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The Plastic and Evolutionary Responses of Fish to Anthropogenic Stressors

Ross D. Breckels, *The University of Western Ontario*

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Biology

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THE PLASTIC AND EVOLUTIONARY RESPONSES OF FISH TO
ANTHROPOGENIC STRESSORS

(Thesis format: Integrated Article)

by

Ross David Breckels

Graduate Program in Biology with Environment and Sustainability

A thesis submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

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Abstract

Ecosystems are being altered at unprecedented rates with little knowledge of the potential impacts on biodiversity. Two of the most pressing contemporary anthropogenic stressors are pollution and global warming. Species can respond to these stressors via dispersal, phenotypic plasticity, or evolutionary adaptation. Many species, especially aquatic organisms, experience ecological or physical barriers to dispersal and will therefore have to respond via phenotypic plasticity or evolutionary responses. I examined the responses of multiple traits associated with fitness in fish to pollution and increased temperature using a 2×2 common garden experimental design. I examined the effects of pollution on behaviour in a natural population of brown bullheads (*Ameiurus nebulosus*), and increased temperature on population demographics, life history traits, reproductive traits, and the immune response in experimental populations of guppies (*Poecilia reticulata*). The plastic responses to pollution were increased locomotion and decreased aggression and the plastic responses to increased temperature were decreased age at maturity, sperm length, sperm velocity, and sperm path linearity. These results are indicative of stress responses by the fish and could potentially decrease reproductive success and survival. Next, I measured reproductive output in experimental populations of guppies and found that, after many generations in elevated temperature, females produced fewer, smaller broods than control populations. However, I found no effect of temperature on census population size, survivorship, sex ratio, size-at-age, or the immune response, indicating that, despite the decreased reproductive output, guppies appear to cope with the increased temperature. Additionally, genetic diversity in the elevated temperature populations decreased more rapidly than control populations, and was

equivalent to one quarter the effective population size relative to the controls. This latter result shows a clear signature of selection. Indeed, I found that sperm length displayed an evolutionary response in an estimated 2-3 generations. And in a natural population of bullheads, after an estimated 33 generations, I found an evolutionary response in locomotion and aggression. However, the reduced genetic diversity could lower the adaptive potential of populations to future stressors. I discuss these results in the context of the scope of organisms to rapidly respond to anthropogenic stressors.

Keywords

Adaptation, anthropogenic stressors, aquatic ecosystems, behaviour, brown bullhead, evolutionary response, genetic diversity, guppy, life history traits, phenotypic plasticity, pollution, population size, reproduction, selection, sperm, temperature

Co-Authorship Statement

A version of Chapter 2 was published in the journal *Ecotoxicology* with Bryan Neff as a co-author. Dr. Neff provided funding for the project, was involved with study design, and contributed editorial comments to the manuscript.

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A version of Chapter 4 has been submitted to *Evolutionary Ecology* with Bryan Neff as a co-author. Dr. Neff provided funding for the project, was involved with study design, and contributed editorial comments to the manuscript.

A version of Chapter 5 was published in *Evolutionary Ecology* with Bryan Neff and Shawn Garner as co-authors. Dr. Neff provided funding for the project, was involved with study design, and contributed editorial comments to the manuscript. Dr. Garner performed parts of the analysis and contributed editorial comments to the manuscript.

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List of Abbreviations

ANCOVA: Analysis of covariance

ANOVA: Analysis of variance

ATP: Adenosine triphosphate

BR: Belle River

BSA: Bovine serum albumin

CaCl₂: Calcium chloride

CT_{\max} : Critical thermal maxima

DDT: Dichlorodiphenyltrichloroethane

DNA: Deoxyribonucleic acid

DR: Detroit River

GDD: Growing degree-days

GLMM: General linear mixed model

HSB: Hue, Saturation, and Brightness

HWE: Hardy-Weinberg equilibrium

ID: Identification

IPCC: Intergovernmental Panel on Climate Change

KCl: Potassium chloride

MgSO₄: Magnesium sulphate

MS-222: Tricaine methanesulfonate

NaCl: Sodium chloride

PAH: Polycyclic aromatic hydrocarbons

PBS: Phosphate buffered saline

PCB: Polychlorinated biphenyl

PHA: Phytohaemagglutinin

Plastic: Phenotypic plasticity

PPM: Parts per million

RGB: Red, Green, and Blue

SD: Standard deviation

SE: Standard error

TC: Trenton Channel

T_{hab} : Mean habitat temperature

T_{opt} : Thermal optimum

Tris: Tris(hydroxymethyl)aminomethane

VCL: Curvilinear velocity

VSL: Straight line velocity

Chapter 1

1 General introduction

In 1992, a joint statement released by Britain's Royal Society and the US National Academy of Sciences concluded that "if current predictions of population growth prove accurate...the future of our planet is in the balance" (Press and Atiyah 1992).

Anthropogenic influences are changing the environment, both locally and globally, through population expansion, industrialisation, and increased intensification of agriculture (Moss 1998). This environmental alteration is occurring at unprecedented levels with little knowledge of the potential consequences these alterations will have on organisms. Pollution and global warming are two of the most pressing anthropogenic stressors - defined here as any human-induced alteration of the environment that elicits a stress response in organisms - which are predicted to have serious negative impacts on biodiversity, especially in aquatic ecosystems (Moyle and Leidy 1992; Ficke et al. 2007). There are suggestions that "we are in the midst of a mass extinction caused by the advancement of one species: *Homo sapiens*" (Angilletta 2009). One of the most pressing contemporary concerns is whether organisms can respond to these stressors on an ecological timescale (Hendry et al. 2008).

In order to avoid widespread extinctions due to anthropogenic stressors, such as pollution or global warming, Fuller et al. (2010) suggested that organisms will have to respond via one, or more, of the following: (1) dispersal - defined here as the movement of populations from a habitat that has become unsuitable or undesirable to inhabit, to a new, more favourable habitat; (2) phenotypic plasticity - where different environmental

conditions trigger a given genotype to display different phenotypes; or (3) an evolutionary response - defined here as genetic changes driven by natural selection on favourable traits. There are many studies documenting dispersal in response to anthropogenic stressors (reviewed in Parmesan 2006). For example, Thomas and Lennon (1999) documented a mean northward shift in 12 bird species in the UK of approximately 20 km over 20 years in response to contemporary climate warming. However, depending on their movement abilities, many species will face physical barriers to dispersal, such as mountain ranges or large water bodies. The dispersal ability of organisms may also be compromised by ecological restrictions, such as food or shelter availability. Dispersal is particularly problematic for aquatic organisms, such as fish, which face a multitude of barriers, such as dams and waterfalls. Additionally, water bodies are often spatially restrictive. Therefore, for those species that cannot disperse in response to anthropogenic stressors, they may instead have to respond via phenotypic plasticity or genetic adaptation. It is these latter two responses that I focus on for my thesis.

1.1 Phenotypic plasticity

The evolution of phenotypic plasticity results in genotypes producing better phenotype-environment matches, in terms of fitness, across a broad range of environments than a trait that does not display phenotypic plasticity (DeWitt et al. 1998). Plasticity includes individual responses to environmental stimuli at all levels of biological organization (Angilletta 2009). Indeed, morphological, physiological, life history, and behavioural traits can all show phenotypic plasticity (e.g. Berry and Bjorkman 1980; Kaufmann and Bennett 1989; Dhillon and Fox 2004). Phenotypic plasticity is best

detailed through a reaction norm which describes the pattern between phenotype expression (i.e. phenotypic performance) over a continuous environmental variable (West-Eberhard 2003). For example, Barlow (1962) detailed the innate capacities for population increase in two species of aphid over a broad range of temperatures and found that the optimum temperature for population growth was approximately 20°C. This plastic response is often the first response of organisms to environmental change, and may be the only response for many long-lived species with long generation times (Bradshaw and Holzapfel 2006; Fuller et al. 2010). Therefore, it is of crucial importance that we better understand the extent of plasticity in nature in order to predict the fate of organisms in changing environments (Somero 2010). A phenotypically plastic response allows an organism to achieve continued performance over a larger range of environmental conditions than they could otherwise. However, phenotypic plasticity has many constraints associated with it which can be categorised into costs (e.g. maintenance and production costs) and limitations (e.g. information reliability and lag-time; reviewed in DeWitt et al. 1998). Plasticity occurs only at the individual level, therefore, each successive generation will have to re-acquire this plastic response which could hinder optimal offspring development and, consequently, fitness as they require energy which could have been channelled towards other somatic processes, such as growth, maturation, and reproduction. Phenotypic plasticity may be advantageous to individuals in that it allows them to adapt to changes in environmental conditions in a manner that will increase fitness, but it may be hindered by these costs and limitations (reviewed in DeWitt et al. 1998). Thus, heritable alternatives would potentially be more advantageous.

1.2 Evolutionary response

Anthropogenic stressors may have large evolutionary consequences by inducing greater selection pressures than would occur naturally (Reznick and Ghalambor 2001). Thus, exposure to these stressors could potentially result in unprecedented rates of evolution. Traditionally, it was believed that an evolutionary response would take thousands of generations to occur (Darwin 1859). More recently, however, evolutionary biologists have recognized that evolution can also occur over much shorter temporal scales. In general, adaptive evolutionary events occurring over a relatively short time-frame are referred to as ‘contemporary’ or ‘rapid’ evolution (Hendry and Kinnison 1999). Perhaps the most notable example of rapid evolution comes from the medium ground finch (*Geospiza fortis*) on the Galápagos Island of Daphne Major, where individuals underwent a severe selection event and, consequently, showed signs of adaptive evolution in just one generation (Boag and Grant 1981; 1984; Grant and Grant 1995; 2003). Since this seminal research on *G. fortis*, interest in rapid evolution has grown considerably, especially over the last decade with the recent realization that most documented examples of rapid evolution are attributed, at least in part, to anthropogenic changes to the environment. Indeed, evolutionary responses are considered essential for population persistence in the face of long-term environmental changes (Lande and Shannon 1996).

Darwin (1859) outlined four postulates which have to be met in order for evolution by natural selection to occur: (1) there must be variation among individuals of a population; (2) this variation must, at least in part, be heritable (i.e. passed on from parent to offspring); (3) this variation would lead to variation in survival between individuals; and (4) those individuals with the most favourable traits would have the highest

reproductive success and consequently pass on their traits to their offspring. Populations can evolve through genetic adaptations by two means; selecting from standing (pre-existing) genetic variation (e.g. Jump et al. 2006), or selecting for new mutations (reviewed in Barrett and Schluter 2008). Evolving from standing genetic variation should occur more rapidly because desirable traits would already be present in the population and at higher frequencies than the rate that mutations could otherwise introduce them. However, such desirable genetic variation is not always present, so mutations may be necessary for some populations to respond genetically to changes in the environment. Favourable mutations likely do not occur that often, even though some stressors increase mutation rates (e.g. pollution; Cachot et al. 2007). Indeed, according to the neutral theory of molecular evolution, advantageous mutations are exceedingly rare (Kimura 1983). Thus, species may require that favourable alleles be present in the population in order to display an evolutionary response to these anthropogenic stressors.

Responding to anthropogenic stressors via evolutionary adaptation may be the best means of adaptation because these beneficial traits do not come at a cost to development, and many species have limited scope for plasticity (e.g. Stillman 2003; reviewed in DeWitt et al. 1998). However, exposure to anthropogenic stressors has been shown to significantly reduce genetic variation in populations (e.g. exposure to pollution in brown bullheads, *Ameiurus nebulosus*, Silbiger et al. 2001; least killifish, *Heterandria formosa*, Athrey et al. 2007; and midges, *Chironomus riparius*, Nowak et al. 2009; and exposure to increased temperature in fruit flies, *Drosophila subobscura*, Santos et al. 2005), demonstrating the significant selection forces these stressors apply on populations (Reznick and Ghalambor 2001). Additionally, increased selection can also lead to

inbreeding and inbreeding depression (Charlesworth and Charlesworth 1987; e.g. Kristensen et al. 2003). Thus, selection can come at a cost to a population, as inbreeding depression and reduced genetic diversity reduces the ability of the populations to respond to other stressors (Meyer and Di Giulio 2003; Vogt et al. 2010; reviewed in Pauls et al. 2013).

1.3 Pollution

1.3.1 Pollution in aquatic environments

Anthropogenically-induced pollution, defined here as the introduction of toxic chemicals into the environment that elicits adverse changes in the local biodiversity, is increasingly becoming a wide-spread environmental stressor and has the potential to cause drastic effects on ecosystems. Aquatic environments are especially vulnerable to pollution because many different forms of anthropogenic waste can enter water systems via multiple means such as dumping, leaching, and run-off (Moyle and Leidy 1992). Now only the most remote rivers and streams remain relatively unpolluted (Moss 1998). Aquatic ecosystems have thus become loaded with chemicals which greatly exceed the concentrations with which they naturally occur. Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals are all found in much higher concentrations in many aquatic environments than considered natural (e.g. Furlong et al. 1988; Arcand-Hoy and Metcalfe 1999). These pollutants have the potential to elicit adverse effects on aquatic biodiversity.

There are three main mechanisms by which pollutants can affect organisms: (1) imposing additional forms of density-independent breeding failure or mortality, such as the reduced levels of protective egg wrapping behaviour observed in dwarf newts, *Triturus pygmaeus* (Ortiz-Santaliestra et al. 2007) or reduced eggshell thickness seen in British sparrowhawks, *Accipiter nisus*, exposed to DDT (dichlorodiphenyltrichloroethane; Newton 1986); (2) reducing food supplies, for example, the decline of the grey partridge, *Perdix perdix*, was associated with declines in its insect prey as a result of insecticide exposure (e.g. Potts 1986); and (3) altering the chemical or physical structure of habitats, for example eutrophication which results in decreased oxygen availability (Newton 1998). For the purpose of my thesis, I focus on the first of these three points; the direct effects of pollution on organisms, and concentrate, where possible, on pollution in aquatic fauna (animal rather than plant life).

1.3.2 The plastic responses to pollution in aquatic organisms

The plastic responses of aquatic organisms to pollution have been well documented. Most examples of these responses involve species being subjected to a single chemical under laboratory conditions (e.g. Milne et al. 2000). Single contaminant exposure has been shown to affect fish behaviour (guppies, *Poecilia reticulata*, Yilmaz et al. 2004), chemosensory functions (pike minnow, *Ptychocheilus lucius*, Beyers and Farmer 2001), sperm traits (e.g. African catfish, *Clarias gariepinus*, Kime et al. 1996; and *P. reticulata*, Tian et al. 2012), reproductive success, ornamentation, sex ratios (e.g. *P. reticulata*, Cardinali et al. 2004; Tian et al. 2012), and survivorship (rainbow trout, *Oncorhynchus mykiss*, Allin and Wilson 2000; rainbow trout and brown trout, *O. mykiss* and *Salmo trutta*, Milne et al. 2000; and *P. reticulata*, Tian et al. 2012). An organism's

ability to respond to multiple pollutants simultaneously, however, is likely to be far more complex. For example, Klerks (1999) examined the acclimatory ability in grass shrimp (*Palaemonetes pugio*) survival to individual and mixtures of contaminants and found that, while the shrimp could acclimate to individual contaminants, they could not acclimate to a mixture. In reality, species in the wild will be exposed to multiple different pollutants simultaneously. For example, the Detroit River, Canada/US, is polluted by various heavy metals, PAHs, PCBs, and numerous other organic chemicals (Furlong et al. 1988; Arcand-Hoy and Metcalfe 1999). Exposure to multiple contaminants can elicit a variety of negative impacts (reviewed in Jones and Reynolds 1997; Harmon and Wiley 2011). Thus, studying the effects of multiple pollutants simultaneously will enable us to better understand and manage natural populations.

1.3.3 The evolutionary responses to pollution in aquatic organisms

To date, few studies have attempted to evaluate the sub-lethal impacts of multiple contaminants on aquatic organisms within a single experiment. Most examples of genetic responses to pollution, such as insects becoming resistant to DDT (e.g. mosquitos, *Anopheles arabiensis*, Jones et al. 2012), plants developing resistance to certain heavy metals (e.g. the grass, *Agrostis stolonifera*, Wu et al. 1975), and rodents becoming resistant to the pesticide warfarin (e.g. house mice, *Mus musculus*, Howe and Redfern 1965), have come from a small subset of exposed species and usually only involve a single chemical (Newton 1998). Of the few studies that do examine the evolutionary responses of species to multiple chemicals combined, the majority only examine survival. For example, wild-caught mosquitofish, *Gambusia affinus*, collected from a polluted river, and their offspring reared in unpolluted water in a laboratory setting, had higher

rates of survival in contaminated water than controls from non-polluted sites, suggesting an evolved resistance of the mosquitofish to pollution (Andreasen 1985). However, these results could also have been confounded by maternal environmental effects (non-genetic information passed on from mother to offspring) or by epigenetics (changes in gene expression). Regardless, the fish from the contaminated site would have an advantage over other individuals that do not possess these maternal environmental or epigenetic effects. Indeed, in another study, killifish (*Fundulus heteroclitus*) from the polluted Elizabeth River in Virginia, US, showed a true genetic response to pollution (i.e. a genetic response was disentangled from maternal environment effects and epigenetics; Meyer and Di Giulio 2003). Laboratory raised F₁ and F₂ generations from Elizabeth River reared in unpolluted water showed normal development and were better adapted to survive than controls when put in polluted water. Clearly, there is an urgent need to determine the sub-lethal evolutionary responses of wild populations, such as behaviour and genetics, to multiple contaminants which are more representative of the pollution currently found in nature.

1.4 Global warming

The occurrence of global warming is now unequivocal, as is evident from current increases in air and water temperatures, snow and ice melts, and sea level rises (IPCC 2007). The level of warming to date has already resulted in: (i) decreased diel (24 hour period) variation as night time temperatures are increasing more than daytime temperatures; (ii) decreased seasonal variation as winter temperatures are increasing more than summer temperatures; (iii) increased frequency of heat waves; and (iv) decreased

frequency of cold snaps (IPCC 2007). This has led to a warmer, less thermally variable climate. Evidence suggests that the current temperature increase is more rapid than, and will surpass all other periods of climate warming known from the fossil record (Allan et al. 2005). In particular, it is the rate of global warming that will be more critical than either the magnitude or the duration of change in temperature to the persistence of species as species will have to adapt to these conditions more rapidly (Davis et al. 2005). Indeed, the rate of warming over the last decade alone exceeded the rate of warming observed over the last five decades (IPCC 2007). The period between 1995 and 2006 had 11 of the 12 warmest years since the commencement of instrumental records in 1880, and this warming trend is only likely to increase (IPCC 2007).

Global warming is accelerated by the release of greenhouse gases (or 'heat-trapping' gases, such as carbon dioxide, methane, and nitrous oxide) and aerosols which affect absorption, scattering, and emission of radiation within the earth's surface and the atmosphere, leading to increased temperatures. Emissions of greenhouse gases are rapidly increasing and the projection for carbon dioxide alone is an increase of 40-110% by 2030 causing a 0.5°C global temperature rise (IPCC 2007). As these emissions are the by-product of energy, transportation, and other essential industries to humans, we cannot expect emissions to cease overnight (Angilletta 2009). Thus, in 2007, the Intergovernmental Panel on Climate Change (IPCC) projected an average global air temperature increase from current levels, which are already 0.74°C higher than the beginning of the 20th century, of 1.8-4.0°C by the year 2100. These predictions were based on the best estimates from six climate change models with likely ranges of 1.1-6.4°C (IPCC 2007). This temperature rise is the equivalent to shifting temperate climatic

belts polewards by 200-300 km or shifting altitudes upwards by 200 m (Newton 1998). As a result, global warming could have startling consequences for both ecosystems and biodiversity, especially for those species that cannot disperse. Indeed, in a review of the potential negative effects of global warming, Thomas et al. (2004) predicted that some 38-52% of species included in the study that cannot disperse would be extinct by 2050 versus 21-32% of the total 1,103 animal and plant species in the study.

1.4.1 The effects of contemporary global warming on organisms

The biological impacts of climate change are already under way (Angilletta 2009). Indeed, the level of warming to date has already resulted in many populations becoming extinct (e.g. Sinervo et al. 2010), and many more experiencing range shifts or altered phenology (the timing of certain life events, such as breeding or migration; reviewed in Parmesan 2006). Almost 60% of 1,598 species exhibited a shift in their ranges and/or phenology over the past 20-140 years, predominantly in the direction expected from climate warming, and 41% of these species have already been impacted by this warming (Parmesan 2006). The phenological response has been an advancement of 2.3 days per decade and species in the Northern Hemisphere generally shifted their ranges 6.1 km northwards or 6.1 m higher in altitude per decade (Parmesan and Yohe 2003). One of the most notable examples of a species responding to global warming is that of the North American red squirrel, *Tamiasciurus hudsonicus* (Réale et al. 2003). Red squirrels from the Yukon, Canada, have shown both phenotypic plasticity and evolutionary responses to the increased spring temperatures and earlier availability of food supplies by shifting their breeding timing 18 days earlier over a 10 year period (Réale et al. 2003). However, there are few examples of evolutionary responses to the future rate of temperature increase

predicted by global warming. Temperature has been described as the ‘ecological master factor’ because so many abiotic factors depend upon it (Brett 1971), thus it is crucial to understand the effects of temperature on organisms, especially tropical species (detailed below), and their responses to this increased temperature.

