of the dissolved oxygen (DO) that is available (Roman *et al.*, 2019). In addition, during the nights when sunlight is not present, the algae can consume oxygen, further decreasing the amount of dissolved oxygen present (Wallace & Gobler, 2021). However, these algae blooms are also seasonal, and as a result, hypoxia is also seasonal.

In addition to the basal metabolic rate (BMR), there is also a maximum metabolic rate (MMR) that fish can attain (Norin & Clark, 2016). MMR is typically measured after extensive exercise at points of exhaustion for the animal (Rosewarne *et al*., 2016). Because increasing water temperatures also can an increase in metabolic rates, it is likely that fish will reach their maximum metabolic rates faster resulting in higher physiological demand for oxygen (Rubalcaba *et al*., 2020).

The difference between basal metabolic rate (BMR) and maximum metabolic rate (MMR) is known as aerobic scope (AS). Aerobic scope indicates how much oxygen is available for additional physiological activities, such as predator avoidance (Norin & Clark, 2017). As water temperatures begin to increase, so does basal metabolic rate, potentially reducing aerobic scope and energy available for routine behaviors (Holt & Jørgensen, 2015). However, past optimal growth rates, the maximum metabolic rate will no longer increase, but the basal metabolic rate will continue to increase, which results in a reduced aerobic scope, thus impacting fitness and survival.

Global warming is also documented as a major influencer to the species composition of a particular ecosystem. As temperatures change, there is a shift in the species inhabiting a particular ecosystem. For instance, a study in Narragansett Bay demonstrated a shift from more vertebrate to invertebrate species and a shift from benthic to pelagic species (Collie *et al*., 2008). For instance, populations of species such as *Pseudopleuronectes americanus* (winter flounder) and *Urophycis chuss* (red hake) decreased, while populations of species such as *Stenotomus chrysops* (scup) and invertebrates, such as *Cancer irroratus* (Atlantic rock crab) and *Cancer borealis* (Jonah crab), increased (Collie *et al*., 2008). However, it should be noted that overfishing or other human activities could also play a role in this change in population distribution. This study looked at data that had been collected over a 47-year period and found that there was a shift towards species that had a temperature preference that was 2 °C higher over the course of the study (Collie *et al*., 2008). Further, this also indicates that many of the species that have a preference towards cooler water temperatures and are currently found in the Bay will be forced to relocate and find habitats that are better suited to their temperature preferences. For instance, cold-water fish such as winter flounder cannot reproduce in warmer waters, and, due to the temperature increases seen in the Narragansett Bay, there has been a sharp decline in such populations (Smith *et al*., 2010). Moreover, North Sea cod have shifted their distribution north and towards deeper cooler waters (Poloczanska *et al*., 2016).

Temperature can also impact the growth of fish. First, one particular study found that there were reported smaller optimal body sizes in herrings as temperatures increased (Ljungström *et al*., 2020). However, it is also important to note that other factors also influenced the growth of these fish, such as how much light they were receiving, where more light was found to help increase growth, and available food sources. Furthermore, Arnott and colleagues

(2006) found that there is a tradeoff between energy demands for growth and for other biological demands in the silversides, *Menidia menidia* (Arnott *et al.*, 2006). A previous study conducted on *Scophthalmus maximus* found that increasing water temperatures resulted in increased growth, but that there was a plateau after a certain temperature (Burel *et al.*, 1996). In addition, a study conducted on *Acanthochromis polyacanthus* found that growth capacity is limited at higher sea temperatures (Munday *et al.*, 2008). Temperature can also affect the performance of fish.

Aspects of performance include sustained swimming and critical swimming speed, which are. It should also be noted that these aspects of performance are species specific. In a study using *Scomber japonicus* (chub mackerel), fish demonstrated recruitment of slow, oxidative muscle fibers at lower temperatures and maintained swimming speeds as temperatures increased (Dickson *et al.*, 2002). Moreover, a study looking at species within a family, Pomacentridae, *Dascyllus, Neopomacentrus,* and *Pomacentrus,* demonstrated severe decrease in critical swimming speed with warmer water temperature (Johansen & Jones, 2011). As a result, fish that do not have a strong swimming ability will be less likely to survive in habitats with higher temperatures and if not able to change their distribution might become locally extinct.

Understanding how planktivorous fish in Narragansett Bay respond to climate change is important to help maintain the biodiversity of the Bay and to protect these animals from becoming endangered. The goal of this research project is to determine how increasing water temperatures affect the metabolic rate and growth of the planktivorous fish *Stenotomus chrysops* (scup) and *Menidia menidia* (Atlantic silversides), commonly occurring in Narragansett Bay.

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Chapter 1

Growth and condition of *Menidia menidia* at different temperatures

Abstract

Rising seawater temperatures can have detrimental effects on marine ecosystems. Narragansett Bay in Rhode Island is an iconic estuary of New England where time series detailing the negative effects of global warming are available. The effects of global warming on intermediate trophic levels including planktivorous species are not completely understood. *Menidia menidia*, a species of planktivorous species of fish commonly found in Narragansett Bay, has received very little attention since it is not a commercially important species. To investigate the effects of temperature on growth and condition, RNA:DNA, Fulton’s index, and otolith radii were analyzed in baseline fish and fish held at ambient temperatures and fish held at 2 °C higher than ambient temperatures. It was expected that *M. menidia* held at lower temperatures have better condition, evidenced by a higher Fulton index, and that fish raised under laboratory conditions have higher growth rates than wild-caught fish. These hypotheses were not supported by the findings. The Fulton index for baseline animals was significantly higher than the other two treatment groups, though there were no statistical differences between RNA:DNA ratios were observed in the three treatments. As the water temperature of Narragansett Bay continues to increase, more studies can investigate how fish compensate for these changes (or respond to temperature stressors). These patterns may be prominent during the summer months.

Introduction

Climate change, which includes global warming, is a threat to many aquatic, both freshwater and marine, ecosystems. Some of the observed effects on ecosystems include shifts in fish species composition (and biodiversity) (Oviatt, 2003), changes in plankton composition and distribution (Benedetti *et al.*, 2021; Volsett *et al.,* 2022), increased harmful algal blooms, or HABs (Trombetta *et al.*, 2019), smaller body sizes at the time of maturation (Niu *et al.,* 2023), increased spinal abnormalities (Han *et al.*, 2020), and decreased growth (Vollset *et al.* 2022). It is abundantly clear that warming of aquatic environments has detrimental effects and is an area of concern for aquatic conservation.

Narragansett Bay, Rhode Island, the largest estuary in New England, is being impacted by both global climate change and human activity (Humphries *et al.*, 2022). Narragansett Bay experiences all four seasons, and, as a result, fluctuations in water temperatures throughout the year with August having the warmest average water temperatures (20-24°C) and February having the coldest average water temperatures (3-6°C). One study by Smith and colleagues (2010) reports that the average temperature of Narragansett Bay has risen by 1.2 °C between the years 1950 and 2010. While projections show that, by 2100, ocean temperatures could increase by 2-3.5°C, it is also projected that water temperature of the northwestern Atlantic Ocean will increase more drastically than the average (Richardson, 2008; Saba *et al*., 2016).

Warming water temperatures play a role in the growth and size of fish. Ljungström and colleagues (2020) found that, at lower temperatures, fish were better able to reach their optimal size, which the authors define as the size at which the animal has the best ability to convert energy for fitness tasks (Ljunström *et al.,* 2020); however, in this study, light and available food also influenced the growth of these fish. One hypothesis that is often used to explain the role of increasing temperature on body size in ectotherms is the temperature-size rule (TSR) (Angilletta & Dunham, 2003; Wootton *et al.*, 2022; Verberk *et al.*, 2021). The TSR states that organisms that are at lower temperatures will reach a larger body size at maturity but will grow at slower rates as juveniles when compared to fish from warmer environments (Wootton *et al.*, 2022). The temperature-size rule postulates that increasing water temperatures also increases the metabolic rates of fishes, which contributes to less allocation of resources to growth (Wootton *et al.*, 2022; Hoefnagel & Verberk, 2015). The study by Hoefnagel and Verberk (2015) found that higher temperatures increased the growth rate and final body size, most notably under hypoxia, but could also be seen under normoxia. However, a study conducted by van Rijn and colleagues using 74 species of fish found that, as temperatures increase under normoxia, body size decreases (van Rijn *et al.*, 2017). This means that these animals are growing at quicker rates in warmer waters, but reaching smaller final body sizes. As a result of global warming, there is an increase in hypoxia (Roman *et al.*, 2019), so it is expected that growth patterns would follow the temperature-size rule and animals at higher temperatures will have faster growth rates but reach a smaller final body size. Arnott and colleagues (2006) found that there was an energy tradeoff between metabolic demands for growth and for other biological functions, such as swimming, in *Menidia menidia*. This same study, which used animals from Nova Scotia as the high-latitude population and animals from South Carolina as the low-latitude population, also found that *M. menidia* at higher latitudes had higher basal metabolic rates and lower aerobic scopes when compared to *M. menidia* found at lower latitudes (Arnott *et al.*, 2006). As such, it would be expected that animals from different populations would have varying metabolic rates.

M. menidia, the Atlantic silverside, are planktivorous fish commonly found in estuaries that are being affected by global warming (Schultz *et al.*, 1998; Bernes *et al.*, 2015). They are most often found in brackish water and can be found in Atlantic waters along the entirety of the U.S. east coast. These animals are commonly found in Narragansett Bay and can potentially serve as keystone species. *M. menidia* at higher latitudes have also been found to have increased growth rates (Present & Conover, 1992), so it can be inferred that different populations may be affected differently. The same study by Present and Conover also found that *M. menidia* have a lower growth capacity at lower latitudes at both high and low temperatures (Present & Conover, 1992). Further, a study done by Yamahira and Conover (2002) found that increased temperatures result in increased growth rates in *M. menidia.* This means that animals of the same species at higher latitudes will commonly have higher growth rates when compared to populations at lower latitudes, and that the higher the temperature in their environment, the more the growth rates will be increased.

According to Bergmann's rule, the body size of organisms can vary with different longitudes and latitudes (Fisher *et al.,* 2010). Those animals at higher latitudes would be larger than those found near the equator to conserve heat (Torres-Romero *et al.,* 2016). It should also be noted that Bergmann's rule is typically applied to the body size of endotherms, not poikilotherms. However, a study by Zamora-Camacho and colleagues (2014) focused on changing body sizes in an ectotherm and found that their body size increased as the elevation also increased. In a study conducted on mesopelagic fish in the Southern Ocean (Antarctic Ocean), it was found that fish will follow Bergmann's rule (Saunders and Tarling, 2018). As a result of these findings, it would be expected that the further north one goes, the larger the animals are and that animals of different populations would have varying body sizes. One simple way to determine if temperature affects growth is to compare weight and length using Fulton

Index under different environmental variables, as fish with access to more food tend to be able to store more fat (Martin *et al.,* 2017; Salmeron, 2018).

Otoliths, the inner ear bones or stones of the vestibular system in bony fish, can be validated for age and growth in many species. When otoliths grow, opaque and translucent zones are formed (Martino *et al.*, 2019; Xieu *et al.*, 2021). In temperate climates, these two zones are paired and each represents one calendar year that the particular animal has lived. The opaque zone is formed throughout the summer months, while the translucent zone is formed in the winter (Heimbrand *et al.*, 2020; Fablet *et al.*, 2011). Poor feeding conditions and less nutrients throughout the winter months can cause slower growth, resulting in narrower bands seen on the otoliths (Geffen, 1982). A study by Otterlei and colleagues (2002) using *Gadus morhua* found that the faster growing animals would have proportionally smaller whole otoliths. Fish are also able to add new material to their otoliths each day, adding daily increments to these structures (Schismenous *et al.*, 2016; Morales-Nin, 2000). The same study by Otterlei and colleagues reported that daily growth of the otoliths increased with increasing temperatures (Otterlei *et al.*, 2002).

Physiological demands of temperature are studied with RNA:DNA ratios, a measure of nutritional and physiological conditions of fish during the development of new protein tissue (Hussna *et al.*, 2020; Chicharo & Chicharo, 2008; Gwak *et al.*, 2003; Vinagre *et al.*, 2008). RNA:DNA ratios rely on the assumption that the amount of DNA present is stable, while the amount of RNA is expected to change with varying conditions (Chicharo & Chicharo 2008; Gwak *et al.*, 2003). Typically, well-fed fish would have a higher RNA:DNA ratio when compared to starving, less active fish (Chicharo & Chicharo 2008; Hussna *et al.*, 2020). RNA:DNA ratios can provide consistent information about an organism's growth rates over a brief period of time and allows researchers to make connections to environmental conditions (Hovenkamp, 1990; Chicharo & Chicharo 2008). Time of collection can also influence RNA:DNA ratios, making it important to take into account (Chicharo & Chicharo 2008).

The goal of this study was to determine how increasing water temperatures affected growth and condition in *M. menidia*. To do this, Fulton Index based on length and weight, RNA:DNA ratios and otolith growth in fish at different temperatures were analyzed and compared. It was hypothesized that lower temperatures would result in higher conditions, meaning an increase in Fulton Index, increased otolith incremental growth, and increased RNA:DNA ratios. It was predicted that the Fulton Index, RNA:DNA ratio, and otoltih radii at the ambient treatment temperature would be better than those found in the IPCC+2 °C treatment. Further, the second hypothesis was that captive fish raised under laboratory conditions would have higher growth rates than wild-caught fish due to less activity and higher food availability. Within laboratory conditions, it was expected that fish reared at higher temperatures would have lower overall growth when compared to those at the lower temperature. It was predicted that the Fulton Index, RNA:DNA ratio, and otolith radii for those in the baseline treatment would be worse than those in the ambient and IPCC+2 °C treatments.

