Behavioural Thermoregulation and Escape Behaviour:

Investigating the Thermal Biology of Round Gobies

By

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Bachelor of Science, 2014, Brock University

Submitted in partial fulfillment

of the requirements for the degree of

Master of Science

Brock University, Faculty of Mathematics and Science

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Abstract

The invasive round goby (*Neogobius melanostomus*) has successfully colonized all of the Great Lakes since its discovery in the region in 1991, yet little is known about its thermal biology. The focus of this thesis was to examine the effect of acclimation to unseasonably warm temperatures on round goby behavioural thermoregulation, as well as behavioural and physiological performance during escapes with warm acute temperatures. Juvenile gobies were acclimated to either 21°C or 24°C for each set of experiments. I first examined goby thermal preference in a shuttlebox through their ability to escape from unfavourable temperatures. I found that escape temperatures were plastic following acclimation to a rise in 3°C rise in temperature (+3°C) and associated positively with acclimation temperature, even though gobies showed slightly lower-than-expected average escape temperatures in each acclimation treatment. Interestingly, acclimation to +3°C leads to lower exploratory behaviour in warm waters and lower overall activity levels during behavioural thermoregulation. In risky situations involving threat of predation, exploratory behaviour is often linked to boldness. Next I investigated exploratory swimming through two behavioural traits: ability to voluntarily enter a tunnel and subsequent swimming activity while being chased in a detour task. Detour tasks require a fish to swim down a narrow space and then detour to the left or right as they approach a barrier. A strong correlation between behavioural traits suggests the two behavioural traits are a good measure of risky swimming behaviour. With respect to the impact of warm acute temperatures, I observed an increase in strength of risky swimming behaviour following a rise in acute temperature of 6°C (+6°C). Finally, I examined burst swimming performance (C- and S-starts) during the detour task. While there was no observed effect of warm acute temperatures on most C- and S-start performance measures, there was a significant increase in S-start time to maximum velocity at +6°C. Along with the observed plasticity in strength of risky swimming behaviour, my

findings showed that acclimation to +3°C shifted physiological performance of escapes. My findings provide a greater understanding of round goby physiological and behavioural functioning during short- and long-term rises in temperature.

Keywords: Round goby; Temperature; Behavioural thermoregulation; Burst swimming; Risky swimming behaviour

Acknowledgements

I would like to give thanks to my supervisor, Dr. Glenn Tattersall, for his support and encouragement throughout my time in the Tattersall Lab. I am grateful for the many opportunities I have had to collaborate on various research projects, and it is my hope that we can continue to do so in the future.

There have been many people that have helped me along the way. I would like to thank all members of my research committee for their input throughout this process. Also, thank you to all current and past members in the Tattersall Lab, including: Ian Black, Curtis Abney, Philip Bartel, Susan Wang, and Kristen Schuppe. A special thanks to Anne Yagi and the MNRF for their expertise in the collection of gobies in the field, as well as Tom Eles for always being available to answer questions regarding animal care. Finally, I would like to acknowledge my fiancée and my family for all the help they provided along the way. I would not be where I am without them. To my late grandmother, Eileen Tomlinson: you have made such a huge impact on my life and I would like to thank you for all your words of wisdom. You inspired me to believe in myself and have the courage to achieve whatever I wanted in life. Thank you for everything.

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Chapter 1: General Introduction

Background

Fish living under varying environmental conditions are required to select temperatures that are optimal for physiological and behavioural functioning. Fish are also adept at learning the thermal characteristics of their environment and have an innate ability to avoid unfavourable environments (Garrity et al, 2010). Conditions of aquatic environments can change drastically over a short period of time. Fish that inhabit small bodies of water such as small rivers and creeks often experience diurnal and seasonal changes in temperature. The source of rapid rises in temperature can be from natural or anthropogenic sources, ultimately impacting fish survival if fish are unable to select appropriate temperatures (Coutant, 1975; Montgomery and Macdonald, 1990).

Aquatic animals must survive in the dynamic conditions associated with life in water, where environmental fluctuations rapidly change the temperature of entire bodies of water (Schmidt-Nielsen, 1997). This is especially true for aquatic ectotherms, such as fish, whose body temperature is equivalent to that of their surroundings (Huey, 1982). Changes in water temperature can thus lead to large changes in body temperature, which leads to impacts on performance traits such as swimming speed and aggressiveness (Crowley and Pietruszka, 1983; Temple and Johnston, 1998). Long-term temperature fluctuations are typically characterized as being diurnal, seasonal, or evolutionary (Lee, 1991). In contrast, short-term fluctuations in temperature occur at the microhabitat level through extreme weather changes or through anthropogenic events such as thermal plumes (Coutant, 1975; Montgomery and Macdonald, 1990). Changing environmental conditions can have detrimental effects on the health of a fish. Thus, depending on the species of fish, dramatic acute temperature changes may need to be avoided to avoid detrimental effects. The temperature that a fish selects is

also an important factor in predator-prey relations, reproduction, and migration (Crawshaw, 1974). Fish may choose to select more favourable temperatures by swimming to deeper water or migrating to larger bodies of water that are not as susceptible to extreme temperature changes (Berman and Quinn, 1991; Neverman and Wurtsbaugh, 1994). Research investigating the temperature a fish selects, and the overall impact of temperature change on behavioural and physiological traits, is vital for understanding fish survival in fluctuating environmental conditions.

Thermal Optimality of Performance Traits

The effect of an acute change in environmental conditions, as in the case of acute temperature change, on physiological performance typically follows what is known as a performance curve (Angilletta and Angilletta, 2009). The performance curve is bound by the critical maximum (CT_{max}) and minimum (CT_{min}) temperatures that define the zone of tolerance of a species (Brett, 1952). The range of temperatures between the thermal limits for a species is known as a species thermal tolerance (Brett, 1952). The impact of temperature on the kinetic energy of biochemical pathways ultimately causes physiological performance rates to change (Hochachka and Somero, 2002), and explains broadly the shape of the thermal performance curve. Performance of physiological functions is typically reduced at low temperatures, followed by a gradual rise in performance as temperature increases towards the optimum temperature (T_{opt}) (Angilletta and Angilletta, 2009).

Peak performance (P_{max}) occurs over a narrow range of conditions, referred to as the performance breadth. Performance breadth is operationally defined as the range of environmental values over which performance is greater than 80 or 90% of maximum (Angilletta et al., 2002). Following peak performance, there is a dramatic drop in performance as temperature approaches the upper thermal limit (Angilletta and Angilletta, 2009). Examples of performance traits that follow an optimality curve with acute

temperature change include heart rate, nerve conduction velocity, enzyme activity, growth rate, maximum swimming speed, and swimming endurance (Randall and Brauner, 1991; Myrick and Cech, 2000; Ojanguren and Braña, 2000; Lee et al., 2003). Rising or falling body temperatures impact metabolic rates, reflecting changes in the balance between ATP consumption and generation as well as the direct effects of temperature on ligand binding, diffusion and enzyme catalysis (Clarke, 2006). Temperatures above T_{opt} can limit oxygen availability through detriments in cardiovascular and ventilation functioning (Pörtner, 2010). Temperatures approaching CT_{max} can then lead to cell damage and denaturation of proteins (Angilletta and Angilletta, 2009). Certain species are able to survive in extreme temperatures that normally limit optimal functioning of performance traits, leading to variability in performance curves between species (Angilletta and Angilletta, 2009).

The thermal tolerance ranges between different fish species are typically defined by the width of their performance breadths, leading to species that are classified as either eurythermal or stenothermal (Pörtner et al, 2000). Generally, eurythermal fish species are able to function across a wide range of temperatures and thus have wider ranges between upper- and lower-avoidance temperatures than stenothermal species (Cherry et al, 1977; Gilchrist, 1995). The reduced sensitivity to environmental change enables eurythermal species to be thermal generalists, and thus potentially survive at extreme temperatures (Johnson and Bennett, 1995). In contrast, individuals of stenothermal species are specialized for more stable thermal conditions (Gilchrist, 1995). It is theorized that low- and high-temperature specialists have a higher maximum performance compared to generalists (Tattersall et al, 2012). Thus, the improvement of fitness at one temperature may theoretically lead to reduced fitness for thermal specialists at other temperatures (Levins, 1968, Palaima, 2007).

Behavioural Thermoregulation

There are two responses animals can have to fluctuating external environmental conditions: modifying behaviour and/or physiology to maintain a relatively constant internal environment (regulators) or allowing their internal environment to shift to that of the external environment (conformers) (Buckley et al., 2015). Regulators attempt to maintain homeostasis, defined as adjustments by an animal to keep the internal environment relatively constant (Angilletta and Angilletta, 2009; Jessen, 2012). In order to regulate body temperature, ambient temperatures are first detected by a sensory process. Behavioural response pathways are regulated through feedback and feedforward loops (Schneck, 1987; Modell, et al., 2015). A feedback loop is described as a pathway that involves a response to a stimulus, which then leads to a behavioural or physiological response to correct any deviation of the internal environment. In instances where there is a change to the ambient temperature, a compensatory response occurs that re-establishes internal thermal homeostasis (Schneck, 1987; Modell, et al., 2015).

Since ectotherms cannot rely on metabolic heat production to maintain thermally optimal body temperatures, ectotherms process the environmental temperature by triggering voluntary and involuntary responses in order to maintain a relatively constant body temperature (Heath, 1970). Regulating body temperature through the choice of thermal microhabitats following acute temperature change is known as behavioural thermoregulation (Crawshaw et al, 1985). Behavioural thermoregulation allows for optimal functioning of catalytic and metabolic processes and is thus a key determinant in ectotherm activity patterns (Grant, 1990). Fish, like other aquatic ectotherms, are particularly adept at selecting microclimates to avoid potential thermal stresses in their natural habitats (Crawshaw, 1979; Crawshaw et al, 1985). Simple taxes are an example of innate responses that help fish avoid lethal temperatures (Garrity et al, 2010). Thermotaxis, for example, described as the ability

to migrate up or down a thermal gradient (Garrity et al, 2010), innately aids young fish in escaping from extreme warm temperatures by swimming into deeper water that is often cooler in a typical body of water (Norris, 1963).

Fish will typically avoid temperatures that are well above or below optimal temperatures if more favourable temperatures are available (Giattina and Garton, 1982). Thermal avoidance behaviours are characterized by escape or aversive reactions that occur when encountering uncomfortable temperatures, typically referred to as upper- or lower-avoidance temperatures (Neill and Magnuson, 1974). Upper and lower avoidance temperatures are also referred to as the voluntary thermal minimum and maximum (Huey et al., 1989). An animal initiates behavioural thermoregulation by responding to sensory input from both peripheral and central thermoreceptors (Caterina, 2007). Neural centers in the hypothalamus are ultimately responsible for body temperature regulation (Berk and Heath, 1976). The concept of thermoregulatory set-points is often used to illustrate how homeostasis is maintained (Cabanac, 2006). According to control theory, temperature-sensitive neurons first send information about temperature senses to a controller in the brain. The current temperatures are then compared with set-points, which are represented by neurons in unknown manner. An animal choosing between warmer and cooler areas has both an upper and lower Tb set-point (USP and LSP, respectively) (Cabanac, 2006). For example, once warm temperatures become unsuitable for an individual, the animal then moves to a cooler location until it reaches its LSP. Thermoregulatory set-points allow an animal to prevent its body temperature from rising above the USP or falling below the LSP, resulting in the selection of temperatures within the optimal range (Cabanac, 2006). Therefore, set-point temperatures have been argued to reflect information about physiological performance breadth.

Shuttleboxes

Studying behavioural thermoregulation in natural settings is challenged by the numerous uncontrolled, non-thermal variables in the environment that can influence animal behaviour. For this reason, behavioural thermoregulatory responses in ectotherms have often been studied by observing thermal shuttling behaviour. The ambient temperature that stimulates shuttling between two temperature regions is referred to as the escape temperature. Lower and upper escape temperatures (LET and UET, respectively) are the lower and upper temperatures voluntarily experienced by an animal before they elect to move to an adjoining warm or cool environment (Black et al., 2019). Shuttleboxes, used to measure escape temperatures, are described as an experimental apparatus that allows an animal to have access to two adjacent thermal compartments separated by an opening (Black et al., 2019).

Early shuttlebox designs assessing avoidance temperatures were instrumental in the experimental design of later shuttlebox studies (Cherry et al., 1975; Cherry et al., 1977). The protocol was essentially the same as used in a shuttlebox, but electronic shuttleboxes eventually allowed the experimenter to continuously raise or lower the temperature of both chambers based on the animal's movement between chambers. The temperatures of the chambers are therefore controlled by the animal's escape temperatures, measuring important information on the upper and lower voluntary temperature thresholds of an individual (Black et al., 2019). Animals shuttle back and forth between temperature chambers to maintain internal body temperature within the limits of its preferred range (Black et al., 2019). While maintaining a constant temperature difference, the shuttlebox heats the water of both chambers at a steady rate when the warm chamber is occupied, and cools when the animal chooses the cool chamber. Through operant conditioning, the movement of the animal between chambers allows it to control the temperature of the environment, ultimately controlling their own body temperature (Black et al., 2019). Due to a minimum level of

activity required to shuttle between the chambers of a shuttlebox, assessing the precision of thermoregulatory behaviour of inactive or unmotivated individuals may be difficult (Black et al., 2019).

Thermal Acclimation

A fish's ability to behaviourally thermoregulate is vital to maintaining optimal body temperature, yet microhabitat selection is ineffective when the temperature of entire bodies of water are altered long-term (Huey, 1991). As temperatures change seasonally and year-to-year in a fish's habitat, long-term physiological adjustments ensure optimal functioning at the new body temperature (Hazel and Prosser, 1974). This long-term adjustment to chronic temperature change is referred to as thermal acclimation (Crawshaw et al, 1985). Thermal acclimation, a form of phenotypic plasticity, is a complex interaction between physiological, hormonal, and cellular processes (Crawshaw, 1979). It acts as a long-term physiological buffer when fish do not have the benefit of selecting different temperatures through behavioural thermoregulation. Thermal acclimation is known to cause shifts in optimal temperatures, lethal limits, temperature preference, and thermal avoidance behaviours (Huey and Berrigan, 1996).

There are several different acclimation responses an animal can demonstrate. Complete compensation involves restoration of physiological functioning from the previous optimal temperature at the new temperature (Bennett, 1990). Thus, complete compensation promotes physiological homeostasis under situations involving long-term fluctuations in temperature. An example of near complete compensation was demonstrated in work done on burst swimming speed in goldfish, with 35°C-acclimated fish shifting maximum burst speed compared to subjects acclimated to 10°C (Johnson and Bennett, 1995). Partial compensation, while demonstrating an improvement in physiological performance following acclimation, results in performance remaining below the original level (Bennett, 1990), and suggests limits

to the capacity for physiological adjustments. In inverse compensation, decreased physiological functioning continues to decline following acclimation. Finally, over-compensation is characterized as improved functioning above the original level (Bennett, 1990).

Escape Responses

Burst swimming during escapes is typically used to measure ectotherm performance in the context of temperature change and predator-prey relationships (Domenici and Blake, 1997). A fish's rapid movement during burst swimming is referred to as a fast-start response (Domenici and Blake, 1997). Fish are able to perform fast-starts when foraging for prey or escaping from predators. Fast-starts are known to thermally acclimate in many different fish species (Domenici and Blake, 1997). This rapid form of swimming is performed anaerobically, strictly through the activation of white muscle fibres (Johnson and Bennett, 1995). As opposed to slow-twitch oxidative "red" muscle use during sustained swimming behaviour, fast-twitch glycolytic "white" muscles are used during anaerobic swimming at high speeds through fast-starts (Marras et al., 2013). Activation of white muscles results in lactate accumulation, glycogen depletion, and ultimately fatigue (Driedzic et al, 1981; Dobson et al, 1987). Therefore, although fish are capable of utilizing fast-starts to swim at high speeds, anaerobic swimming is not sustainable for extended periods of time.

The two different types of fast-starts described in fish, C- and S-starts, differ in the extent of body movement during escapes from a stimulus (Domenici and Blake, 1997). C-starts involve activation of the muscles along one side of the body during an escape, while S-starts require muscles on both sides of the body to be activated (Spierts and Leeuwen, 1999). Fish utilize C-starts to evade predators that attack with high speed. Fish may also utilize S-starts to escape, but also use S-starts to attack prey (Domenici and Blake, 1997). Thus, only

C-starts are normally characterized as escape responses (Domenici and Blake, 1997). Since S-starts are performed when feeding (Harper and Blake, 1991), more precision during strikes is essential for predation success. In contrast, C-starts are performed at a faster speed and the head movement is performed at a much more dramatic angle compared to S-starts (Spierts and Leeuwen, 1999). Fast-starts comprise three stages: pre-propulsion, propulsion, and coastal swimming (Blake, 2004). There are two bursts of acceleration in fast-starts that include two tail-beats, one in each of the first two stages. During the first stage of S-starts, head movement coincides with rapid tail-fin movement that is performed at a considerable angle to the anterior axis of the fish, thereby muscles on both sides of the body produce a large thrust in the desired direction (Spierts and Leeuwen, 1999). Stage 1 of C-starts strictly involves rapid head movement in the direction of the escape (Spierts and Leeuwen, 1999). The absence of a second tail-beat is characterized by a much lower acceleration phase in stage 2 (Foreman and Eaton, 1993).

While C-starts are mediated by a simple neural circuit that is triggered by Mauthner cells, it is unknown what triggers S-starts (Bisazza et al, 1997; Bisazza et al, 2005). Mauthner cells are two large reticulospinal neurons, one on each side of the brain (Bisazza et al, 2005). The Mauthner cell circuit of both fish and amphibians are triggered by Mauthner cells, which is thought of as a simple neural circuit since there is no other sensory information required to complete the circuit once it has been triggered (Eaton and Emberley, 1991; Medan and Preuss, 2014). A single action potential in one of the two Mauthner neurons (MN), stimulated by either auditory or visual stimuli, activates motor networks on the contralateral trunk muscles and simultaneously inhibits ipsilateral muscles (Medan and Preuss, 2014). With respect to C-starts, this motor neuron excitement results in a c-shape bend of the body.

Escape responses are not the only occasions when fish utilise anaerobic muscles. Indeed, white muscle fibres are recruited increasingly at higher swimming speeds, when aerobic muscles are unable to provide the twitch rates required (Rome et al., 1984). These patterns in muscle activation are also influenced by temperature, since muscle fibres themselves have different temperature sensitivities. The nervous system controls the activation of motor units, recruiting more muscle fibres and faster muscle fibre types at low temperatures to compensate for the influence of temperature on muscle function (Rome and Alexander, 1990). This recruitment of muscle fibres while a fish attempts to maintain a constant swimming speed, recruiting red muscles followed by white muscles, occurs in the same order regardless of temperature. Red muscle fibres have higher power output compared to the reduced power of white muscle fibres at low temperatures (Rome et al., 1984). In order to swim at a constant speed while experiencing falling temperatures, motor units are recruited in a compressed manner. With compression of recruitment order, though motor units are recruited in the same order across all temperatures, motor units are recruited over a lower range of speeds at lower temperatures to compensate for the reduced power output (Rome et al, 1984). Thus, white muscles are more utilized in cooler temperatures (Rome et al., 1984). As temperatures increase, both red and white muscle fibres have higher power output. Performance of muscle fibres at these higher temperatures is strictly a function of the muscle properties (Rome et al., 1984).

White muscles require the use of ATP which is provided through anaerobic metabolism and endogenous substrates. There are two phases that occur during activation of white muscles. During the first brief phase, re-phosphorylation of ADP to ATP is catalysed by creatine phosphokinase (CPK) at extremely high rates (Driedzic et al, 1981; Dobson et al, 1987). ATP supply is thought to be functionally limitless during this phase. Consequently, power development of white muscles is restricted by maximal activity of the contractile

elements. During the second phase, a proportion of muscle glycogen stores are used. ATP supply is produced by anaerobic glycolysis of glucosyl residues to lactic acid (Driedzic et al, 1981; Dobson et al, 1987). As opposed to the initial phase, the latter phase of burst swimming activation is likely limited by maximal rates of glycolytic ATP. Therefore, due to power development only being restricted by contractile elements in the first phase, investigations on the impact of temperature change on escape responses in non-exhausted fish are primarily concerned with the first phase of white muscle activation (Kieffer and Tufts, 1996).

Previous research investigating the effect of environmental change on locomotor capabilities have primarily reported escape velocity without addressing the proportion of fish that responded to the threatening stimuli (Grigaltchik et al, 2012). Little is known about the decision-making process behind escape responses, yet the behavioural decision to escape is vital for survival during predator-prey encounters. Responsiveness is a key determinant in the survival of fish during a predatory attack, with unresponsive fish having increased susceptibility to predation (Domenici, 2010). Responsiveness is defined as the propensity of an individual to adjust its behaviour to the prevailing environmental conditions (*i.e*. rapidly responding to predator attacks by performing escape responses) (Wolf et al., 2007). Typically, responsiveness to a simulated predator attack is measured as a binary response, scored as either remaining motionless or rapidly escaping away from the stimulus. In addition to the type of stimulus, factors that influence the probability of escape responses include direction, speed, and distance of the stimulus in relation to the fish (Domenici, 2010). Investigating the responsiveness of fish through determining the likelihood to rapidly escape will give us a greater understanding of the role of behaviour in determining survival of prey during predator attacks.

Animal Personality - Boldness

It is often the ability to successfully forage or assess and respond to a threat that determines survival in fish (Werner et al., 1983). While risky situations require fish to rapidly respond to predators, behavioural flexibility is also crucial for the survival of animals living in complex environments. Behavioural flexibility, a form of plasticity, is defined as the capability of a subject to alter its behaviour by developing new responses to novel stimuli, or by modifying responses to familiar stimuli (Leal and Powell, 2011). Individuals with a high degree of behavioural flexibility are capable of changing their behaviour through learning, leading to modification of existing behaviours or invention of new ones (Reader and Laland, 2003). Behavioural flexibility is often associated with personality (Wolf et al., 2007). The heritable consistent individual differences in whole suites of correlated behaviours are collectively referred to as personality (Wolf et al., 2007) or behavioural syndrome (Sih et al, 2004).

Behavioural syndromes can be broken down into five main temperament traits: shyness-boldness, exploration-avoidance, activity, aggressiveness and sociability (Réale et al, 2007). Boldness is often assessed by the reaction of an individual to a novel object, predator, or conspecific. It is argued that an individual's reaction to a risky situation (*i.e.*, predator) lies within the shyness-boldness continuum, while exploration-avoidance is a reaction specifically to a new situation (Réale et al, 2007). Boldness also characterizes individuals that are 'geared up' in the presence of predators, with bold individuals remaining highly active or having a higher tendency to inspect predators (Sih et al., 2003; Ward et al., 2004; Quinn and Cresswell, 2005). Latency to emerge in a novel environment is most often considered a measure of boldness and not exploration behaviour (Cote et al, 2010). Indeed, boldness, aggression and exploration are thought to be correlated behaviours (Réale et al, 2007). Therefore, there is difficulty in differentiating between exploration and activity since animals

must demonstrate some level of activity during exploration, and bold animals certainly exhibit higher levels of activity (Réale et al, 2010).

An individual's boldness in novel situations may be assessed by exploratory tendency in risky situations, such as under threat of predation (Réale et al, 2007). The correlation between boldness and exploratory behaviour in novel environments, or risky swimming behaviour in open areas, has been demonstrated in numerous fish species (Budaev, 1997; Quinn and Cresswell, 2005; Jones and Godin, 2010; Boulton et al, 2014; Mazué et al, 2015; Moscicki and Hurd, 2015) (Table A.1). Furthermore, investigating exploratory and risky swimming behaviour while being chased in a simple maze may highlight the relevance of decreased freezing behaviour in bold animals by assessing responsiveness to simulated attacks (Quinn and Cresswell, 2005). Research into fish risky swimming behaviour involving more complex tasks could enhance our knowledge of escape behaviour between bold and shy animals and may be expected to produce novel results. Detour tasks require a fish to voluntarily swim down a narrow space and then detour to the left or to the right as they reach the end of the tunnel (Dadda et al., 2010). Making a turn in a detour task while escaping from net chases may resemble natural predator-prey encounters better than many other escape response assays, and thereby acquire data on interindividual differences that reflects a more natural trait.

Bold animals are also described in what is referred to as the proactive-reactive axis (Ward et al, 2004). Proactive individuals demonstrate aggressive behaviour, are bolder in decision-making under novel situations (Verbeek et al, 1994), disperse further distances (Dingemanse et al., 2003), outcompete others for food (Ward et al, 2004), and display dominance over submissive individuals (Careau et al, 2008). Meanwhile, reactive individuals are referred to as docile, submissive, and shy. Depending on the situation, there are costs and benefits to each behavioural type. Proactive individuals have a higher likelihood of engaging

in energetically costly behaviour, such as higher activity levels. They also typically have a higher growth rate and higher energy requirements (Ward et al, 2004). In the presence of predators, bold individuals take greater risks by either maintaining a high activity rate or by proactively inspecting predators (Ward et al, 2004).

Individual differences in physiological state play a vital role in determining different personality types, such as bold and shy animals. It is theorized that positive-feedback mechanisms are capable of maintaining consistent differences in an individual's state over time (Luttbeg and Sih, 2010). Differences in state, such as differences in physiology or experience, lead to behavioural differences among individuals that then act to reinforce initial state differences (Wolf et al., 2007). For example, certain individuals have a higher capability of defence and fleeing during predator attacks due to various factors, such as larger body size and more energy reserves. These individuals then encounter a lower predation risk while exhibiting bold behaviour (Luttbeg and Sih, 2010). Bold personalities can also be explained by the higher metabolic rate of bold animals, perhaps enabling a bold lifestyle that requires higher rates of activity (*i.e.*, exploratory behaviour in a novel environment) (Biro et al., 2009). Bold animals are a good example of the costs and benefits of an aggressive lifestyle as bold individuals have a greater capacity for activities that are more energetically costly (Metcalfe et al., 1995).

Round Gobies

Boldness in novel situations (*i.e.* non-native environments) may provide introduced species with a further advantage when competing for resources (Groen et al., 2012). The wider environmental tolerances of eurythermal species may increase their likelihood of survival and ability to become established as invaders (Olyarnik et al., 2009). As an invasive species in the Great Lakes region, the eurythermal round goby (*Neogobius melanostomus*) is known to survive across a wide range of temperatures (Kornis et al, 2012). The round goby

is a small, benthic freshwater fish that is native to the Black, Caspian and Azov seas (Kornis and Vander Zanden, 2010). The successful establishment of round goby populations in the Great Lakes can be partly attributed to a rapid population growth rate and superior competitive ability (Kornis et al, 2012).

Round gobies have a superior ability to outcompete native fish species in the region, and thus research on goby behavioural and physiological traits may provide insight into their further spread into smaller bodies of water (Kornis et al, 2012). Round gobies have been shown to survive in salinity levels ranging from 5 to 25 ppt (Karsiotis et al, 2012), high habitat contaminant levels (Young et al, 2010), high temperatures (critical thermal maximum 33.4°C) (Cross and Rawding, 2009), and low levels of dissolved oxygen (critical lethal threshold ranges from 0.4 to 1.3 mg/l) (Charlebois et al. 2001). Indeed, the wide environmental tolerance of round gobies enables them to survive and flourish in a range of different habitats. There is little prior research on the impact of acute temperatures on round goby physiology. Food consumption in round gobies increased with rising temperatures with peak performance between 23°C and 26°C (Lee and Johnson, 2005). Also, metabolic rate in round gobies peaks at ~30°C (Lee and Johnson, 2005; Drouillard et al, 2018), while the Q_{10} of round goby oxygen consumption was estimated to be 1.84 (Lee and Johnson, 2005). A recent analysis of the thermal tolerance and standard metabolic rate (SMR) of round gobies shed further light on the physiological functioning of the invasive species (Drouillard et al, 2018). Agreeing with the CT_{max} reported by Cross and Rawding (2009), round gobies demonstrated a thermal tolerance of 33.4°C (Drouillard et al, 2018).