1.4.2 The potential effects of global warming on tropical ectotherms

The level and rate of warming across the globe is highly spatially heterogeneous. The highest level of warming is predicted to occur in high northern latitudes, with the Arctic likely to experience warming rates nearly double those of the global average (Walther et al. 2002), and temperate regions of the Northern hemisphere are expected to warm more than tropical regions (IPCC 2007). The tropics constitute the vast majority of the world’s biodiversity, and, of this biodiversity, the vast majority are ectotherms (Wilson 1992). Ectotherms are organisms that cannot regulate their body temperature via physiological means. Despite the predicted heterogeneity in warming levels across the globe, it is tropical ectotherms that are predicted to be most at risk (Deutsch et al. 2008; but see Walters et al. 2012). This prediction is based on the fact that the basic physiological functions of ectotherms, such as growth and reproduction, are dependent on the ambient temperature. As well, thermal tolerance has been shown to have a positive relationship with temperature variation (e.g. Addo-Bediako et al. 2000). The climate in tropical regions is less thermally variable than temperate regions, so tropical species tend to be adapted to a smaller range of environmental conditions and therefore, tropical species have a narrower thermal performance breadth than temperate species (Deutsch et al. 2008; Angilletta 2009; Dillon et al. 2010). As such, many tropical ectotherms are

currently living close to or at their thermal limits (Deutsch et al. 2008; Angilletta 2009; Dillon et al. 2010), and have a lower capacity for phenotypic plasticity (e.g. Stillman 2003) and evolutionary responses (e.g. Hoffmann et al. 2003). Thus, there has been growing concern regarding the persistence of tropical ectotherms in the context of global warming.

1.4.3 The plastic responses of increased temperature on ectotherms

Increases in temperature can have multiple effects on organisms, including the loss of motor activities, increased metabolism and ventilation rates, protein degeneration, denaturing of enzymes, increased cell division and differentiation, and differential gene transcription (Hochachka and Somero 2002 and references therein). All of these effects can disrupt the equilibrium of internal processes and result in less energy for other somatic (bodily) functions, such as growth, reproduction, and immune responses. Here, I focus on the higher-level, ecological effects of increased temperature on ectotherms.

Studies of the effects of increased temperature have documented changes in life history traits (e.g. small white butterflies, *Pieris rapae*, Kingsolver 2007; and neotropical pseudoscorpions, *Cordylochernes scorpioides*, Zeh et al. 2012), behaviour (e.g. desert night lizards, *Xantusia vigilis*, Kaufmann and Bennett 1989; and marsh frogs, *Limnodynastes peroneii*, Wilson and Franklin 1999), ornamentation (e.g. three-spined sticklebacks, *Gasterosteus aculeatus*, Borg 1982; and fathead minnow, *Pimephales promelas*, Brian et al. 2011), sperm quality (e.g. Siberian sturgeon, *Acipenser baeri*, Williot et al. 2000; eastern mosquitofish, *Gambusia holbrooki*, Adriaenssens et al. 2012; and *C. scorpioides*, Zeh et al. 2012; reviewed in Alavi and Cosson 2005), reproductive

success (e.g. *G. aculeatus*, Hopkins et al. 2011), and immunology (e.g. tench, *Tinca tinca*, Collazos et al. 1996). Most of the plastic effects involving just slight increases in temperature on ectotherms have resulted in a potential decreased fitness (e.g. Hopkins et al. 2011; Zeh et al. 2012). Indeed, while it is known that increasing temperatures in general could have significant and adverse effects on different traits in ectotherms, relatively little is known about the evolutionary responses to counteract the rapid rates of temperature increase associated with global warming. Evolutionary responses to global warming will surely play a role in determining the degree to which ectotherms will be negatively impacted (Angilletta 2009).

1.4.4 The evolutionary responses of increased temperature on ectotherms

Most examples of rapid thermal adaptation in ectotherms come from plants. For example, the European beech tree, *Fagus sylvatica*, in Catalonia, Spain, was able to cope with the ambient temperature increases to date by selecting for heterozygotes at a temperature-linked locus (Jump et al. 2006). This gene pre-existed due to previous climatic fluctuations, thus the trees could evolve rapidly to the recent increase in temperature by selection on standing genetic variation. As well, there are many examples of animals that have become adapted, over hundreds of generations, to their thermal habitat. For example, Dahlgaard et al. (2001) found that both wild-caught and laboratory reared fruit flies, *D. buzzatii*, from a highland, cooler site (Tilcara, Argentina) were significantly larger in size than those caught from a lowland, warmer site (Catamarca, Argentina). However, these flies would have had many thousands of generations to adapt to their climatic conditions. The rate of global warming in the future is expected to

surpass all previous rates in the fossil record (Allan et al. 2005). Thus, species will have to adapt more rapidly to the new climate or face extinction.

There are many examples of wild and laboratory selection experiments involving bacteria, fruit flies, and viruses (see Angilletta 2009). However, there have been few studies documenting rapid evolution to increased temperature in ectothermic vertebrates (but see Leal and Gunderson 2012 who show that lizards introduced to Miami, FL, US from Puerto Rico evolved their critical thermal minimum after just 35 years in response to the cooler climate). Hendry et al. (1998) provides one of the few empirical examples of a wild, ectothermic vertebrate population (sockeye salmon, *O. nerka*) showing rapid genetic thermal adaptation. Embryos from populations of newly diverged Lake Washington sockeye salmon that experienced higher temperatures had evolved to display higher survival rates at increased temperatures than embryos that experienced cooler temperatures after only 9-14 generations (Hendry et al. 1998). This result provides evidence that species can adapt rapidly via evolutionary responses to increased temperature. However, most studies that document a genetic response to increased temperature in ectotherms only detail survivorship (e.g. Nakajima et al. 2009); few have examined the evolutionary response of sub-lethal traits.

1.5 Fish as a model species

For my thesis I focus on the effects of pollution and global warming, currently two of the most severe threats to biodiversity. Pollution is particularly abundant and problematic in aquatic ecosystems (Moyle and Leidy 1992), and global warming is

predicted to have the most severe impact on tropical ectotherms (Deutsch et al. 2008). I chose fish as my study organisms for four reasons. The primary reason for using freshwater fish is that they will likely experience more barriers to dispersal than other organisms. Thus, in the absence of dispersal, they will have to adapt to anthropogenic stressors via phenotypic plasticity or an evolutionary response. Second, aquatic systems are sinks for most forms of anthropogenic waste (Moyle and Leidy 1992), and hence, aquatic organisms are exposed to pollutants in much higher concentrations than the majority of other, terrestrial organisms. Third, most fish, with a few exceptions, such as tuna and sharks of the family Lamnidae, are ectotherms. Some ectotherms can partially control their body temperature through behaviour, a process known as behavioural thermoregulation, via such methods as basking in the sun to warm up or going down a burrow to cool down. However, most freshwater fish are poikilotherms; their ability to behaviourally thermoregulate is constrained by their generally thermally homogeneous aquatic environment. Thus, they are, for the most part, dependent on the ambient temperature for all their physiological processes. Consequently, fish, and especially tropical fish (see Deutsch et al. 2008), will be among the most vulnerable to the predicted level of global warming. Finally, there has been a great deal of research that has documented phenotypically plastic responses of fish to anthropogenic stressors, yet comparatively little work has documented their evolutionary responses. Indeed, evolutionary responses to anthropogenic stressors in vertebrates as a whole have been rare (Nacci et al. 2002). Thus, it is crucial that we detail the evolutionary responses of fish to anthropogenic stressors, as fish constitute a large part of both the diet and economy of most of the human population.

I use two different fish species in my thesis, the brown bullhead (*A. nebulosus*) and the guppy (*P. reticulata*). First, I studied the plastic and evolutionary responses of behaviour in bullheads as these traits tend to be more labile and evolve more rapidly than most other traits (Wcislo 1989; West-Eberhard 2003). I studied bullheads because they are native to north-eastern North America (Wheeler 1978) and are common in southern Ontario (Scott 1955) where they are frequently exposed to high levels of contaminants (e.g. Drouillard et al. 2006). Since as early as the 1960's, water pollution has been recognised as a major problem in North America (Hall et al. 2006). In the catchment areas of the Great Lakes, some 30,000 commercially significant chemicals are manufactured, with about 3,500 new chemicals being manufactured each year (Moss 1998). As a result, many water systems of the Great Lakes have chemical loads that exceed health and safety guidelines (e.g. Drouillard et al. 2006). Most of the pollutants in aquatic ecosystems are accumulated in the sediment which can act as both sinks and secondary sources of pollution (Cachot et al. 2007). These chemically loaded sediments will result in bioaccumulation (the accumulation of substances in an organism) and, consequently, there is concern about the potential impacts of pollution on aquatic organisms, and fish in particular, for both economic and human health reasons. Brown bullheads are benthic and have a high affiliation with the sediment. As they are a game fish and are also commonly used in aquaculture (www.fishbase.org), it is vital that we understand the effects of pollution on brown bullheads.

Second, I studied the plastic and evolutionary responses of multiple reproductive traits and life history traits key to fitness, as well as assessing genetic diversity, in wild caught Trinidadian guppies to increased temperatures. Guppies are a tropical, ectothermic

fish that may be under great threat because of global warming (see Deutsch et al. 2008). They have short generation times (sexually mature at approximately 7 weeks; Reznick et al. 2001), breed readily in a laboratory environment, are highly fecund, and are thus an ideal fish species with which to study both phenotypic plasticity and potential evolutionary responses within the scope of my doctoral thesis. Numerous studies on guppies have documented detrimental short-term effects of increased temperature on aspects of behaviour (e.g. Laudien and Schlieker 1981; Weetman et al. 1998; Muñoz et al. 2012), feeding (e.g. Gonzalez Mayor 2007), sex ratios (Dzikowski et al. 2001), life history, survival, reproduction (e.g. Dzikowski et al. 2001; Karayücel et al. 2008), and thermal tolerance (e.g. Chung 2001). However, to my knowledge, the only studies on guppies to document thermal evolution have examined survival (e.g. Nakajima et al. 2009). Thus, there is an urgent need to better understand the sub-lethal evolutionary response of guppies to increased temperature.

1.6 Thesis framework

The over-arching goal of my thesis is to determine the plastic responses of species to anthropogenic stressors and to determine if, and how rapidly, species could respond to these stressors via an evolutionary response. Throughout my thesis, I utilise a framework that allows for the partitioning of phenotypic plasticity and genetic responses. Tests for evolution which are not confounded by phenotypic plasticity involve placing organisms from different populations into controlled conditions; a common garden experiment (Hendry et al. 1998; Hendry and Kinnison 1999). The framework of my thesis involves subjecting fish from both ‘stressed’ and ‘control’ natal environments to specific fitness

tests in common environments in a 2×2 design (Fig. 1.1). An advantage of the 2×2 design is that it allows for the examination of genotype-by-environment interactions between the fish from a control of stressed natal environment (i.e. determine whether the plastic response by the 'stressed' fish has evolved in a different manner to the 'control' fish). This experimental design enables partitioning of any variance in performance of fitness traits into phenotypic plasticity or genetic responses. If there is variation in the 'control' fish when tested in controlled and stressed experimental conditions, this represents a phenotypically plastic response (i.e. they are showing different phenotypes in different environments). If 'stressed' fish tested in stressed and controlled experimental conditions display similar patterns to control fish, there is no evidence of a genetic response (i.e. this variance in performance was due to plasticity alone). However, if the 'stressed' fish display different levels of performance in the control, stressed, or both control and stressed experimental conditions as compared to the 'control' fish, this suggests a genetic response has occurred.

It is possible that a putative genetic response could be confounded by maternal environmental effects (e.g. increased investment in the yolk of eggs) or by epigenetics. In order to distinguish between these effects, an F_2 generation is required; organisms whose grandparents came from the stressed environment but both their parents and themselves had not been subjected to the stressor. If the F_2 individuals respond in the same way as did their parents, the initial response of the F_1 generation was genetic (including epigenetic effects; e.g. Anway et al. 2005) and not a result of maternal environmental effects.

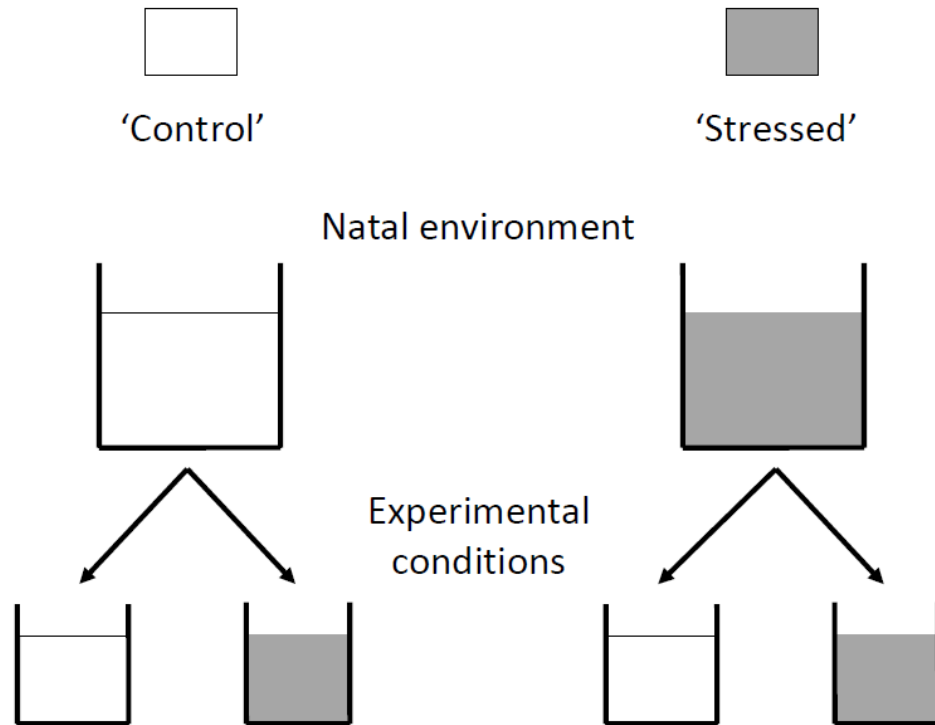


Figure 1.1 An example of a 2 × 2 common garden experimental design.

Shown are two natal environments, 'control' and 'stressed'. Individuals from both environments are then placed into control and stressed experimental conditions. For example, in Chapter 2 (Breckels and Neff 2010) fish from clean and polluted sites were placed in clean and polluted tanks.

1.7 Thesis structure

The four data chapters in my thesis (Chapters 2-5) were prepared as separate research projects, each intended for publication independently yet united by the common theme of plastic and evolutionary responses of fish to anthropogenic stressors. Chapters 2 and 3 have been published and Chapters 4 and 5 have been submitted for publication and are currently under review. I have provided an outline of the objective of each research project below. All the data chapters of my thesis all share a common hypothesis that both short and long-term exposure to pollution and global warming will elicit responses from brown bullheads and guppies respectively. Furthermore, I predicted that fish would display phenotypic plasticity in response to short-term exposure and evolutionary responses after long-term exposure to pollution and global warming.

In Chapter 2 (“Pollution-induced behavioural effects in the brown bullhead (*Ameiurus nebulosus*)”; Breckels and Neff 2010), I examine the potential evolutionary effects of long-term exposure to pollution on brown bullhead behaviour. Specifically, I detail aspects of aggression, locomotion, and escape response and try to partition any variance in behaviour between different treatments into genetic and plastic responses by using the framework detailed above.

In Chapter 3 (“The effects of elevated temperature on the sexual traits, immunology, and survivorship of a tropical ectotherm”; Breckels and Neff 2013), I examine survivorship and the phenotypically plastic effects of various key fitness traits of guppies to elevated water temperatures, as predicted for the end of the century due to global warming. Specifically, I detail brood survivorship and various sperm, ornamentation, and immune response traits at four different temperatures.

In Chapter 4 (“Rapid evolution of a sperm trait in response to increased temperature in an ectothermic fish”; Breckels and Neff *In review*), I examine the potential evolved response of sperm traits in guppies after multi-generational exposure to elevated water temperature, as predicted for the end of the century. Specifically, I detail sperm morphology, velocity, and path linearity at three different time points following exposure to elevated temperature. I distinguish between plastic and genetic responses in sperm traits using the framework detailed above.

In Chapter 5 (“Rapid evolution in response to increased temperature maintains population viability despite genetic erosion in a tropical ectotherm”; Breckels et al. *In press*), I examine the potential evolved response of various demographic and life history traits in guppies after multi-generational exposure to elevated water temperature, as predicted for the end of the century. Specifically, I detail population size, the number of successful pregnancies, brood size, brood survivorship, sex ratio, and age and length at maturity at three different time points following exposure to elevated temperature. I distinguish between plastic and genetic responses in several life history traits using the framework detailed above. Furthermore, I use neutral microsatellite molecular markers to assess the genetic diversity in each of my replicate tanks every six months, starting from time zero and going up to 24 months, and modelled effective population size to best fit the observed genetic data.

Finally, I conclude my thesis (Chapter 6) with a summary of my thesis findings and a discussion on directions for future research which would further our understanding of the effects of anthropogenic stressors on organisms.

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Chapter 2

2 Pollution-induced behavioural effects in the brown bullhead (*Ameiurus nebulosus*)¹

Aquatic ecosystems are major sinks for pollutants which can have adverse effects on biodiversity. Thus, it is important to understand the nature of pollution-induced change in aquatic ecosystems. I show that brown bullheads (*Ameiurus nebulosus*) may have evolved in response to chronic pollution exposure. I collected adults from the Detroit River (polluted site) and Belle River (control site). Both adults and common-garden raised juveniles were tested for aggression, locomotion, and escape response using consecutive unchallenged (clean) and challenged (polluted) trials. Detroit River fish were more aggressive than Belle River fish when challenged. Furthermore, Belle River fish showed increased locomotion when exposed to pollutants, whereas Detroit River fish were unaffected. The consistent difference in adult and juvenile behaviour across trials suggests a genetic response to pollution. Escape response on the other hand, showed inter-population differences, but no consistency between adults and juveniles, indicating that this behaviour is influenced by non-genetic factors. I discuss my data with respect to the potential adaptation of populations to pollution and the implications for prioritizing remediation efforts.

¹ A version of this chapter has been published and is presented here with permission from *Ecotoxicology*.

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2.1 Introduction

The potential effects of pollution on ecosystem health have received increased attention in recent years. Population expansion, industrialisation, and the intensification of agriculture and other industries have led to an increase in the amount and variety of pollutants introduced into the environment (Moss 1998; Hall et al. 2006). As detailed in Chapter 1, aquatic environments and hence aquatic species are especially vulnerable to pollution. For example, 11 out of 19 studies of fish showed adverse changes in reproductive behaviours as a result of pollution (reviewed in Jones and Reynolds 1997). Changes were documented in display frequency, courtship duration, as well as performance of male-specific behaviours by masculinized females. These behavioural changes can decrease reproductive success and, ultimately, population health and viability (Grue et al. 2002). Consequently, individuals and populations must adjust to the stress induced by chronic exposure to contaminants in order to persist in polluted environments (as detailed in Chapter 1). Understanding behavioural differences induced by pollutants can provide insight into the mechanisms that allow individuals to persist in polluted environments.

Anthropogenic stressors, such as pollution, have occurred only recently on an evolutionary timescale. As such, a population that shows a beneficial genetic response to a stressor must have evolved the adaptation in a relatively short period, a process known as rapid evolution (e.g. Grant and Grant 1995; Hendry et al. 1998). Rapid evolution likely occurs through selection on standing (pre-existing) genetic variation, but can also involve selection for beneficial mutations (Barrett and Schluter 2007). Such beneficial mutations can come about from the mutagenic effects of the pollutants themselves. For example,

Cachot et al. (2007) found that Japanese medaka (*Oryzias latipes*) exposed to polluted sediments had higher mutation rates than control fish. Although most of the mutations are likely to be deleterious, increasing mutagenesis can also result in an increased frequency of favourable mutations. Polluted environments thereby provide an exceptional opportunity to study rapid evolution.

In this study, I examine the effects of long-term pollution exposure on the behaviour of brown bullheads, *Ameiurus nebulosus* (LeSueur 1819). Brown bullheads are an ideal species to study the effects of long-term pollution exposure in aquatic habitats. They are native to north-eastern North America (Wheeler 1978) and are most abundant in the lakes and ponds of southern Ontario (Scott 1955). Brown bullheads sexually mature at about 3 years of age and produce large broods of up to 10,000 offspring (Blumer 1985). They are philopatric, benthic fish and have a high sediment affiliation, exposing them to pollutants that occur in the sediment. For example, brown bullheads from the heavily polluted Trenton Channel in the Detroit River have chemical burdens in their tissues similar to those of the sediment (Leadley et al. 1998; Yang and Baumann 2006).