Methods

Fish collection and husbandry

Fifty-one *Menidia menidia* were collected using a seine net from Ninigret State Park Salt Ponds, Charlestown, RI, USA (41.360778°N, 71.656500°W and 41.360111°N, 71.654389°W) on June 19, 2020 when the Bay temperature was 18°C. Temperature was recorded at the time of capture. Fish were placed in Igloo coolers containing salt water from the salt ponds and aerators (0.05 cubic feet per minute of air flow; Marine Metal Bubble Box Portable Air Pump). Fish were transported back to the University of Rhode Island Bay campus, to the Suckling lab and once in the lab, the water in the coolers was gradually replaced with filtered and treated aquaria water. A subset of fish were euthanized immediately after collection and represented the condition of fish at baseline temperatures. Fish were treated with a lethal dose of Syncaine (MS-222) (Syndel, Canada) until mortality was evident. Each *M. menidia* was photographed against a ruler and weighed. The head was severed, and the bodies were frozen at -20°C. RNA:DNA was also measured and quantified. A sub-set of 5-9 fish were used per treatment, euthanized, flash frozen using liquid nitrogen, and stored in a -80 °C freezer until it was time for dissection. For the ambient and IPCC+2 treatments, *M. menidia* were then moved from the cooler to an aerated holding tank using a hand net. Experiments began October 2020 when the Narragansett Bay temperatures were 18.8 °C. The fish were kept in flow-through tanks with either ambient filtered seawater or an elevated temperature by 2 °C as expected by the IPCC scenario (IPCC WGI, 2021, Figure 1). The ambient temperature treatment mimicked the temperature of the Bay at that time, while the IPCC+2 °C treatment was always 2 °C higher than the ambient treatment. For the IPCC+2°C, the temperature was gradually increased to 2 °C above the ambient temperature over the course of several hours. Fifteen fish were used for the baseline treatment, eighteen for the ambient treatment, and eighteen for the IPCC+2 °C treatment. The ambient treatment is the current water temperature of the Bay, while the IPCC+2 °C treatment is the expected water temperature for the year 2100. Ambient and heated seawater was supplied from the University of Rhode Island Marine Science Research Facility using the coastal pumphouse at URI. All seawater was filtered before flowing into tanks. For the IPCC+2 °C treatment, water was mixed with a powerhead pump and heated using submersible heaters and a Profilux 4 computerized controller system (GHL Aquarium Technology, Rheinland-Pfalz, Germany). Ambient water temperature treatment did not contain a submersible heater. Fish were kept under these conditions for 49 days from October through December. Fish were fed Reed mariculture C1 Otohime fish diet *ad libitum.* The starting temperature for the ambient group was 18.8 °C and the starting temperature for the IPCC+2 °C was 20.8 °C. The end temperature for the ambient group was 12.6 °C and the end temperature for the IPCC+2 °C group was 14.6°C. The end temperature was lower than the temperature at which the fish were originally caught because the pumphouse drew the water directly from the Bay, which became cooler during the fall months. The ambient treatment was the current temperature of the Bay, while the IPCC+2 °C was 2 °C higher than the ambient temperature.

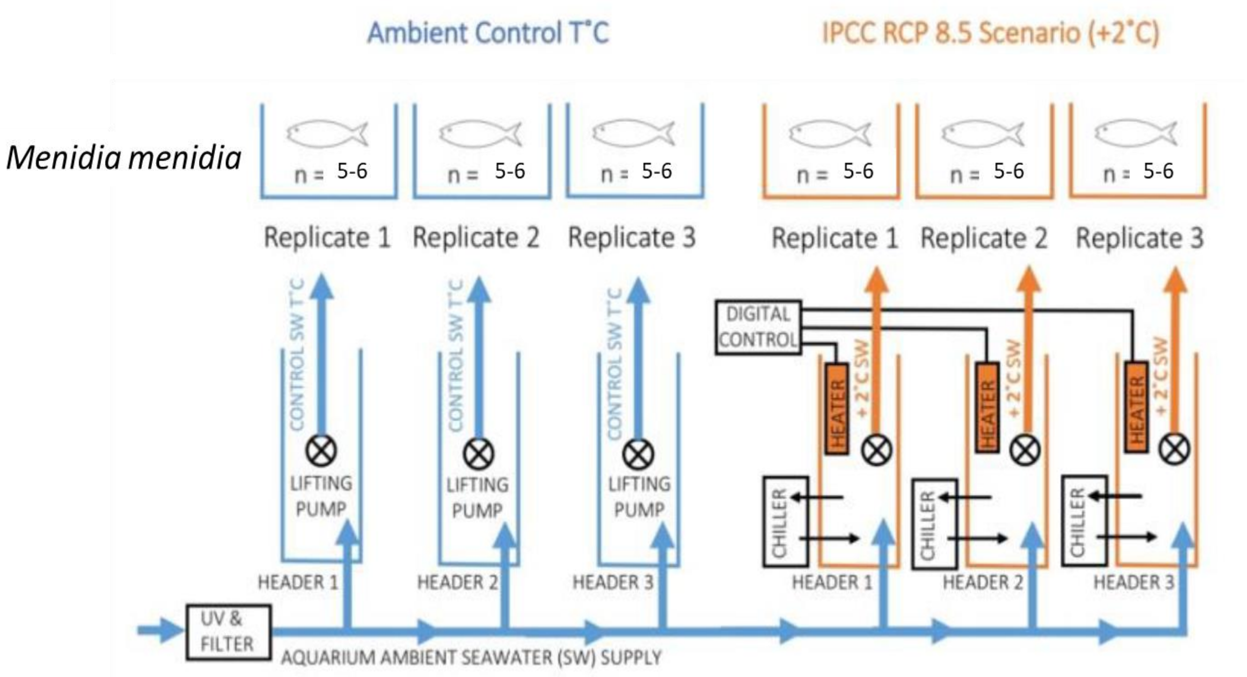

Figure 1: Experimental setup used to house *M. menidia* at two different experimental temperatures. 3 replicates of 5-6 fish were separated for both the ambient and IPPC+2 treatments. All water was filtered and UV treated. For the ambient treatment, a submersible lifting pump was used to mix the water in the tank. The tanks used for the IPCC+2 treatment contained submersible heaters and a Profilux 4 computerized controller system to regulate the temperature of the water.

Relative Condition Factor - Fulton's index

To determine growth and condition, Fulton's index (K) was calculated. Fulton index uses the weight and length of an animal to determine growth and relies on the assumption that growth is isometric (Ragheb, 2023). Fifty-one *M. menidia* were either sedated or euthanized by immersion in a solution of Syncaine, also known as MS-222 (Syndel, Canada). Fifteen fish were from the baseline treatment, eighteen from the ambient treatment, and eighteen from the IPCC+2°C treatment. Each individual fish was weighed and then photographed against a standardized ruler to obtain whole body mass in grams and total length in centimeters. All measurements were taken after sedation or euthanization. Using these values, Fulton's index was calculated using the formula $K = 100*W/L^3$. Fulton's index for each of the three treatments were compared using ANOVA.

RNA: DNA

To determine the nutritional and physiological condition of fish through the development of new protein tissue, RNA:DNA was quantified (Izzo *et al.*, 2015). Five baseline fish, nine ambient, and nine IPCC+2 °C fish were euthanized by immersion in a solution of a lethal dose of MS-222 and flash frozen in liquid nitrogen and stored in a -80˚C freezer until dissection (Vinagre *et al.*, 2008).The baseline group were euthanized immediately after capture in June, while the ambient and IPCC+2 °C groups were euthanized in December. Up to 20 grams of muscle tissue was sampled and then analyzed for RNA:DNA following the methods described by Izzo *et al.* (2015). All fish muscle that was used was dependent on the size of the individual fish. The tissues were ground or pulverized (cells were lysed) using a homogenizer (Pro Scientific Bio-Gen PRO200 and 5 mm diameter probe) in ice-cold tris-EDTA buffered solution containing 1% N-lauroylsarcosine and the supernatant collected for genomic quantification. Homogenization lasted for approximately five minutes. Nucleic acids were quantified using a Qubit 4 fluorometer (ThermoFisher Scientific) using DNA (double-stranded DNA broad range, Q32850 Invitrogen) and RNA (high sensitivity, Q32852 Invitrogen) specific reagents. Standard curves with reference standards for each kit and calculated using the Qubit fluorometer software.

Otolith radii

Sagitta, asterisci, and lapilli otoliths were extracted from sixteen individuals. Five were from the baseline treatment, five were from the ambient treatment, and six were from the IPCC+2 °C treatment. These otoliths were mounted on microscope slides with superglue and polished using sandpaper until the annuli were exposed. The otoliths were then photographed under the microscope for radii analysis. Otolith measurements were made on sagitta otoliths from each treatment group prior to sanding under the same magnification (10x). The length from the center of the otolith (annulus) to the edge of the otolith was found using ImageJ after calibration with a stage micrometer. Each distance was taken using the long edge of the otolith. ImageJ was used to measure radii of otoliths in different treatments. In total, seventeen otoliths from the ambient treatment were used, thirteen otoliths from the baseline treatment, and thirteen otoliths from the IPCC+2 °C treatment were used.

Statistical analysis

The effects of species and temperature was analyzed with linear regression and the following variables: condition index based on length and weight relationships, otolith growth, and RNA:DNA. One-way ANOVAs were run to determine any statistical effect of species and temperature on the aforementioned groups. Tukey’s post-hoc tests were also completed to determine if there was a difference between the groups. All statistical analyses were performed in JASP (Version 0.16.4). When the assumptions of normality and homoscedasticity were rejected, non-parametric tests were used (Zar, 2010).

Results

Relative Condition Factor - Fulton’s index

To determine growth and condition, Fulton’s index was calculated for *M. menidia* at the three different treatments. In a one-way ANOVA, a statistical difference (ANOVA, F(2, 48) = 15.403, p<0.001) in condition when comparing Fulton’s index of all three groups (Figure 2). The baseline treatment (18°C) had the highest Fulton’s index, with both the ambient (12°C), and IPCC+2 °C (14°C) treatments lower indexes. The baseline treatment was significantly higher when compared to the other two treatments. For individual fish, the lowest reported condition factor was 0.347 for the IPCC+2 °C treatment. The highest, within the baseline treatment, was 0.659.

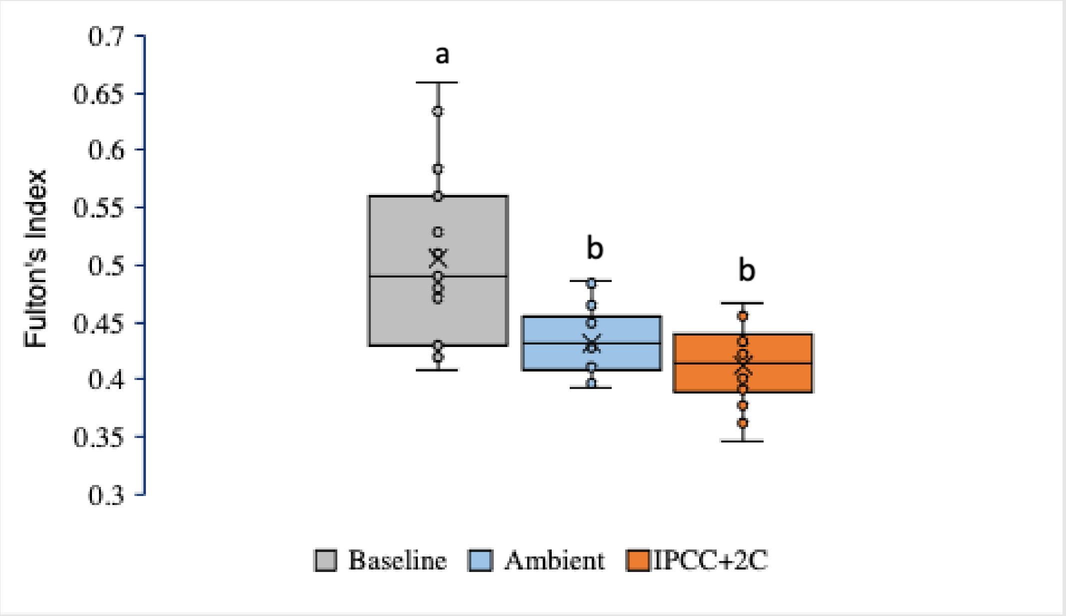

Figure 2: Fulton condition index for *M. menidia* was significantly higher in baseline fish when compared to both ambient and temperature elevated by 2 °C. Fifteen fish were used for the baseline treatment, eighteen for the ambient, and 18 for the IPCC+2.

RNA:DNA

The RNA:DNA ratio of *M. menidia* at each treatment group - ambient (12 °C; n=9), baseline (18 °C; n=5), ambient (12°C), IPCC+2 °C (14 °C; n=9), were not different (Figure 3). A posterior power analysis was done for the sample size of RNA:DNA. Ambient and IPCC+2 sample sizes were found sufficient to yield an asymptotic curve. However, the baseline RNA:DNA sample size was deemed too small. Fish used in this experiment were caught within the baseline temperature, meaning they were caught when the temperature of the Bay was 18°C. The RNA:DNA ratio for the baseline treatment had the greatest variation, though no statistical difference was observed among the groups (one-way ANOVA, F(2, 20) = 1.395, p>0.05). There was a decreasing trend (not statistically significant) between the two treatment groups with the IPCC+2 °C group having a lower condition index.