Thesis Rational and Objectives

Acclimation effects permit temperature preference to track long-term changes in environmental conditions, allowing physiological traits such as burst swimming to remain

optimal (Huey and Berrigan, 1996). The wide thermal tolerance of eurythermal species means they have wider ranges between upper- and lower-avoidance temperatures. Since eurythermal fish species have previously demonstrated plasticity in physiological traits following acclimation to warm temperatures (Otto, 1973; Ford and Beitinger, 2005), I would expect avoidance temperatures in the eurythermal round goby to show plasticity. I therefore hypothesize that escape temperatures of round gobies during behavioural thermoregulation in a shuttlebox are plastic following thermal acclimation to warmer temperatures. I predict that escape temperatures in 24°C-acclimated gobies will be higher compared to gobies acclimated to 21°C.

Maximum burst velocity and acceleration are known to be plastic following acclimation to warmer temperatures with multiple fish species demonstrating complete compensation (Temple and Johnston, 1998; Webb, 1978; Wilson et al, 2000). With the majority of prior research focusing on burst performance at acclimation temperatures rather than acute temperature responses, the impact of warm acute temperatures on burst performance remains unclear. Following acclimation to high temperatures, there is a surprisingly negative impact of moderately warm acute temperatures on fish burst swimming performance at the upper range of thermal performance breadths (Johnson and Bennett, 1995; Temple and Johnston, 1998; Wilson et al, 2000; Grigaltchik et al, 2012) (Table A.2). Acclimation to different warm temperatures will give insight into the impact of warmer long-term temperatures on the preferred method of swimming in round gobies. I hypothesized that fast-start performance in round gobies is acclimatable to warm temperatures, from which emerges the prediction that gobies will show short-term temperature sensitivity in fast-start performance but similar fast-start velocities following acclimation to warm temperatures.

An individual's position along the proactive-reactive axis while experiencing novel situations may be assessed by exploratory tendency in risky situations, such as under threat of

predation (Réale et al, 2007). Furthermore, assessing the exploratory swimming behaviour while under threat of predation may further our understanding of freezing behaviour in risk-taking animals (Quinn and Cresswell, 2005). Assuming that interindividual differences are relatively stable over time, I predicted that there will be consistency in the level of exploratory behaviour while completing a detour task.

While a rise in acute temperatures increases aggressive behaviour, it remains unclear whether increased metabolic rates at warmer acute temperatures will lead to greater levels of exploratory behaviour in round gobies while under threat of predation (Crowley and Pietruszka, 1983; Brodie and Russel, 1999; Herrel et al, 2007; Wolf and Weissing, 2012). Assuming that the effect of rising temperatures on the underlying physiological mechanisms translates to behavioural changes, I predicted that the exploratory behaviour while being chased in a detour task will be impacted by acute temperatures. It is predicted that an increase in acute temperature will lead to greater levels of exploratory behaviour while completing a detour task. If interindividual differences are a fixed property of the individual, I would predict that thermal acclimation to even warmer temperatures would result in consistent changes in exploratory behaviour while being chased in a detour task compared to cooler acclimation temperatures.

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Chapter 2:
Temperature avoidance behaviour in the invasive round goby: impact of warm acclimation on swimming activity during behavioural thermoregulation

Introduction

Climate change may result in altered patterns in mean and extreme temperatures of lakes and streams, potentially providing favourable conditions for non-native aquatic species (Stillman, 2003). Unfavourable weather patterns caused by climate change may lead to further displacement of native fish species in the Great Lakes region. Indeed, the round goby, a non-native fish species to the Great Lakes, has demonstrated a superior ability to survive in a wide range of habitats. Round gobies already outcompete most native fish species of similar size and lifestyle, such as mottled sculpins (Kornis et al, 2012). While round gobies are known to have a wide environmental thermal tolerance based on their diverse habitat range (Charlesbois et al, 1997), research on the impact of temperature change on their behaviour and physiology is rather limited. The broad thermal tolerance of invasive species may facilitate invasions, especially when their thermal tolerances are greater than those of related, non-invasive species (Lockwood and Somero, 2011; Tepolt and Somero, 2014). Investigating round goby physiology and behaviour with respect to short- and long-term temperature changes may provide insight into the underlying reasons for the successful invasion of the Great Lakes region.

Eurythermal fish species, with their wide thermal tolerance, have wider ranges between upper- and lower-avoidance temperatures than that of stenothermal species (Cherry et al, 1977), which enables eurythermal species to survive at extreme temperatures. Fish are likely to preferentially select temperatures that are more optimal for physiological functions, such as feeding and reproduction (Angilletta and Angilletta, 2009). Thermal avoidance

behaviours, typically referred to as upper- or lower-avoidance temperatures (Neill and Magnuson, 1974), are characterized by escape or aversive reactions that occur when encountering uncomfortable temperatures. If there are more favourable temperatures available, fish will then typically avoid temperatures that are well above or below their thermal limits (Giattina and Garton, 1982). These avoidance behaviours are readily examined in the laboratory using dual choice temperature shuttle boxes (Schurmann et al, 1991). During shuttling behaviour, ectotherms alternate between warm and cool chambers in order to maintain internal body temperature within a narrow range. The temperatures that stimulate movement between chambers are known as escape temperatures (Barber and Crawford, 1979). The upper escape temperature (UET) is described as the highest temperature experienced by an ectotherm before moving from a warm to a cooler environment. Conversely, the lower escape temperature (LET) is the lowest temperature experienced by an ectotherm before moving from a cool to a warmer environment (Black et al., 2019).

Although they typically inhabit large bodies of water in their native habitat of the Ponto-Caspian region, round gobies now displace native fish species in small tributaries off of the Great Lakes (Phillips et al, 2003). Being a benthic fish species, the habitat of round gobies consists of a diverse array of structurally-complex aquatic environments (Ray and Corkum, 2001). Round gobies typically are most abundant in rocky habitats, where they spawn and feed (Kornis et al, 2012). Round gobies are also known to be a sedentary species which favours swimming in small bursts (Tierney et al, 2011). Round gobies, along with sympatric bighead gobies (*Ponticola kessleri*), tend to be inactive fish and spend 80 to 100% of their time remaining within shelters during short-term experiments assessing prey capture (Borcherding et al, 2013; Adrian-Kalchhauser and Burkhardt-Holm, 2016). Therefore, the sedentary nature of round gobies may make them difficult study subjects in a shuttlebox

experiment. One particular study investigated the inland dispersal of round gobies into Wisconsin tributaries of Lake Michigan (Kornis and Vander Zanden, 2010) and has suggested that round gobies prefer warm water in small tributaries, with water temperature significantly higher in streams invaded by gobies (23.9°C ± 3.5) compared to streams where they were absent (21.7°C ± 3.5). Interestingly, other field research on round goby abundance has reported nearshore water temperatures of 24°C to be the temperature at which peak abundance occurs (Dopazo et al, 2008). It is thought that many temperate-zone ectotherms live in environments that are cooler than their optimum (Huey et al, 2009), yet the lack of research on round goby physiology in their native habitat makes it difficult to predict the degree of inherent eurythermality or the plasticity in acclimation responses (Tepolt and Somero, 2014).

Questions and Hypotheses

Rising water temperatures may expand the geographical range of round gobies in North America and create more optimal environmental conditions in areas that the gobies already inhabit. Surface water temperature for Lake Erie, the Great Lake with the highest density of round gobies, is projected to rise by as much as 2.5°C to 3.5°C by the year 2100 (Nakicenovic et al, 2000; Trumpickas et al, 2008). The long-term increases in air temperature will especially impact the water temperature of small tributaries, such as the creeks in which round gobies now inhabit in the Great Lakes region. Investigation of the physiology and behaviour of round gobies following acclimation to unseasonably-warm temperatures will help expand our understanding of the potential impacts of climate change on native fish in the Great Lakes region. Since eurythermal fish species have previously demonstrated plasticity in physiological traits following acclimation to warm temperatures (Otto, 1973; Ford and Beitinger, 2005), I would expect avoidance temperatures in the eurythermal round goby to show plasticity. Stenothermal and eurythermal fish species native

to the Great Lakes have previously shown plasticity in thermal preference at the temperature gobies were found to be most abundant (Cherry et al, 1977; Dopazo et al, 2008). I therefore hypothesized that escape temperatures during behavioural thermoregulation in a shuttlebox would be plastic in the round goby, following thermal acclimation to warmer temperatures. I predicted that escape temperatures in 24°C-acclimated gobies would be higher compared to gobies acclimated to cooler temperatures.

Methodology

Subjects

All animals were wild-caught, juvenile round gobies kept at Brock University in an environmentally controlled room. Round gobies (*Neogobius melanostomus*) were caught in the Niagara River as well as creeks that flow into the river from Miller's creek (42.995°N, 78.976°W) and Usher's Creeks (43.052°N, 79.023°W). Fish were housed together in five- and ten-gallon tanks, in groups of between 10 to 20 individuals per tank. Enrichment was given in the form of rocks and polyvinyl chloride (PVC) pipes for hiding areas. Although the housing room was kept lit, the tanks provided cover, ensuring that water light levels were lower than room light. All fish were kept under a 12:12 light/dark cycle. Animals were fed a diet of NorthFin sinking fish pellets (cichlid formula), four times a week.

The focus of the present study was to investigate acclimation capacity of round goby behavioural thermoregulation using a shuttlebox. There were two experimental treatments: 21°C-acclimated subjects (21°C-acc; caught in 2016 and 2017) and 24°C-acclimated subjects (24°C-acc; caught in 2017). Fish were acclimated for ≥ 4 weeks at their respective temperatures. The sample size for 21°C-acc was 50 (2016: n = 31; 2017: n = 19) and for 24°C-acc (2017) was 25. The first cohort of 21°C-acc was caught in August of 2016, and subsequently experimented with in October and November of 2016. In addition, a group of

fish were caught in November and experimented with in December of 2016. The second cohort of 21°C-acc subjects and all the 24°C-acc subjects were caught in June of 2017, and subsequently experimented with in July and August of 2017. The average body length of gobies was 6.2cm ±0.13 (SE) for 21°C-acc (2016: 6.9cm ±0.09; 2017: 5.1cm ±0.12), while body length for 24°C-acc was 5.5cm ±0.09.

Apparatus

The shuttlebox consisted of two symmetrical, opaque white, circular chambers, connected by a narrow opening (3cm x 3cm) large enough for the fish to swim through (Figure 2.1). Each circular chamber had a diameter of 18cm and height of 16cm. Water in each chamber was maintained at a height of 9cm. A short ramp (3.5cm, length and width; height of 1.5cm) was installed to dissuade subjects from idling at the decision point. Black-coloured adhesive paper was applied directly above the narrow opening to provide a visual contrast for the fish to assist it in differentiating the opening from the light-coloured surroundings. Vertical aeration chambers were constructed directly beside the opening between the chambers, providing a space for air stones to be inserted to oxygenate the water during experimentation.

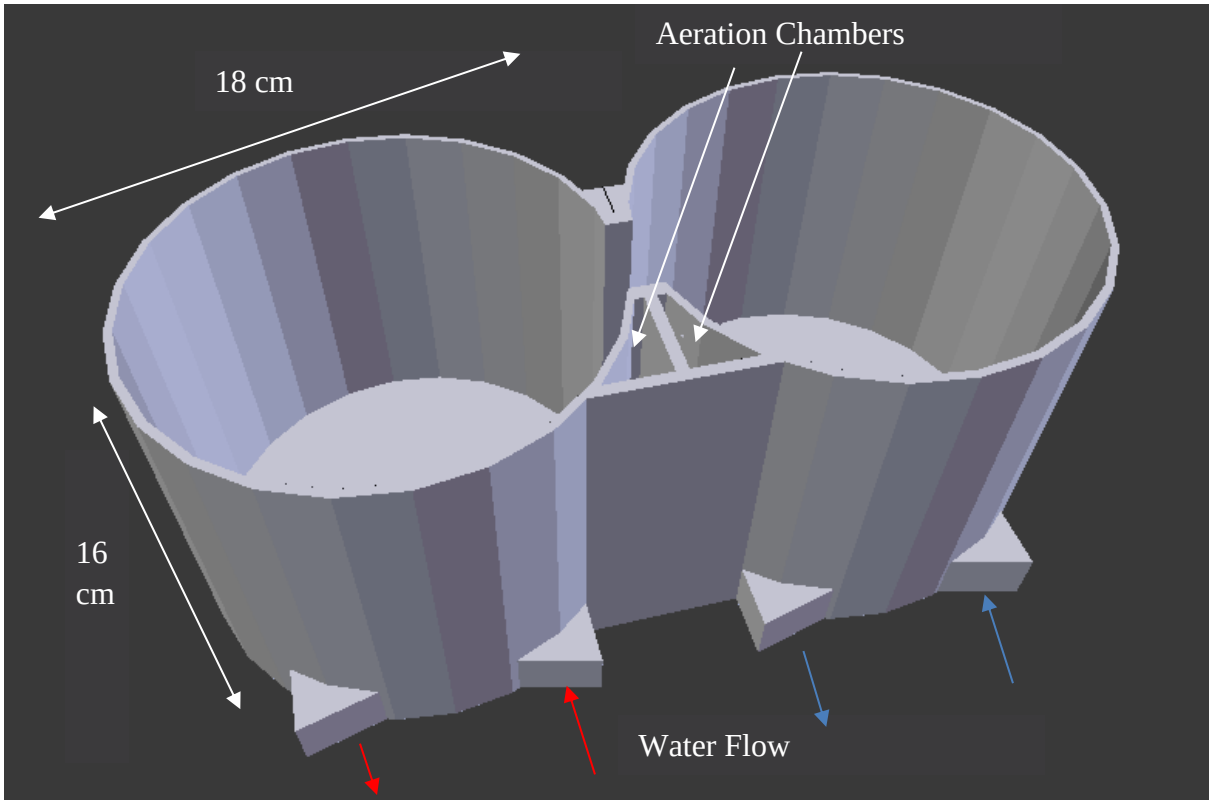


Figure 2.1. Schematic drawing of the shuttlebox used throughout experimentation. Each temperature chamber contains a vertical aeration compartment, constructed directly beside the opening between the chambers, to separate the continual water bubbling from the fish. Water is drawn out of the tank on the left side of each chamber and circulates back in on the right side (blue arrows resemble cool chamber input/output of water flow; red arrows resemble warm chamber input/output). Each chamber contains a temperature probe to allow for continuous regulation of the temperature of chamber. The chambers are separated with a divider (not shown) that only allows for a narrow opening (3cm x 3cm) large enough for fish to swim through. A short ramp (3.5cm, length and width; height of 1.5cm) was installed to dissuade subjects from idling at the decision point. Water was filled in each chamber to a height of 9cm. The interior of each chamber had a white-coloured background. Schematic drawing of the shuttlebox was provided by Mike Delange (Brock University Electronics Shop).

Water temperature was controlled by an automated computerised electronic controller. This system measured continuously the temperature in the shuttlebox through a probe that was placed along the interior wall of each chamber, which allowed for the two chambers to be heated independently (Tattersall et al., 2012). Two 13-Watt light bulbs were placed 20cm on either side of the shuttlebox, but below the top of the apparatus so that light was not directly shining on fish during experimentation. In addition, infrared lights were placed underneath the shuttlebox to help with accurate recordings of fish detection and to avoid glare on the water surface. A webcam was mounted 60cm above the apparatus. The software program ICFish v.2.2 (Brock University Electronics) tracked the fish through the webcam using edge detection. This was enabled by the contrast between the dark-coloured fish and the off-white background of the apparatus interior. The location of the fish was transmitted to the temperature controller (Brock University Electronics). The temperature controller managed the set-point temperature of each chamber and the direction of ramping, based on which chamber the fish occupied (Tattersall et al., 2012). The water temperature in each chamber of the shuttlebox tracked the chamber's set-point temperature, which was set to change at 0.2 °C /min, which provided a temperature change fast enough to elicit a behavioural response by the fish. One chamber was always maintained 3°C warmer than the other. In addition to an external waterbath that provided a capacity to cool the water, the temperature controller regulated the heaters, which provided heat when necessary.

Experimental Design

To investigate round goby avoidance temperatures, subjects were allowed to behaviourally thermoregulate in the shuttlebox by swimming between two temperature chambers (defined as a shuttle). The temperature at which fish shuttle from one chamber into the other was defined as the escape temperature. A shuttle from the warm chamber was defined as an upper escape temperature (UET), while a shuttle from the cool chamber was

defined as a lower escape temperature (LET). After an adjustment period, the temperatures were allowed to ramp up or down, depending on the location of the fish. Once a shuttle occurred, the automated system either continuously heated or cooled both chambers, depending on which chamber was chosen. If the fish was on the warm side, the temperature of both chambers increased; and if the fish was on the cold side, the temperatures decreased. Thus, since a 3°C difference between the two chambers was maintained throughout experimentation, a shuttle from the cold to the warm chamber resulted in continuous warming of both chambers, until a subsequent shuttle occurred into the cool chamber. Therefore, the shuttlebox allowed the fish to be operantly conditioned to associate warmer water with warming and cooler water with cooling, and over time, should learn to behaviourally thermoregulate. The fish constantly had two temperatures to choose from and the combination, or range of temperatures was constantly changing so that, over time, the fish regulated its own temperature.

On the day of experimentation, an individual fish was taken from a housing tank and placed in the shuttlebox. The side of the shuttlebox in which subjects were introduced prior to experimentation was also randomized. Experiments consisted of a one-hour adjustment period, followed by a 3-hour experimentation period (referred to as the ramping period). The adjustment period was provided to the fish to allow it to familiarise itself with the chamber, learn where the junction was located, and begin to discern temperature differences between the two chambers. The temperatures of the warm and cool chambers during adjustment were held constant at 1.5°C above and 1.5°C below acclimation temperature, respectively, to maintain a 3°C difference overall, with minor change from the acclimation temperature. Therefore, the temperature of the two chambers for 21°C-acclimated subjects was 19.5°C and 22.5°C during adjustment. The minimum and maximum allowable temperatures were set to 10 and 30°C.

Data Analysis

Movements of individual fish were first recorded in pixel (x, y) coordinates by the software program ICFish v.2.2 throughout experimentation. These coordinates (sampled every second) were then analysed in R in order to determine exact fish movement between the two temperature chambers of the shuttlebox. Analyses of adjustment and ramping were then broken down into 30-minute segments. When analysing the impact of thermal acclimation on avoidance temperatures, I also investigated the effects of fish size (Length), number of shuttles per 30 minutes (Shuttle Rate), and chamber that fish escaped from (Escape Chamber) on Escape Temperature (ET). The response variable ET consisted of both lower and upper escape temperatures, with Escape Chamber differentiating between the two types of escapes in the statistical analysis. Therefore, ET could be used as an approximation of thermal preference (*i.e.*, the average temperature between LET and UET). In order to investigate the activity levels of subjects during adjustment and ramping, I separately examined the effect of multiple terms on the overall number of shuttles throughout experimentation (Shuttles) and the ratio of distance swam in the warm chamber versus the cool chamber (warm : cool) (Ratio of Distance Swam Between Chambers). For example, a lower number for the ratio signifies shorter distances swam in the warm chamber compared to the cool chamber. Shuttles consisted of the total number of between chamber movements for each experimental phase, irrespective of escape type (LET or UET).

Statistical Methodology

Separate statistical analyses were done investigating the impacts of multiple terms on Escape Temperature, Shuttles, and Ratio of Distance Swam Between Chambers. With the exception of Escape Temperature, a separate analysis was done for both adjustment and ramping on each of the response variables. The data were summarized so that each statistical

analysis was done using a single value of each response variable for each fish averaged within each 30-minute interval. I performed a linear mixed-effects analysis when examining the effect of Acc. Treatment, Length, Escape Chamber, and Shuttle Rate on Escape Temperature using the package lme4 (Bates et al, 2015) in R version 5.3.1 (R Development Core Team, 2012). Linear models were used for the statistical analyses assessing the effect of Acc. Treatment and Length on Ratio of Distance Swam Between Chambers and Shuttles.

I first performed a Type II Wald Chi-square test for linear mixed model analyses on the full model to assess the impact of each fixed effect on Escape Temperature, using the Anova function in the car package (Fox and Weisberg, 2011) in R. F-tests were performed on the full models for the examination of the response variable Shuttles. Results for Ratio of Distance Swam Between Chambers were not normalized, therefore Kruskal-Wallis tests were performed on the full models. In instances where a fixed effect was found to be significant for the response variables Escape Temperature and Shuttles, a comparison of parameter estimates was done. To account for the repeated measures design when using linear mixed models, animal identification was included as random intercepts. Since the response variable Ratio of Distance Swam Between Chambers was not normalized, the data were ranked for the statistical analysis. In addition, the response variable Shuttles was not normalized and was thus logged for the analysis. Visual inspection of residuals and QQ plots from model fits did not reveal any obvious deviations from homoscedasticity or normality for each of the remaining statistical analyses in the present study.

Due to the lack of data on 24C-acc subjects in 2016, the first part of my statistical analysis investigated the effect of Cohort on each response variable. This was done by doing a Cohort comparison between 21°C-acc subjects caught in 2016 and 2017 (with Cohort as the only predictor variable). In instances where Cohort did not have a significant effect on the response variable, 2016 and 2017 21°C-acc subjects were grouped together for subsequent

analyses. Conversely, when Cohort had a significant impact on the response variable, the subsequent analysis was strictly done on 21°C-acc and 24°C-acc subjects caught in 2017.

Results

Shuttles

For the 21°C-acc subjects in the adjustment analysis, there was a significant effect of Cohort on Shuttles demonstrating a significant difference between 2016 and 2017 gobies ($p = 0.00135$, F-value = 10.88, df = 1) (Table 2.1). Thus, analysis was done by strictly comparing 21°C- and 24°C-acc in 2017. During the adjustment period, there was a significant effect of Acc. Treatment (24°C) on Shuttles ($p = 0.00687$, F-value = 7.68, df = 1) (Table 2.2). Further analysis showed that 24°C-acc gobies had a lower number of shuttles during the adjustment period (B = -22.4 Shuttles, $p = 0.011$) (Table 2.3, Fig. 2.2) (B values represent the parameter estimates). There was no significant effect of Cohort on Shuttles for the ramping period (Table 2.1). The results for ramping were similar to the adjustment period as there was a significant effect of Acc. Treatment on Shuttles ($p = 0.00098$, F-value = 11.31, df = 1) (Table 2.2). Further analysis showed that 24°C-acc gobies had a lower number of shuttles during ramping (B = -21.1 Shuttles, $p = 0.002$) (Table 2.3, Fig.2.2). Thus, 24°C-acc gobies exhibited lower activity levels during behavioural thermoregulation in both the adjustment and experimental periods.

Ratio of Distance Swam Between Chambers

There was no significant effect of Cohort on Ratio of Distance Swam Between Chambers, demonstrating no significant difference between 2016 and 2017 gobies (Table 2.1). Thus, I pooled the two cohorts for the subsequent analyses on Ratio of Distance Swam Between Chambers. For the adjustment period, there was a significant negative effect of Acc. Treatment (24°C-acc) on Ratio of Distance Swam Between Chambers ($p = 0.00676$, χ^2

= 7.182, df = 1) (Table 2.4, Fig. 2.3), demonstrating shorter distances swam in the warm chamber compared to the cool chamber for 24°C-acc gobies. Examples of fish detections in the shuttlebox illustrating the differences between individuals that swam short and longer distances in the warm chamber can be seen in Figure 2.4. The results for ramping were similar to the adjustment period as there was a significant negative effect of Acc. Treatment (24°C-acc) on Ratio of Distance Swam Between Chambers ($p = 0.00736$, $\chi^2 = 7.182$, df = 1) (Table 2.4, Fig. 2.3). Thus, 24°C-acc gobies swam shorter distances in the warm chamber compared to the cool chamber in both the adjustment and ramping periods.

Escape Temperature

An analysis of the effect of warm thermal acclimation on round goby behavioural thermoregulation was done by investigating voluntary escape temperatures in a Shuttlebox following acclimation to 21°C and 24°C. For the 21°C-acc subjects in the ramping analysis, there was no significant effect of Cohort on each of the response variables, demonstrating no significant difference between 2016 and 2017 gobies (Table 2.1). Thus, I pooled the two cohorts for subsequent analyses. Acc. Treatment had a significant effect on Escape Temperature ($p = 0.001$, $\chi^2 = 10.915$, df = 1) (Table 2.5). There was a significant effect of Shuttle Rate on Escape Temperature ($p < 0.0001$, $\chi^2 = 34.699$, df = 1). The 24°C-acc gobies had higher escape temperatures compared to 21°C-acc subjects (B = 2.74°C, $p = 0.001$) (Table 2.6, Fig. 2.5). There was a significant positive effect of Shuttle Rate on Escape Temperature (B = 4.43°C, $p < 0.001$) (Table 2.6), signifying that subjects with higher activity levels also had higher escape temperatures. In addition, there was no significant interaction between Acc. Treatment and Escape Chamber on Escape Temperature ($p = 0.0949$, $\chi^2 = 2.789$, df = 1) (Table 2.5).