I partition variation in aggression, locomotion, and escape response behaviour of fish from the polluted Detroit River and fish from a nearby clean site (Belle River) into phenotypic plasticity or evolutionary responses. I chose these behaviours because they are linked to individual performance (fitness) and can thereby affect population viability. For example, alterations in levels of aggression can affect an individual's acquisition of resources such as food, shelter, and mates (e.g. Fero et al. 2007). Locomotion is essential for many activities such as feeding, migration, reproduction, and predator avoidance (Baatrup and Bayley 1993) and is a good indicator of an individual's condition (Martin

and Bateson 1993). Alterations in predator avoidance can result in an increased risk of predation (reviewed in Scott and Sloman 2004). Exposure to pollutants, including polycyclic aromatic hydrocarbons and heavy metals, has been shown to affect these behaviours; (1) aggression: reduced aggression in Nile tilapia, *Oreochromis niloticus* (Almeida et al. 2009) and three-spined stickleback, *Gasterosteus aculeatus* (Bell 2001); elevated aggression in mice (Jaeger et al. 1999), (2) locomotion: e.g. hyperactivity in sea catfish, *Arius felis* and sheepshead, *Archosargus probatocephalus* (Steele 1985), and woodlice, *Oniscus asellus* (Bayley et al. 1997), and (3) predator avoidance: e.g. hyporeactivity in rainbow trout, *Oncorhynchus mykiss* (Ward et al. 2006); hyperactivity in fathead minnows, *Pimephales promelas* (Drummond and Russom 1990).

2.2 Methods

Brown bullheads were collected using electroshocking from two rivers in southwestern Ontario, the highly industrialized Trenton Channel of the Detroit River (42°10'54"N, 83°09'07"W) and the less industrialized Belle River (42°16'57"N, 82°42'50"W). The Detroit River is in the centre of a vast water system, receiving inputs from Lake St. Clair and the St. Clair River as well as from the cities of Detroit, Michigan and Windsor, Ontario. These inputs include effluents from both point and non-point sources including industrial, hazardous, and sewage treatment plant wastes (Drouillard et al. 2006). As a result, the Detroit River is an area of high contaminant loading, primarily by heavy metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), with the sediments on the western side of the river containing over 200 elevated organic chemical concentrations (Furlong et al. 1988; Arcand-Hoy and Metcalfe

1999; Drouillard et al. 2006). Indeed, 93% and 78% of sample stations in Trenton Channel greatly exceed threshold effect level sediment quality guidelines for PAHs and PCBs, respectively (Drouillard et al. 2006) and more than 16% of sample stations in the Detroit River exceed the severe effect level for heavy metals, with the maximum concentrations being confined to the Trenton Channel (Szalinska et al. 2006). Average hydrocarbon levels in the Detroit River are 1,195 parts per million (ppm) whereas these levels are only 77 ppm in the Belle River (Nagy et al. 1984).

2.2.1 Experimental design

First, for the adult behavioural trial, during 17-19 June 2008, 24 adults from Trenton Channel and 25 from Belle River were collected, weighed, measured for total length, and individually marked with a PIT tag. The fish were then transported to Leadley Environmental Corporation (Essex County) (42°06'11"N, 82°55'44"W) where they were held in 2.5 m × 2.5 m × 0.6 m holding tanks with 10 fish from the same site per tank. The fish were then exposed to a behavioural framework involving two different trials that allowed any variation between sites in aggression, locomotion, or escape response to be partitioned into plastic and evolutionary responses (Fig. 2.1). First, an “unchallenged” trial was conducted 3 weeks after capture in unstressed conditions (clean pond water) as direct acute responses to pollution stress from many chemicals are significantly reduced within 3 weeks (e.g. Djomo et al. 1996; Kavitha and Rao 2007). I thus assumed that any difference in behaviour between sites after the 3 week period reflected long-term responses (i.e. plastic or evolutionary responses). Next, immediately afterwards, a “challenged” trial was conducted on the fish by placing them into holding tanks lined

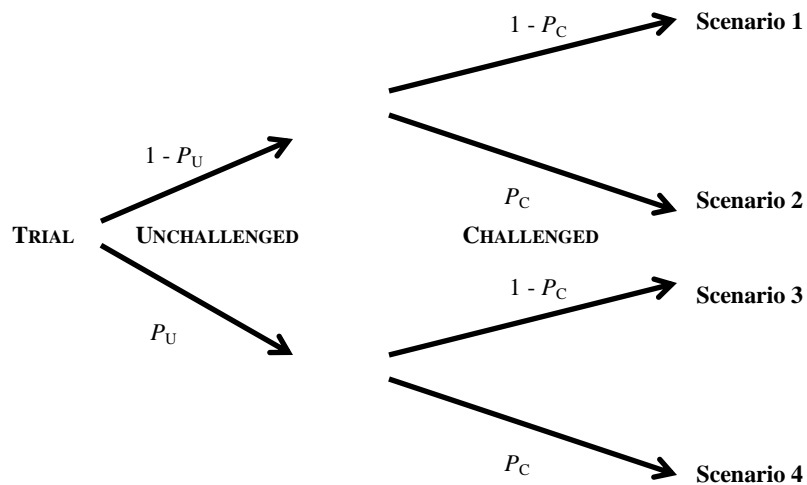


Figure 2.1 A bifurcated tree detailing the four possible scenarios for the behavioural trials in brown bullheads (*Ameiurus nebulosus*).

Arrows pointing upwards represent a difference in that behaviour between the two sites whereas arrows pointing downward represent no difference between the sites. P_U and P_C denote the probability that the null hypothesis is accepted at the unchallenged and challenged trial, respectively. Probability values associated with each arrow are presented in Table 1. The unchallenged trial was conducted after 3 weeks in clean water and the challenged trial was conducted after 24 hours exposure to polluted sediments. As an example, scenario 1 represents a difference between the two sites at both the unchallenged and the challenged trial.

with a 10 cm layer of sediment collected from Trenton Channel (polluted environment) for 24 h. The sediment was collected using Ponar sediment grabs. If fish from the two sites continued to differ in behaviour and also responded differently to the exposure to the polluted sediment, then it would be possible to attribute the long-term response to the pollution (see Fig. 2.1).

Second, for the juvenile behaviour trials, in May 2008, adults were collected and released into site-specific ponds at Leadley Environmental Corporation and allowed to spawn naturally. The ponds were monitored daily for free-swimming juveniles, which were collected and placed into separate site-specific ponds. To ensure a similar age in the experimental juvenile fish, I collected free-swimming individuals at first notice and over only a two day period in July 2008. In September 2008, 20 juveniles from each site-specific pond (40 total) were collected and transported to the Freshwater Ecology Research Facility at the University of Western Ontario, where they were housed in 20 L aquaria with 10 fish per aquarium. The juveniles were kept on a 12h:12h light-dark cycle until experiments commenced in November 2008 (the juveniles were thus about 4 months old). The experiments followed the same framework as the adults. Consistent differences between the populations in the adults and the juveniles would rule-out a plastic response and instead suggest an evolutionary response.

2.2.2 Behavioural trials

Each experimental fish was subjected to the behavioural experiments twice, once for the unchallenged trial and a second time for the challenged trial. The experiments commenced shortly after sunset and were performed under infrared light due to the nocturnal behaviour of the bullheads. Experiments were recorded using SONY DCR-

SR300 video cameras set to night vision mode and placed above the experimental aquaria. Prior to each set of trials, fish were moved from their holding tanks to circular experimental aquaria (150 cm diameter and 20 cm depth for adults, 30 cm diameter and 8 cm depth for juveniles, which ensured similar fish-to-aquarium size ratios). First, locomotion was examined by observing the volitional distance (distance travelled in a given time) of fish from each site. Fish were placed in an experimental aquarium and, after a 15 min acclimatization period, were recorded for the next 15 min. The distance travelled by each fish was measured by extracting a single frame image every 5 s from the video and determining the co-ordinates in a two-dimensional plane using Image J software. The distance travelled between each frame was determined using the Pythagorean Theorem and all the distances were summed to give an estimate of total distance travelled. Bullheads are benthic and consequently rarely leave contact with the sediment and swim into the water column. Thus, the two dimensional analysis provides an accurate measurement of the distance travelled.

Next, the escape response was examined. A stimulus was created by dropping a square weight into the water in the centre of the aquarium. Fish were recorded for 1 min preceding the stimulus and until their response had terminated (i.e. when the fish first ceased progressive forward motion, which typically occurred within 5 s). The distance travelled and maximum burst speed during the response were recorded. The distance travelled was measured by extracting images from the video at 33 frames per second and measuring the total distance travelled (as above). For the burst speed, five single frame images were extracted per second from the video. The greatest distance between

consecutive frames during the entire response was then multiplied by 5 to get an estimate of maximum burst speed (in cm/s).

Finally, after a 30 min rest period, the aggression displayed by fish from the different sites was observed by placing four fish, two from each site, selected to be of similar size, into an experimental aquarium. Fish were individually marked using small marks with liquid paper (Sanford LD, Oakville, Canada) and were initially separated by a cross-shaped barrier measuring 150 cm × 150 cm. The barrier was removed after a 15 min acclimatization period and the aggressive behaviour of the fish was recorded for the next 10 mins. The number of aggressive acts, observed as chases and nudges, initiated by each individual were quantified. Aggression was calculated as the sum of aggressive acts performed by that individual divided by the total number of acts performed by all four individuals in that aquarium (to control for any tank effects and thereby standardize measures across tanks). After the unchallenged trials had finished, fish were moved to new holding tanks containing the polluted Trenton Channel sediment for 24 h, after which the challenged trial commenced following the same procedures as outlined above. Time constraints restricted the aggression trials to 44 adults (22 from each site). The subset of fish was selected haphazardly from the original sample. Throughout the experiments, all fish were fed once daily with commercial fish food (Profishent, Martin Mills, ON).

2.2.3 Statistical analysis

T-tests were used to compare data between each site for all four behaviours and both trials, resulting in two P -values for each behaviour, which I refer to as P_U for the unchallenged trial, and P_C for the challenged trial. Next, to test the biological significance

of the data, these values were converted into the probability that the null hypothesis – that there was no difference in behaviour between the sites – is false by subtracting them from one (i.e. the probability of accepting the null when it is, in fact, true). The later values were multiplied through a bifurcated tree in order to estimate the probability of each of four scenarios: (1) different at both trials; (2) different at the unchallenged trial, but the same at the challenged trial; (3) the same at the unchallenged trial, but different at the challenged trial; (4) the same at both trials (Fig. 2.1). For example, if Trenton Channel fish were significantly more aggressive than Belle River fish across both trials, the most probable outcome would be scenario 1. Additionally, for each behaviour, I assessed the confidence in the probability of the most likely scenario by calculating a log-likelihood ratio (LOD score), which is the likelihood ratio of the most probable scenario compared to that of the next most probable scenario, using the equation:

$$LOD = k \log \left(\frac{P_1 + 1}{P_2 + 1} \right); \quad (1)$$

where P_1 represents the most probable scenario, P_2 represents the second most probable scenario, and k is a normalization constant equal to $1/\log 2$ such that the LOD scores range between 0 and 1. As an example, suppose scenario 1 was the most probable and scenario 2 the second most probable; a score of 1 gives complete support for scenario 1 as the most probable outcome, whereas a score of 0 indicates that the two scenarios are equally likely.

2.3 Results

Adult brown bullheads were of similar sizes between the two sites (total body length: Belle River (BR) = 26.2 ± 5.6 cm; Trenton Channel (TC) = 24.9 ± 8.2 cm; $t_{47} = 1.35$, $p = 0.182$). The juvenile brown bullheads did differ in size between the sites with the individuals from Belle River being longer than those from Trenton Channel (BR = 5.7 ± 0.1 cm; TC = 5.1 ± 0.1 cm; $t_{38} = 3.64$, $p = 0.001$). Consequently, I included body length as a covariate for the analysis of juvenile behaviour, but body length had no effect on any of the recorded behaviours, and was removed from the final analysis. The results for the adult and juvenile behaviours are summarised in Tables 2.1 and 2.2.

2.3.1 Aggression

For the adults, there was no difference between the two sites at the unchallenged trial ($t_{42} = 0.05$, $P_U = 0.960$); however, there was a difference between the sites at the challenged trial, with Trenton Channel fish being more aggressive than Belle River fish ($t_{42} = 2.11$, $P_C = 0.041$) (Fig. 2.2). Indeed, scenario 3 was the most probable outcome with strong statistical support (92.1%; LOD = 0.89). For the juveniles, the difference at the unchallenged trial was not significant ($t_{38} = 0.72$, $P_U = 0.478$). At the challenged trial, similar to the adults, Trenton Channel juveniles were more aggressive than Belle River juveniles, yet this difference was not significant ($t_{38} = -1.66$, $P_C = 0.105$) (Fig. 2.2). The two most probable outcomes for the juveniles were scenarios 1 and 3 with roughly equal support (46.7 % and 42.8% respectively; LOD = 0.04), suggesting that there was a difference at the challenged trial.

Table 2.1 Mean (\pm SE) behavioural measurements and *P* values for both adult and juvenile brown bullheads (*Ameiurus nebulosus*).

	Behaviour	Trial	Belle River	Trenton Channel	<i>P</i> _{U/C}
ADULTS					
Aggression	Relative	Unchallenged	0.249 \pm 0.045	0.251 \pm 0.037	0.960
	Aggression	Challenged	0.212 \pm 0.025	0.288 \pm 0.026	0.041
Locomotion	Volitional Distance	Unchallenged	532 \pm 94.6	1161 \pm 85.9	<0.001
	(cm)	Challenged	1688 \pm 296	1063 \pm 215	0.091
Escape Response	Distance Travelled	Unchallenged	64.9 \pm 13.1	41.4 \pm 8.47	0.188
	(cm)	Challenged	38.9 \pm 12.2	50.3 \pm 12.8	0.542
	Burst Speed	Unchallenged	36.8 \pm 6.10	27.2 \pm 4.99	0.271
	(cm/s)	Challenged	27.3 \pm 6.21	27.5 \pm 7.49	0.984
JUVENILES					
Aggression	Relative	Unchallenged	0.263 \pm 0.028	0.237 \pm 0.023	0.478
	Aggression	Challenged	0.218 \pm 0.030	0.282 \pm 0.024	0.105
Locomotion	Volitional Distance	Unchallenged	1157 \pm 43.1	1321 \pm 65.3	0.043
	(cm)	Challenged	1397 \pm 69.7	1464 \pm 43.8	0.452
Escape Response	Distance Travelled	Unchallenged	19.1 \pm 8.31	11.1 \pm 5.29	0.422
	(cm)	Challenged	15.9 \pm 4.60	7.74 \pm 2.55	0.156
	Burst Speed	Unchallenged	14.1 \pm 3.86	9.35 \pm 3.08	0.344
	(cm/s)	Challenged	20.9 \pm 4.99	6.73 \pm 2.12	0.022

N.B. *P*-values in bold represent significant results ($\alpha = 0.05$).

Table 2.2 Summary of the probabilities of each of four scenarios from the bifurcated tree for adult and juvenile brown bullheads (*Ameiurus nebulosus*). Four behaviours were examined in both adult and common-garden reared juvenile fish.

Behaviour	Scenario 1	Scenario 2	Scenario 3	Scenario 4
ADULTS				
Aggression	0.038	0.002	0.921 (0.886)	0.039
Volitional	0.909 (0.807)	0.091	<0.001	<0.001
Distance				
Burst Speed	0.012	0.717 (0.438)	0.004	0.267
Distance	0.404	0.478 (0.074)	0.054	0.064
Travelled				
JUVENILES				
Aggression	0.467 (0.039)	0.055	0.428	0.050
Volitional	0.524 (0.089)	0.433	0.024	0.019
Distance				
Burst Speed	0.642 (0.297)	0.014	0.336	0.008
Distance	0.487 (0.133)	0.090	0.356	0.066
Travelled				

N.B. Probabilities in bold represent the most probable outcome. Values in parentheses represent the LOD score. As an example, suppose scenario 1 was the most probable and scenario 2 the second most probable; a score of 1 gives complete support for scenario 1 as the most probable outcome, whereas a score of 0 indicates that the two scenarios are equally likely. Scenario 1 represents a difference between the two sites at both trials; Scenario 2 represents a difference at the unchallenged trial, but not the challenged trial; Scenario 3 represents no difference at the unchallenged trial, but a difference at the challenged trial; and Scenario 4 represents no difference between the sites at either trial.

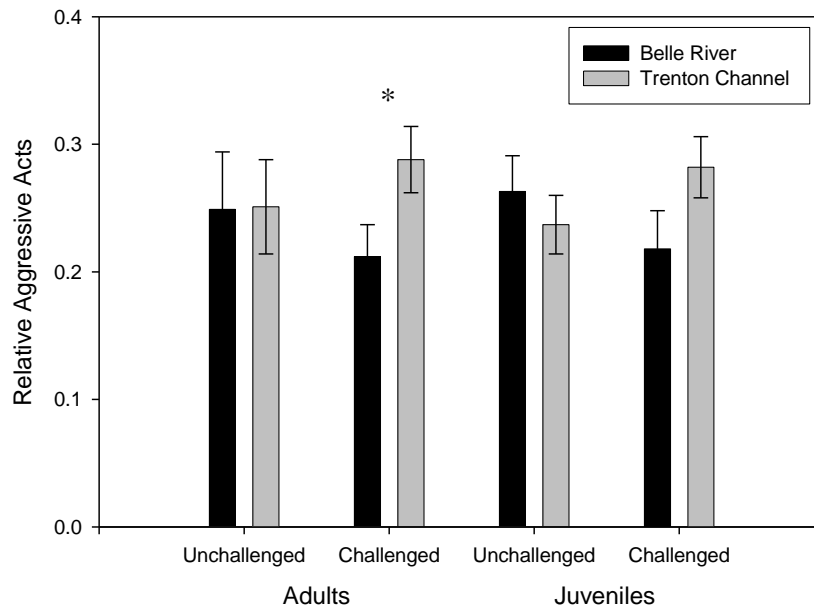


Figure 2.2 Mean aggressive behaviour in brown bullheads (*Ameiurus nebulosus*).

Relative aggressive acts was calculated by the total aggressive acts performed by an individual divided by the sum of the aggressive acts in the tank. Adults and common-garden reared juveniles were tested after 3 weeks in clean water (unchallenged) then 24 h exposure to polluted sediment (challenged). Error bars denote plus or minus one standard error of the mean. Asterisks represent significant differences between sites.

2.3.2 Volitional distance

The most probable outcome for the adults was scenario 1 (90.9%; LOD = 0.81). Trenton Channel fish swam a greater distance at the unchallenged trial than Belle River fish ($t_{47} = -4.91$, $P_U < 0.001$), yet Belle River fish dramatically increased their volitional distance during the challenged trial (paired t-test: $t_{20} = 4.24$, $p < 0.001$). Belle River fish swam farther than Trenton Channel fish during this latter period, consequently the difference between sites was not significant ($t_{42} = 1.73$, $P_C = 0.091$) (Fig. 2.3). The juveniles showed similar results to the adults with scenario 1 being the most probable (52.4%; LOD = 0.09). Trenton Channel juveniles swam a greater distance in the unchallenged trial than Belle River juveniles ($t_{38} = 2.09$, $P_U = 0.043$), yet at the challenged trial, like the Belle River adults, Belle River juveniles significantly increased their volitional distance (paired t-test: $t_{19} = 3.65$, $p = 0.002$) to similar levels as Trenton Channel ($t_{34} = 0.76$, $P_C = 0.452$) (Fig. 2.3).