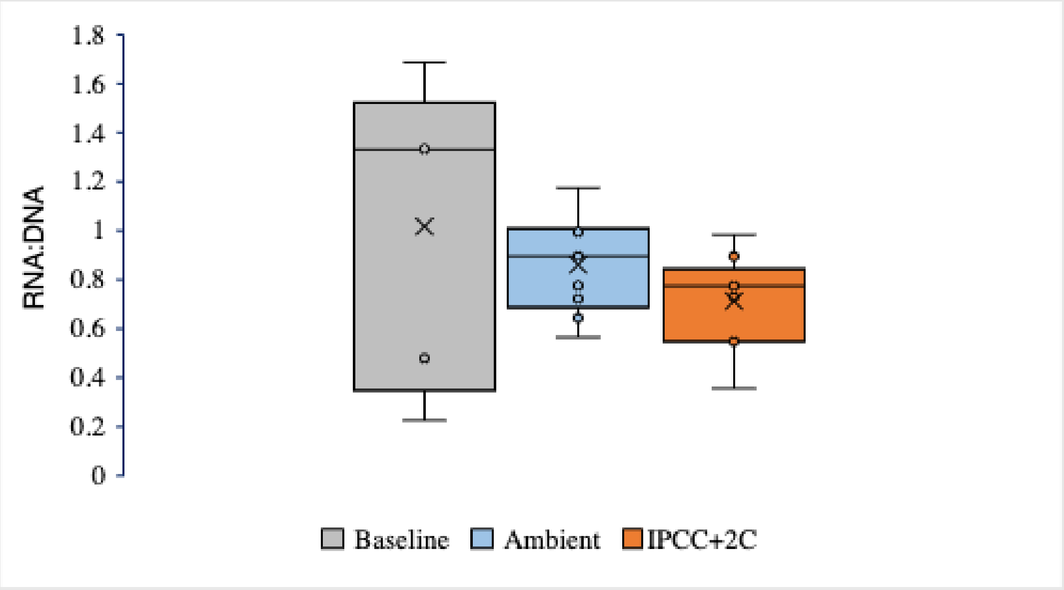

Figure 3: RNA/DNA ratios of *M. menidia* do not differ significantly between treatment groups. Five fish were used for the baseline treatment, nine for the ambient, and nine for the IPCC+2.

<u>Otolith radii</u>

The distance from the center to the edge of the otolith from a representative fish at the baseline treatment was 0.410mm (Table 1, Figure 4). The distance from the center to the edge of the otolith from a representative fish at the ambient treatment was 0.373mm (Figure 5). The distance from the center to the edge of the otolith from a representative fish at the IPCC+2°C treatment was 0.311mm (Figure 6). The average radii of sagitta otoliths were computed after analyzing the measurements for each treatment group (Fig. 7). The average otolith radii for the baseline, ambient, and IPCC+ 2 °C treatments were 0.301 mm, 0.258 mm, and 0.291 mm, respectively (ANOVA, F(2, 40) = 1.101, p=0.342).

Table 1. Distance from center to edge of each otolith (mm) for all three treatment groups.

Ambient	Baseline	IPCC+2°C
0.373	0.203	0.344
0.226	0.348	0.279
0.167	0.229	0.401
0.249	0.236	0.311
0.156	0.334	0.204
0.433	0.410	0.191
0.136	0.324	0.350
0.312	0.185	0.257
0.298	0.180	0.304
0.144	0.252	0.343
0.183	0.381	0.184
0.337	0.376	0.340
0.186	0.460	0.278
0.213		
0.363		
0.318		
0.290		

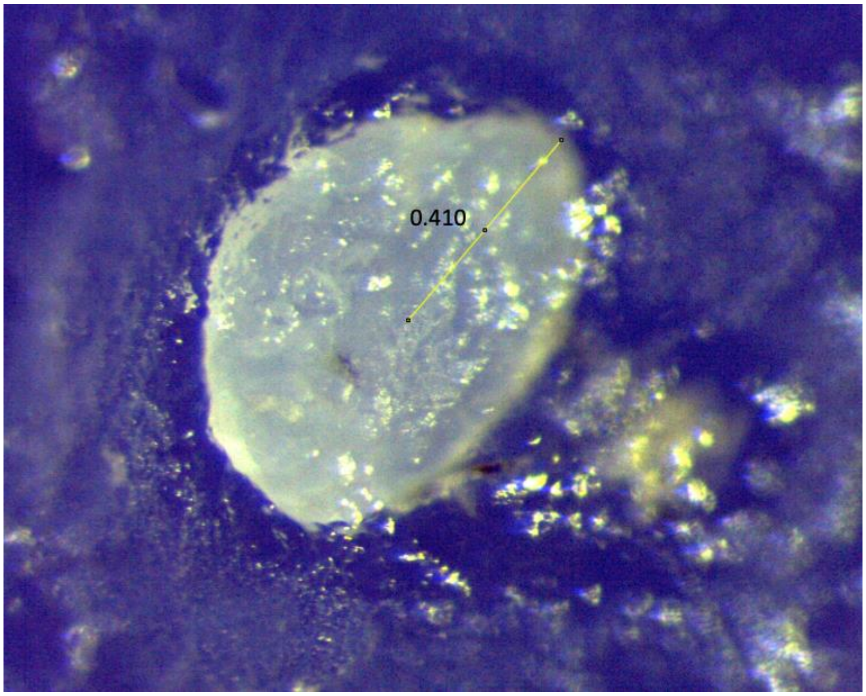

Figure 4. Image of sagitta otolith taken from *M. menidia* at baseline treatment. Radius of this otolith measured 0.410 mm.

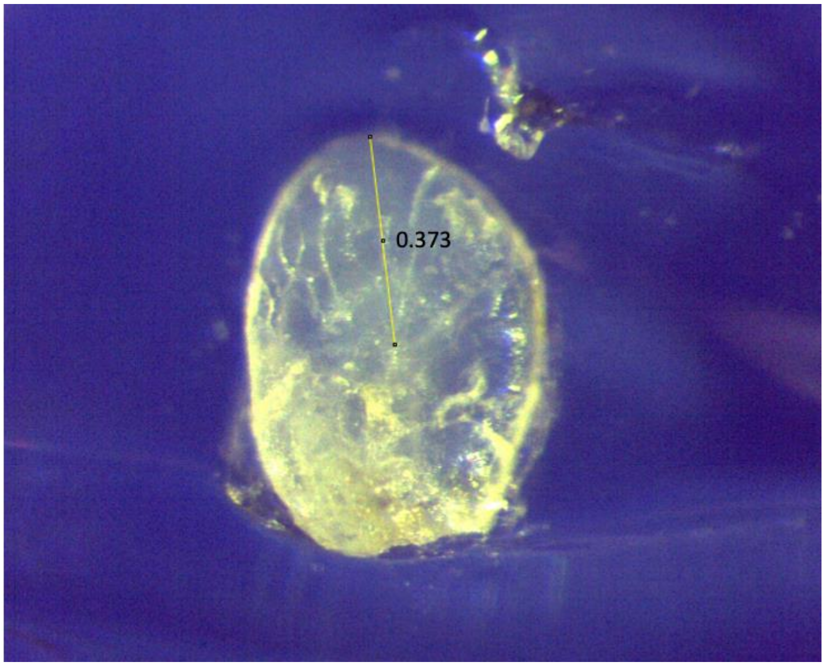

Figure 5. Image of sagitta otolith taken from *M. menidia* at ambient treatment. Radius of this otolith measured 0.373 mm.

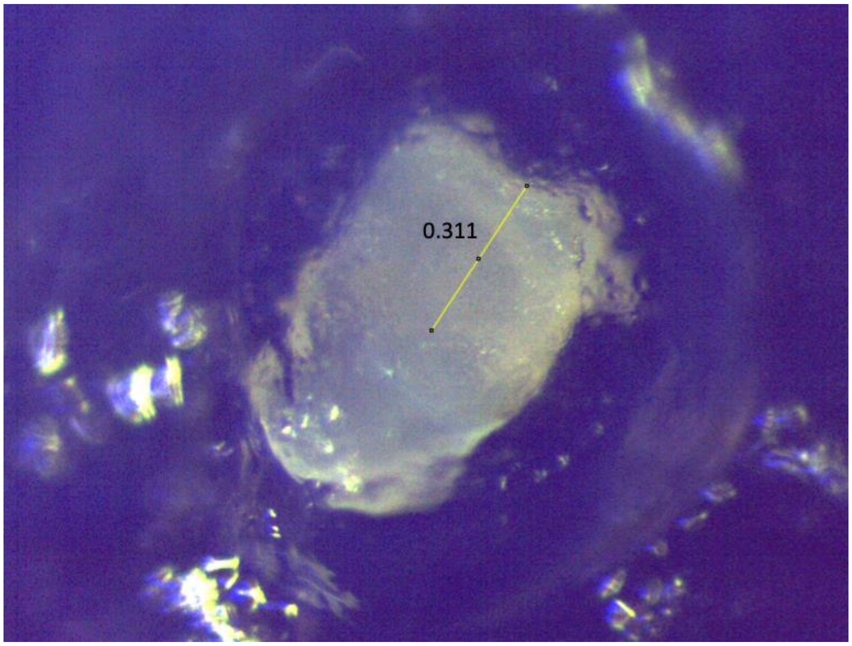

Figure 6. Image of sagitta otolith taken from *M. menidia* at IPCC+2 °C treatment. Radius of this otolith measured 0.311 mm.

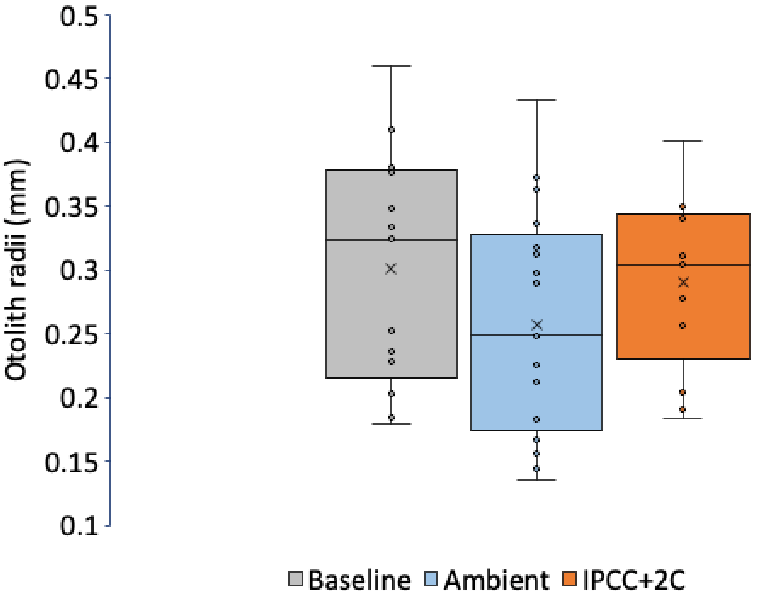

Figure 7. The radii of otoliths of *M. menidia* do not differ significantly between treatment groups. Five fish were used for the baseline treatment, five for the ambient, and six for the IPCC+2.

Discussion

The main objective of this study was to determine how increasing water temperatures affected growth and condition in *Menidia menidia.* To do this, the Fulton index based on length and weight, RNA:DNA ratios, and otolith growth in fish at different temperatures were analyzed and compared. It was hypothesized that lower temperatures would result in higher conditions, meaning a higher Fulton index, larger otolith incremental growth, and higher RNA:DNA ratios. The second hypothesis was that captive fish raised under laboratory conditions would have higher growth rates than wild-caught fish due to less activity and higher food availability. It was expected that fish raised at higher temperatures (ambient +2) within laboratory conditions would have lower overall growth when compared to those at the lower temperature (ambient). These hypotheses were not supported by the data. Fulton index was the highest in wild caught fish (baseline), while RNA:DNA analysis and otolith growth showed no significant differences between treatments.

One way to determine condition is the Fulton index, which is commonly seen as a good measurement of the condition and growth of an organism (Amara *et al.,* 2007). The Fulton index compares the weight and length of the animal to determine its growth and relies on the assumption of isometric growth (Ragheb 2023). Fish with access to more food would likely be able to store more fat, which would result in a higher Fulton index (Martin *et al.,* 2017; Salmeron 2018). Fulton index can vary depending on species and body shape, but typically a higher Fulton index is considered to be a better condition. In this study, animals at the baseline temperature (18°C) had the highest Fulton index, while the ambient (12 °C) and IPCC+2°C (14 °C) treatments were significantly lower. Animals at the baseline treatment were wild-caught and possibly had varying access to nutrients and food when compared to the captive animals, thus resulting in the variation seen in the data. A previous study conducted by Ratz and colleagues (2003) on *Gadus morhua* found that increasing water temperatures resulted in an increase in the Fulton index, similar to the findings of this study when comparing the baseline group to the other two treatment groups. When the water temperature increased, so too did the Fulton index for these *M. menidia*, most noticeably between the ambient and baseline temperatures. There was also no statistical difference between the ambient and IPCC+2°C groups. Contrary to my predictions, temperature effects were not detrimental enough to result in decreased condition, with the best condition being seen at the highest temperature. This could be related to an acceleration of growth due to temperature dependent cellular processes.