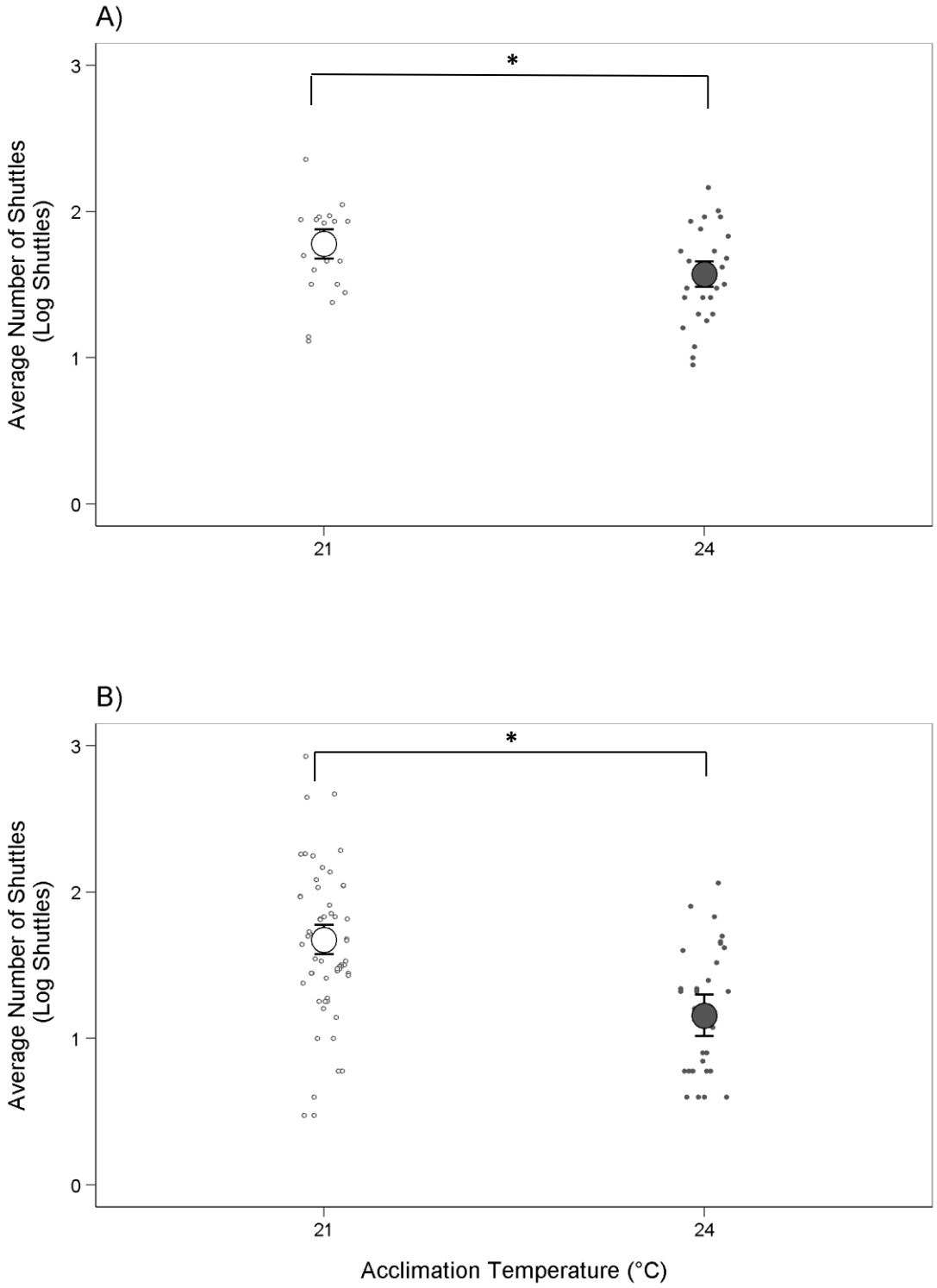


Figure 2.2. Average number of shuttles (Log Shuttles) for 21°C-acc and 24°C-acc round gobies during experimentation in the shuttlebox for the adjustment period (A) and ramping (B). The number of shuttles were observed over 1 hour in the adjustment period followed by the 3-hour duration of the ramping period. Subjects were acclimated to 21°C and 24°C. Error bars depict standard error of the model. Analysis showed a significant effect of Acclimation Treatment (24°C) on Shuttles during both the adjustment (B = -22.4 Shuttles, p = 0.011) and ramping periods (B = -21.1 Shuttles, p = 0.002). * represents significant effect.

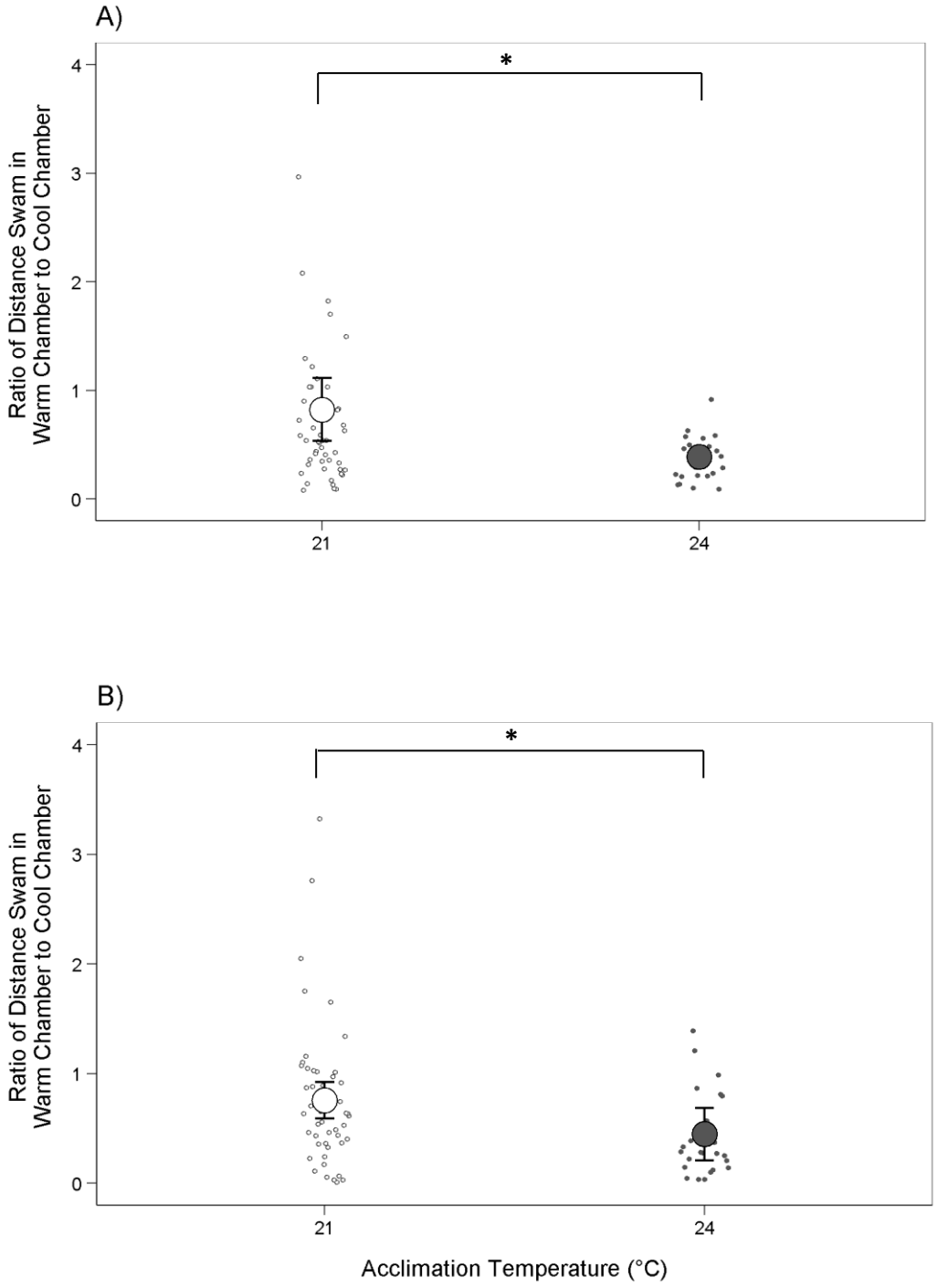
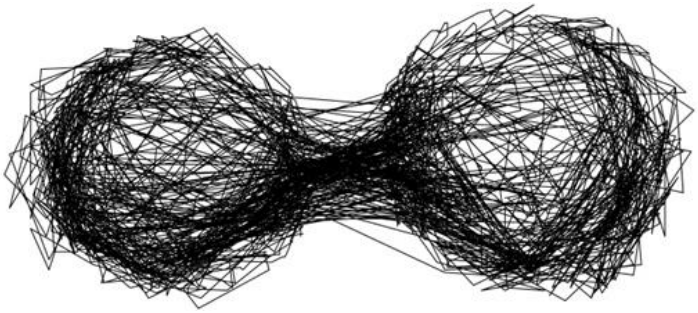
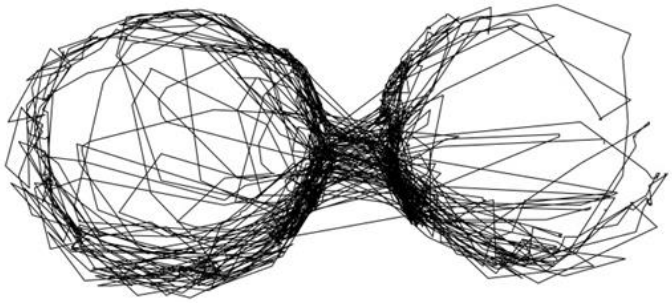


Figure 2.3. Ratio of the distance swam in the warm chamber versus the cool chamber (Ratio of Distance Swam Between Chambers) for 21°C-acc and 24°C-acc round gobies during experimentation in the shuttlebox for the adjustment period (A) and ramping (B). Distance swam was observed over 1 hour in the adjustment period followed by the 3-hour duration of the ramping period. Subjects were acclimated to 21°C and 24°C. Error bars depict standard error of the model. There was a significant effect of Acclimation Treatment on Ratio of Distance Swam Between Chambers during both the adjustment ($p = 0.00676$, $\chi^2 = 7.182$, $df = 1$) and ramping periods ($p = 0.00736$, $\chi^2 = 7.182$, $df = 1$). * represents significant effect.

A) Proportion of distance swam in the warm chamber of ~ 0.5



B) Proportion of distance swam in the warm chamber of ~ 0.3



C) Proportion of distance swam in the warm chamber of ~ 0.1

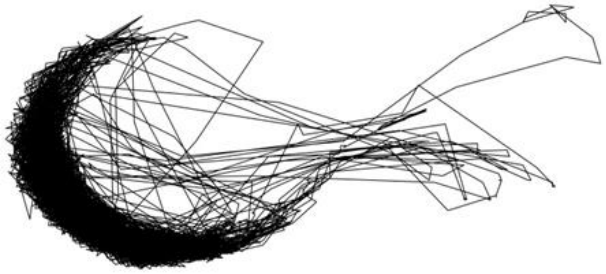


Figure 2.4. Detections of fish movement of each subject in the shuttlebox throughout experimentation. In these examples, the warm chamber is on the right and the cool chamber is on the left. The first example A) shows an active fish exploring the entire apparatus while behaviourally thermoregulating. The second example B) shows a less active fish that is mostly swimming in the area around the junction between the two temperature chambers. The third example C) shows a mostly non-thermoregulating fish . Note that examples B) and C) demonstrate individuals that swam shorter distances in the warm chamber (measured in the response variable Ratio of Distance Swam Between Chambers).

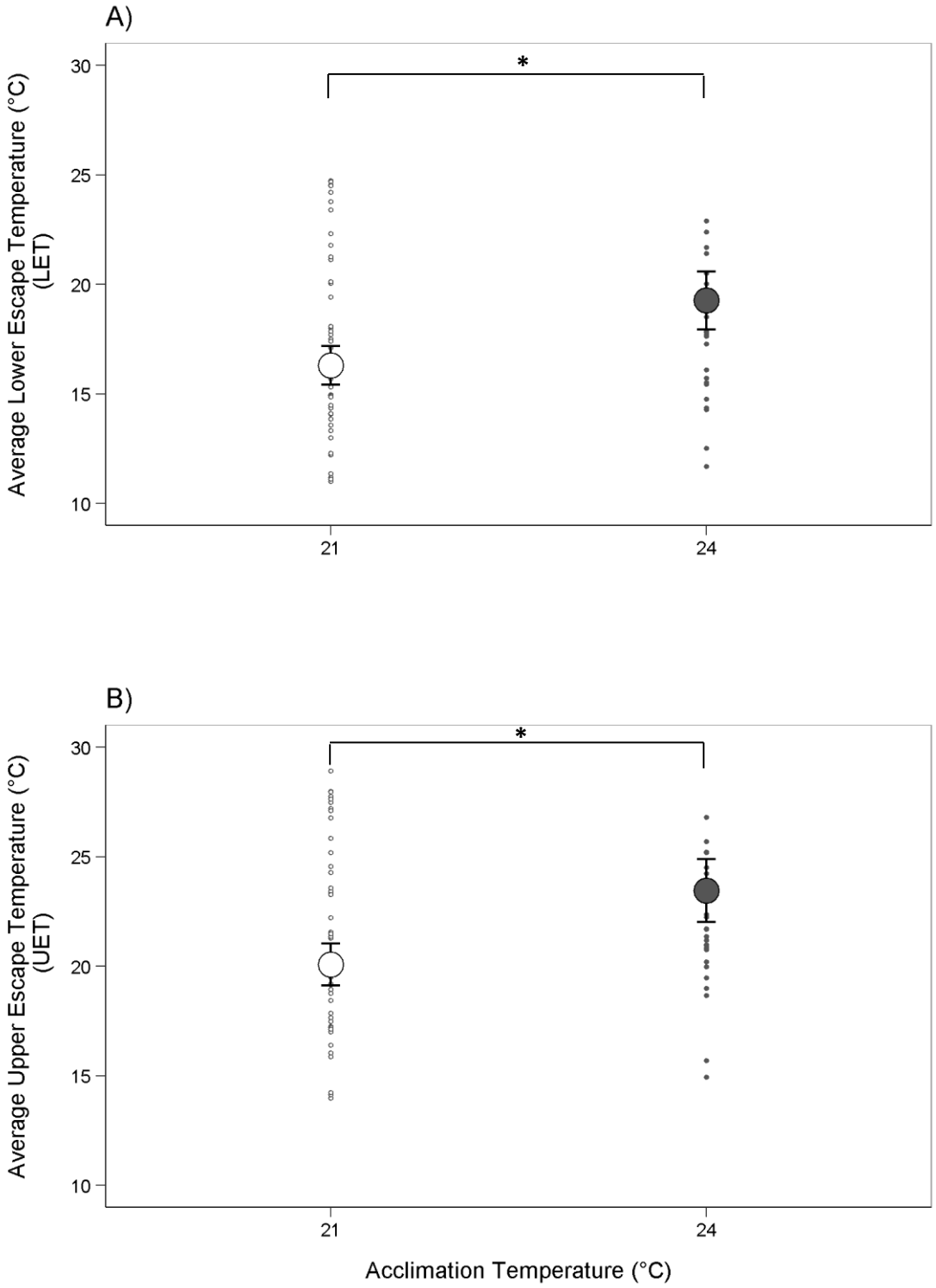


Figure 2.5. Average lower escape temperatures (LET) (A) and upper escape temperatures (UET) (B)for 21°C-acc and 24°C-acc round gobies during behavioural thermoregulation in the shuttlebox during the ramping period. Escape temperatures were observed over the 3-hour ramping period. Subjects were acclimated to 21°C and 24°C. Error bars depict standard error of the model. Statistical analysis of parameter estimates showed a significant effect of Acclimation Treatment (24°C) on Escape Temperature (B = 2.74°C, p = 0.001). * represents significant effect.

Table 2.1. Results for the F-tests and Type II Wald Chi-square tests examining the effect of cohort on round goby escape temperature (ET), Shuttle Rate, and Ratio of Distance Swam Between Chambers during both ramping and adjustment periods. Type II Wald chi-square tests were performed for ET, while F-tests tests were performed for Shuttles and Ratio of Distance Swam Between Chambers. Analysis includes both 21°C-acc gobies from 2016 and 2017. Response variables were observed over 1 hour in the adjustment period followed by a 3-hour ramping period.

			Ramping Period	
			Escape Temperature	
Predictors		*df*	χ^2 *value*	*p-value*
Cohort		1	3.2132	0.0731
			Shuttles	
Predictors	*Sum Sq*	*df*	*F-value*	*p-value*
Cohort	16822	1	0.8115	0.37
			Ratio of Distance Swam Between Chambers	
Predictors		*df*	χ^2 *value*	*p-value*
Cohort		1	0.00168	0.9673
			Adjustment Period	
			Shuttles	
Predictors	*Sum Sq*	*df*	*F-value*	*p-value*
Cohort	16687	1	10.882	0.00135
			Ratio of Distance Swam Between Chambers	
Predictors		*df*	χ^2 *value*	*p-value*
Cohort		1	0.0025	0.9601

Table 2.2. Results for the Type II Wald Chi-square tests examining the effect of warm thermal acclimation and Length on the number of overall shuttles (Shuttles) during the adjustment and ramping periods. Analysis includes both 21°C-acc and 24°C-acc treatments. The number of shuttles were observed over 1 hour in the adjustment period followed by a 3-hour ramping period.

Shuttles				
Ramping Period				
Predictors	*Sum Sq*	df	*F-value*	*p-value*
Acc. Treatment	158539	1	11.312	**0.00098**
Length	25943	1	1.8511	0.1757
Adjustment Period				
Acc. Treatment	12405	1	7.677	**0.00687**
Length	1583	1	0.98	0.325

Table 2.3. Linear model parameter estimates examining effect of warm thermal acclimation on the number of overall shuttles (Shuttles) for 21°C-acc and 24°C-acc round gobies during the adjustment and ramping periods. The number of shuttles were observed over 1 hour in the adjustment period followed by a 3-hour ramping period. Subjects were acclimated to 21°C and 24°C. Parameter estimate confidence limits are also presented.

	Shuttles		
	Ramping Period		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	532.6	236.4 – 828.7	**0.001**
Acc. Treatment (24°C)	-21.09	-34.5 – -7.7	**0.002**
	Adjustment Period		
(Intercept)	70.7	57.9 – 83.5	**<0.001**
Acc. Treatment (24°C)	-22.4	-39.4 – -5.5	**0.011**

Table 2.4. Results for the Kruskal-Wallis test examining the effect of warm thermal acclimation on the ratio of distance swam between chambers (Ratio of Distance Swam Between Chambers) for 21°C-acc and 24°C-acc round gobies during both the ramping and adjustment periods. Distance swam in the warm chamber was observed over 1 hour in the adjustment period followed by a 3-hour ramping period. Subjects were acclimated to 21°C and 24°C.

		Ratio of Distance Swam Between Chambers	
		Ramping Period	
Predictors	*df*	χ^2 *value*	*p-value*
Acc. Treatment	1	7.182	**0.007**
		Adjustment Period	
Acc. Treatment	1	7.337	**0.007**

Table 2.5. Results for the Type II Wald Chi-square test examining the effect of warm thermal acclimation and activity on round goby escape temperature (ET) during ramping. The other predictor variables included in the full model included Shuttle Rate, Escape Chamber, and Length. Analysis includes both 21°C-acc and 24°C-acc treatments. Escape temperatures were observed over the 3-hour ramping period.

	Escape Temperature		
Predictors	*df*	χ^2 *value*	*p-value*
log Shuttle Rate	1	34.699	**<0.0001**
Escape_Chamber (Upper)	1	749.631	**<0.0001**
Acc. Treatment (24°C)	1	10.915	**0.001**
Length	1	12.202	**0.0005**
Escape Chamber*Acclimation Treatment	1	2.789	0.0949

Table 2.6. Linear mixed-effects parameter estimates examining the effect of warm thermal acclimation on round goby escape temperature (ET) (LET and UET combined) during ramping. Analysis includes both 21°C-acc and 24°C-acc treatments. Escape temperatures were observed over the 3-hour ramping period. Parameter estimate confidence limits are also presented.

		Escape Temperature	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	4.61	-0.59 – 9.80	0.082
log Shuttle Rate	4.43	2.94 – 5.91	**<0.001**
Acc. Treatment (24°C)	2.74	1.12 – 4.37	**0.001**
Length	1.37	0.60 – 2.13	**<0.001**
Escape_Chamber (Upper)	3.76	3.48 – 4.03	**<0.001**

Discussion

The focus of this study was to examine the effect of warm thermal acclimation on round goby behavioural thermoregulation. Behavioural thermoregulation was achieved by requiring subjects to voluntarily escape from gradually warming or cooling water temperatures within an operant conditioning environment. I observed that round goby escape temperatures were plastic following acclimation to warmer temperatures, with +3°C acclimation (24°C-acc) leading to a nearly equivalent (2.7°C) shift of escape temperatures towards warmer temperatures (Fig. 2.5). Interestingly, the effect of thermal acclimation was non-significant when shuttle rate was removed from the full model, suggesting that the level of activity between acclimation treatments was an important factor in determining the plasticity of escape temperatures. The shift in escape temperature following warm acclimation agrees with substantial evidence on the impact of thermal acclimation on temperature selection in ectotherms (Cherry et al, 1977). Furthermore, native stenothermal and eurythermal fish, species that co-occur with round gobies in the Great Lakes, raise their thermal preference following acclimation to 24°C compared to 21°C-acc individuals (Cherry et al, 1977). It is important to note that the interaction between acclimation treatment and escape chamber did not have a significant effect on escape temperature, suggesting that 24°C-acc gobies do not show a wider voluntary performance breadth.

In order to successfully behaviourally thermoregulate in a shuttlebox, fish must actively escape temperatures by shuttling to the opposite thermal chamber. Evidence of this was demonstrated by the significant impact of shuttles per 30 minutes on escape temperature. Due to the inherent design of a shuttlebox, stationary fish will cause the shuttlebox to warm or cool to extreme temperatures. Extreme cool temperatures will induce lethargy if an individual remains stationary in the cool chamber for an extended period of time (Black et al., 2019). This slowdown in function may result in the cool chamber becoming a “cold trap”,

thereby increasing the difficulty in attempts to successfully escape to the warm chamber (Cadena and Tattersall, 2009). Therefore, it is expected that low activity drives the apparent low thermal preference.

My observations of individual activity levels throughout the ramping period included a lower number of shuttles in 24°C-acc compared to 21°C-acc (Fig. 2.2). This lower activity level observed in 24°C-acc gobies was supported by a lower ratio of distance swam in the warm chamber compared to the cool chamber during ramping (Fig. 2.3). Interestingly, both of these trends were also observed at stable temperatures as 24°C-acc showed a lower number of shuttles and a lower ratio of distance swam in the warm chamber compared to the cool chamber during the adjustment period (Figures 2.2 and 2.3). Since the shuttlebox is a novel environment, shorter distances swam at stable temperatures implies lower exploratory swimming into warm waters. It is especially surprising that 24°C-acc had lower activity levels overall and lower exploration levels in the warm chamber during adjustment when the warm chamber was only 1.5°C warmer than acclimation temperature. During the experimental period, subjects would often position themselves close to the tunnel between chambers following a shuttle into the warm chamber (personal observations) (Fig. 2.4), which could be explained by individuals preparing to shuttle. Lurking near the entry to the cool chamber in anticipation of the warm side becoming too hot would allow individuals to rapidly escape when deciding to shuttle. Round gobies are well-equipped to employ this behaviour as the fused pelvic fin allows individuals to remain stationary while experiencing rapidly moving waters while on the bottom (Kornis et al, 2012).

It was rather unexpected that acclimation to a moderate change in temperature of +3°C led to decreased activity during behavioural thermoregulation and lower exploration levels in areas with warmer water. These findings were revealed despite no observed statistical differences between 21°C- and 24°C-acc in escape performance and strength of

risky swimming behaviour (see Chapter 3 and 4). Increasing strength of risky swimming behaviour is only possible if fish are capable of adequate aerobic metabolic rates required for sustained swimming (Biro and Stamps, 2010; Quinn and Cresswell, 2005). Thus, in conjunction with known increased ectothermic activity rates in warmer temperatures (Claireaux et al, 1995), it is not likely that behavioural thermoregulation of round gobies following acclimation to 24°C was limited by decreased aerobic functioning. A review of round goby research on physiological functioning at warmer temperatures also supports the view that gobies are not limited aerobically at temperatures above 24°C (Lee and Johnson, 2005; Drouillard et al, 2018). Rate of food consumption in round gobies increases with rising acute temperatures until a drop off in consumption above 26°C (Lee and Johnson, 2005), and standard metabolic rate (SMR) has also been shown to increase exponentially with increasing temperatures up to at least 30°C (Drouillard et al, 2018). In the context of previous findings, I would not expect physiological limitations in round gobies acclimated to 24°C.

It is possible that 24°C-acc subjects demonstrated a trade-off in that reduced activity in warmer water ultimately meant that overall energy expenditure was not increased, given how increased SMR is known to occur in warmer temperatures through Q_{10} effects (Drouillard et al, 2018). By exploring the cool chamber more than the warm chamber, individuals would ultimately be able to lower, or at least, minimize their overall increase in metabolic rates. Alternatively, 24°C-acc gobies may have simply chosen to minimize metabolic rate rather than optimize certain physiological traits during behavioural thermoregulation (Killen, 2014).

Conclusions and Perspectives

Climate change may intensify the spread of invasive species in non-native environments (Gherardi et al., 1987). Surface water temperature for Lake Erie, the Great

Lake with the highest round goby population density, is projected to rise by as much as +2.5°C to +3.5°C by the year 2100 (Nakicenovic et al., 2000; Tremupickas et al., 2008). My findings showed that round gobies had slightly lower-than-expected average escape temperatures in each acclimation treatment. This suggests that gobies prefer cooler temperatures following acclimation to warmer temperatures. Given that round goby physiological functioning remains optimal at temperatures above 21°C, the findings of the present study suggest that goby temperature selection does not coincide with the optimal temperature for certain physiological traits (*i.e.* food consumption rate) (Lee and Johnson, 2005; Drouillard et al, 2018). Furthermore, the lower exploratory behaviour in warm waters and lower overall activity levels both at stable and fluctuating temperatures for gobies acclimated to +3°C suggests that gobies are more suited to acclimate to cooler water temperatures. My findings provide insights into the behavioural thermoregulation strategies of an invasive benthic fish species, furthering our understanding of round goby temperature selection with exposure to long-term temperatures projected with climate change.

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Chapter 3:
Assessing the Effects of Short- and Long-term Temperature Change on Round Goby Fast-start Performance

Introduction

The ability of animals to maintain physiological functioning during environmental fluctuations is vital for survival during predator-prey encounters. This is especially true for eurythermal species living in non-native habitats, where a high level of physiological functioning allows them to potentially outcompete native species (Kornis et al, 2012) with narrower thermal tolerances. Eurythermal fish species are thought to be more capable of withstanding dramatic changes in temperature that would normally be considered thermally stressful (Temple and Johnston, 1998). Such species are able to function across a wide range of temperatures, exhibiting a reduced sensitivity to environmental change, and thus they are often considered thermal generalists (Johnson and Bennett, 1995). In contrast, individuals of stenothermal species are specialized for more stable conditions (Gilchrist, 1995). The tolerance of generalists to environmental fluctuations is thought possible by the higher plasticity in physiological performance compared to stenothermal species (Somero and Dahlhoff, 2008; Jayasundara and Somero, 2013). Generalists also typically have wider thermal performance breadths. Due to a specialist-generalist thermal plasticity trade-off, the improvement of fitness at one temperature may lead to reduced fitness at another temperature (Levins, 1968, Palaima, 2007). Thus, generalists typically have a reduced level of performance at optimal temperatures compared to thermal specialists. As an invasive species in the Great Lakes region, the eurythermal round goby is known to survive across a wide range of temperatures (Kornis et al, 2012). Round gobies have a superior ability to outcompete native fish species in the region, thus research on their physiological capabilities

may deepen our understanding of how gobies have successfully spread to all of the Great Lakes (Kornis et al, 2012).

Fish, like other aquatic ectotherms, are particularly adept at selecting microclimates to avoid potential thermal stresses (Crawshaw, 1979). Small bodies of water can experience short-term fluctuations in temperature through extreme weather changes or through anthropogenic events (Coutant, 1975; Montgomery and Macdonald, 1990). Extreme weather events can change the temperature of the entire body of water within hours. For example, the creek tributaries which round gobies inhabit off the Niagara River have been shown to increase by 6°C over a period of 48 hours (Anne Yagi, unpublished data from MNRF). As ectotherms, fish should select temperatures that are optimal for physiological parameters such as oxygen consumption, muscle functioning during swimming, and cardiovascular functioning (Angilletta and Angilletta, 2009). The impact of temperature on the kinetic energy of biochemical pathways ultimately causes physiological performance rates to change. Biochemical and physiological processes are two major determinants of which temperature range a fish selects in order to function efficiently (Coutant, 1987). Performance traits typically follow an optimality curve in response to acute temperature changes (Angilletta and Angilletta, 2009), wherein performance of physiological function is typically reduced at low temperatures followed by a gradual rise in performance as temperature increases. Peak performance occurs at the optimum temperature (T_{opt}), followed by a dramatic drop in performance as temperature approaches the upper thermal limit (Angilletta and Angilletta, 2009). Thus, the performance curve has a peak performance (P_{max}) that occurs over a narrow range of conditions, referred to as the performance breadth. As rapid increases in temperature are likely to occur more frequently with climate change, it is important to investigate the impact of warm acute temperatures at the upper range of thermal performance breadths.