2.3.3 Escape response

The most probable scenario for burst speed in adults was scenario 2 (71.7%; LOD = 0.44). There was no difference between sites at the unchallenged trial ($t_{38} = 1.12$, $P_U = 0.271$) or at the challenged trial ($t_{30} = 0.02$, $P_C = 0.984$). Unlike the adults, scenario 1 was the most probable outcome for burst speed for the juveniles (64.2%; LOD = 0.30). Belle River and Trenton Channel juveniles showed similar burst speeds at the unchallenged trial ($t_{38} = -0.96$, $P_U = 0.344$). At the challenged trial, Belle River juveniles increased their burst speed while Trenton Channel juveniles showed no difference, resulting in a significant difference between the two sites ($t_{34} = 2.40$, $P_C = 0.022$). The distance travelled in the adults showed scenario 2 as the most probable (47.8%) albeit with low

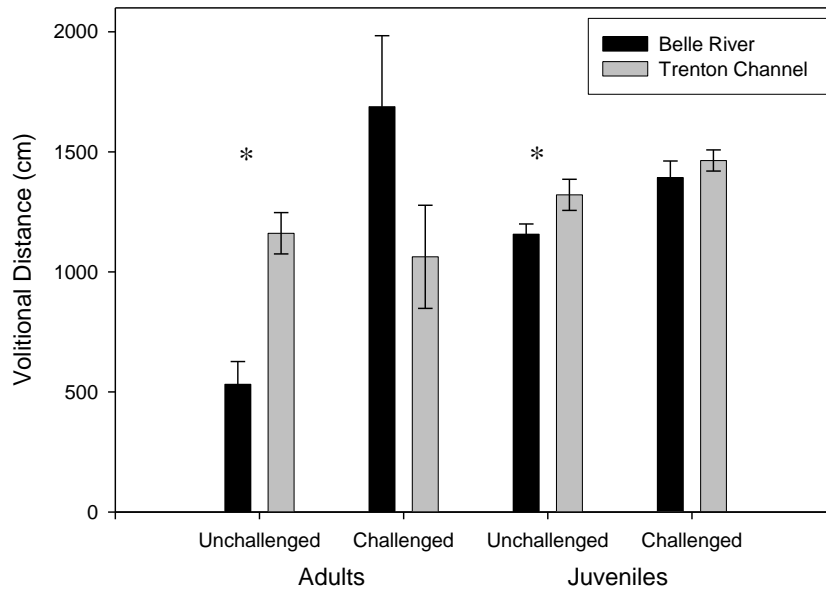


Figure 2.3 Mean volitional distance in brown bullheads (*Ameiurus nebulosus*).

Volitional distance was calculated as the distance an individual swam in 15 mins. Adults and common-garden reared juveniles were tested after 3 weeks in clean water (unchallenged) then 24 h exposure to polluted sediment (challenged). Error bars denote plus or minus one standard error of the mean. Asterisks represent significant differences between sites.

statistical support (LOD = 0.07). There was no difference between the sites at the unchallenged trial ($t_{38} = 1.34$, $P_U = 0.188$) or at the challenged trial ($t_{30} = -0.62$, $P_C = 0.542$). Scenario 1 was the most probable outcome for the juveniles (48.8%; LOD = 0.13). There was no difference between the sites at the unchallenged trial ($t_{38} = 0.21$, $P_U = 0.422$) or the challenged trial ($t_{34} = 1.45$, $P_C = 0.156$).

2.4 Discussion

My study is one of the first attempts to document an evolutionary behavioural response to pollution. I found that bullheads from the highly polluted Detroit River appear to have evolved adaptations to pollution as measured by locomotion and aggressive behaviours. Specifically, Detroit River adults and common-garden reared juveniles were unaffected by the addition of polluted sediments, maintaining the same volitional distance when in clean or polluted water. Belle River fish, on the other hand, displayed an increase in activity with greater volitional distances when exposed to polluted water. Detroit River fish also maintained higher levels of aggression when exposed to pollution than Belle River fish. Increased aggression can be beneficial because individuals typically gain access to more resources, such as food, shelter, and mates (e.g. Fero et al. 2007). Conversely, an increase in locomotion behaviour in response to a stimulus is often a sign of stress in fish (Allin and Wilson 2000). The results from the other behaviours showed differences between the Detroit River and Belle River fish. However, those differences were not consistent between adults and juveniles, suggesting that non-genetic factors may be driving them.

While my results suggest an evolved response in locomotion and aggressive behaviours due to pollution, I cannot rule out other factors. First, parental effects could contribute to the differences I observed. Abnormalities in the offspring could stem from the fathers in the form of damage caused from chemicals in the Detroit River to the DNA carried by the sperm (e.g. Gray et al. 1999). Alternatively, chemicals from the river carried by the mothers could have been directly passed onto the offspring through the eggs. For many chemicals, the amount present in eggs correlates with the amount present in the mother (e.g. mercury: Hammerschmidt et al. 1999; and PAHs: Hall and Oris 1991). However, the burden in eggs is usually considerably lower than in the mothers (Serrano et al. 2008) and the burden in offspring is considerably lower than in the eggs (Beattie and Pascoe 1978). Given that the juveniles in this study were reared in clean water from the egg stage for four months prior to testing, it is likely that any of the pollutants that might have been transferred would have been depurated (e.g. Djomo et al. 1996; Gardinali et al. 2004). Furthermore, it is difficult to understand why pollutants transferred maternally through the egg, or damage done to the germ-line DNA, would enable the Detroit River juveniles to subsequently dominate the Belle River juveniles in the challenge trials. On the other hand, it is possible that pollutants passed from the Detroit River mothers to their offspring is a trigger that ‘turns-on’ genes that allow the offspring to acclimate to the polluted environment. For example, offspring pre-exposed as eggs to cadmium survived longer than naïve offspring when both were later exposed to cadmium (Beattie and Pascoe 1978). Insomuch as those genes remain active or otherwise provide a physiological coping mechanism, the response I observed might not be an evolved response in the Detroit River fish. To test this alternative hypothesis, eggs from Belle

River fish could be pre-exposed to the pollutants to see if a similar effect could be elicited from those fish, or you could conduct a multi-generation study with the Detroit River fish and look at second generation offspring whose parents had also been reared in an unpolluted environment (e.g. Meyer and Di Giulio 2003). Second, epigenetic effects have been shown to play a role in polluted environments through DNA methylation, microRNA, and histone modification (reviewed in Baccarelli and Bollati 2009). However, the importance of epigenetic effects in driving heritable behavioural responses to pollution or other stressors is unknown. Regardless, it is difficult for any study of heritability to definitely rule-out epigenetic effects as an alternative to heritable variation in DNA sequence.

It is also worth noting that although my fish were put in clean water for 3 weeks to remove any effects of the acute response, many chemicals remain stored in the body for a much longer period. I selected three weeks because this duration in clean conditions has been shown as sufficient time to deplete significant amounts of the organophosphate pesticide monocrotophos (Kavitha and Rao 2007), the heavy metals chromium (Parma et al. 2008), cadmium, and copper (Kraemer et al. 2005), the PAHs anthracene, phenanthrene, pyrene, and benzo[a]pyrene (Djomo et al. 1996), and the PCB Aroclor 1254 (Wang 1998). All of these chemicals are present in the Detroit River, although there is little known about the clearance times for many of the other chemicals in the river. Thus, it is possible that some residual chemicals continued to affect the Detroit River fish during the unchallenged trial. Nevertheless, residual burdens would not affect my interpretation of the challenged trial data and, for example, the increased performance of

the Detroit River fish over the Belle River fish during the aggression trials. Thus, my data for aggression and locomotion are most consistent with a genetic response.

Traditionally it was thought that an evolutionary response was a slow process that would take hundreds of generations to occur (Darwin 1859), but more recent evidence suggests that such responses can occur over much shorter timescales (e.g. Grant and Grant 1995). Pollution in the Detroit River dates back to the late 19th century (US EPA 2007) or roughly 100 years ago. Brown bullheads tend to become sexually mature at 3 years of age, so 100 years represents at most 33 generations. Therefore, any evolved response to pollution in the Detroit River brown bullheads has occurred over a relatively short timescale. It is possible that large effective population sizes and large brood sizes contribute to the apparent rapid response. First, a large effective population size should contribute to large amounts of standing genetic variation on which selection can act. Second, a large brood size means that there are increased opportunities for favourable mutations to occur. Additionally, many contaminants can be genotoxic in that they have the ability to be mutagenic. Cachot et al. (2007) found that Japanese medaka (*Oryzias latipes*) exposed to sediment from the upper River Seine, Oissel, France showed significantly higher mutation rates than control fish. The sediments of the Seine in Oissel are known to have contaminant concentrations similar to the Detroit River. Indeed, Maccubbin et al. (1991) found the sediments of the Detroit River to be mutagenic. Increased mutagenesis, while resulting in increased deleterious mutations, could also result in an increased frequency of favourable mutations. Thus, the mutagenic nature of some of the chemicals in the Detroit River may have aided in the adaptation of the brown bullheads to contamination stress. Conceivably, the combination of short generation time,

large brood sizes, large effective population sizes, and mutagenic chemicals, in conjunction with strong selective pressure have contributed to the apparent rapid evolved response in Detroit River bullhead behaviour.

It has been suggested that genetic changes in behavioural traits precede and direct subsequent morphological changes (West-Eberhard 2003). This idea stems from the fact that behavioural traits tend to be more labile than morphological traits (Wcislo 1989). That is, for each morphological state there can be many behavioural states, so the chance of producing a favourable trait is higher in behavioural traits than morphological traits (West-Eberhard 1989). Consequently, adaptive behavioural traits should become established first, followed by adaptive morphological traits. If an individual who resides in a polluted environment displays some heritable behaviour that is associated with a fitness benefit relative to other behaviours in the population, then the phenotype should be rapidly selected for and passed-on to the next generation. For example, in this study I found that Belle River fish significantly increased their locomotion when in polluted water, showing signs of stress, which in turn can lower fitness, whereas Trenton Channel fish were unaffected by the pollution. Additionally, brown bullheads from the Detroit River were more aggressive than their Belle River counterparts when in polluted water. I did not look at morphological traits, so do not yet know if those traits have also responded to pollution, or the relative time scales with which behavioural and morphological traits have evolved in this population. Nevertheless, recently polluted environments do provide an opportunity to examine the relative rates of evolution of behavioural and morphological traits.

There is growing support for the presence of behavioural syndromes in populations. Behavioural syndromes are suites of correlated behaviours that occur in different contexts or situations (e.g. Hedrick 2000; reviewed by Sih et al. 2004). One of the more common behavioural syndromes is the aggressiveness/activity syndrome, where aggression is positively correlated with activity levels (Sih et al. 2004). This relationship has been demonstrated in, for example, the field cricket, *Gryllus integer* in which activity, measured as an individual's latency to leave a glass vial into a novel environment, was positively correlated with aggressiveness, measured as the number of fights won by that individual (Kortet and Hedrick 2007). In my study, bullheads from the Detroit River were more aggressive and were more active than Belle River fish, which is in concordance with Kortet and Hedrick's results. Thus, at least for the aggressiveness/activity syndrome, my data suggest a behavioural syndrome exists in bullhead as well.

Remediation plans aim to restore the ecosystem to some level of acceptable integrity or health. Consequently, many remediation plans target areas with a long history of pollution, such as the Detroit River and Lake Erie (e.g. Heidtke et al. 2002). Between 1993 and 2001, an estimated \$130 million was spent on sediment remediation activities in the Detroit River and western Lake Erie as part of the Detroit River Remedial Action Plan (Heidtke et al. 2002). However, it is apparent that these areas are still loaded with chemicals at levels well above the policy guidelines (Drouillard et al. 2006). The Detroit River has a long history of contamination and my data now show that at least one native species may have evolved in response to the pollution although a more thorough community-level analysis is needed. I suggest that it may be time to start prioritising our remediation action plans with some consideration of potential adaptation to a stressor by

local flora and fauna. Newly-polluted ecosystems or ones that are experiencing rapid population declines ought to be top of our priority list. Conversely, areas with a long history of pollution might be prioritized lower if resident species show signs of adaptation to the current pollution levels. Additionally, if areas such as the Detroit River are fully restored to a non-polluted state, some consideration should be given to ensure that the resident species are fully viable in the 'new' clean environment. For example, it is conceivable that the brown bullhead presently in the Detroit River would be less adapted to the clean environment and would be prone to invasion by other non-local species that occupy a similar niche. As humans continue to pollute aquatic ecosystems, the need for prioritizing remediation efforts will become increasingly important to effectively use conservation resources. My study suggests that consideration of adaptive and physiological responses to stressors should also be considered when prioritizing sites.

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Chapter 3

3 The effects of elevated temperature on the sexual traits, immunology, and survivorship of a tropical ectotherm²

In 2007, the Intergovernmental Panel on Climate Change projected an average global air temperature increase of 1.1-6.4°C by the end of the 21st century. Although the tropics are predicted to experience less extreme temperature increases than regions of higher latitude, tropical ectotherms live close to their thermal limits, and are thus particularly vulnerable to increases in temperature. In this study, I examined how predicted patterns of global warming will affect survival and sexual traits in the Trinidadian guppy (*Poecilia reticulata*). Guppies were exposed from birth to one of four temperature treatments: 23°C, 25°C (control), 28°C, or 30°C. I measured brood survival and at sexual maturity, male ornamentation, sperm traits, and immune response. My results show that increases in temperature result in guppies that have shorter, slower sperm but that there is an optimum temperature for ornamental hue at 28°C. Given the importance of sperm quality for reproduction, these results suggest population viability could be affected by warming. However, I found no difference in brood survival or immune response to a novel antigen across the treatments, indicating that survival may not be as vulnerable as previously thought. Overall, my data suggest that male sexual

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traits, and in particular sperm performance, are more sensitive than survival to a warming environment.

3.1 Introduction

One of the most ubiquitous environmental conditions that broadly impacts organisms is temperature (Dorts et al. 2012). As detailed in Chapter 1, the average global air temperature is predicted to increase by 1.8-4.0°C by the end of the 21st century and this is likely to have severe impacts on organisms, especially tropical ectotherms. The projected increase in temperature is also likely to have ecological impacts, including reduced food availability, which can be confounded by thermally-induced increases in the metabolic rate of ectotherms. Consequently, less energy may be available for other important functions, including reproduction, potentially altering the demographics of populations (Deutsch et al. 2008; Daufrense et al. 2009; Dillon et al. 2010). Therefore, understanding the response of organisms, especially the extent of phenotypic plasticity, is of crucial importance to better understand the fate of organisms in warming environments (Somero 2010).

Sexual traits including sperm performance are key determinants of male reproductive success but exposure to elevated temperatures has the capacity to alter these traits (Alavi and Cosson 2005; Dorts et al. 2012). Increased temperatures have been shown to result in decreased sperm motility (e.g. Williot et al. 2000), decreased sperm number (e.g. Zeh et al. 2012), and, in one study, increased sperm length (e.g. Blanckenhorn and Hellriegel 2002); most other stressors have instead been shown to lead

to decreased sperm length (e.g. Dey et al. 2009; Immler et al. 2010). These changes subsequently can have a significant impact on male reproductive success (Billard 1978; Stoss 1983; Gage et al. 2004; Alavi and Cosson 2005). In addition, temperature may also affect secondary sexual characters which are important sexual traits because they act as an honest signal of male quality and aid females in choosing mates (Kortet et al. 2004). Borg (1982) found that the decline of secondary sexual characters during the summer is accelerated by high temperatures in the three-spined stickleback, *Gasterosteus aculeatus*. As well, Brian et al. (2011) found that an optimum temperature for male secondary sexual characteristics exists in the fathead minnow (*Pimephales promelas*). Therefore, temperature may have the potential to affect both pre- and post-copulatory processes during reproduction.

A rise in temperature is also predicted to result in an increase in the transmission, growth rate, and virulence of parasites and pathogens (Harvell et al. 2002; Marcogliese 2008; Harvell et al. 2009; Dang et al. 2012). The immune system is highly sophisticated and has evolved to defend hosts against the debilitating effects of pathogens and parasites (Møller and Saino 2004). However, variation in temperature can have marked effects on immunological function and effectiveness: increased temperatures can affect the antibacterial activity, antimicrobial activity, and parasite resistance of a host (e.g. Collazos et al. 1996; Lamková et al. 2007; Dang et al. 2012). Indeed, Collazos et al. (1996) found that the immune response to Phytohaemagglutinin (PHA) is compromised at higher temperatures in the tench, *Tinca tinca*. Phytohaemagglutinin, a protein derived from red kidney beans, is commonly used as a novel antigen to test T-cell proliferation (e.g. Collazos et al. 1996; Bayyari et al. 1997; Ardia and Clotfelter 2006).

Phytohaemagglutinin-induced immune response has also been linked directly to parasite resistance (Bayyari et al. 1997). Exposure to PHA thereby provides a simple test of an organism's immune response.

The projected change in air temperature will also result in a change in water temperature (e.g. Stefan and Preudhomme 1993; Caissie et al. 2001). The magnitude of the change in water temperature, however, will depend upon several factors including the location and volume of the water-body. Small, shallow streams are likely to experience similar changes to air temperature, whereas large water bodies, such as oceans, will take longer to respond (Ficke et al. 2007). Indeed, long-term increases in river and stream water temperature are strongly correlated to long-term increases in air temperature (Kaushal et al. 2010). Consequently, global warming will be more problematic for obligate freshwater organisms. For fish, this problem is further compounded due to their poikilothermic nature whereby their basic physiology is directly dependent on the temperature of their environment. Given the potential negative impacts that global warming might cause, studies addressing the short and long-term effects of the increased temperature are needed.

Here, I use the Trinidadian guppy (*Poecilia reticulata*, Peters 1860) as a model poikilothermic fish to examine the effects of increased temperature, as projected for 2100. Guppies are a small, polygamous, live bearing fish native to north-eastern South America and the Caribbean. They inhabit small freshwater streams and pools that flow through lowland and montane rain forests (Houde 1997). Currently, the mean air temperature in Trinidad is 27.7°C and fluctuates by 2.0°C annually between the coldest months (January and February - 26.5°C) and the warmest month (May - 28.5°C), while the diel

temperature fluctuates by approximately 8.4°C (mean values calculated between January 1992 and December 2012; weatheronline.co.uk). Due to its physical nature, short-term temperature variations in water are usually smaller than short-term variations in air temperature (Caissie et al. 2001; Kaushal et al. 2010). The mean water temperature of rivers in Trinidad is approximately 25°C and ranges between 20°C to 28°C (Alkins-Koo 2000). Over the past 60 years, Trinidad has experienced a mean air temperature rise of 1.5°C (Singh 1997), and the temperature is projected to increase by 1.0-3.5°C by the end of the 21st century (Water Resources Agency 2001). However, variation in temperature is set to decrease as night time and winter temperatures are projected to increase more than day time and summer temperature (IPCC 2007). Geographical barriers, such as waterfalls and oceans, mean that natural dispersal for individuals within Trinidadian streams is unfeasible. Therefore guppies, like many other poikilotherms, will largely have to rely on phenotypic plasticity in order to respond to global warming.

The objective of this study was to assess brood survival and to detail the phenotypic plasticity of sperm length, sperm velocity, male ornamentation, and immune response to guppies exposed to increased temperatures. I exposed guppies from birth to one of four temperature treatments: 23°C to represent a cooler climate, 25°C (control), and 28°C or 30°C to represent average or upper projected temperatures for the year 2100, respectively. I hypothesized that there would be an effect of increased temperature on survivorship and reproductive traits. I predicted that exposure to increased temperatures would result in decreased brood survival, sperm length, sperm velocity, male ornament quality, and immune response.

3.2 Methods

Experiments were conducted following ethical guidelines as implemented by the Canadian Council of Animal Care and were approved by the Animal Use Subcommittee at the University of Western Ontario. Guppies used in this experiment were descendants of fish that were collected in 2003 from a tributary of the Paria River in the Northern Range, Trinidad (10°44'42" N; 61°15'42" W). All guppies were kept at a constant temperature of $25 \pm 0.6^\circ\text{C}$ to represent natural conditions (Alkins-Koo 2000). Pregnant females were put into individual 10 L tanks until they gave birth. The number of offspring at birth and again after three months was recorded in order to get an estimate of brood survival. Approximately 24 h after the females gave birth (allowing time for the entire brood to be birthed) they were removed from the tanks so only the offspring remained. The temperature in the tanks was then set to one of four temperatures: 30°C to represent the upper range of future climate predictions for the end of the century, 28°C to represent average future climate predictions for the end of the century, 25°C to act as a control, and 23°C to represent a cooler climate.

3.2.1 Sperm analysis

At three months of age (mean age in days \pm SD: 95.8 ± 7.0), a subset of males were removed from their tanks and put into individual isolation chambers set at the temperature in which they were acclimated for 3 d to ensure full sperm reserves (Pilastro et al. 2002). Males were then anaesthetized with MS-222 and 'pat-dried' to remove all excess MS-222 from their skin. The males were placed under a dissection microscope with their gonopodium swung forward and 40 μl of sperm extender medium (207 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl_2 , 0.49 mM MgSO_4 , 10mM Tris, pH 7.5) held at 25°C

was added to the base of the gonopodium (Evans 2009). Gentle pressure was applied to the side of the abdomen, anterior to the base of the gonopodium to release all sperm bundles into the extender medium. The sperm was then activated using 40 μl of 150 mM KCl solution with 2 mg/l bovine serum albumin (BSA) also held at 25°C which helps to prevent sperm from sticking to the slide. Two 15 μl aliquots of the sperm solution were immediately placed in a 2X-CEL sperm analysis chamber (Hamilton Thorne, Beverly, MA, USA) and put under a microscope. Digital images were recorded using an SI-C400N microscope video camera (Costar Imaging, Lakewood, CA, USA) for velocity analysis. Following methods outlined in Chapter 2 (Breckels and Neff 2010), I extracted images from the recorded video at 10 frames per second and determined the two-dimensional coordinates using NIH Image J software (<http://rsbweb.nih.gov/ij>). Using the Pythagorean Theorem, the distance travelled (μm) by a sperm cell in 1 s was calculated as the sum of the distances travelled between the 11 consecutive frames in that second. The total distance travelled by each sperm in 1 s is called the curvilinear velocity (VCL, $\mu\text{m s}^{-1}$). I then calculated the straight line velocity (VSL) of the sperm by determining the distance travelled between the first and the last of the 11 consecutive frames. Finally, I calculated the path linearity by dividing the VSL by the VCL. A path linearity value of 0 represents a sperm that started and ended at the same point whereas a value of 1 represents a sperm that travelled in a straight line (see Stoltz and Neff 2006 and Kime et al. 2001). I measured the VCL, VSL, and path linearity of 10 sperm per individual.