A common indicator of growth and short-term changes in condition for numerous organisms, from bacteria to fish to even humans, is the RNA:DNA ratio, (Hussna *et al.*, 2020). It is expected that the RNA present will be continuously changing as the animal intakes more nutrients, resulting in a higher RNA:DNA ratio (Chicharo & Chicharo 2008). A previous study by Gwak and colleagues (2001) on *Paralichthys olivaceus* larvae found that starved animals had significantly lower RNA:DNA ratios when compared to animals that had been well-fed. Animals used in this study had been well-fed. While there is a decreasing trend between all of the treatment groups, there were no statistical differences. It would also be expected that animals that had more access to nutrients and a higher RNA:DNA ratio would also have a higher Fulton index. At the baseline (18°C), the Fulton index was the highest, as was the RNA:DNA ratio. This could be because these animals had more available and perhaps more nutritive food sources in the wild and had more energy to expend on growth. In addition to nutrient availability, temperature can also affect the RNA:DNA ratio. We would expect that healthier fish at normal temperatures would produce more proteins while fish exposed to excessive temperatures would have growth and protein synthesis decreased by stress as seen in other studies (Fatma & Ahmed, 2020; Boltaña *et al.*, 2017; Bevelhimer & Bennett, 2000). On the other hand, higher temperatures can lead to increased growth (Audzijonyte *et al.*, 2020) if within the normal range or to overexpression of heat shock and other stress proteins if under heat stress (Liu *et al.*, 2013; Currie & Tufts, 1997; Beemelmanns *et al.*, 2021). While there was no statistical difference between the three treatment groups, there was a decreasing trend between the ambient and IPCC+2 °C treatments. It must be concluded from the analysis of RNA:DNA that either fish were not experiencing heat stress or they were expressing additional proteins to cope with that stress, thus overinflating the RNA:DNA ratio. Future research should include the quantification of heat shock and other stress proteins along with RNA:DNA to disambiguate these results.

Another way to determine growth is otolith analysis. The size of the bands seen in the otoliths and overall otolith size can vary depending on various factors, including temperature and nutrient intake (Geffen, 1982; Otterlei *et al.*, 2002). At the baseline, ambient, and IPCC+2 °C treatments, the average size of the radii of the otoliths were 0.301 mm, 0.258 mm, and 0.291 mm, respectively. It was expected that increasing temperatures would result in larger otoliths. While there was a slight trend for increasing size at higher temperatures, it was not significant. A study conducted by Hovenkamp (1990) found that there was no significant effect of temperature on the incremental growth of otoliths of *Pleuronectes platessa* (Hovenkamp, 1990). Further, another study by Fey (2006) using *Clupea harengus* and *Osmerus eperlanus* found that the growth of the otoliths of the smelt was only slightly affected by varying temperatures (Fey, 2006). It is likely that there were no discernible differences due to the short timeframe of this study and that temperature did have some effect, but that it was not evident yet. It is possible that, given a longer experiment, there would have been more discernible differences in the sizes of the otoliths at the different temperature treatments used in this study. It is possible that there would have been more discernible growth had the daily increments been observed instead of just the radii. It would also be interesting to see if these patterns would have held if the exposure to increased temperature had been done over the growing season – spring/summer – instead of in the fall.

Considering that fish at baseline were exposed to 18°C, there was a statistical difference in Fulton index between the highest temperature and the lower temperatures (ambient), with animals at the highest temperature – the baseline group - having the highest Fulton index, refuting the first aforementioned hypothesis, which stated that lower temperatures would result in higher conditions, such as a higher Fulton index, higher RNA:DNA ratio, and larger otolith growth.. In addition, there were no statistical differences between the RNA:DNA, which also refutes the hypothesis. However, it should be noted that the sample sizes used for the RNA:DNA ratios were quite small, with only five animals being used for the baseline treatment. It is possible that the results would support the hypothesis, had there been more animals in the sample. Further, captive fish raised at higher temperatures (IPCC+2°C) had slightly lower growth rates than those at the ambient temperature, supporting the hypothesis that captive fish raised under laboratory conditions would have higher growth rates than wild-caught fish due to less activity and higher food availability.

Overall, it was concluded that increasing temperature did not negatively affect the condition of *M. menidia.* However, as there was a decreasing trend between the experimental treatment groups for the RNA:DNA ratio, it is possible that a larger sample size would show significance. Also, animals raised within laboratory conditions at higher temperatures had slightly lower growth rates, indicating a potential negative effect of temperature under laboratory conditions.

One limitation of this study was the small sample size, specifically with the RNA:DNA samples. It is possible that the small sample sizes resulted in a type II error, or a false negative result. It is also possible a larger sample size would have resulted in significant differences in the treatments. Another limitation was that the baseline captured mostly the growth season as fish were collected during the summer, but the rest of the experiment took place as the temperatures were decreasing over the fall months. This could contribute to an inflated difference between baseline and the two experimental temperatures. In addition, while there is some thought that increasing temperatures in the winter can displace species (Rindorf & Lewy 2006; Sundby *et al.*, 2016; Barbarossa *et al.*, 2021; Smith *et al.*, 2010; Oviatt *et al.*, 2003), the thermal tolerance of this species might be more challenged in the warmer season (summer months). Repeating these experiments during the summer months and with larger sample sizes might yield better insight in what is affecting growth. Future studies that look at the effects of temperature on the RNA:DNA ratio should also include the quantification of heat or stress proteins to help clarify the results.

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Chapter 2
Effects of temperature on metabolic rate of planktivorous fish in Narragansett Bay

Abstract

Increasing water temperatures have been a worldwide problem for coastal ecosystems. Historical data in Narragansett Bay have shown shifts in fish species composition, likely related to increasing water temperatures. Scup (*Stenotomus chrysops*) and Atlantic silversides (*Menidia menidia*) are two common planktivorous fish species found in the Bay, which remain understudied. The main question of this study asked how the metabolism of these animals would be affected by warming waters. Fish were acclimated to 18 °C over the course of one week and then closed-chambered respirometry experiments were conducted at 18 °C and 22 °C to test for basal (BMR) and maximum metabolic rates (MMR). After completion of these trials, fish were switched to tanks maintained at 22 °C and were acclimated to this temperature for a period of one week and respirometry experiments repeated at both 18 °C and 22 °C. We expected fish to have an increase in metabolic rate with increasing testing temperature. It was also expected that fish acclimated to testing conditions would have higher metabolic rates at higher temperatures. If these species are unable to thrive at elevated temperatures, we would expect metabolic rates to be suppressed. Basal and maximum metabolic rates of both species increased with increasing testing temperatures, while the aerobic scope was unchanged with different held or testing temperatures. Maximum metabolic rate of *M. menidia* was also significantly affected by held temperature, which was the temperature the holding tanks of the animals were prior to experimentation. At the predicted increased temperature for the Bay, we would expect these species to be displaced by other planktivorous species and silversides even more so than scup, since they were also affected by held temperature.

Introduction

Climate change has numerous detrimental effects on aquatic ecosystems, including, but not limited to, coral bleaching (Abdo *et al.*, 2012), increased hypoxia (Roman *et al.*, 2019), increase in harmful algae blooms (Trombetta *et al.*, 2019), and increased energetic costs for biological functions such as migration and spawning (Plumb, 2018). The National Oceanic and Atmospheric Administration (NOAA) has reported that surface temperatures of the oceans rose by approximately 1.3 °C since the year 1979 (Brierly *et al.*, 2009). It is also projected that if this trend in increasing water temperatures continues, oceanic water temperatures will increase by 2-3.5 °C (Richardson, 2008). A study by Rall and colleagues (2010) found that increasing temperatures resulted in increased metabolic rates, while ingestion rates remained lower. This would result in malnutrition across trophic levels, causing disruption to the energy fluxes. In addition, ocean warming is impacting plankton processes, both indirectly and directly (Winder & Sommer 2012; Smith *et al.*, 2010; Nixon *et al.*, 2009). As a result of global warming, there have been changes in algal blooms, plankton species composition, and size (Smith *et al.*, 2010; Ljungstrom *et al.*, 2020; Audzijonyte *et al.*, 2020; Winder & Sommer, 2012). If plankton are removed from the environment, other animals would then either need to find new food sources or migrate to follow their preferred food source. Further, if these animals do not migrate or seek a new food source, it could increase predation (Rosenblatt & Schmitz, 2016).

Narragansett Bay, RI, is the largest estuary in New England and is being impacted by both human activity and global warming (Humphries *et al.*, 2022). A study by Smith and colleagues (2010) reported that the temperature of Narragansett Bay has risen by 1.2° C since the year 1950. It is also projected that, if this trend continues, the ocean temperatures could increase by 2-3.5 °C by the year 2100 (Richardson, 2008). As a result of these increasing temperatures, there has been a species composition shift, with fewer cold-water species being found (Collie *et al.*, 2008; Innes-Gold *et al.*, 2020). Oviatt and colleagues (2003) reported that there was a decrease in fish, such as winter flounder, menhaden, and scup, while animals like lobsters and crabs were thriving in this environment (Oviatt *et al.*, 2003).

Some of the species found in Narragansett Bay function as keystone species, or organisms that an ecosystem heavily depends on, and, if removed from their environment, would cause extreme changes (Valls *et al.*, 2015). Among the fish species in Narragansett Bay are *Stenotomous chrysops* and *Menidia menidia*, or scup and Atlantic silversides, respectively, which are common model organisms to use to learn more about the Bay. Both species are commonly found in Narragansett Bay and are susceptible to changes in their environment. Both species are planktivorous, feeding on zooplankton and phytoplankton (Fry *et al.*, 2008; Gilmurray & Daborn, 1981; Steimle ,1999). The plankton they feed on help maintain the nutrient-rich composition of their environment (Falkowski, 2012). Species such as Atlantic silversides feed heavily on the available plankton in the water column (Fry *et al.*, 2008). If these animals were removed from the Bay, there could be shifts in the abundance of plankton found in Narragansett Bay. Moreover, a study by Innes-Gold and colleagues (2020) used Ecosim to model the trophic web and energy fluxes and projected that mid and upper-trophic level animals would increase if current conditions progress.

As previously mentioned, scup (*Stenotomus chrysops*) and Atlantic silversides (*Menidia menidia*) are two species of planktivorous fish. While these two species are planktivorous, there is a large variation in their body sizes. *S. chrysops* can reach up to eighteen inches in length (Smithsonian Tropical Research Institute, 2023; Commonwealth of Massachusetts, 2023), while *M. menidia* can reach up to seven inches (Connecticut Department of Energy & Environmental

Protection, 2023). This difference in size will result in variations between their metabolic rates, with *M. menidia* having higher metabolic rates. Further, *S. chrysops* are migratory fish that typically remain near rocky areas of the ocean floor and, within the U.S., are most commonly found between North Carolina and Massachusetts. Like *S. chrysops*, *M. menidia* are migratory. They are most commonly found in brackish water and can be found in Atlantic waters along the entirety of the U.S. east coast.

Temperature affects many biological functions and increased water temperatures negatively impact many marine organisms (Alfonso *et al.*, 2021; Volkoff & Rønnestad, 2020; Little *et al.*, 2020). In one study completed on Narragansett Bay, it was found that a 1 °C increase in water temperature caused a reduction in phytoplankton bloom throughout the winter and spring months (Smith *et al.*, 2010). An increase of 1 °C can also cause the release of zooxanthellae, a form of algae, that causes coral bleaching (Brierly *et al.*, 2009), as well as harmful algal blooms (HABs) (Wells *et al.*, 2015). Temperature affects all physiological processes, including metabolic rate. Metabolic rate, in simple terms, indicates how much oxygen a fish is consuming in order to maintain bodily functions (Chabot *et al.*, 2016). These functions include, but are not limited to, growth, reproduction, and digestion (Metcalfe *et al.*, 2016; Farrell, 2015). The majority of fish are ectotherms, which means that they are unable to create their own heat, so it is expected that these fish will be more impacted by higher temperatures. When temperatures increase by 10°C, it has been found that metabolic processes can increase by 2 to 3 fold (Volkoff and Rønnestad, 2020). One particular study found that temperature played a stronger role than CO_2 on the metabolism of *M. menidia* embryos and larvae, with temperature resulting in significantly increased metabolic rates (Schwemmer *et al.*, 2020). Previous studies conducted on fish species such as *Gadus morhua* and *Scophthalmus maximus* have shown that increasing temperatures will result in increased metabolic rates (Tirsgaard *et al.*, 2015; Burel *et al.*, 1996).

Previous studies have found that global warming can also cause an increase in hypoxia (Roman *et al.*, 2019), which they describe as water that has dissolved oxygen concentrations less than 2 mgL^{-1}. As water temperatures increase, there is a decrease in oxygen solubility. Since aquatic environments are already oxygen limited in comparison to terrestrial environments, temperature increases can have even more severe effects. Local hypoxic areas can pose a challenge for fish populations (Roman *et al.*, 2019). The fish must either remain in the hypoxic water, which significantly increases their stress, or they must use behavioral methods to avoid these hypoxic waters. As temperatures increase, so too does hypoxia. In order to survive, fish have to maintain a particular respiration rate (Rubalcaba *et al.*, 2020). This is commonly referred to as basal metabolic rate, or BMR. Basal metabolic rate can be impacted by the temperature and by the amount of oxygen that is in the water. Since fish are so susceptible to changes in their environment, understanding how they react to these areas of lower oxygen is of increasing importance. One report states that, in order to survive hypoxic waters, fish would need to suppress their metabolic rates to regulate their ATP consumption (Richards, 2009). A study using an estuarine fish *Hypomesus transpacificus* found that they increase their metabolic rates at higher temperatures and that there is an upregulation of protein synthesis (Jeffries *et al.*, 2016).

Maximum metabolic rate (MMR) is the maximum amount of oxygen that the fish can consume after a period of exercise or stress (Norin & Clark, 2016; Lapointe *et al.*, 2014; Rummer *et al.*, 2016). Maximum metabolic rate is of particular importance since it indicates when fish are at the limit in energetic terms. A previous study on European sea bass *(Dicentrarchus labrax)* and another comprehensive review of multiple species have found that, as temperatures increase, maximum metabolic rate will increase, but it will then plateau (Claireaux *et al.*, 2006; Norin & Clark, 2016). In some instances, maximum metabolic rate can decrease due to detrimental effects of higher temperatures (Norin & Clark, 2016). Since increasing water temperatures can also increase metabolic rates, it is likely that fish will reach their maximum metabolic rates faster (Rubalcaba *et al.*, 2020).