Previous research has expanded our knowledge of the effect of acute temperature change on escape response (Webb, 1978; Johnson and Bennett, 1995; Temple and Johnston, 1998; Wilson et al, 2000; Grigaltchik et al, 2012), swimming endurance (Ojanguran and Brañta, 2000), and sustainable swimming performance (Fry and Hart, 1948; Randall and Brauner, 1991; Myrick and Cech, 2000; Lee et al, 2003). Increasing rates of oxygen consumption allow for aerobic swimming speeds to increase until a plateau at optimal temperatures before a drop off in performance occurs as temperatures approach the upper critical temperature, CT_{max}, above which survival is zero (Fry and Hart, 1948). One hypothesis for the drop off in aerobic swimming performance at warmer temperatures is ultimately due to a mismatch between oxygen demand and capacity to supply oxygen by the cardiovascular system (Pörtner and Knust, 2007). With respect to anaerobic swimming performance, it is unknown whether burst swimming performance is limited at temperatures in the upper range of thermal performance breadths.

Round gobies prefer to swim by burst swimming, reflective of their bottom-dwelling preference in water bodies (Kornis et al, 2012). Burst swimming is performed anaerobically, powered by white muscle fibres, and involves fish utilizing fast-starts (C- and S-starts) to move at a rapid speed (Domenici and Blake, 1997). Prior studies strictly examined burst performance at acclimation temperatures without considering acute temperature responses (Johnson and Bennett, 1995; Temple and Johnston, 1998; Wilson et al, 2000; Grigaltchik et al, 2012). Thus, the impact of warm acute temperature on burst swimming performance remains unclear. Q_{10} effects on biochemical reaction rates predict that the rate of muscle contraction should increase with a rise in acute temperatures (Johnston, 1980). Burst swimming performance at the upper range of thermal performance breadths reveals a surprisingly negative impact of moderately warm acute temperatures on fish burst swimming performance following acclimation to high temperatures (Johnson and Bennett, 1995; Temple

and Johnston, 1998; Wilson et al, 2000; Grigaltchik et al, 2012) (Table A.2). Australian bass (*Macquaria novemaculeata*) acclimated to 25°C have shown no change in burst velocity following a rise of 5°C in acute temperatures (Grigaltchik et al, 2012), while the eurythermal eastern mosquitofish (*Gambusia holbrooki*) acclimated to the same temperature revealed lower escape velocities when at +5°C above acclimation temperature (Grigaltchik et al, 2012). Results for the eurythermal goldfish also showed a trend to lower C-start maximum velocity at 40°C after thermal acclimation to 35°C (Johnson, 1995) compared to performance when tested at 40°C. While goldfish acclimated to 35°C avoid temperatures between 35°C-40°C, mosquitofish species have an upper avoidance temperature of ~36°C following acclimation to 24°C (Hoyland et al, 1979; Cherry et al, 1976). This suggests that the lower escape velocities of eastern mosquitofish at +5°C above the acclimation temperature of 25°C was not due to exposure to uncomfortable temperatures (Grigaltchik et al, 2012). Thus, the lower escape velocities observed in multiple fish species in Table A.2 cannot be entirely explained by ectotherms being tested at temperatures well above expected preferred temperatures, but rather suggest that burst swimming speed is either temperature insensitive or is expected to generally decline with acute temperature exposure.

Thermal acclimation acts as a long-term physiological buffer when fish do not have the benefit of selecting different temperatures through behavioural thermoregulation (Crawshaw, 1979). Prior studies have shown that thermal acclimation potentially causes shifts in optimal temperatures, lethal limits, temperature preference and thermal avoidance behaviours (Huey and Berrigan, 1996). Specifically, thermal compensation, the process whereby acclimation shifts performance and thereby mitigates the influence of acute temperature changes, allows for the optimal temperature to shift in parallel to the acclimation temperature (Angilletta and Angilletta, 2009). Some of the earliest research on the effect of temperature on burst swimming performance demonstrated that both burst swimming

maximum velocity and acceleration are acclimatable traits (Webb, 1978), meaning that they shift performance when animals undergo long-term exposure to temperatures. Later research expanded on these findings and showed that bottom-dwelling fish species acclimated to 15°C, but tested at 20°C, had similar maximum velocities and accelerations compared to the group acclimated to 20°C and tested at 20°C (Temple and Johnston, 1998). These findings suggested that warm acclimation led to changes in metabolic gene expression and enzyme activity, and thereby supports a complete metabolic compensation (Kingsolver and Huey, 1998). Acclimation of physiological capacity in burst swimming could be considered beneficial since it may compensate for potentially negative influences of environmental change (Tattersall et al, 2012). Complete compensation of burst swimming has also been demonstrated in other aquatic ectotherms, such as in adult striped marsh frogs and Pacific tree frog tadpoles (Watkins, 2000; Wilson et al, 2000).

Temperature has direct impacts on the biomechanical and contractile properties of white muscle fibres (Bennett, 1990; Hochachka and Somero, 2002). Previous research on isolated white muscle fibres at temperatures at the upper range of thermal performance breadths does not clarify why burst swimming performance did not increase following acclimation to higher temperatures (Johnson and Bennett, 1995; Wakeling et al, 2000). Warm acute temperature change does not appear to have a negative impact on myosin ATPase activity in white muscles as Q_{10} values were at or above 2 for both 10°C- and 20°C-acc treatments (Johnson and Bennett, 1995). Increases in acute temperatures result in faster contraction rates in isolated white muscle fibres regardless of acclimation temperature (Wakeling et al, 2000). While there is a lack of research on the impact of acute temperatures on Mauthner neurons, intracellular recordings of MN in 25°C-acclimated fish showed reduced strength and duration of inhibitory inputs compared to 15°C-acclimated subjects (Szabo et al, 2008). Meanwhile, earlier research showed cool acute temperatures leading to

an increase in probability of triggering an escape response, with fish experiencing a 10°C decrease in temperature within a 50-minute time period (Preuss and Faber, 2003). Given the seeming thermal sensitivity of anaerobic swimming performance at the whole-organism level following acclimation to higher temperatures (Table A.2), a discrepancy between white muscle and whole-organism performance may be the result of neurophysiological constraints rather than muscle fibre constraints.

Questions and Hypotheses

Previous research of acute temperature effects on anaerobic swimming performance in aquatic ectotherms at the whole-animal level has shown conflicting results following acclimation to warmer temperatures (Johnson and Bennett, 1995; Temple and Johnston, 1998; Wilson et al, 2000; Grigaltchik et al, 2012). Thermal sensitivity to warm acute temperatures following acclimation to warmer temperatures is surprising considering that isolated white muscle fibres appear to demonstrate enhanced function at warm acute temperatures (Wakeling et al, 2000). By quantifying round goby escape performance at warm acute temperatures, a better understanding of the thermal sensitivity of burst swimming in a eurythermal species can also be gained. Acclimation to different warm temperatures will also give insight into the impact of warmer long-term temperatures on the preferred method of swimming in round gobies. Previous research has demonstrated that burst swimming performance is plastic following acclimation to warmer temperatures (Webb, 1978; Temple and Johnston, 1998). I hypothesized that fast-start performance in round gobies is acclimatable to warm temperatures, from which emerges the prediction that gobies will show short-term temperature sensitivity in fast-start performance but similar fast-start velocities following acclimation to warm temperatures.

Methodology

Subjects

The focus of the present study was to investigate the effect of short- and long-term temperature change on fast-start performance (C- and S-starts). The methodology for collection of subjects is identical to the one described in Chapter 2. There were 3 experimental groups: 21°C-acc, 24°C-acc and a Control (time-based, held at 21°C across the three days of testing). Depending on acclimation treatment, fish were acclimated for ≥ 4 weeks at either 21°C or 24°C. The Control treatment was acclimated to 21°C and served to assess the presence of non-thermally mediated changes in swimming behaviour over the 3 days of measurements. Both thermal acclimation treatments were conducted for two separate years of study. The first cohort was caught in August of 2016 and tested in October and November 2016. A second group of fish was caught in November and tested in December 2016. The second-year cohort was caught in June 2017, and tested in July and August 2017. The sample sizes for each statistical analysis (outlined in Statistical Analysis section) are summarized in the Table 3.1. The average total body length of gobies caught for 21°C-acc was 5.73 cm ± 0.17, 24°C-acc was 5.89 cm ± 0.23, and Control was 5.34 cm ± 0.16.

Table 3.1. Sample sizes of juvenile round gobies for control analysis and analysis of impact of warm acute temperatures on fast-start performance. Analysis includes comparison between 21°C-acc and 24°C-acc treatments. Note: the sample sizes listed here for 21°C-acc and 24°C-acc represent the maximum number of subjects used for the C- and S-start analyses.

Fast-Start Type	Year Caught	Control	21°C-acc	24°C-acc
C-Start	2016	-------	11	7
	2017	11	5	8
S-Start	2016	-------	13	8
	2017	11	4	9

Apparatus

To investigate the effect of short- and long-term temperature changes on burst swimming performance, gobies were observed performing a detour task while being repeatedly chased with a fish net. A detour task can be defined as a task that requires a fish to swim down a narrow space and then detour to the left or to the right as they approach an opaque barrier (Dadda, 2010). The detour task experiment was conducted in a T-maze (Figure 3.1). The apparatus consisted of two open areas on either side of a tunnel (46.9cm x 34.2cm). A removable barrier was placed at the end of the tunnel. The barrier consisted of yellow vertical bars allowing subjects to examine the open area beyond the tunnel, as used in previous studies examining detour behaviour (Bisazza et al, 1997; Bisazza et al, 1998). In order to provide incentive for fish to swim through the tunnel and subsequently make a turn, a PVC pipe was placed on the other side of the barrier. In order to record fish movement in the T-maze, a GoPro camera was used at a height of 60 cm above T-maze. In addition to this, two 13-Watt light bulbs were placed 75 cm above the tunnel of the T-maze.

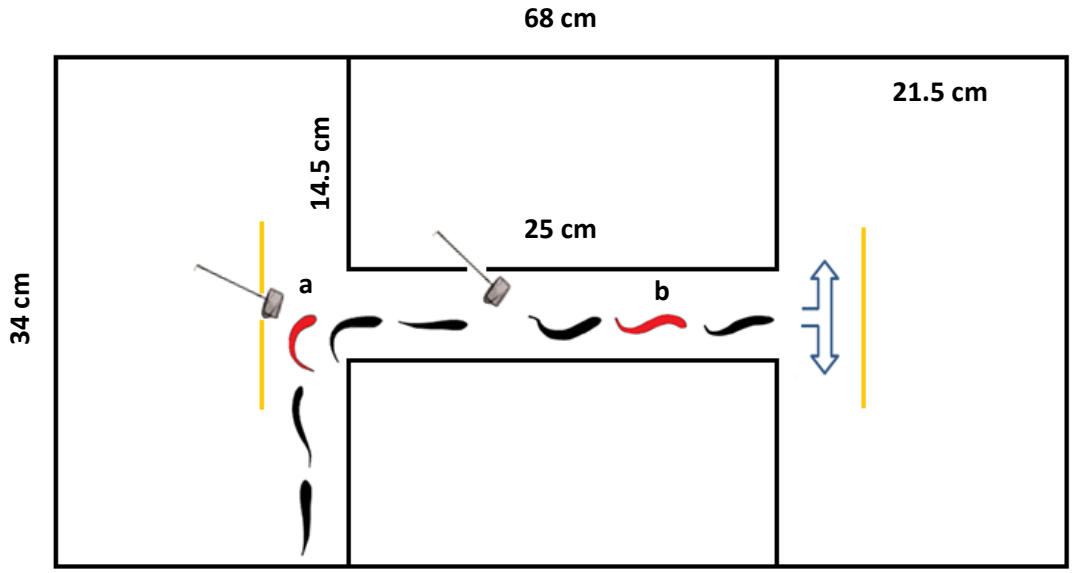

Figure 3.1. Diagram of the T-maze apparatus used during experimentation. Once fish were in the proximity of the tunnel entrance, fish were startled towards the tunnel entrance. Fish responded to net chases near the tunnel entrance by swimming into the tunnel either through a C-start (a) or routine swimming. Subjects were continuously chased until subjects completed a turn at the barrier positioned at the end of the tunnel. Fish often performed S-starts (b) to rapidly escape from net chases while swimming through the tunnel.

Experimental Design

Each fish was subjected to 10 trials per day (referred to as a session) in the detour task. One session was conducted on each of the 3 consecutive days of experimentation. In all treatments, experimentation on Day 1 was performed at the respective acclimation temperature. The acute temperature treatments consisted of: Day 1 at the acclimation temperature, Day 2 at +3^{o}C above acclimation temperature, and Day 3 at +6^{o}C above the acclimation temperature. In total, there were three treatments for this experiment: Control (all 3 days at 21^{o}C), 21^{o}C acclimated (Days 1-3: 21^{o}C, 24^{o}C, 27^{o}C), and 24^{o}C acclimated (Days 1-3: 24^{o}C, 27^{o}C, 30^{o}C). Sample sizes for each experimental treatment are shown in Table 3.1. Temperature was increased over the course of 3 hours before experimentation (exploration period). For the 21°C-acc and 24°C-acc treatments, there was a temperature increase of 1^{o}C/hr throughout the exploration period. Control subjects experienced no change in temperature during the 3-hour period, nor across the 3 days of trials.

On the first day of experimentation, an individual fish was taken from a housing tank and placed in the T-maze. After experimentation on Day 1, subjects were returned to a housing aquarium and maintained overnight at the same temperature experienced on that day of experimentation. The overnight holding tanks held one to two fish. After 24 hours the same subject was exposed to the next acute temperature treatment (Day 2). Again, subjects were housed overnight at the same temperature experienced that day (+3^{o}C for acute temperature change treatments). After 24 hours, the subject experienced +6^{o}C above the original acclimation temperature (Day 3).

An exploration period of three hours was allowed in one of the open areas of the T-maze before detour tasks commenced. Entrance to the tunnel was blocked with a white-coloured partition during this period. The most successful method for getting fish to enter the

tunnel was by guiding the fish towards the tunnel entrance. This was done by chasing the typically wall-hugging subjects with a small fish net along the walls immediately before the tunnel entrance. Once the individual was close to the tunnel entrance, fish were startled into the tunnel. It was at this point where subjects either performed a C-start or swam into the tunnel with routine swimming behaviour. Once fish entered the tunnel, I continually chased the fish with the net until the subject completed a turn upon leaving the tunnel at the opposite end. Multiple net chases were needed in some instances, since gobies prefer to swim in short bursts. In order to encourage continuous movement through the tunnel, net chases were mostly executed when fish slowed down or stopped in the tunnel. Subjects that were the most responsive to net chases either continually swam through the tunnel or performed S-starts to propel themselves to the barrier before making a turn. When S-starts were observed, only a single S-start was performed. A maximum of six chases was done before the trial was terminated and considered a failure. Once a fish made a turn at the barrier and entered the open area, I blocked the tunnel with a partition to prevent the fish from moving through the tunnel between trials. The fish was then given a resting period of two minutes between trials.

Analysis of Fast-Start Maximum Velocity and Time to Maximum Velocity

C-starts, performed at the tunnel entrance, always preceded S-starts, which occurred during swimming behaviour in the tunnel (Figures 3.2 and 3.3). Each trial only ever consisted of a single C-start. Multiple S-starts could occur in the tunnel, but only the first S-start performed was analysed in these instances. Fast-starts with only one tail beat were measured. Measurements were not done on fast-starts that involved an individual hitting the wall at any point during movement. In addition, the occurrence of fast-starts were not quantified if the net accidently touched the body of the fish during a chase.

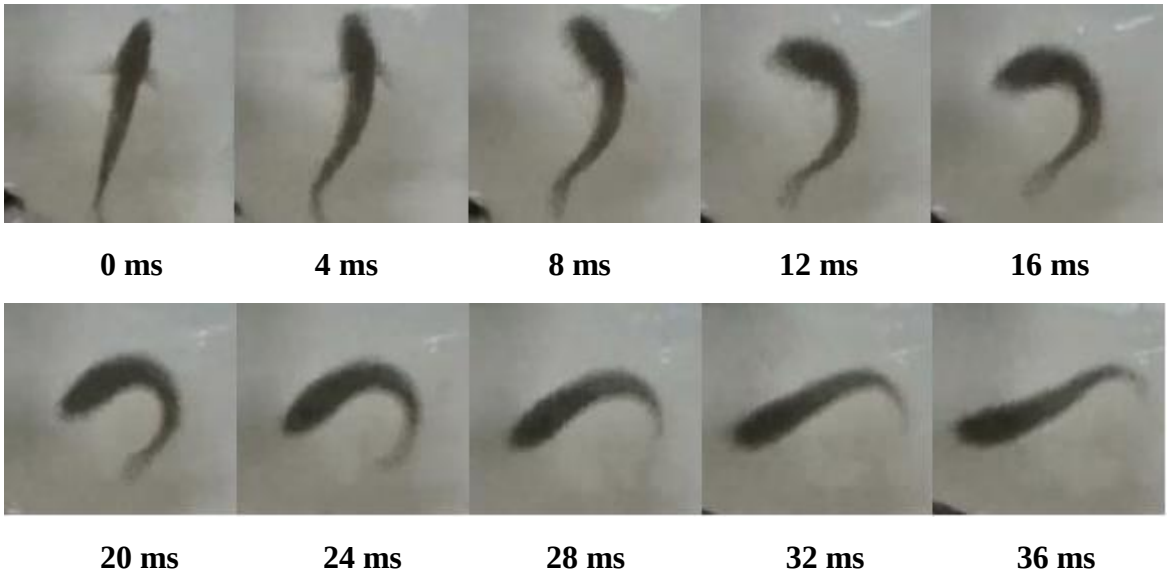

Figure 3.2. The C-start of a round goby during experimentation. The above images demonstrates stages 1 and 2 of a C-start, with the goby escaping to the left in response to an attack from the right side. Stage 1 shows the goby bending into a characteristic C-shape as the head rapidly in the direction of the escape (stage 1 ending at 20 ms), followed by a propulsive stroke in the opposite direction of the predator attack during stage 2. Maximum velocity of C-starts occurred ~20 ms from the initial head movement (during stage 1).

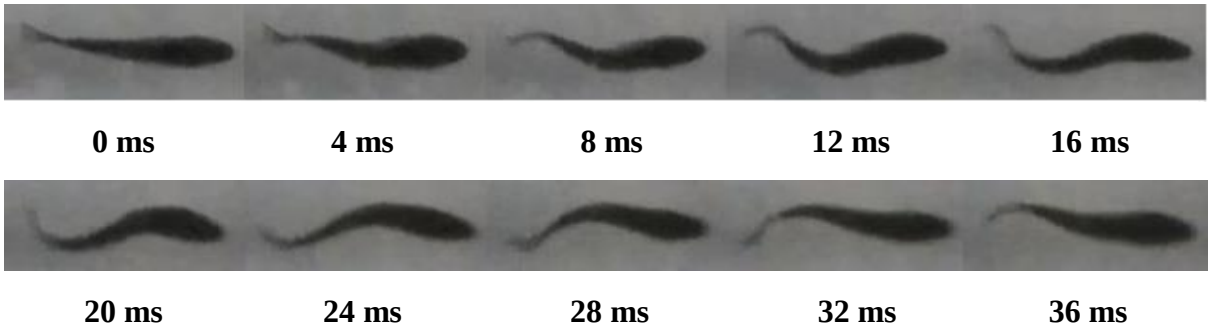

Figure 3.3. The S-start of a round goby during experimentation. The above images demonstrate stages 1 and 2 of an S-start, with the goby escaping to the right in response to an attack from the left side (or attacking a prey to the right). Stage 1 involves both the head and tail-fin movement occurring simultaneously, resulting in a bend of the body into the characteristic S-shape (stage 1 ending at 20 ms). This is followed by a propulsive stroke either in the opposite direction of the predator attack or in the direction of the prey during stage 2. Maximum velocity of S-starts occurred within 40 ms from the initial head movement, immediately following the bending of the fish into an S-shape (during stage 2).

T-maze swimming behaviour was recorded at 240 frames per second using a GoPro camera. Two types of fast-starts, C-starts and S-starts, were analysed for maximum velocity (MV; cm/s) and time to maximum velocity (Time to MV; ms). Time to maximum velocity has previously been considered an inverse measure of acceleration (Wilson et al, 2000). C-starts typically reached maximum velocity in a much shorter period of time compared to S-starts, therefore, only the first 60 ms of S-starts and 20 ms of C-starts were analysed, respectively.

Velocity and Acceleration Measurements

Relative motion of the head of each fish was tracked using the Manual Tracking plug-in in ImageJ in order to obtain a set of cartesian coordinates (x, y) for each frame of the video (4 ms resolution). The x and y coordinates were then subsequently converted to MV and Time to MV measurements. First, the slope and intercept of the tunnel length was calculated using the x and y coordinates and the actual length of the tunnel in the following equations:

$$Slope = \frac{Actual\ Length\ of\ Tunnel\ (cm)}{Change\ in\ Coordinates\ of\ Start\ and\ End\ of\ Tunnel\ (x_2 - x_1)}$$

$$Intercept = y\ Coordinate - (Slope\ \times (x\ Coordinate))$$

Then, utilizing the slope and intercept, x and y coordinates of the displacement that occurred during fish movement each 4 ms were each converted to swimming distance (cm) in the following equation:

$$Distance\ (x\ or\ y\ Coordinate) = Slope\ \times (x\ or\ y\ Coordinate) + Intercept$$

Finally, the displacement between 4 ms time intervals (from i = 2 to the total number of intervals) was calculated as the hypotenuse transcribed over time as:

$$D_i = \sqrt{(x_i - x_{i-1})^2 + (y_i - y_{i-1})^2}$$

where D_i is the total distance swam per time interval. This value was then divided by the video sample rate (4 ms) to calculate instantaneous swimming velocity (cm/s). The

maximum instantaneous swimming velocity and the time taken to reach maximum velocity from the moment of first head movement were then recorded for each experimental session. Thus, since I examined relative motion of the head of each subject, maximum velocity and time to maximum velocity were used as a proxy for muscle contraction rate.

Statistical Analysis

Differences in Performance between Fast-start Types at Acclimation Temperature

The data were summarized so that each statistical analysis was done using a single value of MV or Time to MV averaged across the 10 trials in each session (one session occurred each day). For each analysis in this chapter, I first performed Type II Wald Chi-square tests on the full model to assess the impact of each fixed effect on each Fast-start performance, using the ANOVA function in the car package (Fox and Weisberg, 2011) in R version 5.3.1 (R Development Core Team, 2012). In instances where a fixed effect was found to be significant, a comparison of parameter estimates was done. To account for the repeated measures design, animal identification was included as random intercepts. Visual inspection of residuals and QQ plots from model fits did not reveal any obvious deviations from homoscedasticity or normality for each statistical analysis in the present study.

Statistical analysis was done to compare performances between C- and S-starts when tested at acclimation temperature. Linear mixed-effects analysis was performed on the overall impact of thermal acclimation and fast-start type on MV and Time to MV. Linear mixed effects analysis was performed using the package lme4 (Bates et al, 2015) in R. As part of the analysis, Acute Temperature (Acute Temp), Acclimation Temperature (Acc. Treatment), and Cohort were considered in the full model as fixed effects.

Effect of Warm Acute Temperatures on Fast-Start Performance

Two separate analyses were done on C- and S-start MV and Time to MV: the potential impact of temporal change (day-to-day) on fast-start performance at a constant temperature of 21°C, and the specific impact of warm thermal acclimation with warm acute temperature change. Control data were analysed separately since these subjects were only collected in one season and were not randomized within the timeframe of the acclimation treatments. I then examined the impact of warm thermal acclimation and warm acute temperature change on the performance of both C- and S-starts.

As part of the analysis, I investigated the fixed effect of Day on MV and Time to MV for both Fast-start types. The next part of the analysis investigated the impact of warm acute temperatures on the performance of each Fast-start type. Acute Temperature (Acute Temp), Acclimation Temperature, Cohort, and the interaction between Acclimation Temperature*Acute Temp were considered in the full model as fixed effects. Linear mixed-effects analysis was performed on the overall impact of thermal acclimation and acute temperature change (0, +3, and +6 °C) on C- and S-start performance.

Results

Direct Comparison Between C- and S-start Performance

An analysis of the difference in physiological performance between fast-start types was done by investigating C- and S-start max velocity (MV) and time to max velocity (time to MV) of gobies when tested at their respective acclimation temperature (Day 1). C-starts had an average MV of 144.9 cm/s and S-starts an average MV of 99.5 cm/s (21°C-acc and 24°C-acc combined) when tested at acclimation temperature. There was a significant effect of Fast-Start type (S-start) on Fast-start MV ($p < 0.0001$, $\chi^2 = 132.15$, df = 1) and Fast-start time to MV ($p < 0.0001$, $\chi^2 = 46.47$, df = 1) (Table 3.2). S-starts had lower MV compared to

C-starts (B = -44.90 cm/s, p = <0.001) (Fig. 3.4, Table 3.3). Also, S-starts had slower Time to MV compared to C-starts (B = 61 ms, p = <0.001) (Fig. 3.5, Table 3.3). Therefore, performance of C-starts was better overall compared to S-starts. Acc. Treatment did not have a significant effect on MV (p = 0.547, χ^2 = 0.3636, df = 1) or Time to MV (p = 0.585, χ^2 = 0.298, df = 1) (Table 3.2).

Effect of Constant Temperatures Across Days on Fast-start Performance

In order to investigate the impact of warm acute temperature change on fast-start performance, I first assessed whether performance changed between experimental days with constant temperature conditions (Control). There was no significant effect of Day on C-start MV (p = 0.735, χ^2 = 0.616, df = 2) or C-start Time to MV (p = 0.556, χ^2 = 1.174, df = 2) (Table 3.4). Similarly, there was no effect of Day on S-start MV (p = 0.745, χ^2 = 0.584, df = 2) or S-start Time to MV (p = 0.468, χ^2 = 1.611, df = 2) (Table 3.4). Based on analysis of the trend between trials, there was no apparent learning effect of repeated trials within a session on performance of either fast-start type (Fig.C.1, C.2, C.3, C.4). In summary, fast-start performance did not change within sessions or over a 3-day period under constant temperature conditions.

Effect of Impact of Warm Acute Temperatures on Fast-start Performance

An analysis of the effect of thermal acclimation on physiological performance following exposure to warm acute temperatures was done by investigating C- and S-start performance following a rise in acute temperatures. There were no significant interaction effects between Acc. Treatment and Acute Temp on C- and S-start MV or Time to MV (Table 3.5). There was no significant effect of Acc. Treatment on C- and S-start MV or Time to MV (Table 3.5). While there was no significant effect of Acute Temp on C- and S-start MV (p = 0.1514, χ^2 = 3.775, df = 2; p = 0.1193, χ^2 = 4.25, df = 2) or C-start Time to MV(p =

0.5237, χ^2 = 1.29, df = 2), the effect of Acute Temp on S-start Time to MV was approaching significance (p = 0.0553, χ^2 = 5.79, df = 2) (Figures 3.6 and 3.7, Table 3.5). Thus, I further analysed S-start Time to MV to examine the effect of each rise in acute temperature. While the +3°C acute temperature had no effect (B = 2.12 ms; CI = -3.10 – 7.33; p = 0.426), S-starts had faster Time to MV at +6°C (B = 5.93 ms; CI = 0.52 – 11.35; p = 0.032) (Fig. 3.7, Table 3.6). Thus, 21°C-acc and 24°C-acc subjects are likely to have a 6 ms increase in S-start Time to MV at +6°C compared to testing at acclimation temperature. Interestingly, there was a significant negative effect of Cohort (2017) on C-start Time to MV (B = -7.19 ms, p <0.001) (Table 3.7). Cohort did not have a significant effect on Time to MV when subjects were tested only at their acclimation temperature (p = 0.473, χ^2 = 0.516, df = 1) (Table 3.2).