Next, a 20 μl aliquot of the sperm solution was put onto a slide and covered with a cover slip. The slide was viewed under a microscope at 400 \times magnification and digital images were taken. Images were analysed in UTHSCSA Image Tool software v. 3.0

(<http://compdent.uthscsa.edu/dig/itdesc.html>). The tail length, including flagellum and mid-piece, of 30 sperm per male was measured.

3.2.2 Ornament analysis

Female guppies tend to respond favourably to males with larger and more intense orange spots on their body (Kodric-Brown 1985; 1989; Houde 1997). Thus, I examined the impact of temperature on both orange spot area and colour intensity. At the same time as the sperm analysis measurements, a photograph was taken of each guppy on a white background with a dark blue paint chip and a ruler, which acted as a scale. Images were then analysed using Image J in order to calculate the length of each fish and the proportion of orange on their bodies. For length measurements, fish were measured from the tip of the snout to the end of the caudal peduncle. For the proportion of orange measurements, the outline of the fish was traced in order to get an estimate of the area. Then, each orange spot on the body of the fish was traced and summed to get total orange cover. All measurements were repeated three times and the measures were averaged. The value was then divided by the mean fish area to express the cover as a proportion of body size.

To measure the hue, saturation, and brightness (HSB) of the orange pigmentation, pictures were analysed using Adobe Photoshop CS3 (San Jose, CA, USA). Each photograph was standardized for lighting conditions following Villafuerte and Negro (1998) by recording mean values of red, green, and blue (RGB) for the light background and the dark paint chip. Next, the mean RGB values were recorded for the orange pigmentation on the guppies and standardized. From these values I was able to calculate the standardised HSB values for each guppy (Villafuerte and Negro 1998).

3.2.3 Immune response

To evaluate the immune response, a separate subset of fish from each temperature treatment were injected with phytohaemagglutinin (PHA) and their swelling response was recorded. The PHA swelling response provides a measure of the T-cell proliferation, among other things, and has also been linked to parasite resistance (Bayyari et al. 1997; Ardia and Clotfelter 2006). After roughly 8 months of age (mean age in days \pm SD: 236 ± 43), both male and female guppies were anaesthetized using MS-222 and length measurements were taken as detailed above. Next, the guppies were placed under a dissection microscope and the width of the caudal peduncle, in line with the end of the dorsal fin, was measured independently three times for accuracy with a digital calliper (0.01 mm accuracy). The guppies were then injected in the same area with 4 μ g PHA, in 2 μ l phosphate buffered saline (PBS) using a 10 μ l, 26 gauge syringe (Hamilton Company, NV, USA). Another subset of guppies, reared at 25°C, were either injected with the needle only or received a dose of PBS without the PHA and acted as control groups. The guppies were then put in isolation chambers to avoid contact with other fish, with the temperature set to the temperature that they had been acclimated to, for 24 h. The fish were then anaesthetized again and the caudal peduncle was re-measured as above to determine the swelling response. The immune response of each individual was recorded as the difference in swelling between post- and pre-injection.

3.2.4 Statistical analyses

All statistical analyses were performed using SPSS v. 20 (SPSS Inc., Chicago, IL, USA) or Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and all presented p-values are two-tailed probabilities. Brood survival, orange cover, and sperm

path linearity were transformed using logit transformations. A one-way analysis of variance (ANOVA) was performed to compare brood survival among the four temperature treatments. General linear mixed models (GLMMs) were performed to compare each of male body length, sperm length, VCL, VSL, path linearity, orange cover, HSB, and immune response among the four temperature treatments. Family identification (ID) was included as a random factor and body length was included as a covariate for all tests. Because there was variation in age of the fish tested in the immune response trials and both sexes were used, I included sex as an additional fixed factor and age as a covariate. For post hoc analysis I used a Tukey's b test. Finally, I performed linear contrast analyses for the four different sperm traits in order to determine if there was a linear relationship with temperature.

3.3 Results

The number of families reared at 23°C, 25°C, 28°C, and 30°C was 13, 21, 12, and 21, producing mean brood sizes of 6.7, 4.9, 4.8, and 5.5 offspring, respectively. There was no difference in brood survival among the four temperature treatments (mean brood survival, % \pm SD: 23°C: 0.81 ± 0.21 , 25°C: 0.72 ± 0.29 , 28°C: 0.94 ± 0.12 , and 30°C: 0.74 ± 0.28 ; $F_{3,63} = 1.4$, $p = 0.258$). A total of 82 fish were used for the sperm trials and ornament analysis (23°C: $N = 11$, 25°C: $N = 27$, 28°C: $N = 19$, and 30°C: $N = 25$). Family ID had a significant effect on male body length at three months of age ($F_{13,65} = 2.0$, $p = 0.040$) and there was also a significant effect of temperature ($F_{3,65} = 5.0$, $p = 0.003$). Interestingly, males in the 23°C and 28°C treatments were significantly longer than the

25°C and 30°C treatments (mean length, mm \pm SE: 23°C: 15.2 \pm 0.6, 25°C: 13.9 \pm 0.3, 28°C: 15.1 \pm 0.4, 30°C: 13.9 \pm 0.2).

3.3.1 Sperm analysis

Male body length had no effect on either sperm length, VCL or VSL, and neither body length nor family ID had an effect on path linearity across the four treatments ($p > 0.05$ for all). However, family ID had an effect on sperm length, VCL, and VSL ($F_{13,64} = 2.3$, $p = 0.008$; $F_{13,59} = 1.9$, $p = 0.047$; and $F_{13,59} = 2.2$, $p = 0.023$, respectively). There was a significant decrease in average sperm length with increasing temperature ($F_{3,64} = 38.3$, $p < 0.001$), with the 30°C acclimated fish producing significantly shorter sperm than the 28°C acclimated fish, which in turn produced significantly shorter sperm than both the 23°C and 25°C acclimated fish (Fig. 3.1 A). Similarly, there was a significant decrease in VCL and VSL with increasing temperatures ($F_{3,59} = 7.8$, $p < 0.001$ and $F_{3,59} = 8.0$, $p < 0.001$, respectively), with 30°C acclimated fish showing significantly decreased VCL and VSL than fish from the other three temperatures (Fig. 3.1 B, C). The path linearity also decreased significantly with increasing temperature ($F_{3,59} = 3.8$, $p = 0.015$; Fig. 3.1 D), with the 23°C acclimated fish displaying a greater path linearity than both the 28°C and 30°C acclimated fish. Additionally, sperm length, VCL, VSL, and path linearity all declined linearly with increasing temperature ($F_{1,78} = 75.9$, $p < 0.001$; $F_{1,73} = 15.7$, $p < 0.001$; $F_{1,73} = 17.7$, $p < 0.001$; and $F_{1,73} = 12.0$, $p = 0.001$, respectively).

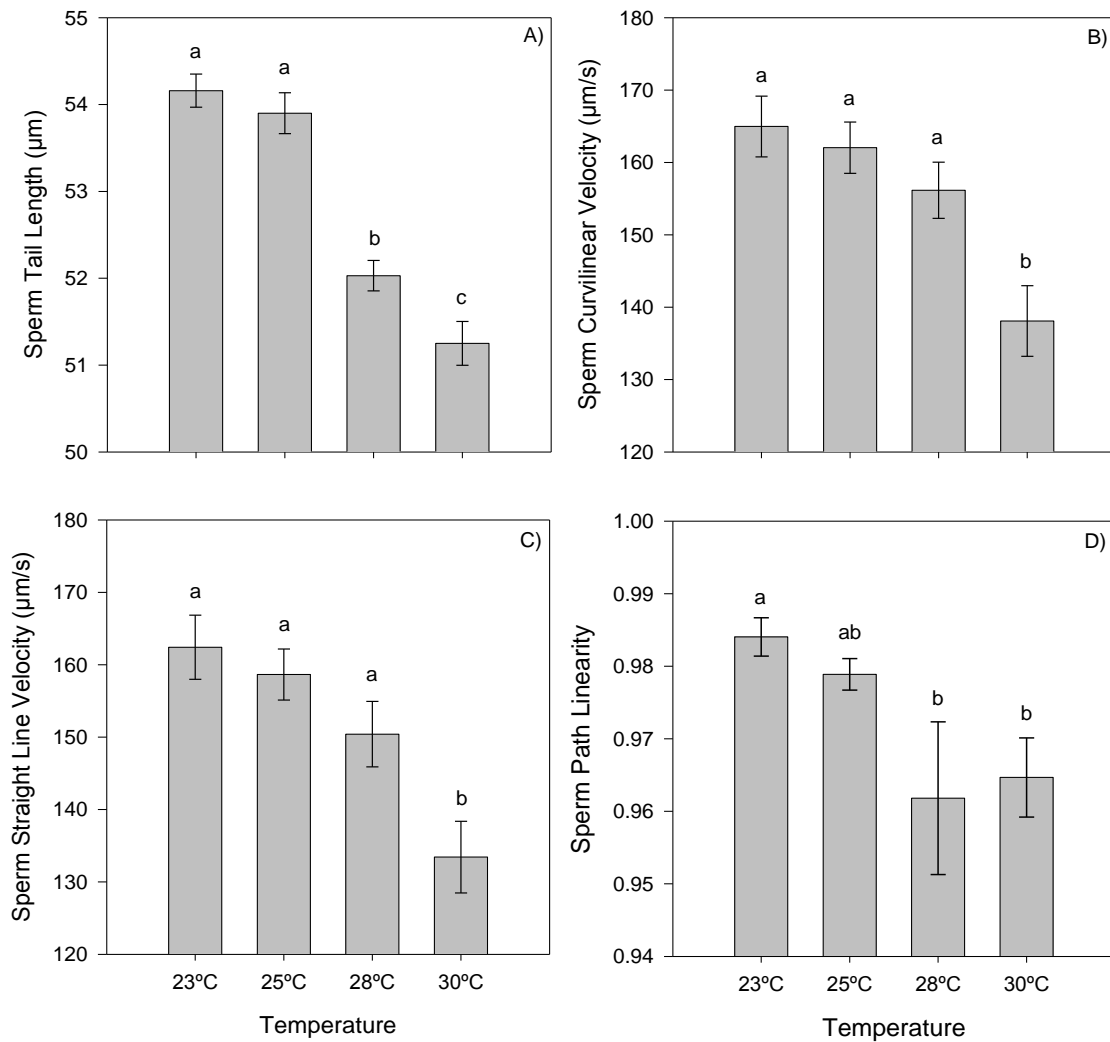


Figure 3.1 Sperm measurements of guppies (*Poecilia reticulata*) reared from birth at one of four temperatures.

Shown are means (\pm SE) for (A) sperm length, (B) curvilinear velocity, (C) straight line velocity, and (D) path linearity. Error bars with the same letter are not significantly different ($p > 0.05$) according to a Tukey's b HSD test.

3.3.2 Ornament analysis

There was no effect of family ID on orange cover, saturation, or brightness, nor was there an effect of body length on orange cover, hue, or saturation ($p > 0.05$ for all). There was also no effect of temperature on orange cover or saturation (mean orange cover, % \pm SE: 23°C: 5.2 ± 0.6 , 25°C: 6.3 ± 0.5 , 28°C: 5.6 ± 0.6 , and 30°C: 6.3 ± 0.6 , $F_{3,64} = 0.7$, $p = 0.548$; mean saturation \pm SE: 23°C = 0.87 ± 0.01 , 25°C = 0.91 ± 0.02 , 28°C = 0.82 ± 0.03 , and 30°C = 0.86 ± 0.02 , $F_{3,64} = 2.0$, $p = 0.120$). There was, however, an effect of family ID ($F_{3,64} = 2.9$, $p = 0.002$) and temperature on hue ($F_{3,64} = 17.5$, $p < 0.001$; Fig. 3.2), with the 28°C fish displaying a significantly greater hue than all other treatments. The 25°C displayed significantly greater hue than the 30°C fish whereas the 23°C fish were not significantly different from either the 25 or 30°C fish. There was an effect of body length on brightness ($F_{1,64} = 7.2$, $p = 0.009$), but temperature had no effect (mean brightness \pm SE: 23°C: 0.45 ± 0.02 , 25°C: 0.45 ± 0.01 , 28°C: 0.44 ± 0.01 , and 30°C: 0.41 ± 0.01 ; $F_{3,64} = 1.0$, $p = 0.487$).

3.3.3 Immune response analysis

A total of 156 fish were used from 65 families in the immune response trials (control: $N = 13$, PBS control: $N = 10$, 23°C: $N = 38$, 25°C: $N = 35$, 28°C: $N = 27$, and 30°C: $N = 33$). Age, length, and family ID had no effect on PHA swelling response ($p > 0.05$ for all). Although, there was a significant increase in PHA swelling response between the two controls and the four temperature treatments ($F_{5,121} = 4.4$, $p = 0.001$; Fig. 3.3), there was no difference in swelling response among the four temperature treatments. Additionally, males produced a significantly larger swelling response than did females ($F_{1,121} = 4.8$, $p = 0.031$).

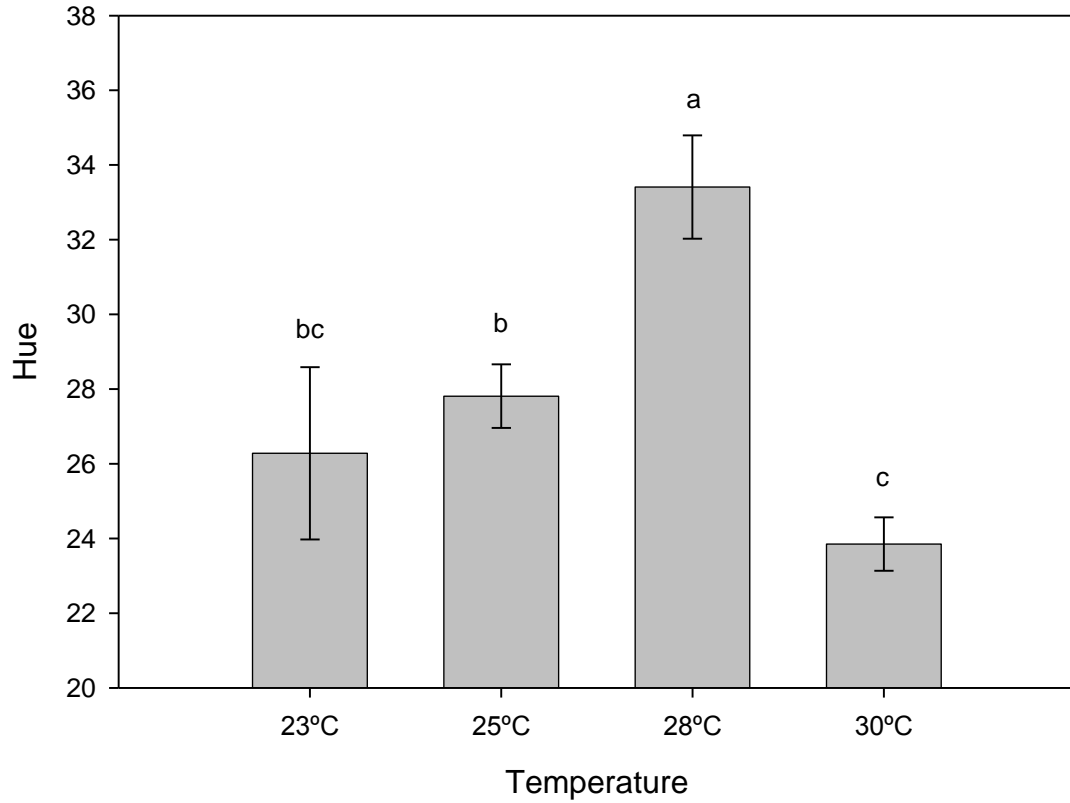


Figure 3.2 Ornament hue of guppies (*Poecilia reticulata*) reared from birth at one of four temperatures.

Shown are means (\pm SE). Error bars with the same letter are not significantly different ($p > 0.05$) according to a Tukey's b HSD test.

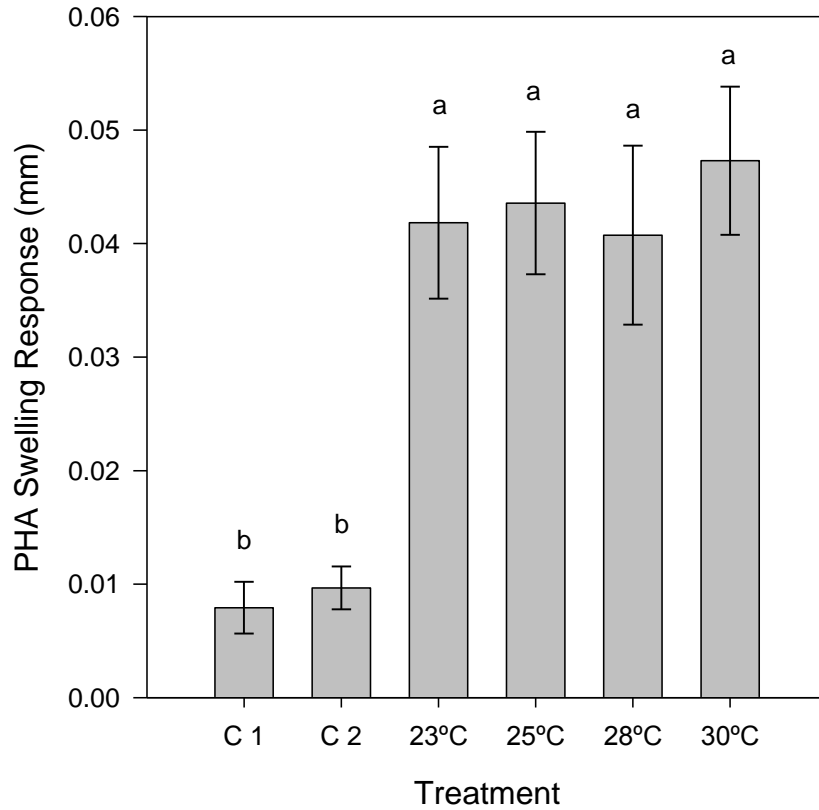


Figure 3.3 Phytohaemagglutinin (PHA) swelling response of guppies (*Poecilia reticulata*) reared from birth at one of four temperatures or the controls (C1 - needle only; C2 - phosphate buffered saline injection).

Shown are means (\pm SE). Error bars with the same letter are not significantly different ($p > 0.05$) according to a Tukey's b HSD test.

3.4 Discussion

Climate change, particularly the increased temperature predicted for the end of the century, has the potential to alter many life history traits, including juvenile survival (e.g. Zeh et al. 2012; reviewed in Pepin 1991). Although temperature can impact many aspects of natural ecosystems (reviewed in Ficke et al. 2007; IPCC 2007), its direct effect on physiology and survival is a critical first step in discerning the impact of climate change on natural populations. A previous study suggested that guppies have lower juvenile survival rates at temperatures of 29°C and above (Karayucel et al. 2008). However, in my study I found no difference among temperature treatments in brood survival. This discrepancy may be because Karayucel et al. (2008) used commercial aquarium fish that had been selectively bred for their elaborate pigmentation and fins (Karayucel et al. 2006), whereas I used guppies caught from the wild and maintained in a large stock population without any intentional, directional artificial selection. Taken together, the two studies suggest that the elaboration of sexual ornaments affects survival, particularly in warmer environments, indicating that they are costly (Andersson 1994). The discrepancy between the two studies may also reflect differences in genomic diversity as aquarium guppies tend to be highly inbred due to selective breeding whereas wild caught guppies have a much higher level of genetic variation (e.g. Bleakley et al. 2008). Thus, wild caught guppies could potentially have broader thermal limits than aquarium fish allowing them to survive at higher temperatures. Regardless, I found no evidence to suggest that temperature increases as predicted for the end of the century will have a significant effect on brood survival in guppies.