Aerobic scope (AS) can be experimentally determined as the difference between basal metabolic rate and maximum metabolic rate. Aerobic scope indicates how much oxygen is available for routine behaviors, such as predator avoidance (Norin & Clark, 2017). As temperatures increase, the basal metabolic rate is likely to also increase, however maximum metabolic rate is likely to be limited. This potentially reduces the aerobic scope and the energy available for routine behaviors (Holt & Jørgensen, 2015). If the basal metabolic rate is lower at cooler temperatures, there is likely a larger aerobic scope, and, thus, a greater ability to deal with any stressors (Killen, 2014). Past optimal growth rates, the maximum metabolic rate will no longer increase and aerobic scope will be reduced. This will impact both the fitness and survival of the fish.

The goal of this study was to determine how acclimation and increasing temperatures affected oxygen consumption in planktivorous fish in Narragansett Bay. To do this, basal metabolic rate, maximum metabolic rate, and aerobic scope were evaluated. It was hypothesized that increasing water temperatures would result in increasing both basal and maximum metabolic rates and depressed aerobic scope in both *Stenotomous chrysops* and *M. menidia* and that fish acclimated to each testing temperature would be less negatively affected, meaning that their metabolic rates would not be as changed, than fish not acclimated to that testing temperature.

Methods

Fish collection and husbandry

Twenty-nine *Menidia menidia* were collected from Ninigret State Park in Charlestown, RI, USA (41.3649°N, 71.6564°W) using a seine net in accordance with IACUC protocol #50F-2/3 and existing DEM Scientific Collection Permits (Fig. 1). 12 of these silversides were used for experimentation, while the remaining were housed for possible use in case of mortalities. Twelve *Stenotomus chrysops* were collected from several locations in Narragansett Bay using a rod and reel in accordance with IACUC protocol #50F-2 and existing DEM Scientific Collection Permits. Scup were collected from Pier 5 in Narragansett (41.4222°N, 71.4549°W), Camp Cronin Fishing Area in Narragansett (41.3623°N, 71.4873°W) and a boat dock in Tiverton (211 Riverside Drive, Tiverton, RI; 41.6391°N, 71.2112°W).

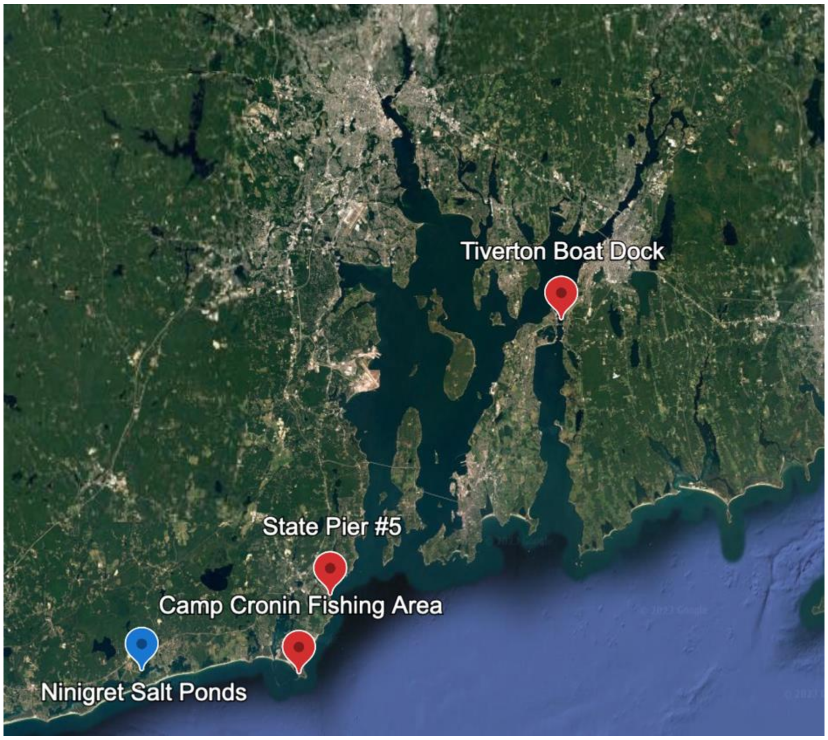

Figure 1: Collection sites of *M. menidia* and *S. chrysops*. Blue markers represent areas where *M. menidia* were collected, while red markers represent locations where *S. chrysops* were collected. Map made using Google Earth.

Fish were transported from their respective location to Rhode Island College (RIC) using a cooler and aerator. Fish were transferred from the cooler to the saltwater tanks in the seawater facility at RIC. All tanks were initially held at 18°C±0.5 °C (Fig. 2). Fish were only removed from their tanks for measurement and to conduct respirometry experiments. Fish were left to acclimate at this temperature for a week before respirometry experiments began. After completion of the experiments done with the animals acclimated to 18°C, the temperatures of the tanks were gradually increased to 22°C±0.5 °C over the course of 3 hours. Once the temperature reached 22°C, fish were left to acclimate for a week. Temperature and salinity were recorded at 10-minute intervals for 24 hours a day using HOBO dataloggers for the duration of the experiment. Light and dark cycles were maintained throughout the experiment. Fish were kept in a well-lit room for 12 hours a day and then in darkness for 12 hours. Fish were fed brine shrimp cubes *ad libitum* during the acclimation and food levels were then maintained throughout the experiment.

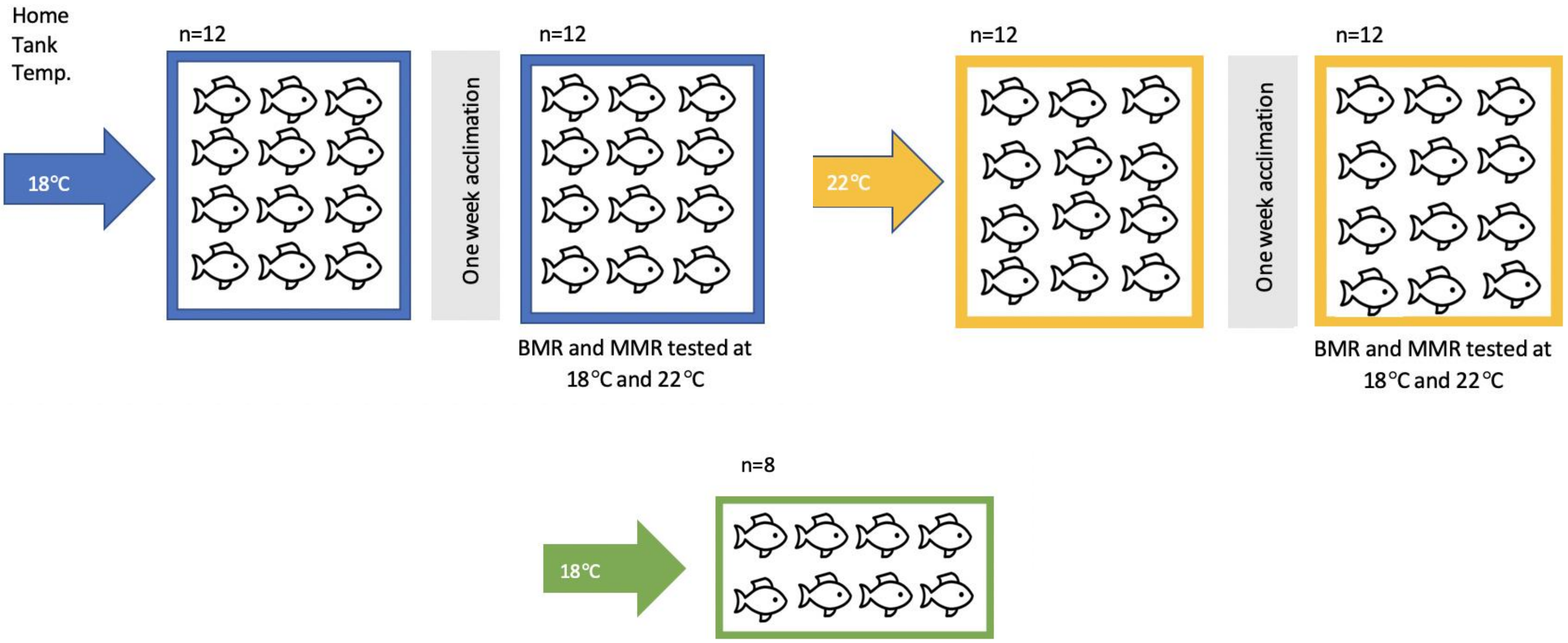

Figure 2: Experimental setup to determine metabolic rate of planktivorous species at different temperatures. Fish were placed in tanks kept at 18 °C and were acclimated for one week. Respirometry trials were run on these fish at 18 and 22 °C to record both their basal and maximum metabolic rates. Fish were then placed in tanks at 22 °C and acclimated for one week and respirometry trials run at 18 and 22 °C to record their basal and maximum metabolic rates. Moreover, additional fish (green) were housed in another tank at 18 °C, only for the eventuality that fish died of natural causes before the end of the first acclimation.

<u>Respirometry</u>

Respirometry experiments were conducted to determine how much oxygen the fish were consuming at increasing temperatures. Fish were tested at either their holding temperature or the other treatment temperature to determine basal and maximum metabolic rates. Experiments were done in a random order, although it was ensured no fish was run at both temperatures on the same day. Before the fish were moved to the respirometry chamber, the salinity and temperature were checked and fish were weighed. To weigh the animals, a container of saltwater was placed on top of a scale and tared. Fish were then placed in the container and the weight was recorded.

This was done for each individual trial. Salinity was kept between 28-32 ppt in holding tanks and experiments were conducted at 31 ppt. Temperature depended on the particular trial being conducted but was maintained at either 18°C±0.5 °C or 22°C±0.5 °C. Salinity and temperature were checked daily using the YSI 5200A Multiparameter Monitor (YSI; Yellow Springs, OH, USA). Fish were not fed until after the experiments were completed each day. A dissolved oxygen optical probe, ProOdo or ProSolo (YSI; Yellow Springs, OH, USA) was fit into a tight custom-built respirometry chamber inside a large bath. This chamber was chosen based on the size of the fish to account for 10x the fish volume. The respirometry chamber volumes were 5500mL for *Stenotomous chrysops* and 970mL for *M. menidia.* Each fish underwent two respirometry experiments at each temperature. The order of these trials were random. The fish were placed in the large bath and left to acclimate for 15 minutes before basal metabolic rate (BMR) was measured. An optical probe was fit into the lid of the respirometry chamber and then sealed with parafilm to ensure there was no gap excess oxygen could move through. After 15 minutes, the lid was placed on the chamber with the fish inside and sealed with parafilm. The optical probe collected dissolved oxygen and temperature every second during the duration of the trial. The trials were run for 30 minutes or for the time needed for the oxygen concentration in the tank to deplete to 80% saturation. To collect the maximum metabolic rate (MMR), the fish were chased around the bath tank for 10 minutes and then placed in the respirometry chamber. The process was then repeated. These data were then fitted to a linear regression to obtain the mass-corrected oxygen consumption for basal and maximum metabolic rates at each temperature. The aerobic scope was then determined by taking the difference between the maximum and basal metabolic rates.

Statistical analysis

All statistical analysis was completed using Microsoft Excel. The amount of oxygen consumed versus time for each trial was graphed and a linear line regression was fit. The oxygen consumption was then corrected for the mass of each fish. Two-way ANOVAs with interactions were run with testing and acclimation (held) temperature as predictors for basal metabolic rate, maximum metabolic rate, and aerobic scope. Additional ANOVAs were run with species, testing and holding temperatures as predictors. Interaction effects were also tested for. All analyses were verified using JASP (Version 0.16.4).

Results

Increased testing temperature had a significant effect on the basal metabolic rate and maximum metabolic rate of both scup, *Stenotomus chrysops* and silversides, *Menidia menidia*. Temperature at which the fish were held for a week prior to testing also had an effect on the maximum metabolic rate of *M. menidia*. There was no effect of held temperature on any of the three variables- basal metabolic rate, maximum metabolic rate, and aerobic scope- of *S. chrysops.* There was also no effect of held temperature or testing temperature on the aerobic scope of *M. menidia*.

Scup, *Stenotomus chrysops*

Basal Metabolic Rate

Basal metabolic rate of scup, *S. chrysops* was significantly increased by increased water temperature, but not by held (or acclimation) temperature (ANOVA, F(1,44)=4.822, p=0.033, Fig. 3). When tested at 18°C, fish that had been held at 18°C had an average basal metabolic rate of 309 $mgO_2kg^{-1}hr^{-1}$, while fish that had been held at 22°C had an average basal metabolic rate of

265mgO$_2$kg^{-1}hr^{-1} (Table 1). Fish tested at 22°C had an average basal metabolic rate of 344 mgO$_2$kg^{-1}hr^{-1} and 343 mgO$_2$kg^{-1}hr^{-1} when held at 18°C and 22°C, respectively. Acclimation temperature had no effect on the basal metabolic rate of *Stenotmus chrysops*, while there was a significant effect of testing temperature (p=0.033, Fig. 3). Interaction effects were also tested, but none were found to be statistically significant.