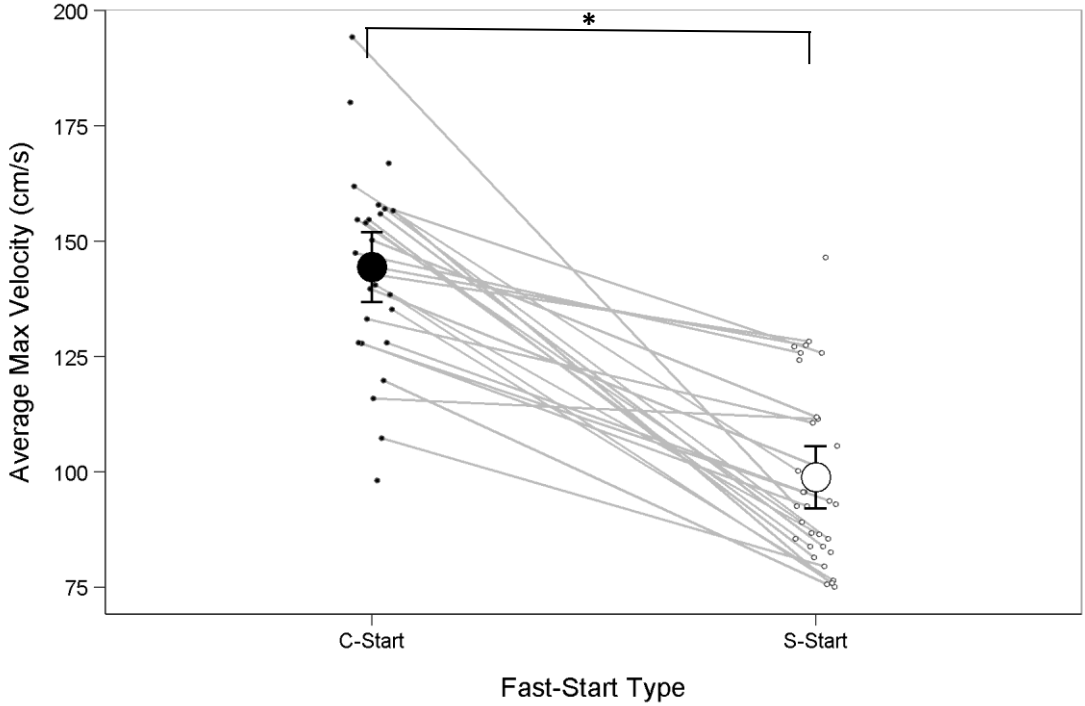

Figure 3.4. Average Maximum Velocity (MV) between C- and S-starts for both 21°C-acc and 24°C-acc treatments when tested at the respective acclimation temperatures (Day 1). Lines depict the difference between C- and S-start performance for each individual fish. Error bars depict standard error of the model. Statistical analysis of parameter estimates showed a significant effect of Fast-Start type (S-start) on MV (B = -44.90 cm/s, p = <0.001). * represents significant effect.

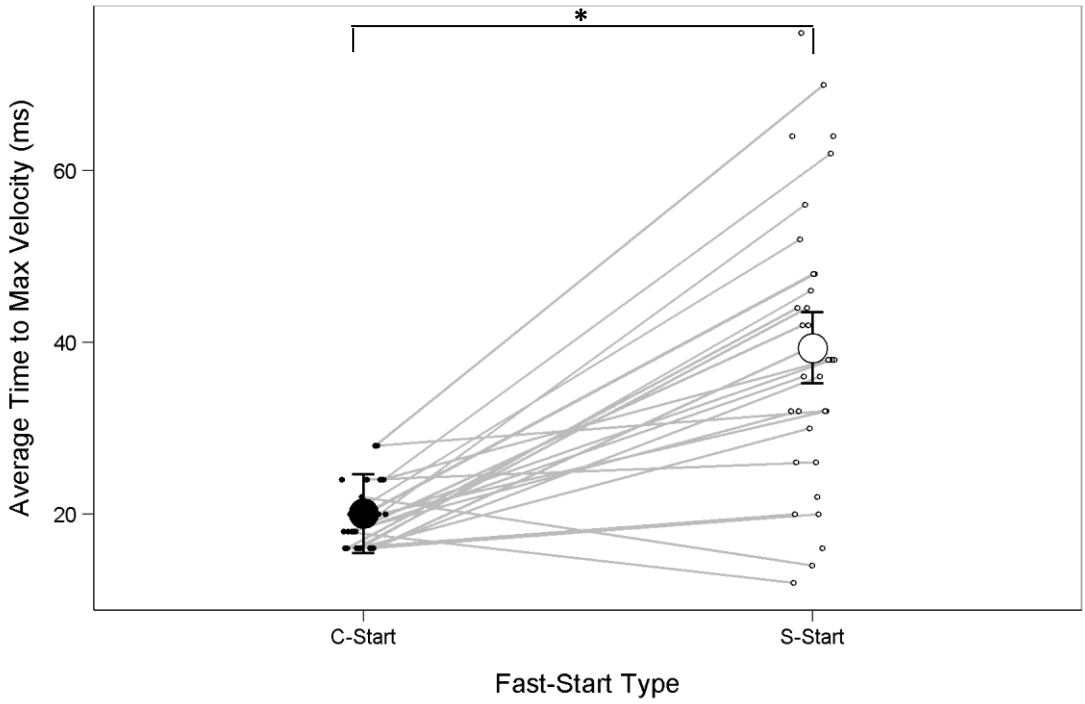

Figure 3.5. Average Time to Maximum Velocity (Time to MV) between C- and S-starts for both 21°C-acc and 24°C-acc treatments when tested at the respective acclimation temperatures (Day 1). Lines depict the difference between C- and S-start performance for each individual fish. Error bars depict standard error of the model. Statistical analysis of parameter estimates showed a significant effect of Fast-Start type (S-start) on Fast-start Time to MV (B = 61 ms, p = <0.001). * represents significant effect.

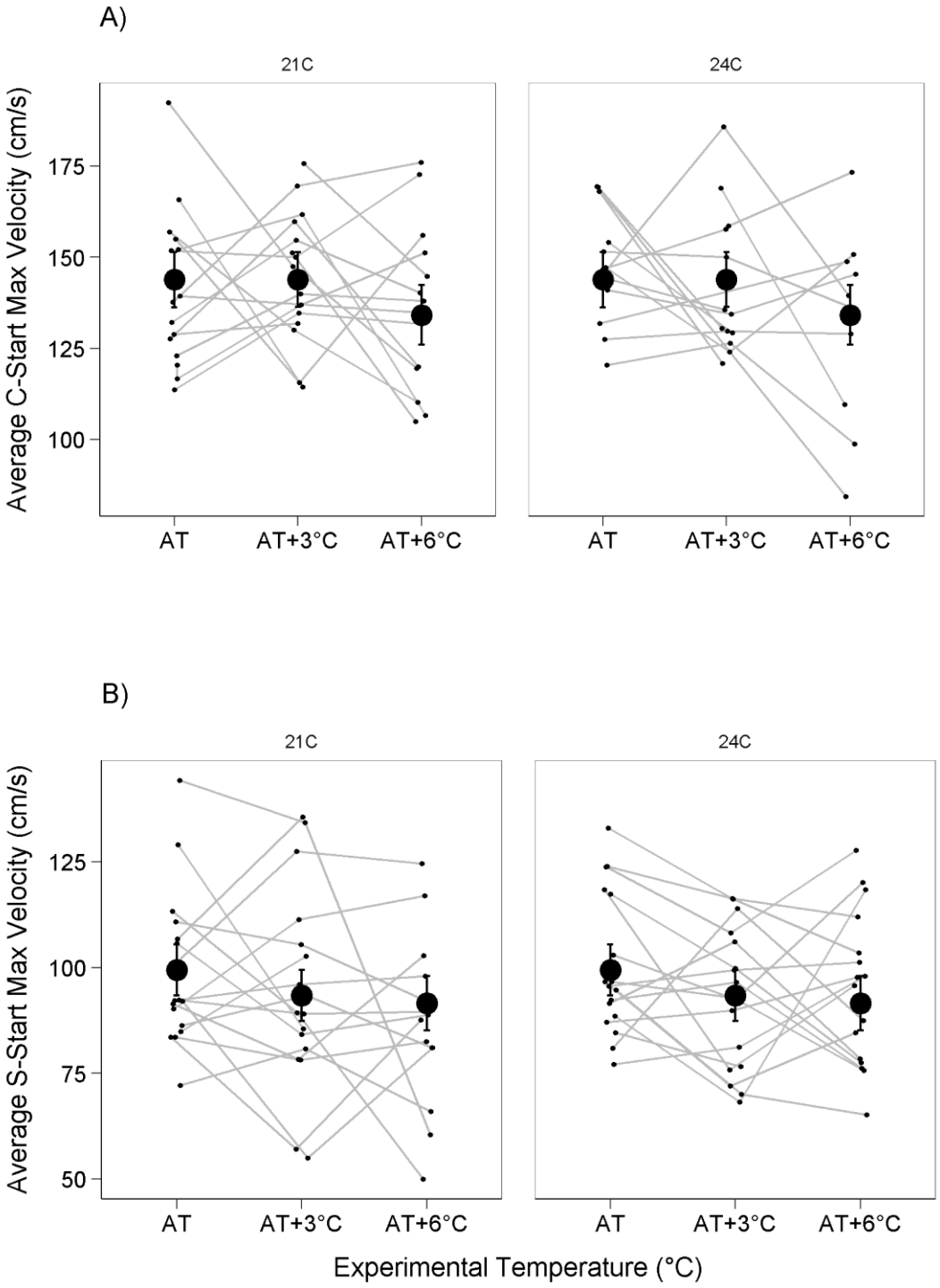

Figure 3.6. Average C-start Maximum Velocity (MV) (A) and S-start MV (B) following a rise in acute temperatures between 21°C-acc (left figures) and 24°C-acc (right figures) treatments. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C). Error bars depict standard error of the model. There was no significant effect of Acclimation Treatment on C- and S-start MV ($p = 0.9742$, $\chi^2 = 0.001$, $df = 1$; $p = 0.6981$, $\chi^2 = 0.1504$, $df = 1$). There was also no significant effect of Acute Temperature on C- and S-start MV ($p = 0.1514$, $\chi^2 = 3.775$, $df = 2$; $p = 0.1193$, $\chi^2 = 4.25$, $df = 2$).

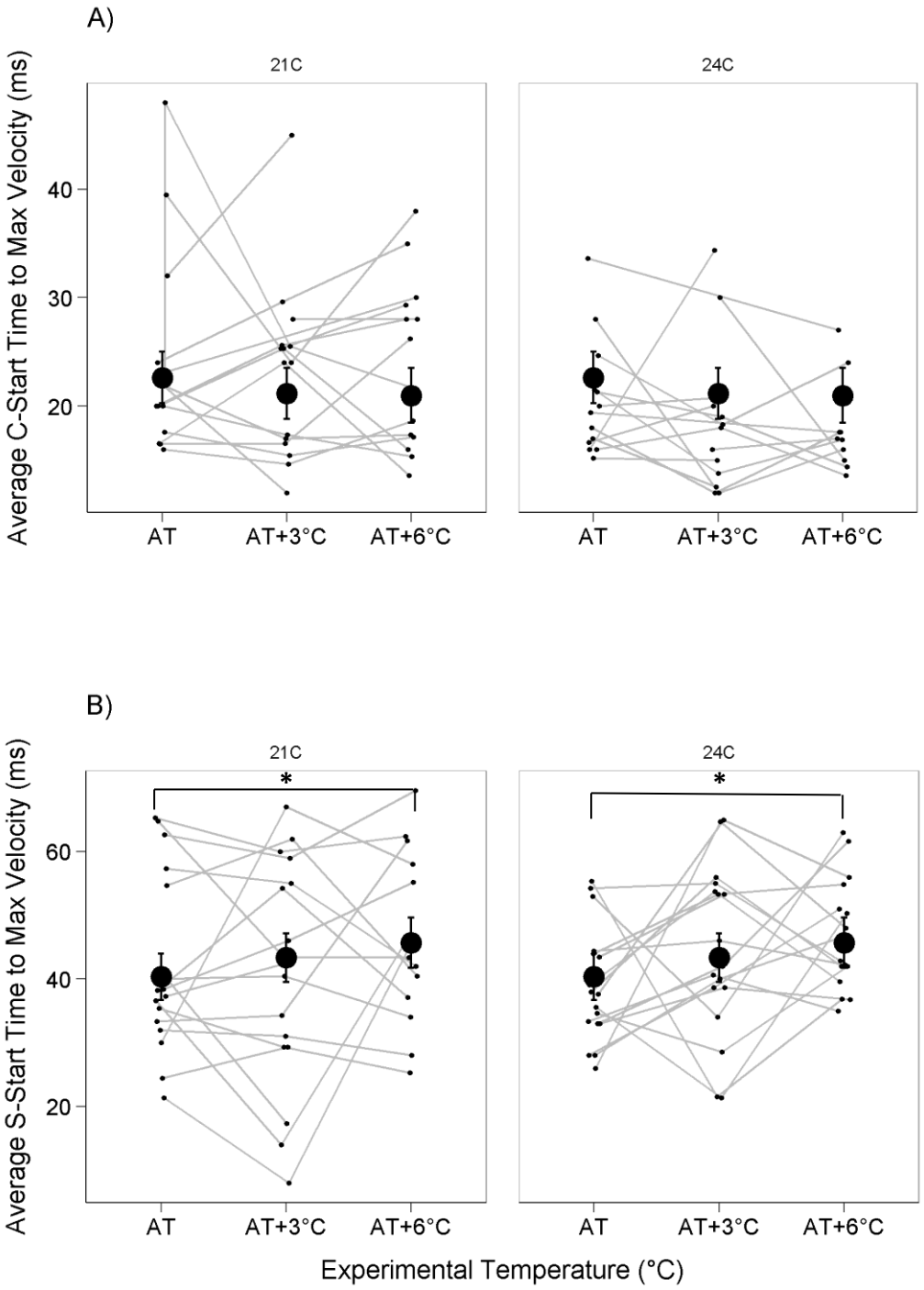

Figure 3.7. Average C-start Time to Maximum Velocity (Time to MV) (A) and S-start Time to MV (B) following a rise in acute temperatures between 21°C-acc (left figures) and 24°C-acc (right figures) treatments. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C). Error bars depict standard error of the model. There was no significant effect of Acclimation Treatment on C- and S-start Time to MV ($p = 0.0644$, $\chi^2 = 3.4192$, $df = 1$; $p = 0.9237$, $\chi^2 = 0.0092$, $df = 1$). While the +3°C acute temperature had no effect (B = 2.12 ms; CI = -3.10 – 7.33; $p = 0.426$), the acute temperature of +6°C had a significant effect on S-start Time to MV (B = 5.93 ms; CI = 0.52 – 11.35; $p = 0.032$). * represents significant effect.

Table 3.2. Results for the Type II Wald Chi-square tests examining Maximum Velocity (MV) and Time to Maximum Velocity (Time to MV) between fast-start types on Day 1. Statistical analysis includes MV and Time to MV measurements for both C- and S-starts. Analysis includes both 21°C-acc and 24°C-acc treatments of round gobies.

		Maximum Velocity	
Predictors	*df*	χ^2 *value*	*p-value*
Fast-start Type	1	132.1494	**<0.0001**
Cohort	1	0.0001	0.9924
Acc. Treatment	1	0.3636	0.5465
		Time to Maximum Velocity	
Fast-start Type	1	46.4710	**<0.0001**
Cohort	1	0.0113	0.9155
Acc. Treatment	1	0.2387	0.6252

Table 3.3. Linear mixed-effects parameter estimates examining Maximum Velocity (MV) and Time to Maximum Velocity (Time to MV) between fast-start types on Day 1. Statistical analysis includes Time to MV measurements for both C- and S-starts. Analysis includes both 21°C-acc and 24°C-acc treatments of round gobies. Parameter estimate confidence limits are also presented.

	Max Velocity		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	144.57	138.06 – 151.08	<0.001
Fast-Start Type (S-Start)	-44.90	-52.51 – -37.29	<0.001
	log(Time to Max Velocity)		
(Intercept)	3.06	2.95 – 3.16	<0.001
Fast-Start Type (S-Start)	0.61	0.48 – 0.73	<0.001

Table 3.4. Results for the Type II Wald Chi-square tests examining the control fish for C- and S-start Maximum Velocity (MV) and Time to Maximum Velocity (Time to MV) across 3 days. Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1.

		C-start MV	
Predictors	*df*	χ^2 *value*	*p-value*
Day	2	0.6164	0.7348
		S-start MV	
Day	2	0.5842	0.7467
		C-start Time to MV	
Day	2	1.1736	0.5561
		S-start Time to MV	
Day	2	1.6112	0.468

Table 3.5. Results for the Type II Wald Chi-square tests examining the Control for C- and S-start Maximum Velocity (MV) and Time to Maximum Velocity (Time to MV) with a rise in acute temperatures across 3 days. Analysis includes both 21°C-acc and 24°C-acc treatments of round gobies. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C).

		C-start MV	
Predictors	*df*	χ^2 *value*	*p-value*
Acute Temp	2	3.7754	0.1514
Cohort	1	0.0244	0.8759
Acc. Treatment	1	0.0010	0.9742
Acute Temp*Acc. Treatment	2	1.1396	0.5656
		S-start MV	
Acute Temp	2	4.2520	0.1193
Cohort	1	6.2172	0.0127
Acc. Treatment	1	0.1504	0.6981
Acute Temp*Acc. Treatment	2	0.8406	0.6568
		C-start Time to MV	
Acute Temp	2	1.2937	0.5237
Cohort	1	16.0641	0.00006
Acc. Treatment	1	3.4192	0.0644
Acute Temp*Acc. Treatment	2	0.9848	0.6112
		S-start Time to MV	
Acute Temp	2	5.7911	0.0553
Cohort	1	0.0003	0.9871
Acc. Treatment	1	0.0092	0.9237
Acute Temp*Acc. Treatment	2	1.3	0.5221

Table 3.6. Linear mixed-effects parameter estimates examining S-start Time to Maximum Velocity (Time to MV) following a rise in acute temperatures. Analysis includes both 21°C-acc and 24°C-acc treatments of round gobies. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C). Parameter estimate confidence limits are also presented.

	S Start Time to Max		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	40.40	36.12 – 44.68	<0.001
Acute Temperature (+3°C)	2.12	-3.10 – 7.33	0.426
Acute Temperature (+6°C)	5.93	0.52 – 11.35	0.032

Table 3.7. Linear mixed-effects parameter estimates examining C-start Time to Maximum Velocity (Time to MV) following a rise in acute temperatures. Analysis includes both 21°C-acc and 24°C-acc treatments of round gobies. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C). Parameter estimate confidence limits are also presented.

	C-Start Time to Max		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	24.67	22.50 – 26.83	**<0.001**
Cohort (2017)	-7.19	-10.51 – -3.87	**<0.001**

Discussion

Impact of Thermal Acclimation and Differences in Performance Between C- and S-starts

The focus of this study was to examine the effect of warm thermal acclimation on fast-start performance in round gobies at warm acute temperatures. In order to measure fast-start performance, gobies were required to escape from net chases while completing a detour task in a T-maze. I observed no significant difference between acclimation treatments in either fast-start velocity or S-start time to maximum velocity when tested at acclimation temperature. This finding suggests a plasticity in round goby fast-start performance at warmer temperatures following a +3°C thermal acclimation. Complete compensation has also been observed in rainbow trout fast-start max velocity and acceleration at warmer temperatures (Webb, 1978). Similar fast-start maximum velocities between acclimation treatments at warm acute temperatures have also been shown in studies investigating differences in warmer acclimation treatments 5°C apart (Temple and Johnston, 1998) and in studies with wide differences in acclimation temperatures (Wilson et al, 2000).

Acclimation effects on muscle function has revealed changes in myosin heavy chain isoform expression and myofibrillar ATPase activity (Johnson and Bennett, 1995). Altering the expression of myosin isoforms influences muscle contraction kinetics and power output primarily through changes in maximum shortening speed (Johnston et al, 1985; Johnston and Temple, 2002). For example, the expression of significantly faster myosin light chains in cold-acclimated carp (*Cyprinus carpio*) resulted in increased contraction velocity of abductor superficialis muscle compared to warm-acclimated subjects (Langfeld et al, 1991). Myofibrillar ATPase activity is a key determinant of the maximum shortening velocity in muscle fibres (Edman et al, 1988). Thermal effects have shown that cold acclimation

enhances myosin ATPase activity in white muscles when tested at both cool and warm acute temperatures (Johnson and Bennett, 1995; Watkins, 2000).

In my investigation of differences in performance between fast-start types, C-start maximum velocity was approximately 10 cm/s faster overall compared to S-starts when tested at acclimation temperature (Fig. 3.4). C-starts were likely to have an 18 ms faster time to maximum velocity than S-starts (Fig. 3.5), which is consistent with previous research showing slower acceleration rates in S-starts compared to C-starts (Spierts and Leeuwen, 1999). As opposed to stage 1 of C-starts that only consist of rapid head movement in the direction of the escape, acceleration of head movement in stage 1 of S-starts coincides with a tail-fin movement of high lateral velocity (Spierts and Leeuwen, 1999). While both fast-start types have similar total power output upper range values, S-starts have much lower total power outputs overall compared to C-starts (Frith and Blake, 1995). Additionally, C-starts are less variable in total power output compared to S-starts (Frith and Blake, 1995). S-starts may show more variability in performance between individuals not only due to the added complexity of tail-fin movement compared to C-starts, but also may be due to the different variations of S-starts that are utilized for different prey distances during attacks (Spierts and Leeuwen, 1999; Webb and Skadsen, 1980). This is illustrated in my study where there was much larger variability in S-start Time to Maximum Velocity compared to C-starts (Fig. 3.5).

Impact of Warm Acute Temperatures on Fast-Start Performance

In addition to the impact of thermal acclimation, the present study also examined the effect of rise in acute temperatures on fast-start performance. Results revealed no significant effect of warm acute temperatures on maximum velocity for both C- or S-starts (Fig. 3.6). Although velocity was seemingly unaltered with moderate acute temperature change, my results were similar to previous findings of aquatic ectotherms experiencing moderate warm

acute temperatures following acclimation to high temperatures (Johnson and Bennett, 1995; Temple and Johnston, 1998; Wilson et al, 2000; Grigaltchik et al, 2012) (Table A.2). Similarly, I did not observe a change in C-start time to maximum velocity following a rise in acute temperatures (Fig. 3.7). In contrast, a rise in acute temperature of +6°C resulted in a slowing of the time to reach maximum velocity for S-starts (Fig. 3.7). The results of the present study are surprising since muscle contraction rate and force in white muscle fibres of common carp (*Cyprinus carpio)* remains unaffected by warm acute temperatures following acclimation to higher temperatures (Wakeling et al, 2000). The trends to slower time to maximum velocity in S-starts and to lower velocity in both fast-starts types with warm acute temperatures is also surprising since the warmest acute temperature (+6°C) was not close to the CT_{max} previously shown in round gobies (CT_{max} of 33.4°C when acclimated to ~21°C) (Cross and Rawding, 2009; Drouillard et al, 2018). Future research should focus on the underlying mechanisms affecting fast-start performance at high acute temperatures following acclimation to warmer temperatures.

Conclusion

The results of the present study showing no change in fast-start maximum velocity with warm acute temperatures is similar to what was found in previous studies (Johnson and Bennett, 1995; Temple and Johnston, 1998; Watkins, 2000; Wilson et al, 2000; Grigaltchik, 2012). Thus, when compared to previous research investigating aerobic swimming performance, there is a potential difference in thermal sensitivity between aerobic and anaerobic swimming performance following acclimation to high temperatures (Fry and Hart, 1948; Johnson and Bennett, 1995). This conclusion was further substantiated by the increase in time to maximum velocity for round goby S-starts following a rise in acute temperatures, temperatures that coincided with increasing oxygen consumption in previous work on the round goby (Lee and Johnson, 2005). Future research focusing on the thermal sensitivity of

burst swimming to warm acute temperatures following acclimation to higher temperatures will deepen our understanding of environmental fluctuations on anaerobic swimming performance.

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Chapter 4:
An Investigation of Exploratory Behaviour While Being Chased as a Measure of Risky Swimming Behaviour During a Detour Task

Introduction

Investigations into individual behavioural differences within populations of various taxa have become more numerous in the last few years (Careau and Garland, 2012; Biro and Adriaenssens, 2013). Behavioural plasticity in response to environmental change highlights the need to understand how individual differences develop in the wild. Phenotypic plasticity is known as the capacity of an organism to express environmentally-influenced behavioural, physiological, or morphological phenotypes to adapt to environmental changes (Garland and Kelly, 2006). Animals that have flexibility in their responses are more likely to outcompete other individuals or species (Sol et al, 2002). In contrast to physiological responses, initial behavioural responses to altered conditions potentially allow for avoidance of specific variations in environmental extremes (Cherry et al, 1977). In contrast, physiological responses occur at slower time scales in response to prolonged experience or exposure (Briffa et al, 2008). Although the majority of plasticity research is physiological (Johnston and Temple, 2002), it is behaviour that is thought to be highly plastic thus requiring further investigation (Dingemanse et al, 2010).

Animal personality, or behaviour type, can be defined as the consistent individual tendencies over time or across situations within a population (Wolf and Weissing, 2012). Recent research has illustrated the differential impact of environmental change on personality types (Biro et al., 2010b) and have noted three key features of personality: individual variation, consistency, and correlations between behavioural traits (Wolf and Weissing, 2012). Consistency in behaviour over time is vital for maintaining and describing

behavioural traits. One example of correlated behavioural traits is boldness and aggressiveness, in which individuals that demonstrate boldness reveal more aggressive behaviours (Wolf and Weissing, 2012). While behavioural traits involve personality differences, groups of correlated traits are known as behavioural syndromes (Wolf and Weissing, 2012). An example of a behavioural syndrome is the 'proactive-reactive axis' (Quinn and Cresswell, 2005). Proactive individuals have been described as animals that demonstrate aggressive behaviour, are bolder in decision-making under novel situations (Verbeek et al, 1994), disperse further distances (Dingemanse et al., 2003), outcompete others for food (Ward et al, 2004), and display dominance over submissive individuals (Careau et al, 2008) (Quinn and Cresswell, 2005). Meanwhile, reactive individuals are considered docile, submissive and shy.

Predation is thought to be one of the key mechanisms driving differential selection of personality types (Sih et al., 2004), yet the link between anti-predator behaviours and interindividual differences is poorly understood. Assessment of an individual's boldness in novel situations involving a predator attack may be explained by the exploratory tendency and bold behaviour of that individual in risky situations (Réale et al, 2007). A previous study assessed boldness in the European sea bass by observing the time taken to emerge from cover following a predator attack (Killen et al, 2011). The boldness assessment was unique in requiring individuals to escape down a runway towards cover. Research into fish boldness involving more complex tasks could enhance our knowledge of escape behaviour between bold and shy animals and may be expected to produce novel results. Requiring a fish to make a choice between two escape routes would add an extra level of complexity when assessing risky swimming behaviour. For example, detour tasks commonly used to examine behavioural lateralisation require a fish to swim down a corridor and then detour to the left or to the right as they approach an opaque barrier (Dadda et al, 2010; Bisazza et al, 2001).