Secondary sexual characters influence female mate choice because they can act as an honest signal of male quality (Andersson 1994). Brian et al. (2011) found that there was an optimum temperature for ornamentation in fathead minnows which was slightly higher than the native temperature. My results show that ornament hue was highest at 28°C, higher than the mean natural temperature of 25°C. While it has been documented that hue is an important factor in female mate choice for many species (e.g. Chinook salmon, *Oncorhynchus tshawytscha* [Neff et al. 2008] and the blue crab, *Callinectes sapidus* [Baldwin and Johnsen 2009]), its role in mate choice for guppies is less well known. One study at least suggests that female guppies instead prioritize the area of orange and colour saturation over hue (Karino et al. 2010), yet I found no effect of temperature on those two aspects of ornamentation. It is conceivable that ornamentation traits subject to intense sexual selection become canalized from environmental stressors such as the increased temperature in my study. This then brings into question whether the signals are, in fact, honest. Indeed, Candolin (1999) found that the condition of male three-spined sticklebacks displayed a curvilinear relationship with ornament quality; males of both good and poor condition had larger ornaments than males of intermediate condition. My results show that male guppies reared at higher temperatures had lower quality sperm (a key component of fertility in the guppy: e.g. Boschetto et al. 2011) but their ornament, as measured by orange colour and saturation, was unaffected, suggesting that these aspects of the secondary sexual character may not be honest signals of quality.

Zeh et al. (2012) have claimed that the “Achilles’ heel” for tropical ectotherms will be reproduction in a warming climate. Zeh et al. (2012) found that with slight increases in temperature (3.5°C) male neotropical pseudoscorpions, *Cordylochernes*

scorpioides produced half the sperm loads as controls and females failed to reproduce at all. Lahnsteiner and Mansour (2012) similarly found that sperm velocity decreased in both brown trout, *Salmo trutta* and burbot, *Lota lota* as temperature increased across a biologically relevant range. I found that increased temperature significantly decreased sperm length, and curvilinear and straight line velocity. Sperm length and velocity are key determinants of fertilization in many ectotherms (Billard 1978; Stoss 1983; Gage et al. 2004; Alavi and Cosson 2005). Indeed, Gage et al. (2004), Casselman et al. (2006), and Gasparini et al. (2010) found that there was a positive relationship with sperm velocity and fertilization success in internally or externally fertilizing fish. Additionally, sperm length is often positively correlated to sperm velocity (e.g. Gomendio and Roldan 1991; Malo et al. 2006; Fitzpatrick et al. 2009). Thus, my results indicate that reproduction could be compromised in a warmer environment, supporting the claim made by Zeh et al. (2012) that reproduction is the “Achilles’ heel” for tropical ectotherms.

Many studies suggest that global warming has the potential to negatively affect the immune system (e.g. Collazos et al. 1996; Dang et al. 2012). Indeed, Collazos et al. (1996) found that seasonal variation in temperature affects the immune response to PHA in the tench with the increased summer temperatures experienced by the fish causing a decreased immunological response compared to winter temperatures. However, Le Morvan-Rocher et al. (1995) found no effect of increased temperature on the PHA response in carp, *Cyprinus carpio*. My results agree with those of Le Morvan-Rocher et al. (1995) as I found no evidence of a reduced PHA swelling response at increased temperatures. This apparent difference in results with those of Collazos et al. (1996) may reflect the experimental manipulation of my study whereas Collazos et al. studied the

effects of natural, seasonal variation. Tench breed in the summer so their immune system may be down-regulated during this period as resources are shifted to reproduction (e.g. Fedorka et al. 2004; Whitton 1982; Moret and Schmid-Hempel 2000 and references therein). Regardless, my results suggest that the innate immune system of guppies may be able to cope with the projected temperature increase for the end of the century, at least as measured by the swelling response to a novel antigen.

In my study, I found that increased temperatures affected some sexual traits (sperm characteristics and ornament hue), but not aspects of immune function or survival. It is possible that, at the elevated temperatures, guppies channel resources to up-regulate their immune system, which then leaves their reproductive system more susceptible to immunological attack (Folstad and Skarstein 1996). Indeed, the immunocompetence handicap hypothesis (Folstad and Karter 1992) states that sperm cells are considered non-self and subject to attack from the immune system (reviewed in Kosuda and Bigazzi 1987). To counter attacks on sperm cells, males can release elevated levels of gonadal androgens which act to down-regulate the immune system (Folstad and Skarstein 1996). I did not, however, directly measure immune cell proliferation or circulating androgen levels in my fish and therefore cannot confirm if my data support the immunocompetence handicap hypothesis. My results might also reflect a trade-off between reproduction and immunity (and potentially other life history traits) with the latter taking precedence over reproductive traits in guppies when thermally stressed.

In conclusion, the results of my study suggest that the temperature rise predicted by the end of the century had no effect on immunity or survival in the guppy, a tropical ectotherm. Conversely, the increased temperature could have a significant impact on

reproduction in this fish. I found that increased temperatures resulted in decreased sperm length and motility, which are key aspects of fertility. My study thereby indicates that key sexual traits are more sensitive to elevated temperatures than traits linked to survival. Future work might emphasize long-term experiments that examine potential maternal environmental effects (e.g. McAdam et al. 2002), epigenetic effects (e.g. Miller et al. 2012), and genetic adaptations (e.g. Réale et al. 2003) that could all help to ameliorate the negative impacts of climate change.

3.5 References

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Chapter 4

4 Rapid evolution of a sperm trait in response to increased temperature in an ectothermic fish

The Intergovernmental Panel on Climate Change predicts an average global temperature increase of 1.8-4.0°C by 2100. Tropical ectotherms are expected to be particularly sensitive to this temperature increase because they live close to their thermal limits. I investigated the phenotypic plasticity and evolutionary responses of sperm traits in guppies (*Poecilia reticulata*) to increased temperatures after 6, 18, and 24 months. Guppies with experimental population temperatures of 25°C (control) or 28°C were reared in either 25°C or 28°C in a 2 × 2 common garden design. The plastic response to increased temperature was a decreased sperm length, velocity, and path linearity. The evolutionary response was a subsequent increase in sperm length, resulting in complete compensation after just 6 months in 28°C water. Sperm velocity and linearity showed no sign of evolution even after 24 months. This study provides evidence that some reproductive traits can respond via rapid evolution to the temperature increase associated with climate change.

4.1 Introduction

Changes in the environment can have marked effects on organisms (e.g. Endler 1980; West and Packer 2002), with temperature being one of the most ubiquitous environmental conditions with broad impacts on virtually all species (Dorts et al. 2012). As detailed in Chapter 1, there is concern about the potential impact of global warming on species composition and ecosystem health. Species have the capacity to respond to a

warmer environment by phenotypic plasticity or genetic adaptation (detailed in Chapter 1). Genetic adaptations to temperature can occur via natural selection acting on either phenological mechanisms or thermal physiology (Angilletta 2009). There are a number of examples of species showing genetic based adaptations in phenology (detailed in Chapter 1). However, little is known about rapid genetic adaptations of thermal physiology with the predominant view being that adaptation of thermal physiology is not likely over ecological timescales (Leal and Gunderson 2012). Yet, more recent evidence suggests that thermal adaptation can occur more rapidly than once believed (e.g. Leal and Gunderson 2012).

The effects of temperature on plasticity in developmental and life history traits have been well documented, yet less is known about reproduction, particularly reproductive morphology, despite these latter traits being crucial to population health and persistence (Angilletta 2009; Berger et al. 2011). In males, sperm length and velocity have been linked to fertilization success, particularly under competition (reviewed in Snook 2005; Simmons and Fitzpatrick 2012; but see Humphries et al. 2008). However, sperm traits have been shown to be sensitive to changes in temperature, with even slight increases in temperature resulting in reduced sperm numbers (Zeh et al. 2012), longevity (Binet and Doyle 2013), motility (Williot et al. 2000), length (Adriaenssens et al. 2012; Chapter 3; Breckels and Neff 2013), and velocity (Chapter 3; Breckels and Neff 2013; but see Adriaenssens et al. 2012). Such studies have led Zeh et al. (2012) to claim that reproduction is a potential “Achilles’ heel” for many species in the face of global warming. Certainly, more studies are needed to examine the plastic and genetic responses in reproductive traits to increases in temperature.

Here, I use Trinidadian guppies (*Poecilia reticulata*, Peters 1860) as a model ectotherm to detail the effects of long-term exposure to increased temperature, as projected for the end of the 21st century. Guppies are a small, live bearing fish, native to north-eastern South America and the Caribbean that inhabit small freshwater streams (Houde 1997). They tend to be highly polyandrous with males experiencing high levels of sperm competition. Males mature at approximately 7 weeks of age or younger (Reznick et al. 2001). Guppies have overlapping generations; as such generation time has been estimated between 1.5 and 6.9 months (e.g. Endler 1980; Reznick et al. 1997). Over the past six decades, Trinidad has experienced a mean air temperature increase of 1.5°C (Singh 1997), and is set to increase by a further 1.0-3.5°C by the end of the 21st century (Water Resources Agency 2001). This projected increase in air temperature will result in similar increases in stream and small river water temperatures (Stefan and Preudhomme 1993; Caissie et al. 2001; Kaushal et al. 2010). In Trinidad, the current mean daily air temperature is 27.7°C with daily fluctuations of up to 8.4°C (calculated between January 1992 and December 2012; weatheronline.co.uk). Mean river water temperatures are approximately 25°C and fluctuate between 20°C and 28°C (Alkins-Koo 2000). Although, guppies periodically experience temperatures of 28°C, I have previously shown that prolonged exposure to 28°C affects sperm traits (Chapter 3; Breckels and Neff 2013). Thus multi-generational exposure to increased temperature could negatively affect reproduction. Additionally, geographical barriers, such as waterfalls and oceans, mean that natural dispersal is unfeasible. Therefore guppies, like many other species, will have to rely on phenotypic plasticity or genetic adaptation in order to respond to a warming environment.

Specifically, I have previously shown that exposure to elevated temperatures during development results in decreased sperm length, velocity, and path linearity (Chapter 3; Breckels and Neff 2013), but that study measured only the initial plastic response and thus could not address the multi-generational, evolutionary response. In the present study, I exposed guppies to elevated temperatures for many generations to evaluate the scope of the genetic response. My objective was to examine whether sperm length, velocity, or path linearity would respond genetically and if that response was compensatory (returned to baseline levels). These sperm traits typically show high levels of heritability (Simmons and Moore 2009; Evans 2011), so I predicted that a genetic response would occur, resulting in partial or full compensation.

4.2 Methods

Guppies used in this experiment were descendants of fish caught from the Paria River, Trinidad in 2003. Guppies were held in the Freshwater Ecology Research Facility room at the University of Western Ontario in tanks lined with bottom layers of gravel and artificial plants to provide cover. Fish were kept on a 12h:12h light-dark cycle with the water temperature set to 25°C, using internal heaters, to simulate current natural conditions (Alkins-Koo 2000). Fish were fed twice daily, once with Tetramin® flake food and once with brine shrimp.

On May 1st 2010, six, 250 L experimental populations were seeded with 55 adult fish (25 males and 30 females). The initial water temperature in all six experimental populations was set to 25°C. The temperature in three of these experimental populations was raised gradually, at a rate of 1°C every 45 days, up to 28°C (SD: $\pm 1.2^\circ\text{C}$) to simulate

average levels of global warming by the end of the century (IPCC 2007). The three other experimental populations remained at 25°C (SD: $\pm 0.5^\circ\text{C}$) throughout the experiment and acted as controls. To produce families for the common garden treatments, after 6, 18, and 24 months, eight pregnant females (evident from enlarged abdomens and darker anal regions; Houde 1997) were removed from each experimental population and put into separate, individual 10 L rearing tanks with the water set at the same temperature as the experimental population that the female had come from (i.e. if the female came from an experimental population set at 25°C she was put into a rearing tank with the water set at 25°C). Females were allowed to give birth, after which they were returned to their original experimental population, leaving only their offspring in the rearing tanks.

Next, I created four treatments in a common garden experimental design by switching the water temperature in four of the eight rearing tanks, 24 hours after the first offspring was born, to that of the alternate experimental populations: (1) 25-25 (*control*), fish that had an experimental population and rearing temperature of 25°C; (2) 25-28, fish that had an experimental population temperature of 25°C but a rearing temperature of 28°C; (3) 28-28, fish that had an experimental population and rearing temperature of 28°C; and (4) 28-25, fish that had an experimental population temperature of 28°C but a rearing temperature of 25°C (see appendix A). There were a total of 12 tanks in each treatment, four from each of the three different experimental populations with the corresponding temperature. From the offspring in these rearing tanks (i.e. the F₁ generation) I estimated sperm traits as detailed below.

In addition, for the 18 month trial, a breeding design was used to generate an F_2 generation (F_2) of the 28-25 treatment. The breeding design used fish from the four rearing tanks of each experimental population. Males and females from the 28-25 treatment were separated into individual rearing tanks before they became sexually mature. After approximately four months, males and females were paired in a design that ensured brothers and sisters were not mated. The guppies were given 3 days to copulate and then the males were removed. When the females gave birth, the offspring were removed and put into separate rearing tanks. The water temperature remained at 25°C and sperm traits were examined on the offspring as detailed below.

4.2.1 Sperm characteristics

When fish were 3 months of age (mean age in days \pm SD: 96 ± 10), males were removed from their rearing tanks and put into individual isolation tanks for 3 days to ensure full sperm reserves (Pilastro et al. 2002), with the water temperature set to the same as they had been reared in. I then followed the methods for sperm analysis outlined in Chapter 3 (Breckels and Neff 2013). I did not measure sperm number here because of logistical constraints, but previous analysis revealed no change in numbers with increased temperature (sperm count \pm SD; 25°C: $2.2 \times 10^6 \pm 0.8 \times 10^6$; 28°C: $2.5 \times 10^6 \pm 1.0 \times 10^6$; $t_{17} = 0.87$, $p = 0.398$).

4.2.2 Statistical analysis

All presented p-values are two-tailed probabilities and all statistical analyses were performed using IBM SPSS v. 20 (SPSS Inc., Chicago, IL, USA). Metrics from individuals within the same family were averaged in order to get family means which

were used for all statistical analyses except when noted. Sperm path linearity was transformed using a logit transformation to normalize the data. General linear mixed models (GLMMs) were performed on family means of body length, sperm length, VCL, and path linearity. I included time point (6, 18, or 24 months) and experimental population and rearing temperatures as fixed factors and experimental population identification nested within experimental population temperature as a random factor in all tests. For the 18 month trial, I also used a one-way ANOVA and a subsequent tukey's post hoc test to compare body length, sperm length, velocity, and path linearity among the *control* (25-25), 28-28, 28-25, and F_2 treatments.

Variation in sperm length, velocity, or path linearity due to rearing temperature would suggest a phenotypic plastic response. Variation due to experimental population temperature suggests either a genetic response or maternal environmental effects. If this latter variation persists in the F_2 treatment, a genetic response is indicated.

4.3 Results

A total of 92 families were used across the three time periods (Table 4.1). This number is lower than the maximum expected of 144 families because some females did not give birth ($N = 23$), females gave birth to female only broods or males in the brood died before sperm analysis was conducted ($N = 26$), or no sperm could be taken from males in a family ($N = 3$). There was no effect of time, experimental population or rearing temperature, or the interaction between experimental population and rearing temperature on body length ($F_{2,80} = 1.5$, $p = 0.226$; $F_{1,4.4} = 0.5$, $p = 0.500$; $F_{1,80} = 1.5$, $p = 0.224$; and

Table 4.1 Metrics for the families used in analyses of sperm characteristics in the guppy (*Poecilia reticulata*).

Variable	Treatments				
	<i>Control</i>	<i>25-28</i>	<i>28-28</i>	<i>28-25</i>	<i>F₂</i>
<i>6 Month</i>					
No. families	10	9	9	4	
Males per family	1-10	1-5	1-5	2-7	
Body length (mm)	15.0 ± 0.9	14.8 ± 1.3	14.0 ± 0.9	14.0 ± 1.7	
<i>18 Month</i>					
No. families	9	7	9	7	7
Males per family	1-5	1-5	1-9	1-3	1-4
Body length (mm)	15.4 ± 0.7	14.6 ± 1.1	14.8 ± 0.8	14.9 ± 1.1	15.1 ± 0.6
<i>24 Month</i>					
No. families	9	10	5	4	
Males per family	1-4	1-4	1-2	1-4	
Body length (mm)	14.9 ± 0.9	14.4 ± 0.8	15.2 ± 1.3	15.0 ± 1.0	

N.B. Experimental populations and rearing temperatures were either 25°C or 28°C, in a 2 × 2 design (see text). Means are plus or minus one standard deviation. Numbers of families represent those families that were used in the analysis (see text).

$F_{1,80} = 0.5$, $p = 0.467$, respectively; Table 4.1). Similarly, there was no significant difference in male length in the F_2 treatment and the *control*, 28-28, and 28-25 after 18 months ($F_{3,28} = 1.0$, $p = 0.390$).

4.3.1 Sperm length

There was a significant effect of time, experimental population temperature, and rearing temperature on sperm length over the three time periods (Table 4.2; Fig. 4.1 A-C). Across the three sampling times, fish reared at 28°C (25-28 and 28-28) produced sperm that were about 3.5% shorter than fish from the corresponding experimental populations but were reared at 25°C (*control* and 28-25). Conversely, all treatments with fish from the 28°C experimental populations (28-28 and 28-25) had sperm that were over 4% longer than fish from the 25°C experimental populations with the corresponding rearing temperature (25-28 and *control*). After 18 months, males from the 28-25 and F_2 treatments had sperm that were similar in length but significantly longer than the *control* and 28-28 treatments ($F_{3,28} = 31.5$, $p < 0.001$; Fig. 4.2 A).

4.3.2 Sperm velocity

There was a significant effect of rearing temperature on sperm velocity (Table 4.2; Fig. 4.1 D-F). Treatments where fish were reared at 28°C produced sperm that were 11.5% - 12.4% slower than fish from the same experimental population but reared at 25°C. There was no effect of time or experimental population temperature on velocity (Table 4.2). Similarly, there was no significant difference between the F_2 treatment and the *control*, 28-28, and 28-25 after 18 months ($F_{3,26} = 0.9$, $p = 0.446$; Fig. 4.2 B).

Table 4.2 General linear mixed model results of sperm traits in families of guppies (*Poecilia reticulata*).

Factor	Sperm Length	Velocity	Path Linearity
Time	$F_{2,80} = 87.1, p < 0.001$	$F_{2,74} = 2.2, p = 0.120$	$F_{2,74} = 1.6, p = 0.210$
Population temperature	$F_{1,4.8} = 142, p < 0.001$	$F_{1,7.0} = 0.5, p = 0.487$	$F_{1,4.8} = 0.4, p = 0.539$
Rearing temperature	$F_{1,80} = 198, p < 0.001$	$F_{1,74} = 18.9, p < 0.001$	$F_{1,74} = 14.3, p < 0.001$
Population \times Rearing temperature	$F_{1,80} = 2.1, p = 0.154$	$F_{1,74} = 0.0, p = 0.938$	$F_{1,74} = 2.1, p = 0.148$
Random factor	$F_{4,80} = 1.7, p = 0.160$	$F_{4,74} = 0.5, p = 0.762$	$F_{4,74} = 1.7, p = 0.164$

N.B. Experimental population and rearing temperatures were either 25°C or 28°C, in a 2 \times 2 design (see text). Time denotes the three sampling periods of 6, 18, and 24 months. The random factor was experimental population identification nested within experimental population temperature.