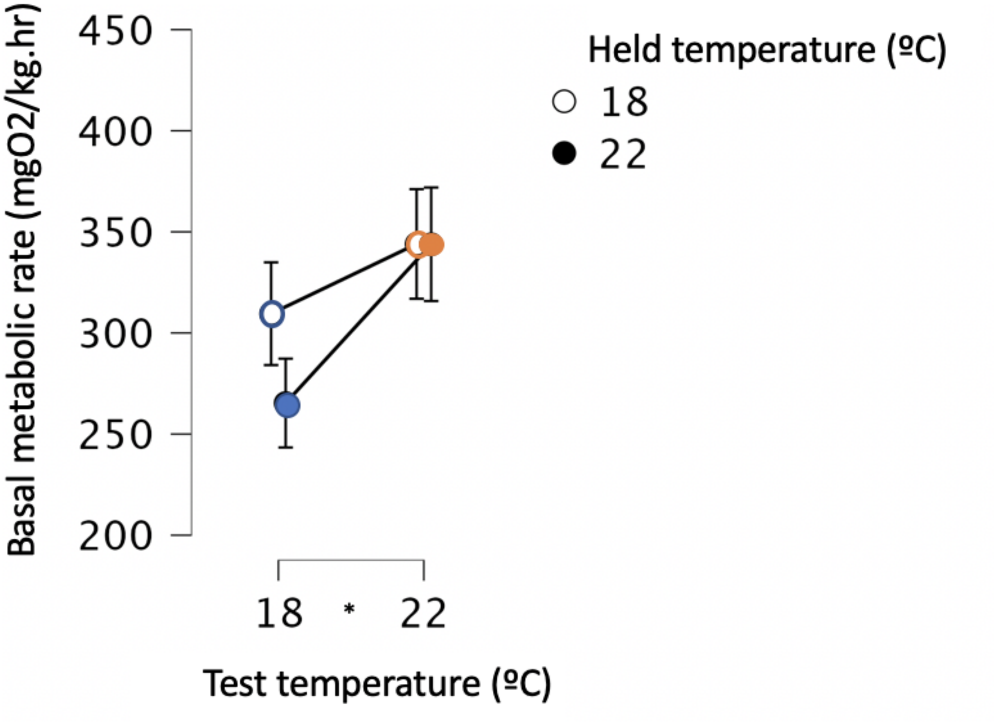

Figure 3. Basal metabolic rate of *S. chrysops* increased at increasing testing temperatures, but not with holding temperature. * denotes significance. Increasing testing temperature resulted in a p-value of 0.033 and held temperature resulted in a p-value of >0.05.

Maximum Metabolic Rate

Maximum metabolic rate of scup, *S. chrysops* increased significantly with increasing testing temperature, but remained unchanged with changing held temperatures (ANOVA, F(1,44)=4.768, p=0.034, Fig. 4). When tested at 18°C, fish that had been held at 18°C had an average maximum metabolic rate of 349 mgO$_2$kg^{-1}hr^{-1}, while fish that had been held at 22°C had an average maximum metabolic rate of 345mgO$_2$kg^{-1}hr^{-1} (Table 1). Fish tested at 22°C had an average maximum metabolic rate of 402 $mgO_2kg^{-1}hr^{-1}$ and 421$mgO_2kg^{-1}hr^{-1}$ when held at 18°C and 22°C, respectively. There was a statistical difference ($p=0.034$) between the maximum metabolic rates of *Stenotomous chrysops* at different temperatures but not between held temperatures. Interaction effects were also tested, but none were found to be statistically significant.

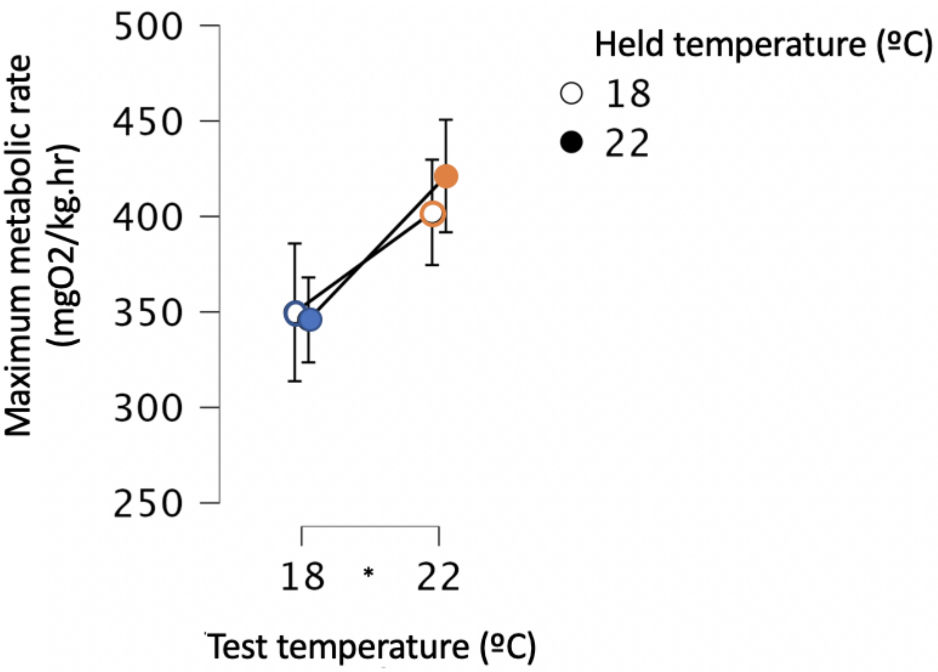

Figure 4. Maximum metabolic rate of *S. chrysops* significantly increased with increased testing temperatures, but not with holding temperature. * denotes significance. Increasing testing temperature resulted in a p-value of 0.034 and held temperature resulted in a p-value of >0.05.

Aerobic Scope

There was no significant effect of testing temperature or acclimation temperature on the aerobic scope of *S. chrysops* (ANOVA, Fig. 5). When tested at 18°C, fish that had been held at 18°C had an average aerobic scope of 40$mgO_2kg^{-1}hr^{-1}$, while fish that had been held at 22°C had an average aerobic of 80mgO_2kg^{-1}hr^{-1} (Table 1). Fish tested at 22°C had an average aerobic scope of 58mgO_2kg^{-1}hr^{-1} and 77mgO_2kg^{-1}hr^{-1} when held at 18°C and 22°C, respectively. Interaction effects were also tested, but none were found to be statistically significant.

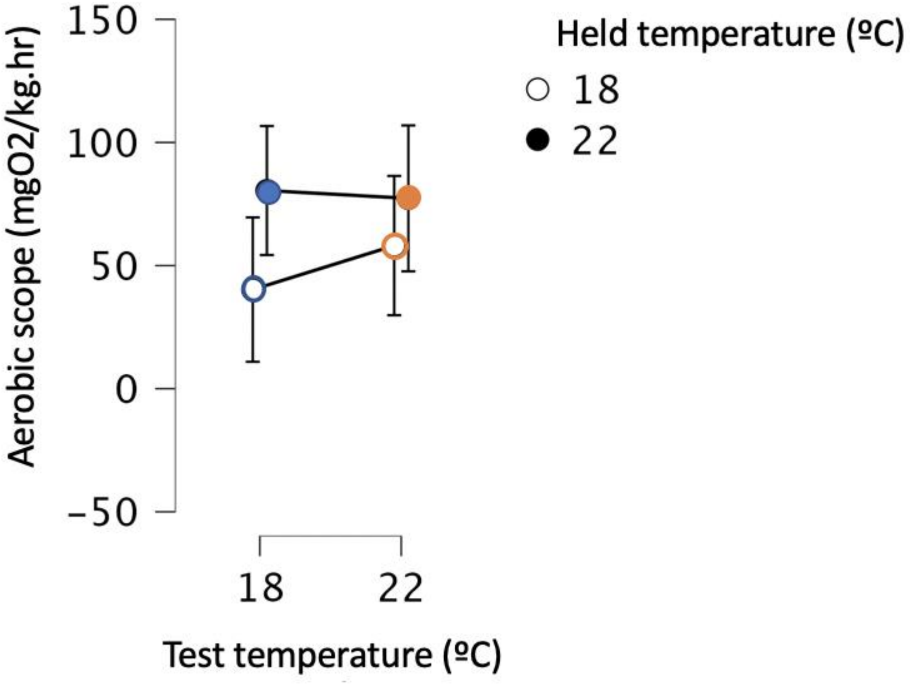

Figure 5. Aerobic scope of *S. chrysops* did not differ significantly with either acclimation or testing temperatures. Increasing test temperature resulted in a p-value of >0.05 and acclimation temperature resulted in a p-value of >0.05.

Table 1. Average basal metabolic rate, maximum metabolic rate, and aerobic scope of *S. chrysops.*

Variable and testing temperature	Acclimated- held at testing temp. (mgO_2kg^{-1}hr^{-1})	Non-acclimated- not held at testing temp. (mgO_2kg^{-1}hr^{-1})
Basal metabolic rate at 18°C	309	265
Basal metabolic rate at 22°C	343	344
Maximum metabolic rate at 18°C	349	345
Maximum metabolic rate at 22°C	421	402
Aerobic scope at 18°C	40	80
Aerobic scope at 22°C	77	58

Atlantic silversides, *Menidia menidia*

Basal Metabolic Rate

Basal metabolic rate of Atlantic silversides, *M. menidia* increased with increasing testing temperature (ANOVA, F(1,44)=12.008, p=0.001), but not with held or acclimation temperature (Fig. 6). When tested at 18°C, fish that had been held at 18°C had an average basal metabolic rate of 539$mgO_2kg^{-1}hr^{-1}$, while fish that had been held at 22°C had an average basal metabolic rate of 512$mgO_2kg^{-1}hr^{-1}$ (Table 2). Fish tested at 22°C had an average basal metabolic rate of 657$mgO_2kg^{-1}hr^{-1}$ and 620$mgO_2kg^{-1}hr^{-1}$ when held at 18°C and 22°C, respectively. The metabolic rate was significantly higher at increasing temperatures, and, when held at higher temperatures, the metabolic rate of *M. menidia* is reduced. Interaction effects were also tested, but none were found to be statistically significant.

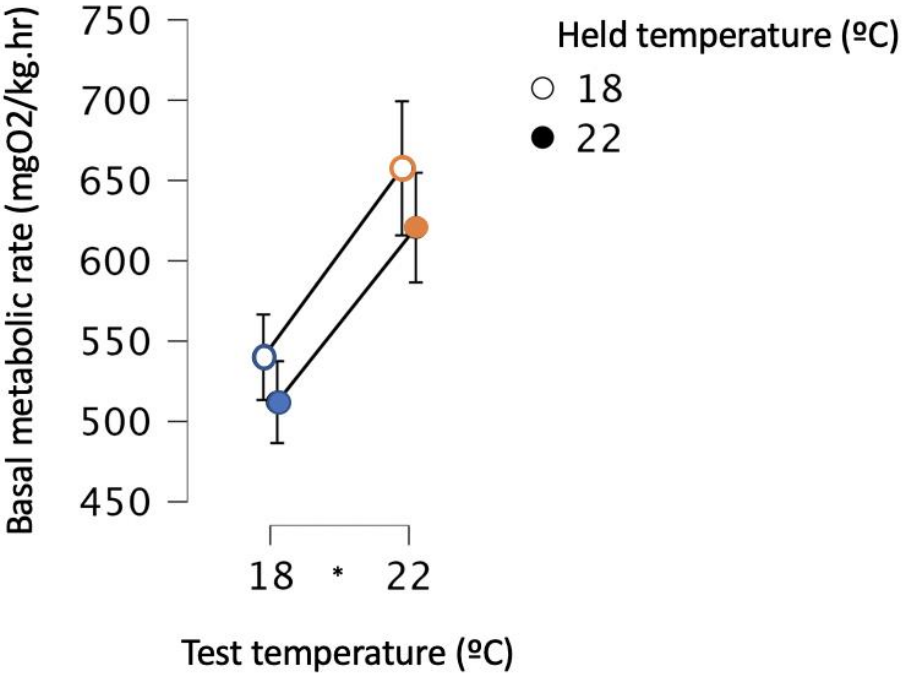

Figure 6. Basal metabolic rate of *M. menidia* significantly increased at increasing testing temperatures, but not with holding temperature. * denotes significance. Increasing testing temperature resulted in a p-value of 0.001 and held temperature resulted in a p-value of >0.05.

Maximum Metabolic Rate

Increasing testing temperatures increased the maximum metabolic rate of Atlantic silversides, *M. menidia* (ANOVA, F(1,44)=19.181, $p<0.001$; Fig. 7). When tested at 18°C, fish that had been held at 18°C had an average maximum metabolic rate of 508mgO_2kg^{-1}hr^{-1}, while fish that had been held at 22°C had an average maximum metabolic rate of 465mgO_2kg^{-1}hr^{-1} (Table 2). Fish tested at 22°C had an average maximum metabolic rate of 687mgO_2kg^{-1}hr^{-1} and 522mgO_2kg^{-1}hr^{-1} when held at 18°C and 22°C, respectively. An increase in testing temperature resulted in an increase in maximum metabolic rate in silversides ($p<0.001$). Maximum metabolic rate of *M. menidia* was also affected by held temperature (ANOVA, F(1,44)=14.955, $p<0.001$). Held temperature affected maximum metabolic rate by depressing it at higher temperatures, particularly when the testing temperature was also higher. The interaction between held and testing temperatures also had a significant effect ($p=0.029$) on the maximum metabolic rate of *M. menidia*.