Zebrafish chased with a net learned to escape to another chamber more rapidly compared to individuals that only experienced a still net (Arthur and Levin, 2001) suggesting that escaping fish need to discriminate the spatial and non-spatial visual cues to solve the task, and have sufficient motor function to successfully escape when chased and sufficient levels of motivation to escape from the simulated predator attack (Arthur and Levin, 2001). Escapes from net chases while completing a detour task may resemble natural predator-prey encounters better than many other escape response assays, and I will thereby acquire data on interindividual differences that reflect a more natural trait.

Anti-predator behaviour between bold and shy individuals, assessed through individual exploration and activity levels while under threat of predation, has been previously investigated in birds and fish (McLean and Godin, 1989; Quinn and Cresswell, 2005). These studies assessed the relationship between activity and boldness, while revealing that shy behaviour can be assessed in anti-predator contexts based on the tendency to freeze (McLean and Godin, 1989; Quinn and Cresswell, 2005). Freezing in response to a predator attack may also be characterized as a higher level of responsiveness to a threatening stimulus (Eilam, 2005). Responsiveness can be described with three possible responses occurring: remaining motionless, swimming away at low speed, or by eliciting an escape response (Domenici, 2009). In the studies by McLean and Godin (1989) and Quinn and Cresswell (2005), less active individuals at the beginning of a trial were more likely to freeze than flee in response to a predator attack (McLean and Godin, 1989; Quinn and Cresswell, 2005). Interestingly, the more responsive shy subjects demonstrated longer latencies to emerge from shelter following an attack (McLean and Godin, 1989; Quinn and Cresswell, 2005). With respect to shy animals in high-risk situations (*i.e.* in the presence of a predator), research suggests that shy individuals exhibit a greater degree of behavioural plasticity when in the presence of a predator (Quinn and Cresswell, 2005). When an animal freezes in the presence of a predator,

a prey may remain immobile in order to evade the attention of the predator (Eilam, 2005). Therefore, the greater behavioural flexibility of shy animals allows for variability in responses to risky situations. Furthermore, bold animals have a greater tendency to exhibit superficial exploration of a novel environment (Sih et al, 2004). One study showed that shy cichlids were faster at responding to a predator attack while foraging (Jones and Godin, 2010). This finding suggests that bold fish demonstrated less attention to anti-predatory vigilance while foraging, resulting in a delayed response to predator attacks (Jones and Godin, 2010). This decreased reactivity, or lower responsiveness, of fish to a predator could be thought of as superficial exploration while foraging.

In addition to performing escape responses to avoid predators during attacks, some fish species employ alternative anti-predator defensive behaviours (Domenici, 2010). It has been suggested that individual fish that rely on camouflage may have higher responsiveness to predatory attacks (Domenici, 2010), allowing these fish to conserve energy while remaining motionless (Domenici, 2010). Previous research on guppies demonstrated that shy fish exhibited much stronger antipredator responses in comparison to bold ones when exposed to a high-risk alarm cue (Brown et al, 2014). Understanding the role of responsiveness in animals that demonstrate cryptic behaviour is important in understanding predator-prey relations and is crucial for gaining insight into the role of energetics and temperature on fish physiology.

Ectothermic animals must seek out optimal temperatures for optimal physiological functioning (Dreisig, 1984). While behavioural thermoregulation allows individuals to avoid sub-optimal temperatures, acute changes in temperature also have wide-ranging effects on animal behaviour. Increased aggressive behaviour, a behavioural trait associated with bold animals, occurs following increases in warm acute temperatures (Crowley and Pietruszka, 1983; Brodie and Russel, 1999; Herrel et al, 2007). Coral reef fish demonstrated an overall

increase in boldness following an acute rise in temperature (Biro et al, 2010b). Bold animals also have a higher metabolic rate (Finstad et al, 2007), enabling a lifestyle that involves higher rates of activity. A fish's ability to behaviourally thermoregulate is vital to maintaining optimal body temperature during acute temperature changes, yet microhabitat selection is ineffective when the temperature of entire bodies of water are altered over the long-term (Crawshaw, 1979). As temperatures change seasonally and year-to-year, fish must make long-term physiological adjustments to ensure optimal functioning at the new body temperature (Hazel and Prosser, 1974). This long-term adjustment to chronic temperature change, called thermal acclimation, allows an individual fish to maintain activity levels and metabolic functioning (Crawshaw et al, 1985). One study showed that bass attack more frequently when acclimated to warmer temperatures (Grigaltchik et al, 2012), though the reason for the enhanced behaviour at higher temperature was unexplained. It is unknown whether warm acclimation impacts behaviours associated with risky swimming behaviour (*i.e.* aggressiveness, boldness), although given that warm temperatures generally speed up movement and reaction times, on the whole, ectotherms as a group would be expected to become bolder. Whether individual risky swimming behaviour is influenced by short- and long-term changes in temperature remains an open question.

Questions and Hypotheses

It remains unclear whether risky swimming behaviour can be assessed by observing escape behaviour in a detour task, and whether differences in responsiveness to net chases helps to differentiate strength of risky swimming behaviour between individuals. Previous research investigating boldness has found correlations between individual boldness and exploratory behaviour in novel environments or risky swimming behaviour in open areas (Budaev, 1997; Quinn and Cresswell, 2005; Jones and Godin, 2010; Boulton et al, 2014; Mazué et al, 2015; Moscicki and Hurd, 2015) (Table A.1). While some studies investigated

the impact of the presence of a predator, there is a gap in research on these swimming behaviours while being chased by a predator. Furthermore, assessing exploratory and risky swimming behaviour while being chased may shed further light on the relevance of decreased freezing behaviour previously shown in bold animals. By quantifying fish exploratory behaviour in a simple maze while under threat of being caught, a better understanding of anti-predator strategies between individuals can be gained. Assuming that interindividual differences are heritable and relatively stable over time, I hypothesized that there would be consistency in risky swimming behaviour in gobies while completing a detour task under threat. It is predicted that subjects with heightened exploratory behaviour will demonstrate greater willingness to enter a T-maze tunnel and less evidence of freezing, the latter which can be assessed by scoring the presence of S-start behaviours. If interindividual differences in swimming behaviour are a fixed property of an individual, I would predict that thermal acclimation to even warmer temperatures would still result in consistent changes in exploratory behaviour while being chased in a detour task compared to cooler acclimation temperatures.

Bottom-dwelling round gobies are a sedentary species that prefer to swim in small bursts (Tierney et al, 2011). It is unknown whether there will be greater levels of exploratory behaviour in round gobies while completing a simple task under threat of predation. This study aims to further our understanding of the short- and long-term temperature effects on risky swimming behaviour in response to net chases. Assuming that the effect of rising temperatures on the underlying physiological mechanisms translates to behavioural changes, I predicted that the exploratory behaviour while being chased in a detour task will be impacted by acute temperatures. It is predicted that an increase in acute temperature will lead to greater levels of exploratory behaviour while completing a detour task.

Methodology

Subjects

To investigate the effect of short- and long-term temperature change on round goby risky swimming behaviour during net chases, gobies (Control, 21°C-acc, and 24°C-acc) were observed performing a detour task while being repeatedly chased with a fish net. The detour task experiment was conducted in a T-maze (Figure 4.1). The data for this chapter was based on video analysis of fish behaviour derived from the videos that recorded swimming velocity in Chapter 3. No additional fish were tested during this experiment. The methodology for collection of subjects, apparatus design, and experimental design are covered in detail in Chapters 2 and 3. The sample sizes for each of the acclimation treatments were as follows: Control (Day 1: n=11, Day 2: n=11, Day 3: n=10), 21°C-acc (Day 1: n=19, Day 2: n=19, Day 3: n=18), and 24°C-acc (Day 1: n=20, Day 2: n=20, Day 3: n=19).

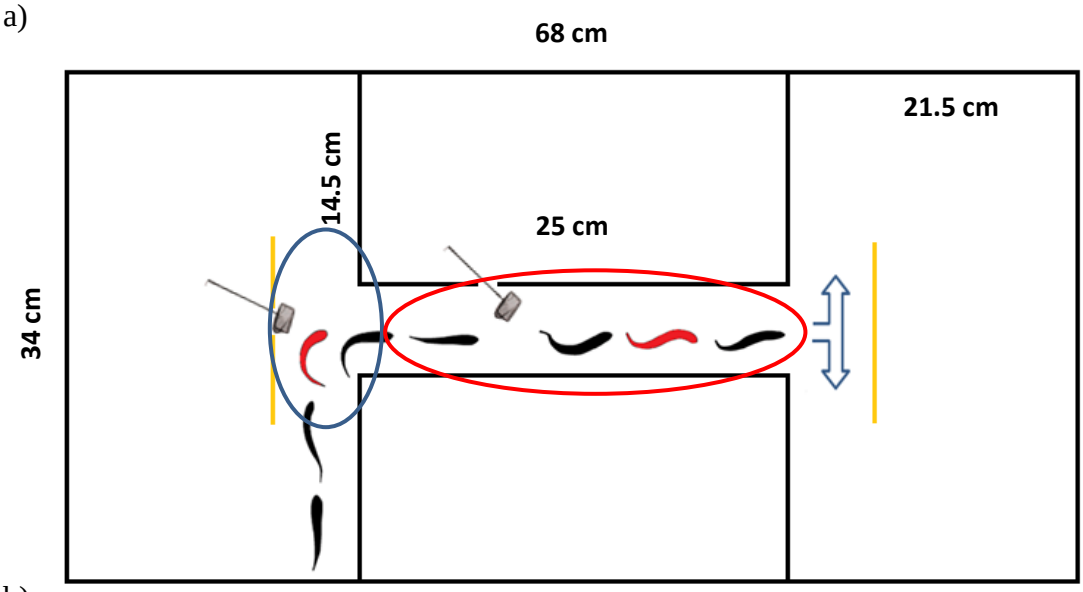
Experimental Procedure

Round gobies swim with short bursts of speed and normally live along the bottom of lakes and small bodies of water and may prefer darker areas of water bodies. Previous research has demonstrated that fish not only prefer darker tanks, but that latency to exit white-coloured compartments is significantly shorter compared to dark-coloured areas (Serra, 1999). Nevertheless, preliminary testing in the present study determined that gobies needed to be chased in order to successfully complete the detour task. Thus, the floor and walls of the majority of the open area were coloured black to persuade gobies to promptly leave the white-coloured tunnel while being chased. Gobies were chased with a net during the detour task to determine interindividual differences, as net chases have been shown to be a stressor (Sopinka et al, 2016). To investigate detour behaviour at high swimming speeds, I first chased gobies from the open area into the tunnel. To visually guide fish towards the tunnel entrance, the walls immediately before the tunnel entrance were covered in white contact

paper. The walls within the tunnel were covered in black contact paper to contrast from the white walls before the tunnel entrance. In order to encourage fish not to linger in the tunnel and to complete the detour task, the floor of the tunnel was covered in white contact paper.

The most successful method for getting fish to enter the tunnel was by guiding fish with nets towards the tunnel entrance. This was done by chasing the typically wall-hugging subjects along the walls immediately before the tunnel entrance. Once the individual was close to the tunnel entrance, fish were startled into the tunnel. Some individuals were not as active in their response to net chases (swimming away from the net at a low or high speed). These subjects would occasionally perform burst swimming behaviour when chased by the net but seemed particularly averse to entering the tunnel on their own, often remaining motionless along the walls immediately before the tunnel entrance. Less active fish in the open area, wall-hugging fish that were averse to entering the tunnel or not responding to net chases overall, were required to be gently pushed along the wall towards the entrance of the tunnel to allow task completion. This was termed involuntary tunnel entry. In contrast, generally swimming away from net chases and subsequently voluntarily swimming into the tunnel was defined as voluntary tunnel entry (VTE). VTE in this study was defined as a behavioural trait, scored as 1 (voluntary tunnel entry) or 0 (involuntary tunnel entry) for each net chase trial conducted. A depiction of both behavioural traits assessed in the present study can be seen in Figure 4.1.

a)



b)

Figure 4.1. Diagram of the T-maze apparatus used during experimentation (a). Once fish were in the proximity of the tunnel entrance, fish were startled towards the tunnel entrance. Individuals that demonstrated higher activity levels in the open area and near the tunnel entrance voluntarily swam into the tunnel (defined as Voluntary Tunnel Entry (VTE); observations recorded within blue circle). Individuals that showed higher activity levels during net chases in the tunnel completed a turn while requiring two or fewer net chases (defined as Activity during Net Chases (ANC); observations recorded within red circle). Fish often performed S-starts to rapidly escape during net chases while swimming through the tunnel (defined as S-start Presence), denoted by the red-coloured fish within the tunnel. Picture of the T-maze apparatus (b). The tunnel that the fish enters consists of a white-coloured bottom and black-coloured walls. The barrier consists of vertical yellow bars to allow subjects to visualize the PVC pipe on the opposite side, as well as the rest of the open area beyond the barrier.

Once individuals that were less active in the open area were positioned at the tunnel entrance, I waited a maximum of five seconds before attempting to startle the fish into the tunnel. After fish entered the tunnel, I continually chased the fish until the subject completed a turn. Multiple net chases were needed in some instances since gobies prefer to swim in short bursts. In order to encourage continuous movement through the tunnel, net chases by the experimenter were mostly executed when fish slowed down or stopped in the tunnel. Once in the tunnel, success in making a turn while repeatedly being chased required a high level of activity. This resulted in continuous movement through the tunnel. Individuals that failed to complete a turn (by remaining in the tunnel) were gently pushed to the end of the tunnel. A maximum of six chases was done before the trial was terminated and considered a failure. The number of net chases required, or the number of net chases before a fish made a turn at the barrier, was then defined as Activity during Net Chases (ANC). The number of net chases in ANC ranged from 0 to 5. To differentiate between subjects that required a lower number of chases versus those that were not as active, two subject groups were created: more active subjects (0-2 chases) and less active subjects (3-5 chases). The decision to group the data was based on an observed bimodal distribution in the responses. Since there was a large number of subjects that did not complete the detour task (thereby remaining in the tunnel and not making a turn), these subjects were grouped with the less active subjects. For each of the 10 trials, more active subjects in ANC were assigned a score of 1 and less-active subjects were assigned a score of 0. More active subjects either continually swam through the tunnel or performed successive S-starts to propel themselves to the barrier before making a turn. Occurrence of S-starts in the tunnel was defined as S-start Presence. When S-starts were performed, only a single S-start was performed. It is important to note that S-starts occurred either from a still position or in mid-movement while an individual was continuously swimming through the tunnel. Once a fish made a turn at the barrier and

entered the open area, I blocked the tunnel with a partition to prevent the fish from moving through the tunnel between trials. The fish was then given a resting period of two minutes between trials.

Analysis of Fast-Start Presence

Multiple S-starts could occur in the tunnel, but only the first S-start performed was analysed in these instances. Fast-starts with only one tail beat were verified from video capture. Measurements were not done on fast-starts that involved an individual hitting the wall at any point during movement. In addition, the occurrence of fast-starts was not quantified if the net accidently touched the body of the fish during a chase. T-maze swimming behaviour was recorded at 240 frames per second using a GoPro camera. Observations of fish exploratory behaviour while being chased was done by observing videos of behaviour in detour task. Determination of S-start occurrence was done by quantifying S-start occurrence as analysed in Chapter 3.

Statistical Analysis

Relationship between Behavioural Traits and S-Start Presence

Statistical analysis was done to investigate whether there was consistency between the behavioural traits VTE and ANC. A Spearman's correlation test was used to assess the correlation between VTE and ANC. In order to investigate the relationship between the occurrence of fast-starts in the tunnel and individual scores in VTE and ANC, I ran a Spearman's correlation test between S-Start Presence and VTE and ANC. Each correlation analysis of these two investigations involved data from both acclimation treatments at each warm acute temperature. The effect of constant temperatures across days was analysed separately as a control for experience across sessions. For these analyses, the data were summarized so that the statistical analysis was done on pooled results across all 3 sessions for

each fish. The resulting values were the proportion of trials (0 to 1) each fish exhibited either voluntary tunnel entry (VTE), activity during net chases (ANC),or S-Start presence.

Impact of Warm Thermal Acclimation and Acute Temperatures on Strength of Behavioural Traits

Two separate analyses were done on the behavioural traits VTE and ANC: the impact of temporal change (day-to-day) on fast-start occurrence at a constant temperature, and the specific impact of warm thermal acclimation with warm acute temperature change. The first part of the analysis assessed the change in strength of VTE and ANC between days at a constant temperature of 21°C, acting as a constant-temperature control for changes in performance with experience. Control data were analysed separately since these subjects were only collected in one season and were not randomized within the timeframe of the acclimation treatments. I then examined the impact of warm thermal acclimation and warm acute temperature change on both VTE and ANC. For these analyses, the data were summarized so that the statistical analysis was done using a single value of ANC or VTE that was the total number of trials in each session (one session occurred each day).

I performed a generalized linear mixed-effects analysis on VTE and ANC for the control experiments. Since the data were count data and followed a Poisson distribution, generalized linear mixed-effects analysis was performed on the overall impact of constant temperatures across days on each behavioural trait using the package lme4 (Bates, 2015) in R version 5.3.1 (R Development Core Team, 2012). As part of the analysis, I investigated the fixed effect of Day on VTE and ANC. I performed Type II Wald Chi-square tests on each behavioural trait to assess the impact of temperature, using the ANOVA function in the car package (Fox and Weisberg, 2011) in R version 5.3.1 (R Development Core Team, 2012).

The next part of the analysis investigated the impact of warm acute temperatures on each behavioural trait. Acute Temperature (Acute Temp), Acclimation Temperature, Cohort,

and the interaction between Acclimation Temperature*Acute Temp were entered into the global model as fixed effects. To account for the repeated measures design, animal identification was included as random intercepts. Generalized linear mixed-effects analysis was performed on the overall impact of thermal acclimation and acute temperature change on each behavioural trait (number of trials out of 10 in each session). I first performed Type II Wald Chi-square tests on the full model to assess the impact of each fixed effect on VTE and ANC. In instances where the effect of Acute Temperature was found to be significant, a comparison of incidence rate ratios (IRR) was done to determine the impact of +3°C and +6°C. Visual inspection of residuals and QQ plots from model fits did not reveal any obvious deviations from homoscedasticity or normality for each statistical analysis in the present study.

Results

Relationship between Behavioural Traits and S-Start Presence

An analysis of the consistency in risky swimming behaviour while being chased during detour tasks was done by investigating the relationship between behavioural traits. Thus, I analysed the correlation between the behavioural traits Voluntary Tunnel Entry (VTE) and Activity during Net Chases (ANC). Results from the impact of warm acute temperatures analysis involving subjects from both acclimation treatments showed a strong positive correlation between VTE and ANC ($r_s = 0.6050$, df = 40, p = 0.000036) (Fig. 4.2). Thus, gobies that demonstrated a greater willingness to enter the T-maze tunnel (VTE) were more likely to complete the task with fewer net chases (ANC). For the control group, there was no significant correlation between VTE and ANC ($r_s = -0.2023$, df = 11, p = 0.5508). I also investigated whether individuals with high ANC and VTE scores would rapidly escape from net chases more often (by performing S-starts; S-start Presence) compared to less active

subjects. Results from the impact of warm acute temperatures analysis for both 21°C-acc and 24°C-acc subjects showed a strong positive correlation between VTE and S-Start Presence (r_s = 0.5491, df = 40, p = 0.0002) (Fig. 4.3). For ANC, there was a moderate positive correlation between ANC and S-Start Presence (r_s = 0.3642, df = 40, p = 0.0209) (Fig. 4.4). Therefore, active individuals in both behavioural traits are more likely to perform S-starts during escapes. Results for the control revealed no significant correlation between VTE and S-start Presence (rs = 0.0093, df = 11, p = 0.9783) or between ANC and S-start Presence (r_s = 0.1363, df = 11, p = 0.6895).

Impact of Warm Thermal Acclimation and Acute Temperatures on Strength of Behavioural Traits

An investigation of the effect of constant temperatures on both behavioural traits was done as a control for learning or habituation across days. There was no significant effect of Day on ANC (p = 0.9996, χ^2 = 0.0009, df = 2) (Fig. 4.5, Table 4.1). For the VTE control analysis, there was no significant effect of Day on VTE (p = 0.9983, χ^2 = 0.0035, df = 2) (Fig. 4.5, Table 4.1). Based on analysis of the trend between trials, there was no apparent learning effect of repeated trials within a session on the score of either of the behavioural traits (Fig. D.1, D.2). The second part of the analysis was to assess the effect of thermal acclimation on ANC and VTE when tested at warm acute temperatures. The interaction of Acc. Treatment*Acute Temp did not have a significant effect on VTE or ANC (Table 4.2), suggesting that acclimation treatment did not have an impact on VTE and ANC with acute temperature change. Results showed no significant effect of Acute Temperature on VTE (p = 0.9144, χ^2 = 0.1790, df = 2) (Fig. 4.6, Table 4.2). I next analysed the impact of warm acute temperatures on ANC. There was a significant effect of Acute Temperature on ANC (p = 0.0447, χ^2 = 6.2156, df = 2) (Fig. 4.7, Table 4.2). While there was no significant effect of +3°C acute temperature on ANC (IRR = 1.15, 95% CI = 0.94 – 1.39, p = 0.170), results showed that greater activity levels in response to net chases was more likely to occur at +6°C

(IRR = 1.28, 95% CI = 1.05 – 1.55, p = 0.013) (Fig. 4.7, Table 4.3). Thus, ANC was expected to increase by 1.28 trials out of 10 for both 21°C-acc and 24°C-acc subjects when tested at +6°C compared to testing at acclimation temperature.

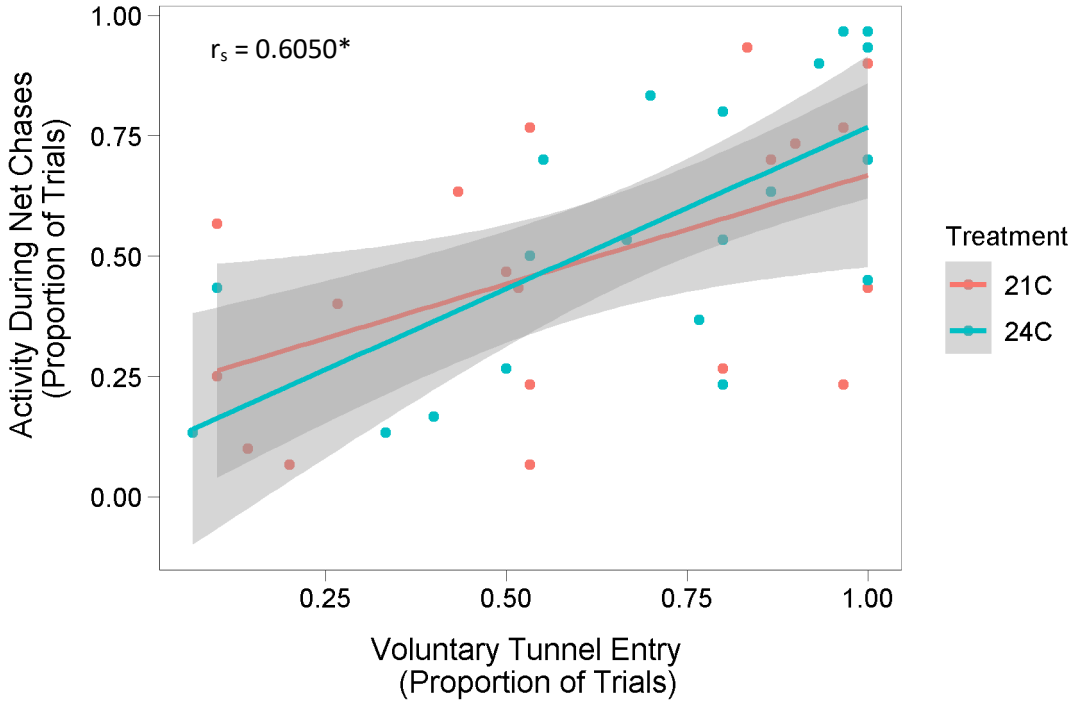


Figure 4.2. Correlation between the two behavioural traits assessing willingness to enter the tunnel of the T-maze (Voluntary Tunnel Entry) and activity during chases while completing the detour task (Activity during Net Chases) for both 21°C-acc and 24°C-acc treatments. Following testing at their acclimation temperature on Day 1, subjects experienced +3°C acute temperature change on each of Days 2 and 3. A proportion of 1.0 signifies an individual that required two or fewer net chases (ANC) and voluntarily entered the tunnel (VTE) in each of the 10 trials in a session. Shaded areas around mean depict 95% confidence limits. There was a significant positive correlation between VTE and ANC (r_s = 0.6050, df = 40, p = 0.000036). * represents significant effect.

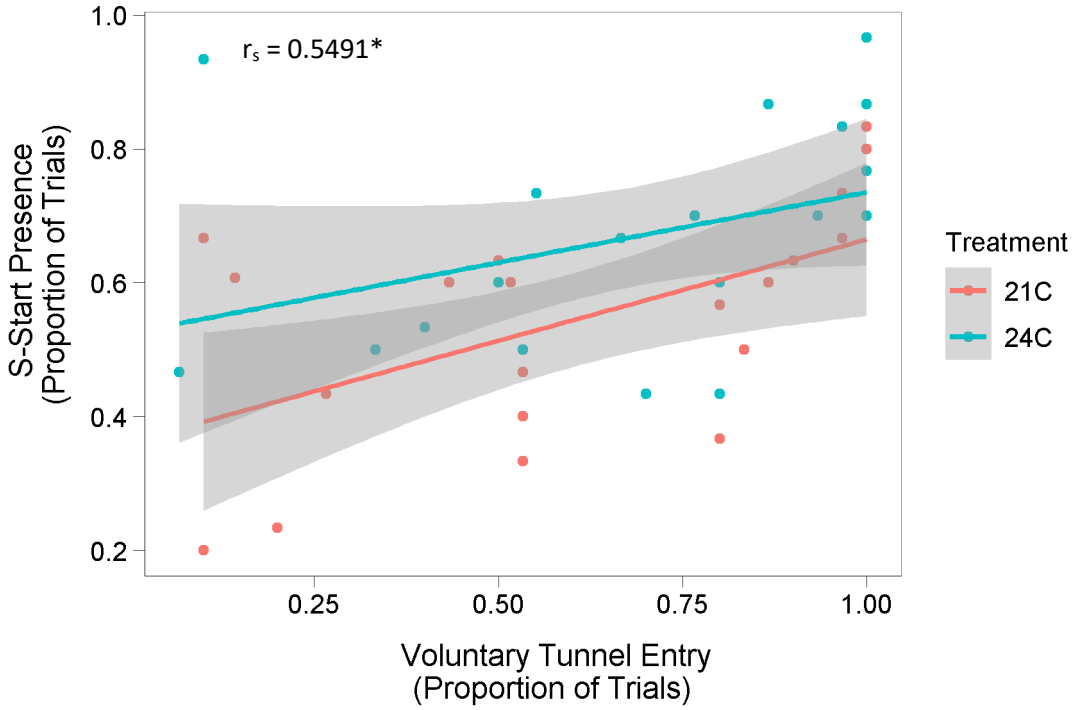


Figure 4.3. Correlation between the behavioural trait assessing the willingness to enter the tunnel of the T-maze (Voluntary Tunnel Entry) and S-start Presence for both 21°C-acc and 24°C-acc treatments. Following testing at their acclimation temperature on Day 1, subjects experienced +3°C acute temperature change on each of Days 2 and 3. A proportion of 1.0 signifies an individual that voluntarily entered the tunnel (VTE) and performed an S-start (S-start Presence) in each of the 10 trials in a session. Shaded areas around mean depict 95% confidence limits. There was a significant positive correlation between VTE and S-Start Presence (r_s = 0.5491, df = 40, p = 0.000243). * represents significant effect.