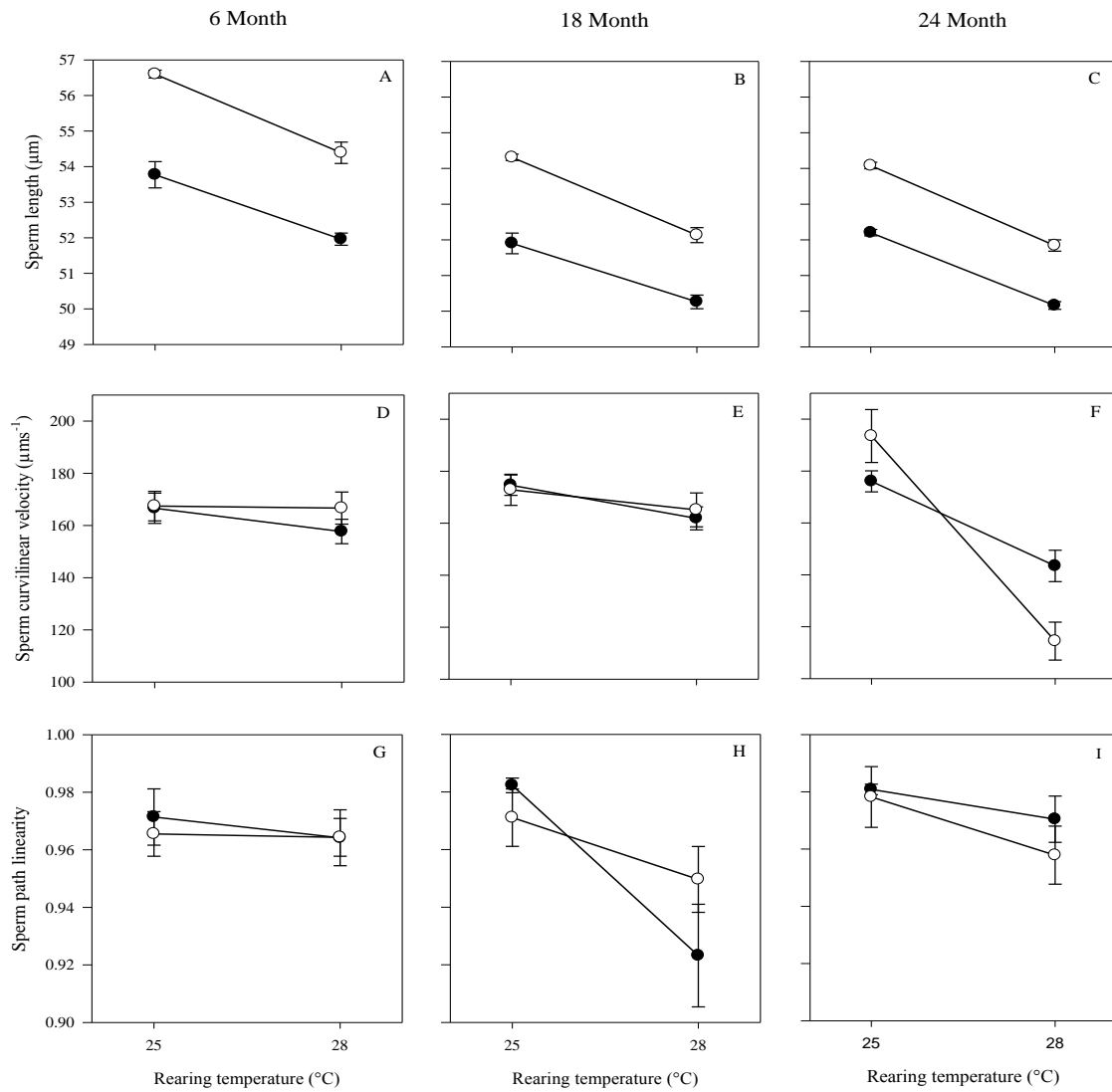


Figure 4.1 The effects of experimental population and rearing temperature on sperm traits in the guppy (*Poecilia reticulata*).

Offspring from experimental populations at 25°C (black circles) or 28°C (open circles) were sampled at 6, 18, and 24 months and reared at either 25°C or 28°C. Shown are means (\pm SE) for sperm length (A-C), velocity (D-F), and path linearity (G-I).

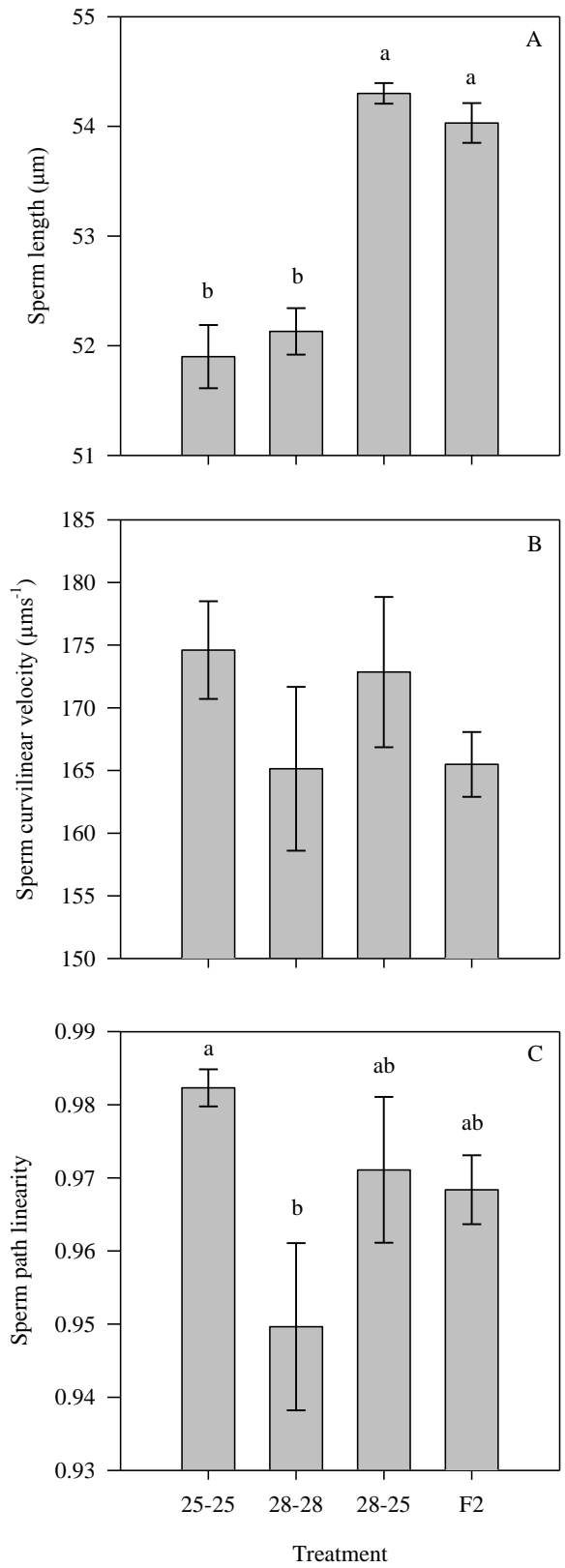


Figure 4.2 The effects of temperature treatment after 18 months on sperm traits in the guppy (*Poecilia reticulata*).

Treatments include fish from the 25°C and 28°C experimental populations (25-25 and 28-28), fish that were from the 28°C experimental populations but reared at 25°C (28-25), and the offspring of the fish that were from the 28°C experimental populations but reared at 25°C for 2 generations (F_2). Shown are means (\pm SE) for sperm length (A), velocity (B), and path linearity (C). Error bars with the same letter are not significantly different ($p > 0.05$) according to a Tukey's b HSD test.

4.3.3 Sperm path linearity

There was a significant effect of rearing temperature on sperm path linearity (Table 4.2). Treatments where fish were reared at 28°C (25-28 and 28-28) produced sperm that travelled about 2% less linearly than fish from the same experimental population temperature but reared at 25°C (*control* and 28-25; Fig. 4.1 G-I). There was no effect of time or experimental population temperature on sperm path linearity (Table 4.2). There was a significant difference between the *control*, 28-28, 28-25, and F_2 treatments at 18 months in sperm path linearity, with the *control* displaying significantly greater path linearity than the 28-28 treatment ($F_{3,26} = 4.8$, $p = 0.009$; Fig. 4.2 C).

4.4 Discussion

Previous research shows that reproduction in species could be significantly affected by climate change (e.g. Zeh et al. 2012; Chapter 3; Breckels and Neff 2013). Here I found that rearing temperature had a significant effect on all sperm traits that I measured; the phenotypically plastic response to increased temperature was decreased sperm length, velocity, and path linearity. These traits may be critical for male competitiveness during reproduction (Simmons and Fitzpatrick 2012). My results corroborate other studies that have similarly shown that an increase in temperature leads to a decrease in sperm length (e.g. Adriaenssens et al. 2012) and velocity (e.g. Beirão et al. 2011; Lahnsteiner and Mansour 2012). Some of those studies suggest that even small changes in temperature can elicit a stress response and negatively affect reproduction. Collectively, these studies suggest that the projected increase in temperature due to climate change could be detrimental to ectotherms, at least in the short term, because it

may significantly reduce sperm quality and performance, and consequently, reproductive success and population viability.

Given the potentially negative phenotypically plastic response observed, sperm traits must instead respond genetically via natural selection to overcome the effect of increased temperature. Sperm traits in several species have been shown to be highly heritable (e.g. Simmons and Moore 2009 and references therein), including in an introduced population of the guppy (Evans 2011), so these traits should have the potential to evolve rapidly. Here, male guppies from the 28°C experimental populations showed an evolved response with sperm length returning to the same size as the 25°C experimental populations after only 6 months (about 2-3 generations). The F_2 offspring (i.e. fish from the 28°C experimental populations reared at 25°C for two generations) had sperm lengths similar to the 28°C experimental populations reared at 25°C, indicating that the response to the increased temperature was indeed genetic (including epigenetics; e.g. Anway et al. 2005). Sperm velocity and path linearity, on the other hand, showed no sign of an evolved response even after 24 months (about 8-12 generations). Interestingly, fish from the 28°C experimental populations reared at 25°C produced sperm that were significantly longer than fish from the 25°C and the 28°C experimental populations, following the ‘hotter-is-better’ hypothesis (Huey et al. 1999), whereby fish from the 28°C experimental populations produce longer sperm than fish from the 25°C experimental populations, whatever temperature they are reared at. The fact that sperm traits have a high additive genetic component, yet I found that only length evolved, suggests that length may have been under stronger selection than velocity or path linearity. Thus, at least in guppies,

sperm length may play a more important part in reproductive success than previously thought (see Boschetto et al. 2011).

In several species, it has been documented that sperm length co-evolves with different aspects of females' reproductive tract (e.g. Briskie and Montgomerie 1992; Presgraves et al. 1999; Pitnick et al. 1999; Morrow and Gage 2000; Miller and Pitnick 2002). For example, in *Drosophila melanogaster*, females that were artificially selected to have longer sperm storage organs preferentially used longer sperm for fertilization, and consequently males evolved longer sperm (Miller and Pitnick 2002). There was no difference in my experimental populations of guppies in female body length between fish that were acclimated to 25°C or 28°C (Chapter 3; Breckels and Neff 2013) or after 6 months in the experimental populations ($F_{1,39} = 1.1$, $p = 0.294$). Assuming that body length is an indicator of female reproductive tract length or sperm storage organ size (micropockets in guppies, Kobayashi and Iwamatsu 2002), females from the two experimental population temperatures should not differ in those traits. Consequently, female reproductive morphology might impose strong stabilizing selection and drive the evolutionary response in sperm length. Interestingly, the 28-25 treatment males produced significantly longer sperm than either the 25°C or the 28°C experimental population males. It remains to be seen if those males gain higher reproductive success than the 25°C or 28°C experimental population males or whether their sperm are in fact too long and selected against via the female's reproductive tract.

Understanding the genetic covariance between traits is fundamental because it can determine the response of the traits to selection (Lynch and Walsh 1998). At the phenotypic level, sperm length is often correlated with sperm velocity (e.g. Gomendio

and Roldan 1991; Malo et al. 2006; Fitzpatrick et al. 2009; but see Humphries et al. 2008). However, little is known about the genetic covariance between these two traits (Mossman et al. 2009 and Evans 2011). My results suggest that there is minimal genetic covariance between sperm length and velocity as length responded to my temperature treatment independent of velocity. Furthermore, my results indicate that the traditional kinematics associated with sperm length and velocity can be easily disassociated, perhaps mediated by a reduced beat frequency of the flagellum in sperm from the higher temperature populations. Regardless, if sperm length and velocity are correlated, my study suggests that they can be rapidly disassociated both phenotypically and genetically.

In conclusion, the results of my study show that the short-term effects of the increased temperature predicted for the end of the century could have negative impacts for reproduction in a tropical ectotherm. However, I found evidence of an evolved response in sperm length after only 6 months or about 2-3 generations. This genetic response indicates that guppies can respond to climate warming via rapid evolution, at least for some reproductive traits.

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Chapter 5

5 Rapid evolution in response to increased temperature maintains population viability despite genetic erosion in a tropical ectotherm³

Climate change is predicted to increase the average global air temperature by up to 4.0°C by the end of the century. This increased temperature could have negative effects on many life history traits that are closely linked to fitness. Many species will therefore have to adapt to the warmer environment, but life history traits often have limited additive genetic variance. Here, we investigated population demographics and the evolutionary response of life history traits, as well as genetic diversity in guppies (*Poecilia reticulata*), in response to an experimentally increased temperature. There were fewer successful pregnancies, smaller brood sizes, and males matured earlier at a higher temperature as compared to control populations. However, there was no sign of an evolutionary response in these traits after 24 months of exposure to the increased temperature. We also found that population size, brood survivorship, sex ratio, and male length at maturity were unaffected by the increased temperature. Genetic diversity decreased rapidly in the increased temperature populations at a rate equivalent to an effective population size of only one quarter of the controls, revealing a clear signature of selection in response to

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increased temperature. This genetic erosion, however, could hamper the adaptive potential of the populations to other environmental changes associated with climate change.

5.1 Introduction

As detailed in Chapter 1, global warming is projected to increase the average global temperature by 1.8-4.0°C by the end of the century. In response to this warming, many species will have to disperse or adapt, or face the risk of extinction (as detailed in Chapter 1). Life history traits are a major determinant of both individual and population fitness and may be an especially important target for selection and genetic adaptation in response to a warming environment (reviewed in Crnokrak and Roff 1995; Roff and Emerson 2006). Life history traits include individual growth rate, age and size at maturity, reproductive investment, such as brood or clutch size, sex ratio, and survivorship (Stearns 1992). Recent evidence indicates that increases in temperature have negative impacts on many of these traits (e.g. guppies, *Poecilia reticulata* Dzikowski et al 2001; Karayücel et al 2008; neotropical pseudoscorpions, *Cordylochernes scorpioides* Zeh et al 2012; and grayling, *Thymallus thymallus* Wedekind et al 2013). For example, the effects of short-term increases in temperature on life history traits include reduced successful parturition (e.g. Karayücel et al 2008; Zeh et al 2012), reduced brood sizes (e.g. Dzikowski et al 2001; Karayücel et al 2008), decreased survival (e.g. Zeh et al 2012), and altered sex ratios resulting from sex-specific differences in survival (e.g. Karayücel et al 2008; Wedekind et al 2013). However, because life history traits are so closely linked to fitness, they often have little additive genetic variance and therefore

cannot respond to selection, at least until new mutations arise (reviewed in Crnokrak and Roff 1995; Roff and Emerson 2006). On the other hand, more recently there has been evidence for cryptic genetic variation in many traits (reviewed in Gibson and Dworkin 2004), which is expressed as a result of changes in the environment (i.e. genetic variation among individuals in phenotypic plasticity). Thus, some life history traits may show higher levels of additive genetic variance in a warmer environment, which could help species respond to global warming and could facilitate evolutionary adaptation.

Of particular relevance to global warming is the temperature-size rule which is a taxonomically widespread relationship between temperature and life history traits (Atkinson 1994; Angilletta 2009). According to this rule, for ectotherms, age at maturity and size at maturity decrease with increasing temperature (Atkinson 1994). This rule can be largely explained by a direct effect of the environment on physiological processes, which are dependent on the ambient temperature in ectotherms. Increased temperature leads to a phenotypically plastic response of earlier maturation because it causes more rapid cell division and differentiation, and smaller size at maturity when the rate of cell division and differentiation exceeds the rate of growth (van der Have and de Jong 1996; Angilletta et al 2004). The specific relationship between size or age at maturation and temperature (i.e. the reaction norm) for a population or species is generally understood to be genetically controlled and influenced by the relationship between size and reproductive success, as well as selection from ecological factors such as predator-prey interactions and competition (Neuheimer and Taggart 2007; Daufresne et al 2009). Global warming may therefore push populations off their optimum trait value for size or

age at maturation until selection can act on new mutations or existing genetic variation in the reaction norm.

Here, we use the Trinidadian guppy (*Poecilia reticulata*, Peters 1860) as a model ectotherm to determine the effects of multi-generational exposure to an elevated temperature. We exposed replicate experimental populations of guppies over two years (approximately 8 generations) to the temperature predicted for the end of the century, and measured multiple life history traits and population demographics, as well as levels of genetic diversity. Specifically, we measured population size, the number of successful pregnancies, brood size, brood survivorship, sex ratio, age and length at sexual maturation, and genetic diversity using microsatellite loci. We compared these traits to control experimental populations and partitioned variation between phenotypic plasticity and genetic responses.

5.2 Methods

5.2.1 Study species

Guppies inhabit shallow pools in streams and rivers of north-eastern South America and the Caribbean (Houde 1997). Currently, the mean water temperature in Trinidad is approximately 25°C with annual fluctuations between 20°C and 28°C (Alkins-Koo 2000; Grether et al 2001). Trinidad is projected to have an average air temperature increase of 1.0-3.5°C by the end of the century (Water Resources Agency 2001), which will likely result in similar increases in water temperature (e.g. Stefan and Preudhomme 1993; Caissie et al 2001; Kaushal et al 2010). The current natural variation

in temperature experienced by guppies may mitigate the potential negative effects of global warming, yet they rarely experience temperatures of 28°C for prolonged periods of time. Thus, the predicted temperature increase for the end of the century could be detrimental to guppies.

Guppies are sexually dimorphic, and males can be differentiated from females after 5-6 weeks as their anal fin develops into a rod like structure known as a gonopodium (Houde 1997). Males are mature when the gonopodial hood extends beyond the main part of the gonopodium, which typically occurs at approximately 7 weeks (49 days) of age or younger (Houde 1997; Reznick et al 2001). Breeding occurs throughout the year and females have a gestation period of approximately 3-4 weeks (Houde 1997). Generation times in guppies have been estimated to range between 1.5 and 6.9 months (e.g. Endler 1980; Reznick et al 1997).

5.2.2 Experimental set-up

Guppies and the experimental set-up for this experiment were the same as those in Chapter 4 (Breckels and Neff *In review*). Population size was counted three times for each population (repeatability $r^2 = 0.994$; $F_{23,71} = 712$, $p < 0.001$) every 6 months up until 24 months. After 6, 18, and 24 months, pregnant females were removed from each experimental population. If the female did not give birth within 2 months, she was replaced by a new female from the same experimental population. If the second female did not give birth within 2 months, she was not replaced and no data were collected from that rearing tank.

As outlined in Chapter 4 (Breckels and Neff *In review*), I created four treatments in a 2×2 common garden experimental design. From the offspring of the females in the rearing tanks I estimated life history traits at each time point (6, 18, and 24 months) as outlined below. Additionally, after conducting the 18 month trial, to examine maternal versus genetic effects on the offspring traits, I also generated an F_2 generation as detailed in Chapter 4 (Breckels and Neff *In review*).

5.2.3 Demographics and life history traits

I measured the number of successful pregnancies, brood size, brood survivorship, and sex ratio for the broods in each rearing tank. For the male offspring in each brood, we also measured male age and body length at maturation. The number of successful pregnancies was calculated as the number of females that produced a brood within 2 months. Brood size was calculated by counting the number of offspring that each female produced within the first 24 h of birthing her first offspring (females that did not produce broods were not included in this analysis). Brood survivorship was calculated as the proportion of the offspring born in a rearing tank that survived to 3 months of age. Sex ratio was calculated as the proportion of each brood that were male (determined at 3 months of age when all fish had reached maturity). Male age at maturity was calculated as the number of days from birth until a given male offspring first reached sexual maturity. At maturation, we also measured male body length from the tip of the snout to the end of the caudal peduncle.

5.2.4 Genetic analysis

At the baseline and every 6 months, fin clips were taken from 30 adults per experimental population and stored in 95% ethanol for microsatellite analysis of genetic

diversity. DNA was first isolated from each fish using a proteinase K digestion (Neff et al 2000). Eight previously described microsatellite loci were then PCR amplified for each individual (*Pr36*, *Pr39*, *Pr80*, *Pr92*, and *Pr171*; Becher et al 2002 and *Pre8*, *Pre9*, and *Pre17*; Paterson et al 2005). The resulting microsatellite products were visualised using an ABI 3730S DNA analyzer and manually sized using GENEMAPPER v. 4.0 (Applied Biosystems).

I checked for linkage disequilibrium between pairs of loci using GENEPOP v. 4.1 (Rousset 2008) at each time point, resulting in 840 comparisons; a Bonferroni correction method was used. I checked for the presence of non-amplifying ('null') alleles using MICRO-CHECKER v. 2.2 (van Oosterhout et al 2004). Null alleles were detected in our data, so we used FREENA (Chapuis and Estoup 2007) to correct the allele frequencies. Next, for the loci without null alleles, we assessed whether each locus from each experimental population at each time point was in Hardy-Weinberg equilibrium (HWE) using GENALEX v. 6.5 (Peakall and Smouse 2012), again applying a Bonferroni correction. Allelic richness was also estimated at these times as the average number of alleles observed at the eight microsatellite loci based on the sample of 30 fish. Finally, I estimated Nei's standard genetic distance between the experimental populations at each time point using GENALEX.

5.2.5 Assessing a signature of selection

We used a simulation approach to determine if declines in allelic richness over the course of the experiment could be explained by genetic drift, given the observed population sizes in each tank. I used the combined allele frequencies across all six tanks at the initial time point, correcting for and incorporating the null alleles, to seed my

simulated populations. To mirror my experimental design, I then simulated six replicate populations of 25 males and 30 females with genotypes chosen at random based on the initial allele frequencies. I first modeled the behaviour of each population assuming random mating in each generation, with the parents for each individual chosen at random from all individuals of the appropriate sex in the previous generation. The sex of each offspring was assigned probabilistically based on a 45% male sex ratio (see results; Table 1). The population size of the simulated populations was altered between generations to match the observed values in the experimental populations. I assumed non-overlapping generations and a 3 month generation time (similar results were obtained with a 6 month generation time). For each simulated population, I sampled 30 individuals at each time point to calculate allelic richness as in the experimental populations. I then repeated this simulation 1000 times to produce an expected distribution of allelic richness from which the 99% confidence intervals in the 25°C and 28°C simulated populations could be estimated at each time point. I used 99% confidence intervals to correct for repeated comparisons at the five time points (0, 6, 12, 18, 24 months). Ultimately, these simulations allowed us to determine if genetic drift could explain the declines in allelic richness that were observed in the 25°C and 28°C experimental populations.