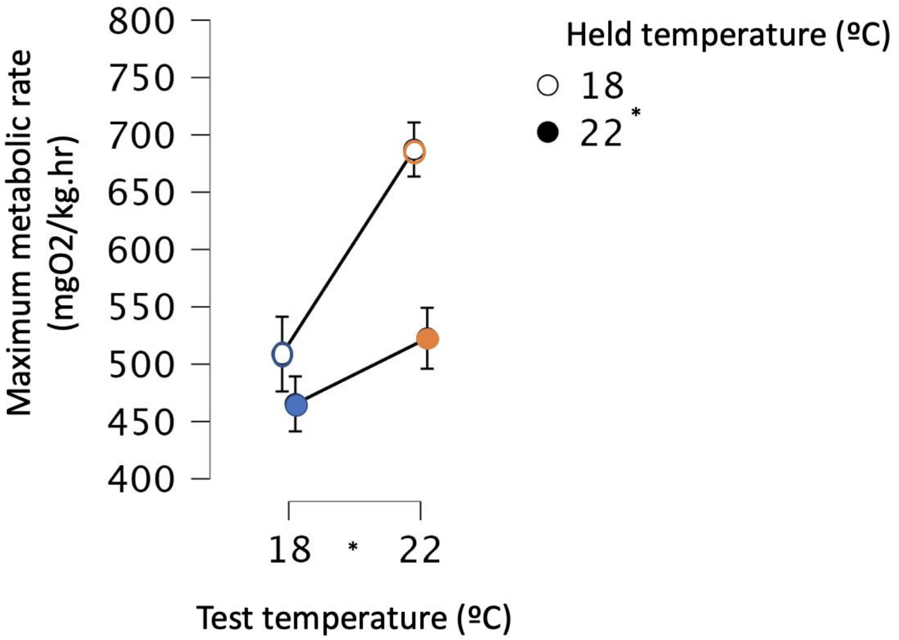

Figure 7. Maximum metabolic rate of *M. menidia* significantly increased at increasing test temperature and decreased with increasing acclimation temperatures. * denotes significance. Increasing testing temperature resulted in a p-value of <0.001 and acclimation temperature resulted in a p-value of <0.001.

Aerobic Scope

There were no statistical differences between the aerobic scopes at different testing or acclimation temperatures for *M. menidia* (ANOVA, Fig. 8). When tested at 18℃, fish that had been held at 18℃ had an average aerobic scope of -31$mgO_2kg^{-1}hr^{-1}$, while fish that had been held at 22℃ had an average aerobic of -46$mgO_2kg^{-1}hr^{-1}$ (Table 2). Fish tested at 22℃ had an average aerobic scope of 29$mgO_2kg^{-1}hr^{-1}$ and -98$mgO_2kg^{-1}hr^{-1}$ when held at 18℃ and 22℃, respectively. Aerobic scope was negative for fish held at higher temperatures, particularly when the testing temperature was also higher. There were no statistical differences between testing temperatures or between held temperatures.

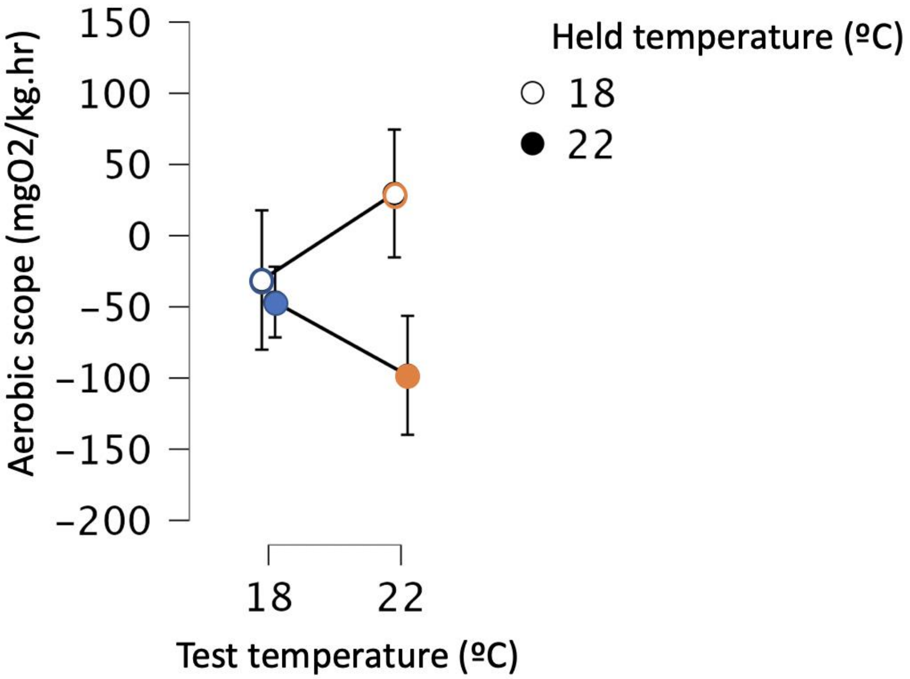

Figure 8. Aerobic scope of *M. menidia* did not differ significantly with either acclimation or testing temperatures. Test temperature resulted in a p-value of >0.05 and acclimation temperature resulted in a p-value of >0.05.

Table 2. Average basal metabolic rate, maximum metabolic rate, and aerobic scope of *M. menidia.*

Variable and testing temperature	Acclimated- held at testing temp. ($mgO_2kg^{-1}hr^{-1}$)	Non-acclimated- not held at testing temp. ($mgO_2kg^{-1}hr^{-1}$)
Basal metabolic rate at 18°C	539	512
Basal metabolic rate at 22°C	620	657
Maximum metabolic rate at 18°C	508	465
Maximum metabolic rate at 22°C	522	687
Aerobic scope at 18°C	-31	-46
Aerobic scope at 22°C	-98	29

Interspecific Comparisons

Species had a highly significant effect on basal metabolic rate (ANOVA, F(1,88)=164.790, p<0.001), maximum metabolic rate (ANOVA, F(1,88)=70.007, p<0.001), and aerobic scope (ANOVA, F(1,88)=16.203, p<0.001) (Figs. 9, 10, 11). At testing temperatures of 18°C and 22°C, both basal and maximum metabolic rates were significantly higher for Atlantic silversides, *Menidia menidia* than for scup, *Stenotomous chrysops* (Figs. 9, 10). The aerobic scope was lower in *M. menidia* than it was for *S. chrysops* (Fig. 11). Increased testing temperature also had a highly significant effect on the basal metabolic rate (ANOVA, F(1,88)=16.653, p<0.001) and maximum metabolic rate (ANOVA, F(1,88)=20.904, p<0.001) of both *M. menidia* and *S. chrysops*. Both the basal and maximum metabolic rates were higher in *M. menidia* at higher testing temperatures. The held temperature (ANOVA, F(1,88)=5.896, p=0.017) and interaction between species and held temperature (ANOVA, F(1,88)=7.884, p=0.006) also had a significant effect on the maximum metabolic rate, with *M. menidia* higher than *S. chrysops*. The interaction between species and held temperature also had a significant effect on the aerobic scope of these species.

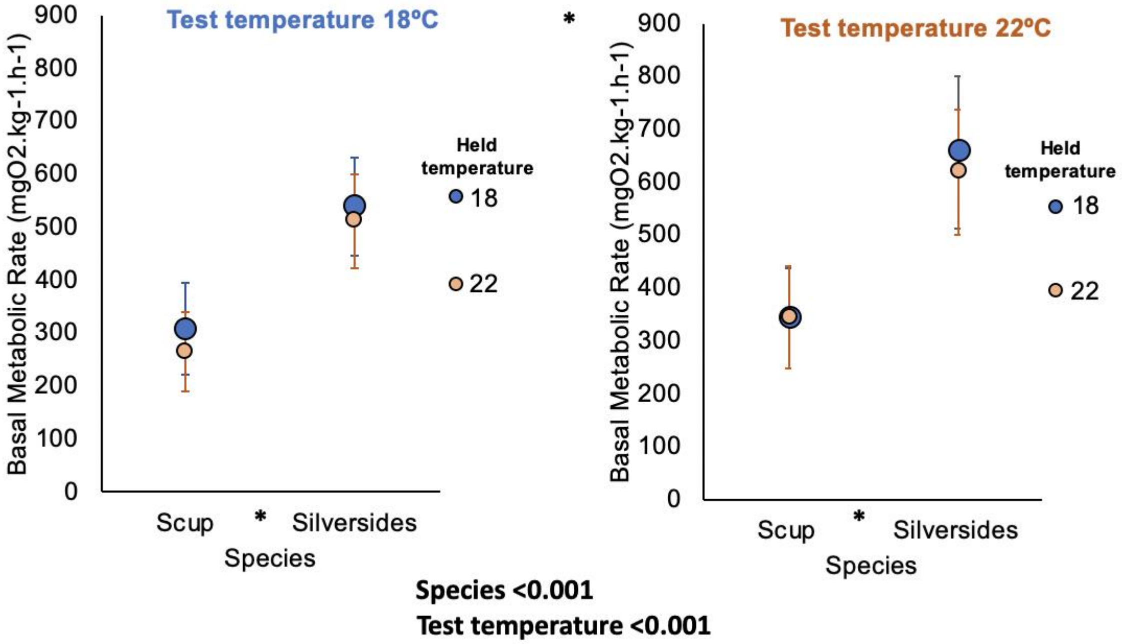

Figure 9. Species and testing temperatures had a significant effect on basal metabolic rate. Left shows basal metabolic rate of animals tested at 18 °C and right shows the basal metabolic rate of animals tested at 22°C. Species had a p-value of <0.001, test temperature had a p-value of <0.001, and held temperature had a p-value >0.05.

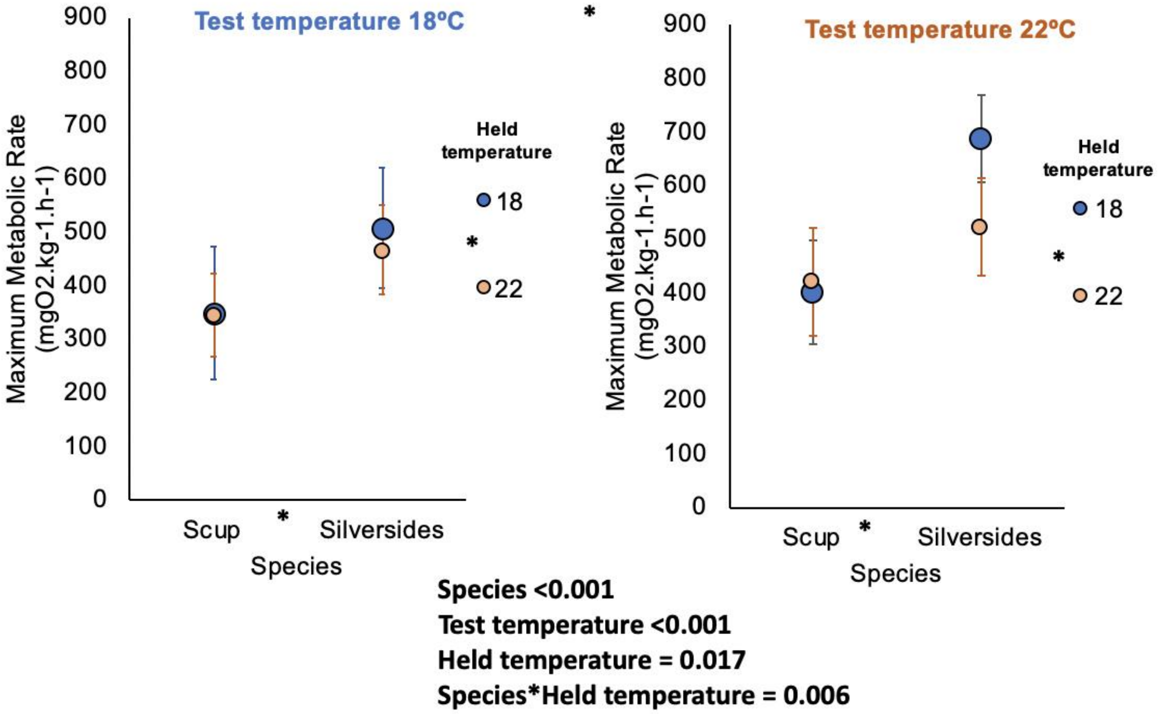

Figure 10. Species, test temperature, and held temperature were all predictors of maximum metabolic rate. Increasing testing temperature resulted in higher maximum metabolic rates, while the opposite was true for acclimation temperature. The most pronounced effects were seen at the higher holding temperature where cold acclimated silversides had a higher maximum metabolic rate. Left shows maximum metabolic rate of animals tested at 18 °C and right shows the maximum metabolic rate of animals tested at 22 °C. Species had a p-value of <0.001, test temperature had a p-value of <0.001, and acclimation had a p-value >0.05.

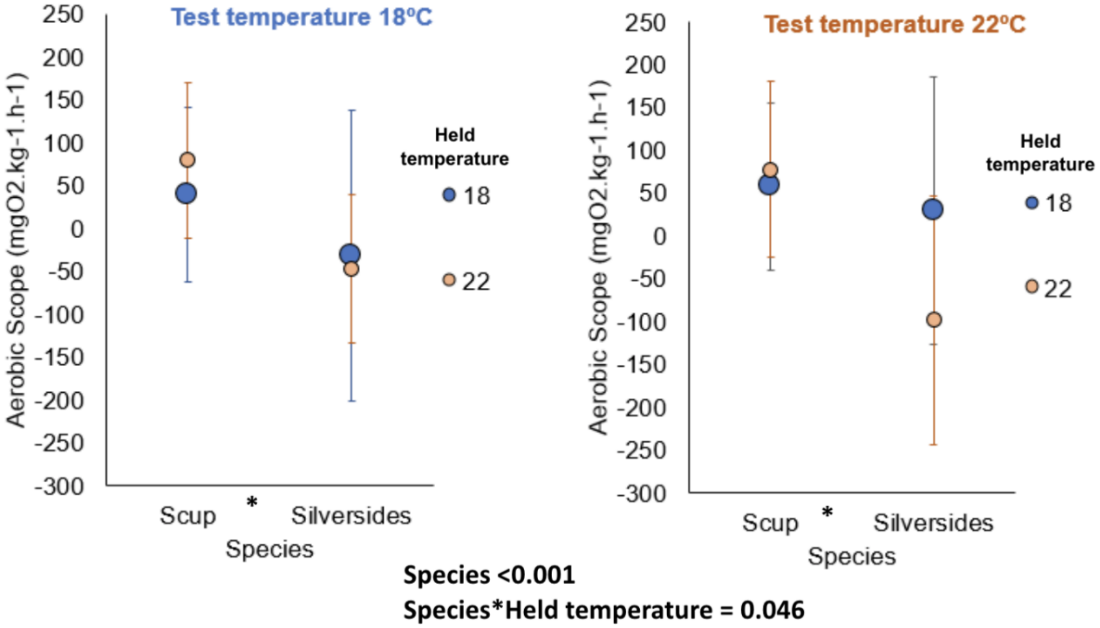

Figure 11. Species and the interaction between species and held temperature had a significant effect on aerobic scope, but held temperature did not. Left shows aerobic scope of animals tested at 18 °C and right shows the aerobic scope of animals tested at 22°C. Species had a p-value of <0.001, the interaction had a p-value of 0.046, and held temperature had a p-value of >0.05.