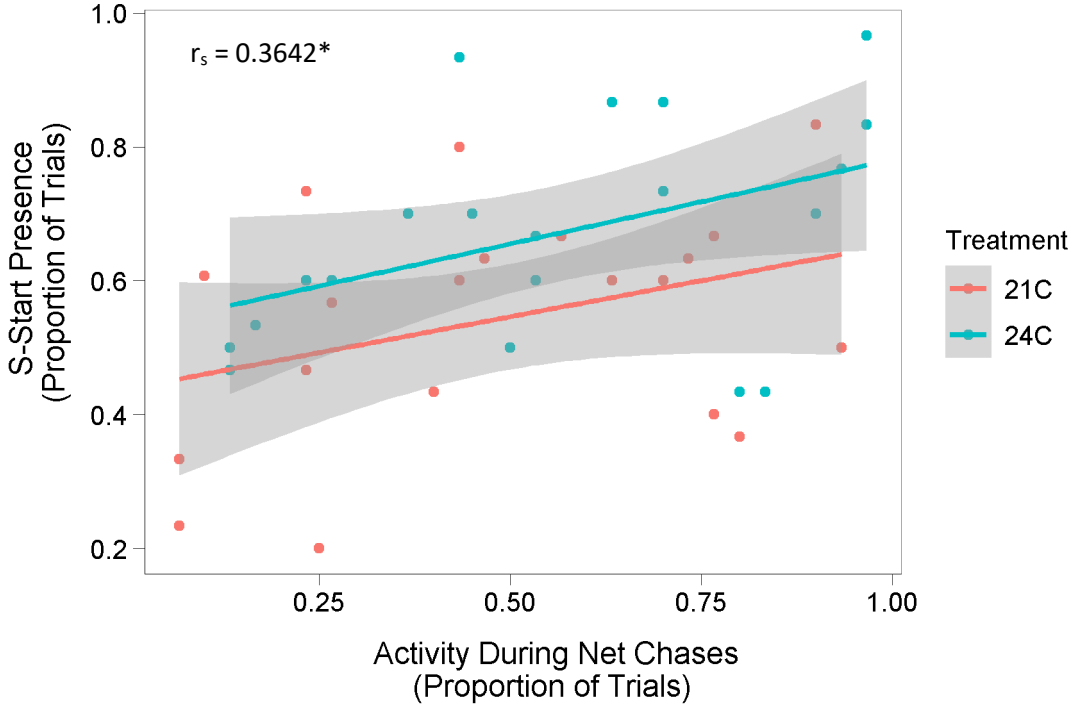


Figure 4.4. Correlation between the behavioural trait assessing the activity during chases while completing the detour task (Activity during Net Chases) and S-start Presence for both 21°C-acc and 24°C-acc treatments. Following testing at their acclimation temperature on Day 1, subjects experienced +3°C acute temperature change on each of Days 2 and 3. A value of 1.0 signifies a trial that required two or less net chases (ANC) and performed an S-start (S-start Presence) in each of the 10 trials in a session. Shaded areas around mean depict 95% confidence limits. There was a significant positive correlation between ANC and S-Start Presence (r_s = 0.3642, df = 40, p = 0.0209). * represents significant effect.

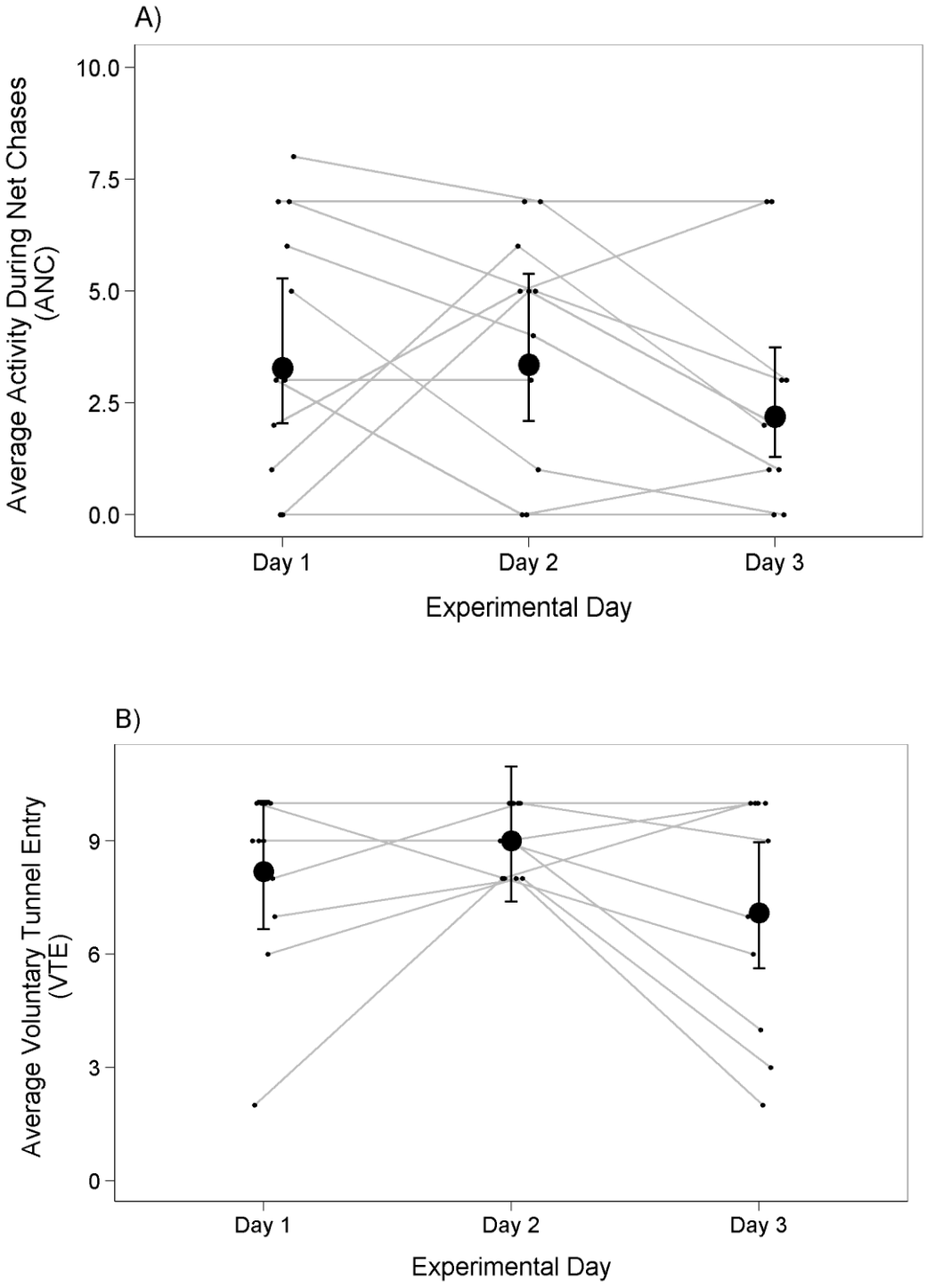


Figure 4.5. Average number of trials across 3 days of constant temperature (21°C) in which round gobies, A) were more reactive to chases in the behavioural trait Activity during Net Chases (ANC), or B) voluntarily entered the tunnel in the behavioural trait Voluntary Tunnel Entry (VTE). Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1. A value of 10 signifies an individual that required two or fewer net chases (ANC) and voluntarily entered the tunnel (VTE) in all 10 trials. Error bars depict standard error of the model. There was no significant

effect of Day on ANC ($p = 0.9996$, $\chi^2 = 0.0009$, $df = 2$). There was also no significant effect of Day on VTE ($p = 0.9983$, $\chi^2 = 0.0035$, $df = 2$).

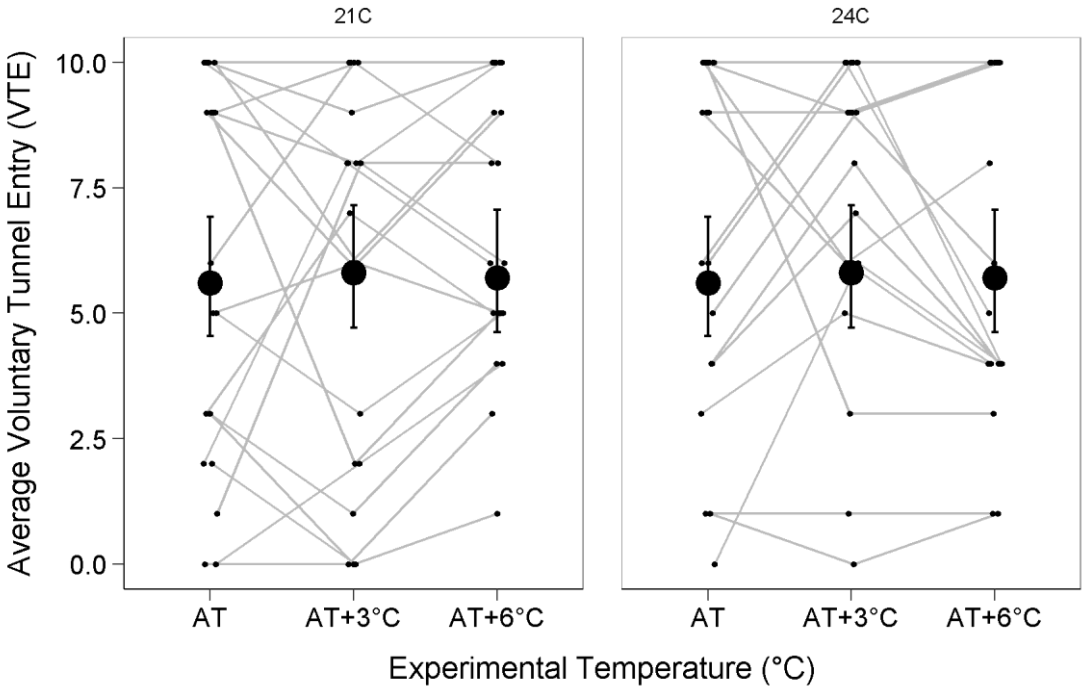


Figure 4.6. Average number of trials across 3 days in which round gobies voluntarily entered the tunnel in the behavioural trait Voluntary Tunnel Entry (VTE) following a rise in acute temperatures between 21°C-acc (left figure) and 24°C-acc (right figure) treatments. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C). A value of 10 signifies sessions where a fish voluntarily entered the tunnel in all 10 trials. Error bars depict standard error of the model. There was no significant effect of Acclimation Treatment on VTE ($p = 0.3514$, $\chi^2 = 0.8686$, $df = 1$). Results showed no significant effect of Acute Temperature on VTE ($p = 0.9144$, $\chi^2 = 0.1790$, $df = 2$).

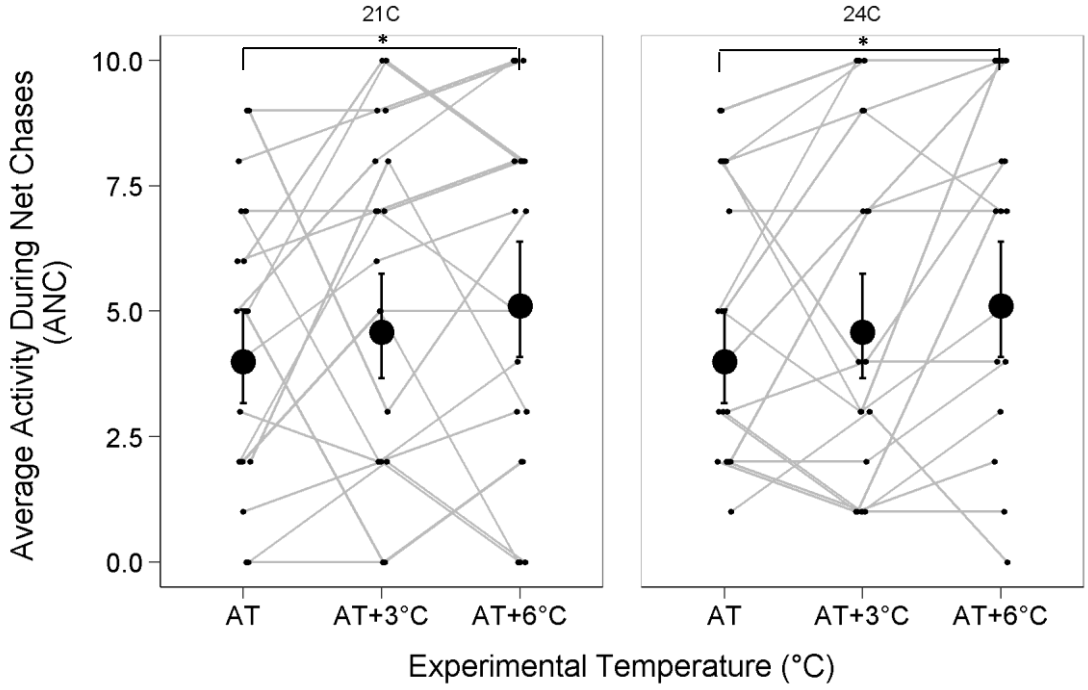


Figure 4.7. Average number of trials across 3 days in which round gobies were more responsive to chases in the behavioural trait Activity during Net Chases (ANC) following a rise in acute temperatures between 21°C-acc (left figure) and 24°C-acc (right figure) treatments. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C). A value of 10 signifies sessions where a fish required two or fewer net chases in ANC in all 10 trials. Error bars depict standard error of the model. There was no significant effect of Acclimation Treatment on ANC ($p = 0.5073$, $\chi^2 = 0.4397$, $df = 1$). While there was no significant effect of +3°C acute temperature on ANC (IRR = 1.15, 95% CI = 0.94 – 1.39, $p = 0.170$), statistical analysis of parameter estimates showed a significant effect of+6°C on ANC (IRR = 1.28, 95% CI = 1.05 – 1.55, $p = 0.013$). * represents significant effect.

Table 4.1. Type II Wald Chi-square test results examining the Control for Voluntary Tunnel Entry (VTE) and Activity during Net Chases (ANC) across 3 days. Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1.

		Voluntary Tunnel Entry	
Predictors	*df*	χ^2 *value*	*p-value*
Day	2	0.0035	0.9983
		Activity during Net Chases	
Day	2	0.0009	0.9996

Table 4.2. Type II Wald Chi-square test results examining Voluntary Tunnel Entry (VTE) and Activity during Net Chases (ANC) following a rise in acute temperatures. Analysis includes both 21°C-acc and 24°C-acc treatments of round gobies. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C).

		Voluntary Tunnel Entry	
Predictors	*df*	χ^2 *value*	*p-value*
Acute Temp	2	0.1790	0.9144
Cohort	1	0.0779	0.7802
Acc. Treatment	1	0.8686	0.3514
Acute Temp*Acc. Treatment	2	2.8625	0.2390
		Activity during Net Chases	
Acute Temp	2	6.2156	0.0447
Cohort	1	0.5800	0.4463
Acc. Treatment	1	0.4397	0.5073
Acute Temp*Acc. Treatment	2	0.9979	0.6072

Table 4.3. Generalized linear mixed-effects parameter estimates examining the effect of Acute Temperature on Activity during Net Chases (ANC). Analysis includes both 21°C-acc and 24°C-acc treatments of round gobies. Following testing at their acclimation temperature (AT) on Day 1, subjects experienced +3°C acute temperature change on Day 2 (AT+3°C) and Day 3 (AT+6°C). Parameter estimate confidence limits are also presented.

	Activity during Net Chases		
Predictors	*Incidence Rate Ratios*	*CI*	*p-value*
(Intercept)	4.00	3.17 – 5.04	**<0.001**
Acute Temperature (+3°C)	1.15	0.94 – 1.39	0.170
Acute Temperature (+6°C)	1.28	1.05 – 1.55	**0.013**

Discussion

Exploratory and Risk-Taking Behaviour During the Detour Task

The focus of this study was to examine the consistency in round goby exploratory swimming behaviour in two behavioural traits while being chased throughout a simple maze task. I then investigated the impact of warm acute temperatures on the exploratory and risky swimming behaviour. While the control data show no relationship between behavioural traits, subjects of both acclimation treatments revealed a strong correlation between Voluntary Tunnel Entry and Activity during Net Chases when experiencing warm acute temperatures. The combination of heightened exploratory behaviour and risky swimming behaviour in both behavioural traits has been similarly documented in prior studies that show consistency in exploratory behaviour in fish under the threat of predators (Budaev, 1997; Quinn and Cresswell, 2005; Jones and Godin, 2010; Boulton et al, 2014; Mazué et al, 2015; Moscicki and Hurd, 2015) (Table A.1). In my study, gobies that demonstrated a greater willingness to enter the T-maze tunnel (VTE) were more likely to complete the task with fewer net chases (ANC). Thus, more active gobies demonstrated a greater willingness to explore the T-maze while being chased. Based on previous research measuring similar behaviour in fish, consistency between behavioural traits in round gobies while being chased during detour task confirms that each behavioural trait can be considered as a measure of risky swimming behaviour. It is unknown why I observed no such correlation in the control subjects. Perhaps the lower sample size of the control was not robust enough to obtain a proper depiction of the relationship between the two behavioural traits.

The high metabolic rates typically seen in bold fish allow more aggressive individuals to have a more active lifestyle (Huntingford et al, 2010; Finstad et al, 2007). In addition to the findings in Biro et al (2010b), previous findings in birds (chaffinches) have shown a correlation

between activity and boldness (Quinn and Cresswell, 2005). Boulton et al (2014) shows a significant correlation between time spent moving at 1.5 cm/s or higher and area covered ($r = 0.653$), demonstrating a consistency in interindividual differences between higher activity levels and exploration of an open environment. A correlation between exploratory behaviour and boldness has been demonstrated both in the presence and absence of a predator (Mazué et al, 2015, Jones and Godin, 2010). In the present study, higher activity rates (assessed in the behavioural trait ANC) could have given risk-taking gobies a physiological advantage when employing aerobic swimming behaviour and thus allowed for heightened exploratory behaviour while being chased.

Spending more time away from the outer edges of an open area could be thought of as higher risk (*i.e.* from predation). Boldness, assessed as latency to emerge from a shelter, has been shown to correlate with time spent in the middle of a novel environment without the presence of predators (Boulton et al, 2014). Furthermore, boldness was also correlated with exploratory behaviour (area of tank covered when swimming). Based on previous findings, I suggest that subjects that scored high in the VTE behavioural trait are individuals that had a greater strength of risky swimming behaviour since prior studies have linked boldness to both exploratory and risky behaviour in open environments (Boulton et al, 2014). A study on convict cichlids (*Amatitlania nigrofasciata*) demonstrated a significant positive relationship between increased tendency to emerge from shelter and decreased time spent in the corners of an open environment (Moscicki and Hurd, 2015). Time spent in corners is characterized as freezing behaviour, suggesting that freezing behaviour is also associated with shy individuals (Moscicki and Hurd, 2015).

Occurrence of S-starts as a measure of freezing behaviour

The findings quantifying the decision to perform S-starts in response to net chases supports the hypothesis that more active subjects would show less freezing behaviour. I observed a strong positive correlation between VTE and S-Start Presence (Fig. 4.3). In addition, although not as strong a correlation, there was a moderate positive correlation between ANC and S-Start Presence (Fig. 4.4). The present study did not quantify the amount of time spent freezing in the tunnel, however, the significant correlations between both behavioural traits and S-Start Presence suggested that shy gobies often exhibited freezing behaviour in response to net chases. Based on the ANC observations, shy individuals not only were less likely to perform rapid escapes when under threat but also often chose to remain in the tunnel (and thus not completing the task). Risk-taking gobies responded to most net chases by performing an S-start or swimming away at a fast rate. Similar to the correlations between behavioural traits, the correlations between both behavioural traits and S-start Presence for control subjects were non-significant.

In chaffinches, less active individuals, assessed immediately after introduction to a novel environment, were more likely to freeze than flee in response to a predator attack (Quinn and Cresswell, 2005). Interestingly, these shy subjects demonstrated longer latencies to emerge from shelter following an attack, demonstrating a link between shyness and tendency to freeze (Quinn and Cresswell, 2005). Although the positive correlation between ANC and S-start Presence in round gobies is not as strong as the relationship between VTE and S-Start Presence, the correlation between the two behavioural traits provides further evidence that risk-taking fish are less likely to exhibit freezing behaviour in response to a simulated threat.

The consistency in decision-making of shy gobies was demonstrated in choosing to not rapidly escape from a threat and in many instances not completing the detour task. In addition to

fleeing from a predator attack, the decision to flee in the presence of a non-attacking predator has also been correlated with shy behaviour (Budaev, 1997). Guppy freezing behaviour in the presence of a predator is negatively correlated with two measures of activity in the open apparatus (Budeav, 1997). Overall, the findings showing freezing behaviour in the presence of an attacking (Mclean and Godin, 1989) and non-attacking predator (Budaev, 1997) suggests that freezing behaviour is an anti-predator behaviour.

Freezing may be associated with fear and stress, as shown in paradise fish in the presence of a non-attacking predator (Gerlai and Csányi , 1990), in addition to unwillingness to explore in the presence of a predator (Budaev, 1997). Deciding to escape may reflect a fish's assessment of predation risk. Remaining immobile when inhabiting a covered area may be less costly than fleeing, since an area with even slightly better cover is likely to provide better camouflage (Mclean and Godin, 1989). Fleeing runs the risk of alerting the predator and thus is contingent on the surroundings of the environment in which the attack occurs. This has been illustrated in *Anoline* lizard individuals that were more likely to remain immobile in the presence of a nearby refuge compared to a lack of cover (Hennig et al, 1976; Mclean and Godin, 1989).

Shy gobies remained immobile in the tunnel despite repeated attempts to startle the fish with a net. Although there was a PVC pipe available for shelter following completion of the detour task, freezing in the shade of the tunnel or along the dark walls of the open area might be seemed as optimal for shy gobies during repeated net chases. It is possible that shy subjects learned that repeated chasing occurred in the open area in the following trial, thus remaining in the tunnel for longer periods of time (or remaining for the duration of experimentation). Furthermore, the use of artificial light could have had an adverse effect on fish swimming behaviour. In an examination of the proportion of fish remaining still in the presence of artificial light, the findings showed variability in the impact of artificial light on swimming activity between the various fish species studied (Marchesan et al, 2005). Future research may reveal

different predation risks between risk-taking and risk-adverse individuals when under threat, supporting prior findings revealing bold individuals taking greater risks in the presence of predators (Bell, 2005; Quinn and Cresswell, 2005; Sih et al., 2004; Ward et al., 2004).

Impact of Warm Acute Temperatures on Behavioural Traits

In the present study, there was no significant effect of thermal acclimation on the strength of each behavioural trait following a rise in acute temperatures. Further analysis showed that only one of the behavioural traits was significantly impacted by warm acute temperatures, regardless of acclimation temperature. While I observed no effect of warm acute temperatures on VTE, ANC increased by 1.28 trials out of 10 when tested at +6°C (~32% rise in strength of Activity during Net Chases) (Fig. 4.7). The findings for control subjects of either behavioural traits demonstrated no impact of constant temperatures across days of experimentation (Fig. 4.5), indicating that the expected increase in strength of ANC at +6°C was not due to learning over the time course of the experiments. The reported positive correlation between the two behavioural traits (Fig. 4.2), along with results showing individuals with higher scores in VTE and ANC exhibiting higher tendencies to rapidly escape (Fig. 4.3 and 4.4), implies that the behavioural traits could be a good measure of risky swimming behaviour. Thus, my findings suggested that round goby risky swimming behaviour was expected to increase in strength at the acute temperature of +6°C. Biro et al. (2010b) also illustrate the impact of acute temperatures on the strength of boldness traits, with a 2.5-fold increase in boldness in warmer temperatures and an increase in activity of juvenile coral reef fish under acute temperature change. Due to the known increase in ectothermic metabolic rates with warm acute temperatures and the resulting impact on aerobic swimming activity, individual differences in energy metabolism may contribute to interindividual differences in swimming behaviour (Claireaux et al, 1995; Biro and Stamps, 2010a). Thus, I would expect round gobies to have increased voluntary swimming activity in

warmer temperatures since gobies have optimal oxygen consumption at warm acute temperatures, similar to what subjects experienced in the present study (Lee and Johnson, 2005).

Conclusion

My results suggested that short-term temperature change impacted behaviour, while no effect of long-term changes in temperature were observed. My findings showed that ANC increased in strength at the acute temperature of +6°C, agreeing with previous research showing an increase in boldness with warm acute temperatures (Biro et al, 2010b). The present study investigated two behavioural traits assessing exploratory and risky swimming behaviour in response to chases during a detour task. Results showed significant correlation between behavioural traits following a rise in acute temperatures. Based on previous research on fish (Killen et al, 2011; Boulton et al, 2014; Mazué et al, 2015; Moscicki and Hurd, 2015), I suggest that individual gobies with higher scores in both behavioural traits required higher activity levels. Previous findings on levels of fish boldness in risky situations supports the conclusion that variability in activity levels exhibited by gobies is explained by differences in strength of risky swimming behaviour (Killen et al, 2011; Boulton et al, 2014; Mazué et al, 2015; Moscicki and Hurd, 2015). Furthermore, the lower number of S-starts performed by individuals with lower levels of exploratory behaviour, similar to increased freezing behaviour by shy animals in prior studies (McLean and Godin, 1989; Quinn and Cresswell, 2005), provides additional evidence that both behavioural traits are a good measure of risky swimming behaviour in round gobies. My findings in the present study are important in presenting new methodology for assessing exploratory behaviour in fish while performing a detour task while being chased. Future research will shed further light on whether these measures of swimming behaviour are applicable to both bottom-dwelling and free-swimming fish species.

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Chapter 5:
Summary and Future Directions

Invasive round gobies have resulted in a decline in populations of native fish species in the Great Lakes since their discovery in the region in 1991, resulting in extremely high densities (>100 fish/m^2) in the warmest Great Lake, Lake Erie (Steinhart et al, 2004; Kornis et al, 2012). With climate change, lakes and streams will see dramatic fluctuations in temperature with an increased likelihood of extreme weather events. Long-term global warming is also likely to increase the surface temperature of the Great Lakes by as much as +3°C by the year 2100 (Nakicenovic et al, 2000; Trumpickas et al, 2008). Bottom-dwelling round gobies do not experience the higher temperatures that fish living closer to the surface of the water experience. It is possible for smaller water bodies to experience some degree of thermal stratification (Tate et al, 2006). The bottom of small tributaries that gobies now inhabit in the Great Lakes region are more impacted by air temperatures compared to that of larger bodies of water. The wider thermal performance breadths of eurythermal fish compared to stenothermal species could also provide an advantage to round gobies with climate change. It is important to understand the degree of phenotypic plasticity in round gobies since thermal acclimation is crucial for withstanding the temperature fluctuations typical for eurythermal species (Sokolova and Pörtner, 2003). In the present study, I observed plasticity in escape temperatures following acclimation to +3°C. Similar to the observed round goby escape temperatures, native stenothermal and eurythermal fish that co-occur with round gobies in the Great Lakes shift their thermal preference following acclimation to 24°C compared to 21°C (Cherry et al, 1977). My findings show that round gobies have slightly lower-than-expected average escape temperatures in each acclimation treatment, which suggests that gobies may not prefer warm temperatures, or at least this may be the case following acclimation to warmer temperatures. The escape temperatures are surprising since round goby physiological functioning is optimal at temperatures above 24°C

(Lee and Johnson, 2005). Gobies are able to increase their oxygen consumption up to ~28°C and have peak feeding rates at 26°C (Lee and Johnson, 2005). Thus, my results suggest a contradiction between goby thermal preferences and temperatures that are optimal for other traits.