Discussion

The main objective of this study was to determine if increasing water temperature and acclimation had an effect on the basal and maximum metabolic rates and aerobic scope of scup, *Stenotomus chrysops*, and Atlantic silversides, *Menidia menidia*, from Narragansett Bay. It was hypothesized that increasing water temperatures would increase metabolic rates and decrease aerobic scope in both *S. chrysops* and *M. menidia* and that fish held at each testing temperature would be less negatively affected than fish non-acclimated to that temperature. For both species, increased testing temperatures caused an increase in both basal metabolic rate and maximum metabolic rate. Acclimation, or the temperature at which fish were held for a week prior to respirometry, did not have any effect on any of the variables, except for the maximum metabolic rate of *M. menidia.* For each individual species, aerobic scope was not significant with increased temperatures, indicating that aerobic scope was not affected by increasing temperatures. When comparing the two species, *M. menidia* also had significantly higher basal and maximum metabolic rates than *S. chrysops,* but lower aerobic scope. It was expected that *M. menidia* would have higher metabolic rates than *S. chrysops* due to the size discrepancies. The hypotheses were partially supported, in that higher testing temperatures resulted in increased metabolic rates. Acclimation did not affect most variables, refuting the second aforementioned hypothesis.

Increased testing temperature (22°C) had a significant effect on the basal metabolic rate and the maximum metabolic rate, but not the aerobic scope of *S. chrysops* and *M. menidia*. This indicates that these fish are experiencing stress when exposed to these higher temperatures. When tested at 18°C, acclimated and non-acclimated *S. chrysops* had an average basal metabolic rate of $309mgO_2kg^{-1}hr^{-1}$ and $265mgO_2kg^{-1}hr^{-1}$. Acclimated and non-acclimated *S. chrysops* had an average basal metabolic rate of $343mgO_2kg^{-1}hr^{-1}$ and $344mgO_2kg^{-1}hr^{-1}$, respectively, when tested at 22°C. At 18°C, acclimated and non-acclimated *M. menidia* at an average basal metabolic rate of 539 $mgO_2kg^{-1}hr^{-1}$ and 512 $mgO_2kg^{-1}hr^{-1}$, while the averages for acclimated and non-acclimated animals were 620 $mgO_2kg^{-1}hr^{-1}$ and 657 $mgO_2kg^{-1}hr^{-1}$ when tested at 22°C. At both testing temperatures, *M. menidia* had a significantly higher basal metabolic rate than *S. chrysops.* For both species, increased acclimation temperature resulted in a slightly lower basal metabolic rate at both testing temperatures for both species, though this was not significant. Since these two species of fish are ectotherms and cannot generate their own heat, it was expected that increasing testing temperatures would result in increased metabolic rates. Previous studies conducted on Atlantic cod, *Gadus morhua* and lumpfish, *Cyclopterus lumpus* also found that basal metabolic rate increased with increasing temperatures within their optimal range, although the mass-corrected metabolic rates for these animals were much lower than the ones found in our study (Tirsgaard *et al.,* 2015; Hvas *et al.,* 2018). These are benthic species that are less active than scup or Atlantic silversides and larger in size, which are expected to affect metabolic rate. Another possible explanation would be that the holding and testing temperatures used in this study are already stressful for these two species *M. menidia* and *S. chrysops*.

The maximum metabolic rate of both *S. chrysops* and *M. menidia* was significantly affected by increasing water temperatures, while the maximum metabolic rate of *M. menidia* was also significantly affected by acclimation temperature. At the testing temperature of 18°C, the maximum metabolic rates of acclimated and non-acclimated *S. chrysops* were 349 $mgO_2kg^{-1}hr^{-1}$ and 345 $mgO_2kg^{-1}hr^{-1}$ while the average maximum metabolic rate for *M. menidia* were 508 $mgO_2kg^{-1}hr^{-1}$ and 465 $mgO_2kg^{-1}hr^{-1}$, respectively. At the testing temperature of 22°C, the maximum metabolic rates of acclimated and non-acclimated *S. chrysops* were 421 $mgO_2kg^{-1}hr^{-1}$ and 402$mgO_2kg^{-1}hr^{-1}$ while the average maximum metabolic rate for *M. menidia* were 522$mgO_2kg^{-1}hr^{-1}$ and 687$mgO_2kg^{-1}hr^{-1}$, respectively. Maximum metabolic rate of *M. menidia* was significantly higher at both testing temperatures when compared to *S. chrysops*, but lower at increasing acclimation temperatures. A study conducted by Claireaux and colleagues (2006) on the European sea bass, *Dicentrarchus labrax,* found that as temperatures continued to increase, maximum metabolic rate also increased up to 24 °C and would level off after this temperature. Further, the lower metabolic rate at increasing acclimation temperature found in this study is likely a result of a decreased stress response. A study done by Davis and Parker (1990) using striped bass, *Morone saxatilis,* found that acclimation to testing temperatures can reduce stress-related responses (David & Parker, 1990).

The aerobic scope of both species used in this study were unaffected by both increased testing temperature and acclimation temperature. For acclimated and non-acclimated *S. chrysops* tested at 18°C, the average aerobic scopes were $40mgO_2kg^{-1}hr^{-1}$ and $80mgO_2kg^{-1}hr^{-1}$. When these same animals were tested at 22°C, the average aerobic scope was $77mgO_2kg^{-1}hr^{-1}$ for acclimated *S. chrysops* and $58mgO_2kg^{-1}hr^{-1}$ for non-acclimated *S. chrysops.* In *M. menidia* tested at 18°C, the average aerobic scope was $–31mgO_2kg^{-1}hr^{-1}$ and $–46mgO_2kg^{-1}hr^{-1}$ for acclimated and non-acclimated animals. When these animals were then tested at 22°C, the average aerobic scopes of acclimated and non-acclimated *M. menidia* were $–98mgO_2kg^{-1}hr^{-1}$ and $29mgO_2kg^{-1}hr^{-1}$, respectively. At both testing temperatures, *M. menidia* had lower aerobic scopes than *S. chrysops.* Most notably, *M. menidia* had a very depressed aerobic scope at the testing temperature of 22 °C after being held at 22°C. A study by Hvas and colleagues (2017) looked at the aerobic scope of *Salmo salar* at 13°C, 18°C, and 23 °C and also found that there were no statistical differences between the varying temperatures, although 23 °C trials did have the highest aerobic scope among treatments. Their findings also reported a much higher aerobic scope than the ones found in this study. This suggests that the animals used in this study were consuming less oxygen after being exercised, which could be indicative of a stress response affecting especially their basal metabolic rate.

Testing temperature had a significant effect on the basal and maximum metabolic rates of both species used in this study. This was expected, as these animals are ectotherms and cannot generate their own heat. Basal and maximum metabolic rates increased as the testing temperature increased. Despite this study's predictions, acclimation had no effect on the basal metabolic rate of *S. chrysops* or on the basal metabolic rate of *M. menidia;* however, it affected the maximum metabolic rate of *M. menidia*. In general, cellular processes increase with increased temperatures up to a certain level when cell physiology becomes disrupted (Boscolo-Galazzo *et al.,* 2018; Somero, 2020; Little *et al.,* 2020). Clark (1993) found that protein synthesis is increased at higher temperatures, which could also influence metabolic rates (Clarke & Fraser, 2004; Clarke, 1993). Thus, increasing temperatures are bound to increase metabolic rate, especially if the fish are not near their limits.

While basal and maximum metabolic rates of both species increased with higher temperatures, the same pattern was not observed for aerobic scope. In *S. chrysops*, aerobic scope was higher at both testing temperatures when the animals had been held at 22°C. Also, the average aerobic scope was significantly lower for *M. menidia* than for *S. chrysops*, with almost all values being negative. This means that exercising these animals resulted in the consumption of less oxygen when compared to non-exercised animals. It is possible that forcing the *M. menidia* to rest increased their stress levels, seeing as these are very active fish, constantly swimming (Conover & Ross, 1982; Griffen & Valiela, 2001; Connecticut Department of Energy & Environmental Protection, 2023). A study conducted on Atlantic salmon, *Salmo salar*, found that confinement stress can result in increased cortisol levels (Webster *et al.,* 2020). While *M. menidia* used in this experiment were only subjected to small areas of confinement for brief periods of time during experimentation, it is possible that it was long enough to result in increased stress levels. However, since hormone levels were not quantified in this experiment, there is no way to know how cortisol affected these animals.

While temperature had a noticeable effect on both species, acclimation only affected the maximum metabolic rate of *M. menidia.* One possible explanation is the production of heat shock proteins, which are in stabilizing cytoplasmic membranes at higher temperatures (Balogi *et al.*, 2019). In one study conducted using doctor fish, *Garra rufa*, researchers found that animals acclimated to warmer temperatures had higher levels of heat shock proteins in response to stressors (Oksala *et al.*, 2014). This same study also found that increased levels of heat shock protein resulted in lower levels of oxidative stress (Oksala *et al.*, 2014). Further, Beitinger and Bennett (2000) found that acclimation affects tolerance to lower temperatures more than higher temperatures, while another study by Davis and Parker (1990) using *Morone saxatilis* found that acclimation to testing conditions resulted in decreased stress responses. Acclimation appears to be more important for Atlantic silversides than for scup, further supporting the higher stress that increased temperatures cause in silversides.

S. chrysops were completely unaffected by acclimation, while *M. menidia* were only partially affected. It is possible that these animals were not given enough time to properly acclimatize to these testing conditions. A recent study conducted by Stewart and colleagues (2023) found that brook trout (*Salvelinus fontinalis*) exposed to increased temperatures for longer periods of time had an increase in their thermal tolerance, but that they had not completely acclimated to the testing temperatures by 30 days (Stewart *et al.* 2023). It was found that acclimation can occur over the span of several days but could take continue to change for up to at least a month (Stewart *et al.*, 2023). Other studies also found that full acclimation could take up to 36 days in certain fish species. Based on this understanding, it is possible that the *S. chrysops* and *M. menidia* in this study had not been given enough time to completely acclimate to their new environmental conditions. As a result, their basal and maximum metabolic rates may have been more affected in a longer-term study, with longer periods of acclimation.

These results are important for the conservation of the species in Narragansett Bay. If the temperatures of the Bay continue to increase as expected, *S. chrysops* and *M. menidia* will be affected. *M. menidia* will be particularly impacted by these increasing temperatures. It is likely that these animals will be displaced by others that are more tolerant of higher temperatures. Future studies should look at the effects of long-term acclimation or multigenerational studies to see if that would have different results. In addition, more drastic temperature differences could be used for future studies.

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Conclusions

Understanding how global warming affects the marine life in Narragansett Bay is necessary to predict what will happen to these animals in the future. Water temperatures in Narragansett Bay have increased by 1.2 °C in the past several decades. If current trends persist, Narragansett Bay will continue to get warmer and warmer. As a result, there will be an array of negative impacts on this ecosystem. It has already been observed that plankton and fish, such as flounder, are decreasing or being displaced, the sea level of the Bay is rising, and increased salinity has been reported. In this study, it was found that the physiology of *Menidia menidia* and *Stenotomus chrysops* is impacted by increasing temperatures, with *M. menidia* more susceptible to temperature increases. *M. menidia* also exhibited a decreasing trend in the RNA:DNA ratio when the temperature was elevated under experimental conditions, which also indicates that warming of the waters even in the winter will negatively impact cellular responses. Basal and maximum metabolic rates of both species increased with increasing testing temperatures, while the aerobic scope was unchanged with different held or testing temperatures. Maximum metabolic rate of *M. menidia* was also significantly affected by held temperature. Future studies should look at other species common in Narragansett Bay, should quantify stress proteins to see if these animals were indeed experiencing heat shock or were adequately dealing with it, should look at the annuli of the otoliths for growth, and should acclimate the fish for longer periods of time. This study provides important knowledge on the present marine ecosystem of the Bay. *M. menidia* are an important bait fish and food source for many animals and if they are displaced or harmed, there will likely be disruptions to present ecosystems. This would also disrupt local fishing operations, which could hinder the Rhode Island economy as fishing generates millions of dollars each year (RI DEM, 2021). Due to the location of the Bay, displacement of species northwards can be impacted by the biogeographic barrier that is Cape Cod. This is expected to function as a population fragmentation barrier especially for smaller species like *M. menidia*. It is recommended that impacts on local fish species be addressed not only through abundance surveys but also through laboratory-controlled experiments to test the temperature tolerance of the species.

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