Round gobies are known to survive in a wide range of environmental conditions, yet little is known about their superior competitive abilities compared to native fish species (Kornis et al, 2012). Round gobies co-occur with bluntnose minnows (*Pimephales notatus*) and white suckers (*Catostomus commersoni*) in the Great Lakes region and have displaced benthic species such as mottled sculpins (*Cottus bairdii*) and johnny darters (*Etheostoma nigrum*) (Ruetz and Strouse, 2009). White suckers and bluntnose minnows prefer temperatures slightly above their acclimation temperature of ~24°C (Cherry et al, 1975; Cherry et al, 1977; Reynolds and Casterlin, 1978; Cincotta and Stauffer, 1984). Demonstrating a correlation between temperature preference and the optimal temperature of a physiological trait, white suckers acclimated to ~24°C select temperatures similar to their optimal temperature for growth rate (Cincotta and Stauffer, 1984; McCormick et al, 1977). Similarly, the temperature preference of rainbow trout below the acclimation temperature of 21°C seems to correlate with the optimal temperatures for growth rate (Hokanson et al, 1977; Myrick and Cech, 2000) and maximum food consumption rate (Cherry et al, 1977; Myrick and Cech, 2000). Thus, rainbow trout seem to prefer cooler temperatures but differ from round gobies in that their optimal temperatures for physiological functioning also occur at those cooler temperatures. Therefore, what does the thermal preference in round gobies imply about its swimming behaviour in the wild?

Ectotherms may have different optimal temperatures, and different physiological traits may have slightly different thermal sensitivities (Martin and Huey, 2008). It is possible that round gobies select temperatures that prioritize different behavioural or physiological functions (Clark et al, 2013). The stenothermal yellow perch, known to feed on round gobies, have

optimal food consumption rates at 24°C (Schneider, 1973) and optimal growth rates at 23-24°C (Schneider, 1973; Tidwell et al, 1999; Hart, 1947). However, yellow perch acclimated to 24°C show preferred temperatures of ~22°C (Cherry et al, 1977; McCauley and Read, 1973). There is still much to understand about round goby physiology, and future research may show physiological traits agreeing with the preference for cooler temperatures.

Observations during behavioural thermoregulation also show that acclimation of +3°C leads to lower exploratory behaviour in warm waters and lower overall activity levels both at stable and fluctuating temperatures. These findings were revealed despite plasticity in escape performance and risky swimming behaviour, with no observed statistical differences observed between 21°C- and 24°C-acc in escape performance and strength of behavioural traits. Furthermore, the present study observed an increase in the strength of risky swimming behaviour at acute temperatures of +6°C. In addition to the known increased ectothermic activity rates in warmer temperatures, increasing strength of risky swimming behaviour is only possible if fish are capable of adequate aerobic metabolic rates required for sustained swimming (Claireaux et al, 1995; Biro and Stamps, 2010; Quinn and Cresswell, 2005). Therefore, it is not likely that behavioural thermoregulation of round gobies following acclimation to 24°C was limited by decreased aerobic functioning. The lower exploratory behaviour in warm waters and lower overall activity levels could be explained by choosing to minimize metabolic rate rather than optimize certain physiological traits during behavioural thermoregulation (Killen, 2014). For example, stickleback fish are known to alter their thermal preference and prioritise group behaviours over individual thermoregulatory preference (Cooper et al, 2018). A simpler explanation could be that round gobies are more suited to acclimate to cooler water temperatures, reflective of their benthic lifestyle . Nonetheless, my findings provide insight into the behavioural responses of round gobies acclimated to temperatures projected with climate change.

Unlike their native habitat in the Ponto-Caspian region, round gobies are now found in creeks and small rivers in the Great Lakes region where there are known species-at-risk hotspots (Phillips et al, 2003; Poos et al, 2009; Kornis et al, 2012). Diurnal fluctuations in temperature are especially evident in streams and rivers. Native fish may be at a disadvantage with the increase in severity and occurrence of extreme weather events through climate change, as at-risk species may be thermally stressed with rapid rises in temperature (Coutant, 1987). Furthermore, the effects of climate change may be amplified with the competitive abilities associated with invasive species (Rahel and Olden, 2008). Aggressiveness, a key behavioural component of bold animals, seems to be associated with the ability of non-native species to establish and rapidly grow in new habitats following introduction (Lodge, 1993). Therefore, personality type may enhance colonization success for non-native animals (Cote and Clobert, 2006).

The strong correlation between behavioural traits observed in a detour task under threat of predation, along with the strong correlation between lower scores in voluntary tunnel entry and freezing behaviour, suggests that both behavioural traits are good measures of risky swimming behaviour (McLean and Godin, 1989; Quinn and Cresswell, 2005; Mauze, 2015). As previously mentioned, the observed increase in strength of risky swimming behaviour at +6°C requires adequate aerobic metabolic rates for sustained swimming (Claireaux et al, 1995; Biro et al, 2010b; Quinn and Cresswell, 2005). Prior research has suggested that individual differences in energy metabolism may contribute to animal personality since warmer temperatures are known to increase metabolic rates in ectotherms (Biro and Stamps, 2010a). The increase in metabolic rates at warmer temperatures has a direct impact on aerobic swimming activity, with increased voluntary swimming activity at higher temperatures (Claireaux et al, 1995). Round gobies have previously demonstrated a greater number of aggressive behaviours towards native mottled sculpins, both when gobies were the resident in a shelter and also as intruders in a laboratory territorial experiment (Dubs and Corkum, 1996). The aggressive behaviour of male

gobies when guarding nests also suggests an inherent heightened aggressiveness that has ultimately facilitated the displacement of native fish species (Meunier et al, 2009). Thus, with an observed increase in risky swimming behaviour with warm acute temperatures, the competitive abilities of round gobies may become enhanced with the rapid temperature increases known to occur during extreme weather events.

Observations of anaerobic swimming performance demonstrated no significant effect of warm acute temperatures on maximum velocity for both C- or S-starts or C-start time to maximum velocity. In contrast, a rise in acute temperature of +6°C resulted in an increase in S-start time to maximum velocity. Following acclimation to high temperatures, the increase in S-start time to maximum velocity agrees with previous findings on the negative impact of warm acute temperatures on burst swimming performance, suggesting that above 27 - 30°C, round gobies may begin to demonstrate decreased performance. As with my findings on round gobies acclimated to warmer temperatures, burst swimming performance at the upper range of thermal performance breadths reveals either no change or a decrease in performance in multiple eurythermal fish species (Johnson and Bennett, 1995; Temple and Johnston, 1998; Wilson et al, 2000; Grigaltchik et al, 2012). While previous research has demonstrated very similar maximum S-start velocities of round gobies compared to the present study (~100 cm/s for 21°C-acc subjects tested at acclimation temperature) (Tierney et al, 2011), there is no previous research on how proficient gobies are when performing C-starts.

In addition to lower overall activity levels during behavioural thermoregulation, decreased exploratory behaviour in warm waters for gobies acclimated to warmer temperatures may reflect a suitability for benthic fish species to acclimate to cooler water temperatures. Furthermore, a preference for cooler temperatures was observed in both experimental treatments following acclimation to higher temperatures. Based on my findings of increased risky swimming behaviour at higher temperatures, while also considering previous literature

examining round goby physiology, it is possible that round goby behavioural and physiological functioning would remain optimal in warmer temperatures when the environment does not allow for selection of cooler temperatures (Lee and Johnson, 2005; Drouillard et al, 2018). Further examination of other physiological traits, such as whether round goby escape temperatures coincide with temperatures that are optimal for other performance traits, would allow for insight into the possible further spread of round gobies in non-native habitats.

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Appendix

Table A.1. Previous research investigating the relationship between exploratory behaviour and various behavioural traits. Studies using various species demonstrate a significant positive correlation between exploratory behaviour and boldness, responsiveness, and freezing behaviour.

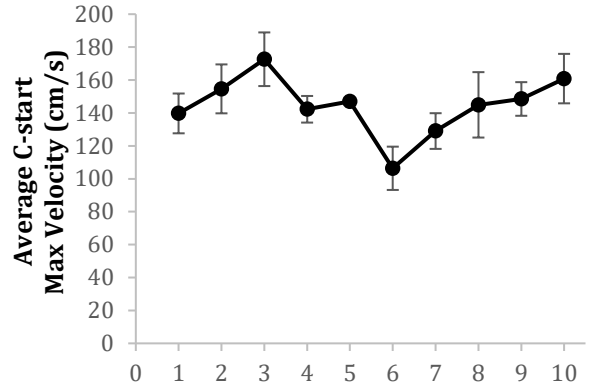
Reference and Species	Exploratory Behaviour		Behavioural Trait Correlated with Exploratory Behaviour
	Exploratory Trait(s)	**Methodology**	
Killen et al (2011) European sea bass *(Dicentrarchus labrax)*	● Time spent in open area of tunnel ● Number of transitions between sections of tunnel	● Simulated predator attack leading subjects down tunnel towards cover ● Food placed where attack occurred to entice subjects to swim back through tunnel	**Boldness** Time taken to emerge from cover following a simulated predator attack
Boulton et al (2014) Sheepshead swordtail *(Xiphophorus birchmanni)*	● Time spent in middle of tank ● Percentage of area covered during exploration ● Percentage of time swimming at minimum of 1.5 cm/s* *Activity trait	● No use of predator ● Open environment	**Boldness** Tendency to emerge from a refuge in a novel apparatus No use of predator
Mazué et al (2015) Convict cichlids *(Amatitlania nigrofasciata)*	● Proportion of novel environment explored ● Time spent in novel environment	● No use of predator ● Open environment	**Boldness** Latency to emerge in an open environment in the presence/absence of a live predator
Moscicki and Hurd (2015) Convict cichlids *(Amatitlania nigrofasciata)*	● Time spent in corners of the tank	● No use of predator ● Open environment	**Boldness** Latency to emerge from shelter in a novel open environment No use of predator

Jones and Godin (2010) Convict cichlids *(Amatitlania nigrofasciata)*	• Number of squares explored in tank	• No use of predator • Open environment	**Responsiveness** Response latency to the presence of a predator model while foraging
Quinn and Cresswell (2005) Chaffinches *(Fringilla coelebs)*	• Number of times a row of straws was crossed during flight	• No use of predator • Open environment	**Freezing behaviour** In response to a simulated predator attack
Budaev (1997) Guppies *(Poecilia reticulata)*	• Number of squares crossed per minute • Proportion of time spent swimming* *Activity trait	• In the presence of a predator • Open environment	**Freezing Behaviour** In response to the presence of a live predator

Table A.2. Burst swimming performance (velocity and acceleration) from previous research on ectotherms suggests acclimation to high temperatures leads to no change or a trend to lower maximum velocity and acceleration following moderate increases in acute temperature. Each study measured burst swimming performance at an acute temperature of 5°C above acclimation temperature. Observed trends in each study were not statistically analysed.

Species	**Acclimation Temperature**	**Impact of +5°C acute temperature on velocity**	**Impact of +5°C acute temperature on acceleration**	**Reference**
Goldfish *Carassius auratus*	35°C	Trend to lower velocity	----------	*Johnson and Bennett 1995*
Eastern Mosquitofish *Gambusia holbrooki*	25°C	Trend to lower velocity	----------	*Grigaltchik et al 2012*
Australian Bass *Macquaria novemaculeata*	25°C	No observed change	----------	*Grigaltchik et al 2012*
African Clawed Frog (adults) *Xenopus laevis*	25°C	No observed change	*Trend to increase in time to maximum velocity	*Wilson et al 2000*
Pacific Tree Frog (tadpoles) *Hyla regilla*	25°C	Trend to lower velocity	----------	*Watkins 2000*
Long-spined sea scorpion *Taurulus bubalis*	15°C	Trend to higher velocity	No observed change	*Temple and Johnston 1998*
Short-horn sculpin *Myoxocephalus scorpius*	15°C	Trend to lower velocity	No observed change	*Temple and Johnston 1998*

A)



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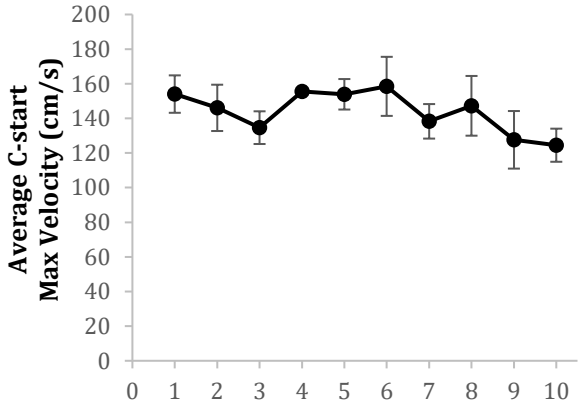

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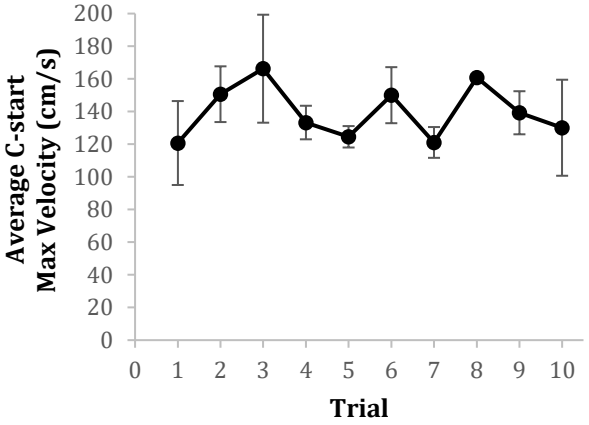
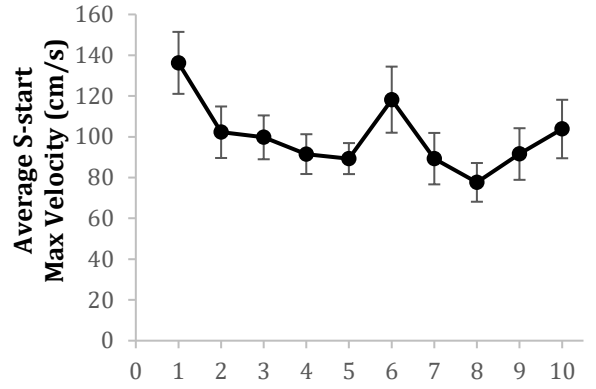


Figure C.1. Average C-start Maximum Velocity (MV) of gobies between subjects across trials in a session for, A) Day 1, B) Day 2, C) Day 3. Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1. Error bars depict standard error of the mean.

A)



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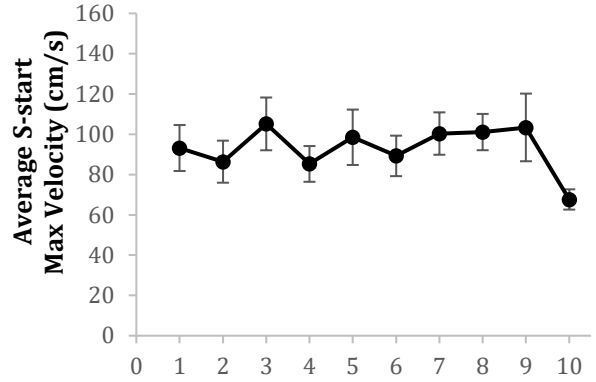

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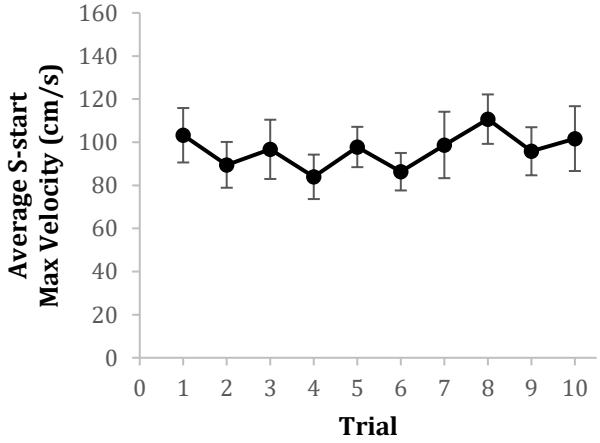

Figure C.2. Average S-start Maximum Velocity (MV) of gobies between subjects across trials in a session for, A) Day 1, B) Day 2, C) Day 3. Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1. Error bars depict standard error of the mean.

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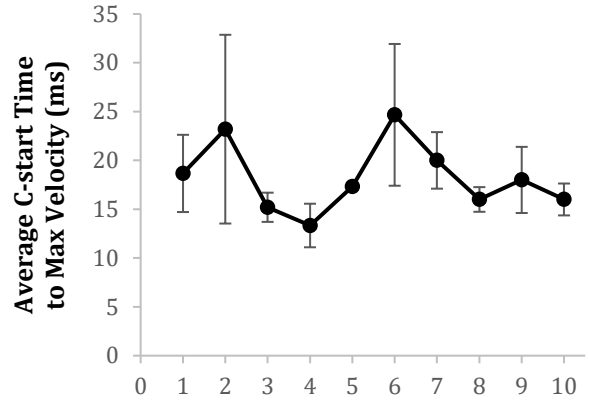

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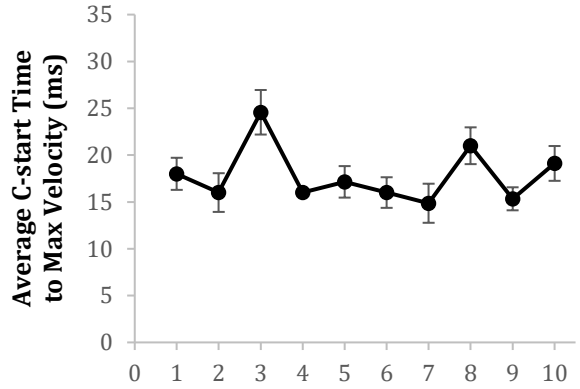

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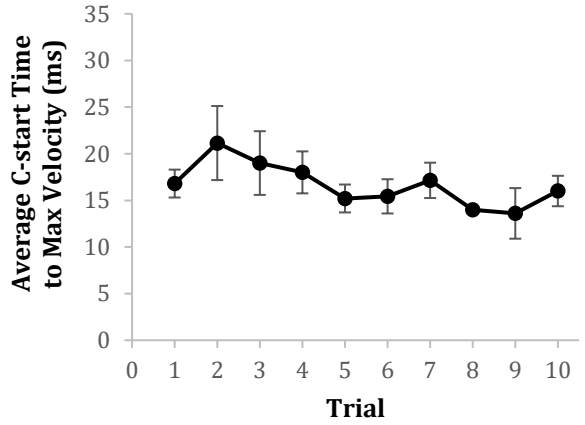

Figure C.3. Average C-start Time to MV of gobies between subjects across trials in a session for, A) Day 1, B) Day 2, C) Day 3. Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1. Error bars depict standard error of the mean.

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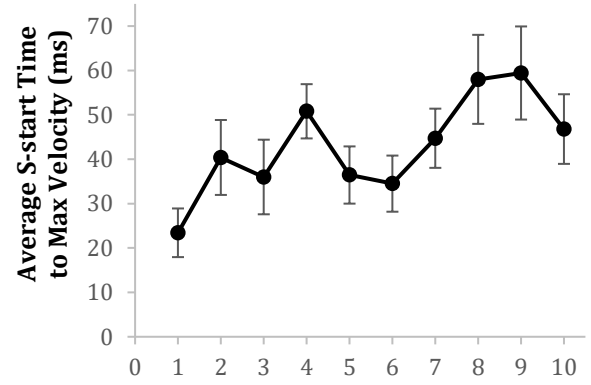

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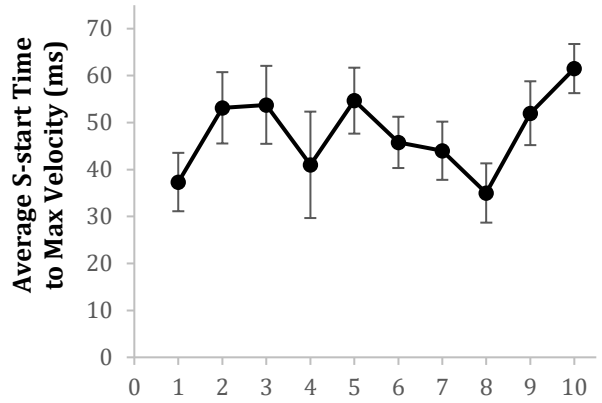

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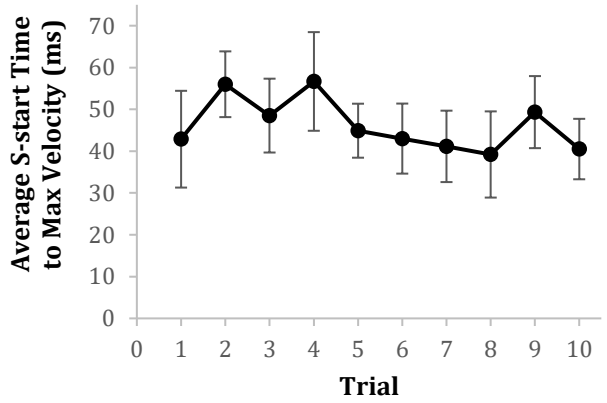
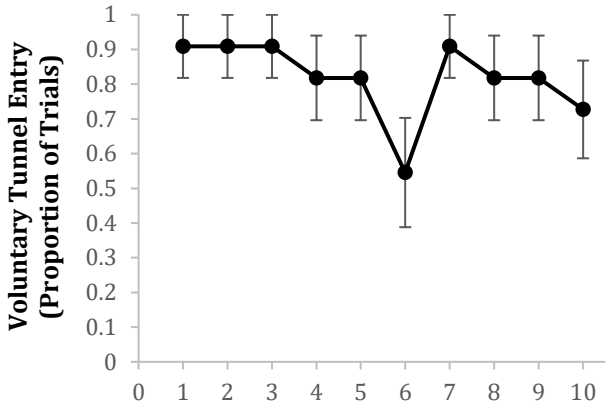


Figure C.4. Average S-start Time to MV of gobies between subjects across trials in a session for, A) Day 1, B) Day 2, C) Day 3. Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1. Error bars depict standard error of the mean.

A)



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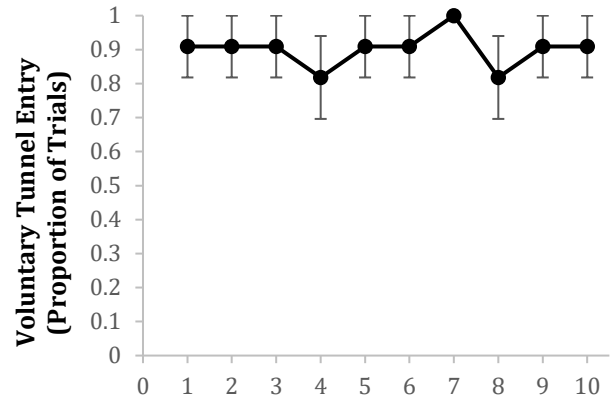

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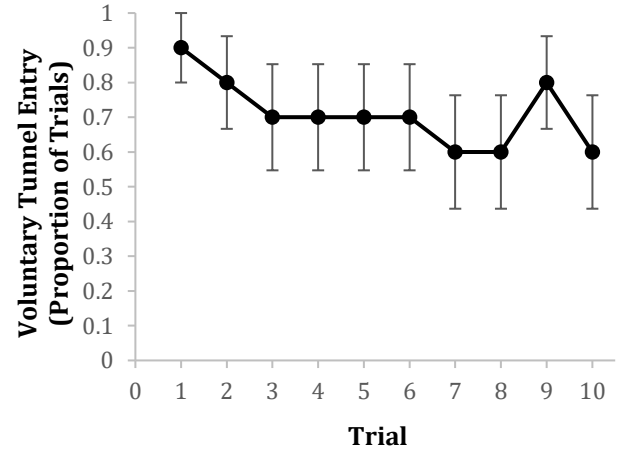

Figure D.1. Average proportion of gobies that voluntarily entered the tunnel in the behavioural trait Voluntary Tunnel Entry (VTE) between subjects across trials in a session for, A) Day 1, B) Day 2, C) Day 3. Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1. Error bars depict standard error of the mean.

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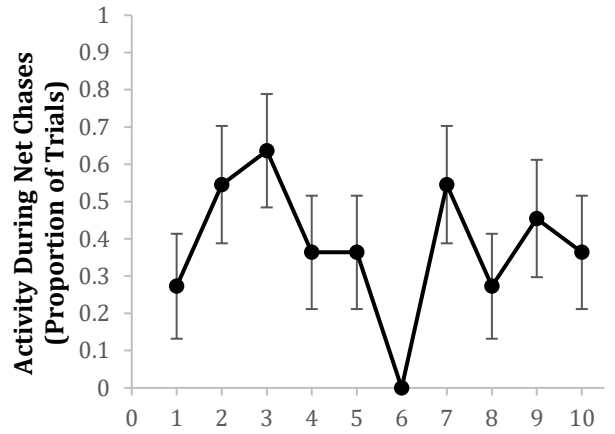

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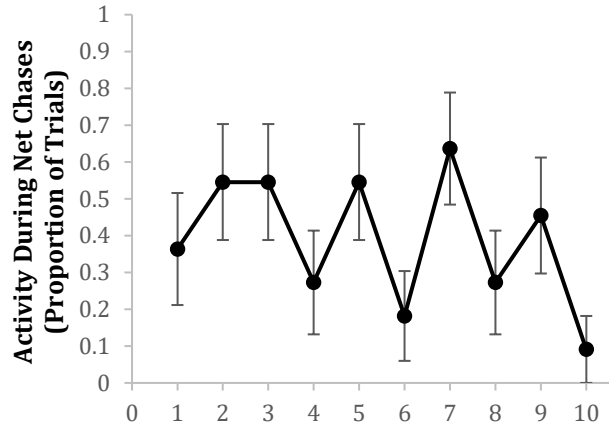

C)

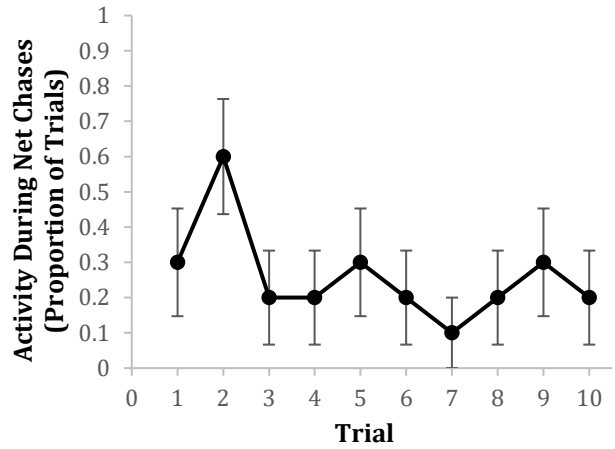

Figure D.2. Average proportion of gobies that were more reactive to chases in the behavioural trait Activity during Net Chases (ANC) between subjects across trials in a session for, A) Day 1, B) Day 2, C) Day 3. Subjects experienced constant temperatures across 3 days of experimentation following testing at their acclimation temperature (21°C) on Day 1. Error bars depict standard error of the mean.