Additionally, when the simulations indicated that genetic drift alone could not explain the decline in allelic richness, I estimated how much smaller the effective population size would need to be relative to the census population size to produce the allelic richness values that I observed at 24 months. I did this by allowing only a fixed proportion of the individuals to breed in any generation. For example, if this proportion was set at 0.5, then only 50% of the individuals in any generation were included as

potential parents in the next generation. The simulations were repeated in 0.05 increments for each value of this proportion between 0.1 and 1.0, from which I selected the proportion that best matched the observed data. I considered the proportion that best fit the data to be the proportion that produced an average allelic richness that was most similar to the observed allelic richness at the 24 month point.

5.2.6 Statistical analysis

The sex ratio and brood survivorship data were transformed using a logit transformation. All other variables were normality distributed (Kolmonov-Smirnov test; all $p > 0.091$). A log-linear model was performed to compare the prevalence of successful and unsuccessful pregnancies between the two experimental population temperatures across the three time points (6, 18, and 24 months). General linear mixed models (GLMMs) were then used to analyse differences among brood size, sex ratio, brood survivorship, age, and length at maturity at each time point. For brood size, we included experimental population temperature as a fixed factor and, for all other tests, we included experimental population temperature and rearing temperature as fixed factors. Experimental population replicate number (tank ID) nested within experimental population temperature was included as a random factor for all tests. When significant effects were found, we performed linear contrasts between fish from different population temperatures with the same rearing temperature to determine whether (1) adaptation to high temperature compromises performance at the control temperature (i.e. 25-25 > 28-25), and (2) adaptation to high temperature increases performance at high temperatures relative to the controls (i.e. 25-28 < 28-28). Additionally, for the 18 month trial, we used GLMMs to compare brood size among fish born in 25, 28, and the F_2 and to compare the

other demographic and life history traits among the *control*, 28-28, 28-25, and F_2 treatments. We included treatment as a fixed factor and tank ID as a random factor in the GLMMs.

T-tests were performed to compare allelic richness between the 25°C and 28°C experimental populations at the baseline level. Then, repeated measure analysis of variance (ANOVA) tests were performed to compare the estimated population size and allelic richness among experimental populations from different temperatures across all sampling times. One-way ANOVAs were performed at each time point to compare Nei's standard genetic distance among the three pair-wise comparisons treatments: the two intra-temperatures and the inter-temperature experimental population pair-wise comparisons (i.e. all three 25°C vs. 25°C pair-wise comparisons, all three 28°C vs. 28°C pair-wise comparisons, and all nine 25°C vs. 28°C pair-wise comparisons). All statistical analyses were performed using the statistical software packages IBM SPSS v. 20 (SPSS Inc., Chicago, IL, USA) or JMP v. 4 (SAS Inc., Cary, NC, USA).

5.3 Results

5.3.1 Demographics and life history traits

Although the experimental populations nearly doubled in size during the experiment, the change in size over time was not significant ($F_{4,16} = 2.5$, $p = 0.086$) and there was no difference in population size between the two temperatures ($F_{4,16} = 0.7$, $p = 0.598$; Fig. 5.1). There was no difference in the number of successful pregnancies across the three time periods (loglinear model: $\chi^2 = 4.0$, $df = 2$, $p = 0.135$). However, the proportion of successful pregnancies was greater in the 25°C ($68/88 = 77\%$) than the

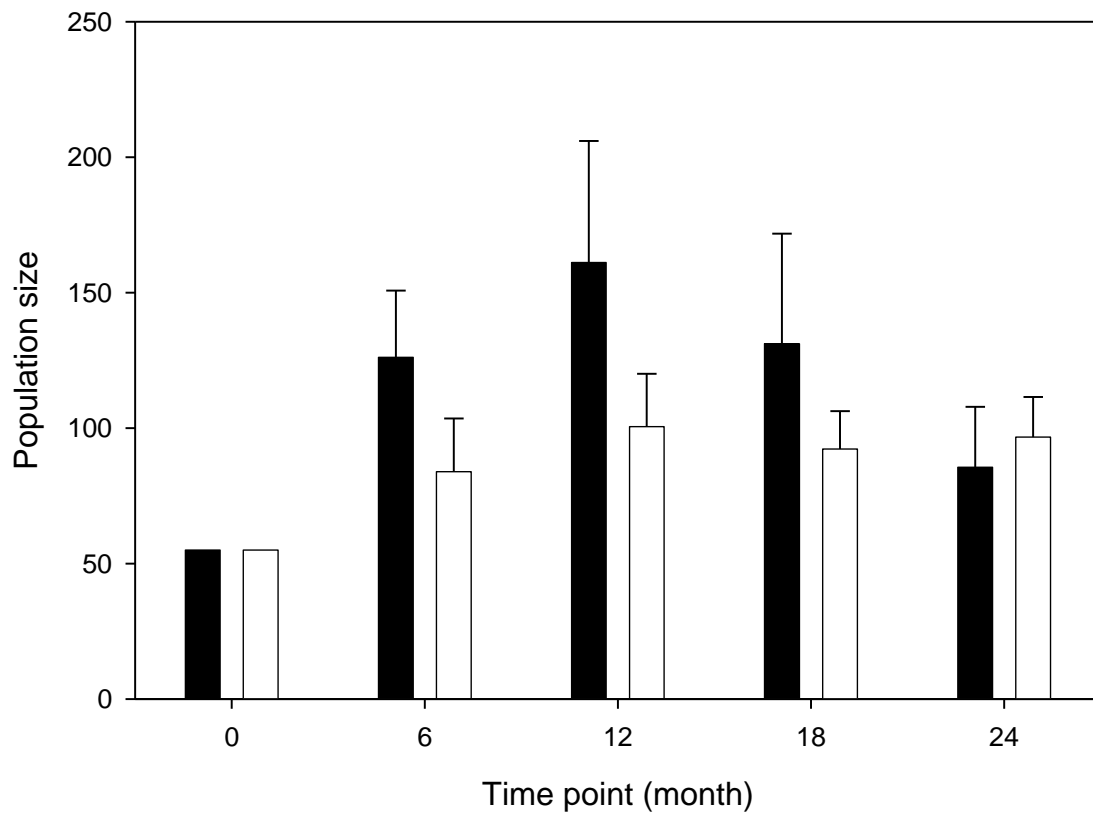


Figure 5.1 Size of experimental populations of guppies (*Poecilia reticulata*).

Shown are means (\pm SE) for populations at 25°C (black bars) or 28°C (open bars) over five time points. Error bars are based on three replicates within each temperature.

28°C (56/100 = 56%) experimental populations ($\chi^2 = 10.0$, $df = 1$, $p = 0.002$) and this difference increased over time ($\chi^2 = 6.7$, $df = 2$, $p = 0.035$). There was no effect of experimental population temperature on mean brood size at the 6 and 18 month time points ($F_{1,13.6} = 0.0$, $p = 0.986$ and $F_{1,17.4} = 0.1$, $p = 0.705$, respectively; Table 5.1). At the 18 month time point, there also was no difference in brood size among fish from the 25°C and 28°C experimental populations and the F_2 treatment ($F_{2,44} = 0.2$, $p = 0.819$; Table 5.1). However, at the 24 month time point, females from the 28°C experimental populations produced approximately half as many offspring as fish from the 25°C experimental populations ($F_{1,14.3} = 11.6$, $p = 0.004$; Table 5.1). There was no effect of experimental population or rearing temperature on brood survivorship or sex ratio at any time point (Table 5.1, 5.2), nor was there a significant difference between the *control*, 28-28, 28-25, and the F_2 treatments in brood survival or sex ratio for the 18 month trial ($F_{3,36} = 0.6$, $p = 0.598$ and $F_{3,35} = 0.5$, $p = 0.706$, respectively).

Males reared at 28°C matured approximately 7, 8, and 11 days sooner than fish reared at 25°C at the 6, 18, and 24 month time points, respectively (Table 5.2; Fig. 5.2 A-C). However, there was no effect of experimental population temperature on male age at maturity. There was also no difference at any time point between the 25-25 and 28-25 treatments (linear contrasts, 6 month: $F_{1,26} = 2.3$, $p = 0.142$; 18 month: $F_{1,19} = 0.9$, $p = 0.34$; 24 month: $F_{1,20} = 1.5$, $p = 0.233$) or between the 25-28 and 28-28 treatments (linear contrast, 6 month: $F_{1,26} = 0.1$, $p = 0.811$; 18 month: $F_{1,19} = 0.5$, $p = 0.481$; 24 month: $F_{1,20} = 1.1$, $p = 0.300$). Age at maturity also did not differ between males from the *control*, 28-28, 28-25, and the F_2 treatments at 18 months ($F_{2,26} = 2.4$, $p = 0.108$).

Table 5.1 Metrics for the families used in analyses of life history traits in the guppy (*Poecilia reticulata*).

Variable	Treatments				
	<i>Control</i>	<i>25-28</i>	<i>28-28</i>	<i>28-25</i>	<i>F₂</i>
<i>6 Month</i>					
No. families	12	12	11	7	
Brood size	6.7 ± 5.8	4.2 ± 2.8	4.9 ± 3.0	6.6 ± 5.9	
Brood survivorship (%)	89.2 ± 13	93.8 ± 12	90.2 ± 23	95.4 ± 6.6	
Sex ratio (% males)	0.36 ± 0.2	0.43 ± 0.3	0.48 ± 0.3	0.33 ± 0.4	
<i>18 Month</i>					
No. families	12	9	12	8	10
Brood size	4.3 ± 3.4	4.4 ± 2.3	5.6 ± 4.1	4.0 ± 3.0	4.4 ± 2.8
Brood survivorship (%)	87.5 ± 31	90.1 ± 13	84.4 ± 31	100 ± 0.0	86.4 ± 30
Sex ratio (% males)	0.38 ± 0.3	0.43 ± 0.3	0.47 ± 0.2	0.52 ± 0.3	0.45 ± 0.4
<i>24 Month</i>					
No. families	11	12	9	7	
Brood size	7.9 ± 4.0	4.5 ± 3.1	2.1 ± 0.8	3.6 ± 2.5	
Brood survivorship (%)	88.4 ± 30	91.0 ± 17	96.3 ± 11	90.5 ± 19	
Sex ratio (% males)	0.40 ± 0.2	0.43 ± 0.3	0.46 ± 0.5	0.71 ± 0.4	

N.B. Experimental population and rearing temperatures were either 25°C or 28°C, in a 2 × 2 design (see text). Means are plus or minus one standard deviation.

Table 5.2 General linear mixed model results of the life history traits in families of guppies (*Poecilia reticulata*).

Factor	Experimental Population Temperature	Rearing Temperature	Experimental Population × Rearing Temperature	Nested Factor
<i>6 month</i>				
Brood Survival	$F_{1,4.3} = 0.0, p = 0.837$	$F_{1,34} = 1.3, p = 0.268$	$F_{1,34} = 0.2, p = 0.694$	$F_{4,34} = 1.2, p = 0.301$
Sex Ratio	$F_{1,4.4} = 0.7, p = 0.461$	$F_{1,34} = 1.2, p = 0.280$	$F_{1,34} = 0.6, p = 0.434$	$F_{4,34} = 1.1, p = 0.366$
Age at Maturity	$F_{1,4.5} = 0.4, p = 0.555$	$F_{1,26} = 10.7, p = 0.003$	$F_{1,26} = 1.9, p = 0.185$	$F_{4,26} = 3.6, p = 0.017$
Length at Maturity	$F_{1,5.3} = 3.1, p = 0.136$	$F_{1,26} = 0.0, p = 0.893$	$F_{1,26} = 2.0, p = 0.165$	$F_{4,26} = 1.4, p = 0.254$
<i>18 month</i>				
Brood Survival	$F_{1,4.5} = 0.3, p = 0.615$	$F_{1,33} = 1.4, p = 0.243$	$F_{1,33} = 0.2, p = 0.634$	$F_{4,33} = 0.7, p = 0.599$
Sex Ratio	$F_{1,4.1} = 0.4, p = 0.585$	$F_{1,31} = 0.1, p = 0.803$	$F_{1,31} = 0.1, p = 0.794$	$F_{4,31} = 3.0, p = 0.033$
Age at Maturity	$F_{1,7.6} = 0.0, p = 0.829$	$F_{1,19} = 4.6, p = 0.045$	$F_{1,19} = 1.6, p = 0.219$	$F_{5,19} = 1.4, p = 0.276$
Length at Maturity	$F_{1,14.9} = 8.3, p = 0.011$	$F_{1,19} = 3.9, p = 0.063$	$F_{1,19} = 3.6, p = 0.072$	$F_{5,19} = 0.9, p = 0.860$
<i>24 month</i>				
Brood Survival	$F_{1,5.5} = 1.9, p = 0.225$	$F_{1,31} = 0.8, p = 0.374$	$F_{1,31} = 0.0, p = 0.834$	$F_{4,31} = 0.7, p = 0.617$
Sex Ratio	$F_{1,4.9} = 0.5, p = 0.519$	$F_{1,31} = 0.6, p = 0.458$	$F_{1,31} = 1.6, p = 0.219$	$F_{4,31} = 1.1, p = 0.352$
Age at Maturity	$F_{1,8.2} = 2.3, p = 0.166$	$F_{1,20} = 16.1, p < 0.001$	$F_{1,20} = 0.0, p = 0.962$	$F_{4,20} = 1.0, p = 0.416$
Length at Maturity	$F_{1,11.1} = 0.4, p = 0.527$	$F_{1,20} = 0.3, p = 0.605$	$F_{1,20} = 0.7, p = 0.422$	$F_{4,20} = 0.6, p = 0.642$

N.B. Experimental population and rearing temperatures were either 25°C or 28°C, in a 2 × 2 design (see text). The nested factor was experimental population replicate identification nested within experimental population temperature.

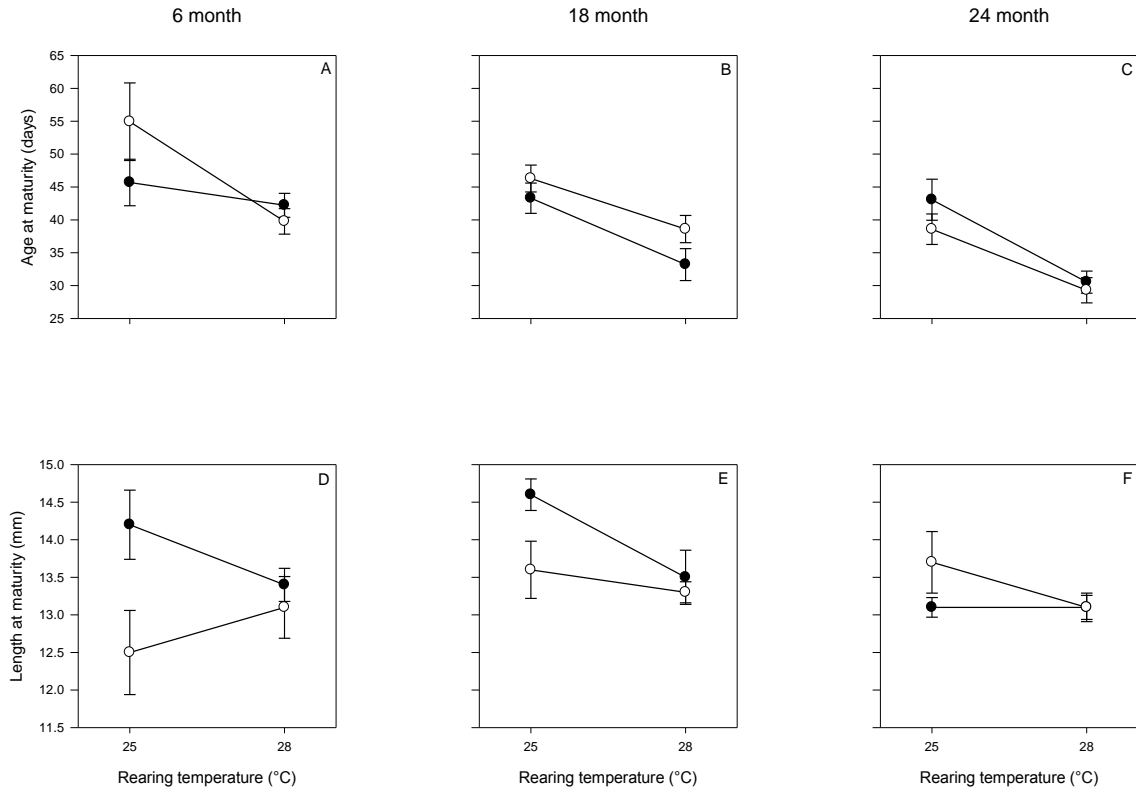


Figure 5.2 The effects of experimental population and rearing temperature on life history traits in families of guppies (*Poecilia reticulata*).

Offspring from populations at 25°C (black circles) or 28°C (open circles) were sampled at 6, 18, and 24 months and reared at either 25°C or 28°C. Shown are means (\pm SE) for male age (A-C) and body length (D-F) at maturity.

There was no effect of either experimental population or rearing temperature on male body length at maturity at the 6 and the 24 month time points (Table 5.2; Fig 5.2 D, F). At the 18 month time point, males reared at 28°C were approximately 0.8 mm shorter at maturity than fish reared at 25°C (Table 5.2; Fig. 5.2 E). Males from the 28°C experimental populations were shorter than fish from the 25°C experimental populations, but this difference was not statistically significant ($p = 0.053$). Fish from the 28-25 treatment were approximately 1.7 mm and 1.0 mm shorter at maturation than fish from 25-25 treatment after 6 and 18 months, respectively (6 month: $F_{1,26} = 4.4$, $p = 0.046$; 18 month: $F_{1,19} = 7.1$, $p = 0.015$). However, this effect was not observed after 24 months ($F_{1,20} = 1.0$, $p = 0.322$). There was no difference between the 25-28 and 28-28 treatments in size at maturity at any time point (linear contrast, 6 month: $F_{1,26} = 0.0$, $p = 0.629$; 18 month: $F_{1,19} = 0.1$, $p = 0.766$; 24 month: $F_{1,20} = 0.0$, $p = 0.905$). Additionally, there was no difference in length at maturity between males from the *control*, 28-28, 28-25, and the F_2 treatments at 18 months ($F_{2,26} = 1.0$, $p = 0.366$). Consequently, the differences in body length observed between the *control* and 28-25 treatments cannot be explained by a genetic response.

5.3.2 Genetic diversity

After applying a Bonferroni correction, approximately 2% of the pair-wise comparisons between microsatellite loci showed significant linkage disequilibrium (18 of 840). However, the pair-wise comparisons that did show linkage disequilibrium did not include the same pairs of loci across different tanks or time points, suggesting that the deviations do not reflect actual linkage between the loci. Only 72% of the microsatellite loci were in Hardy-Weinberg equilibrium (HWE; 173 out of 240) after controlling for

multiple comparisons. However, when excluding loci with null alleles present (four out of eight loci), 92% of the remaining loci were in HWE (110 out of 120) and there again was no consistent pattern across tanks or time points.

Mean allelic richness did not differ between experimental populations based on temperature at the baseline ($t_4 = 0.1$, $p = 0.923$). Mean allelic richness decreased over time in all tanks ($F_{4,16} = 55.3$, $p < 0.001$), but the 28°C experimental populations decreased significantly more rapidly than the 25°C experimental populations ($F_{4,16} = 12.0$, $p < 0.001$; Fig. 5.3). From my simulation model of genetic drift, I determined that drift alone could explain the decrease in allelic richness in the 25°C experimental populations (Fig. 5.3). However, the 28°C experimental populations experienced a greater decline in allelic richness than could be explained by drift. I calculated that the 28°C experimental populations lost allelic richness at a rate equivalent to populations that had an effective size that was only 25% of the observed size.

There was no significant difference in Nei's standard genetic distance among treatments for the pair-wise comparisons of intra- or inter-temperature experimental populations (i.e. all 25°C vs. 25°C, 28°C vs. 28°C, and 25°C vs. 28°C pair-wise comparisons) at the 0, 6, 12, or 18 month time points ($p > 0.084$ for all). However, at the 24 month time point, the 28°C experimental populations were significantly more diverged from each other than were the 25°C experimental populations ($F_{2,14} = 4.5$, $p = 0.034$), with the inter-temperature pair-wise comparisons not significantly different from either the 25°C or 28°C pair-wise comparisons ($p > 0.137$; Table 5.3). This result is consistent

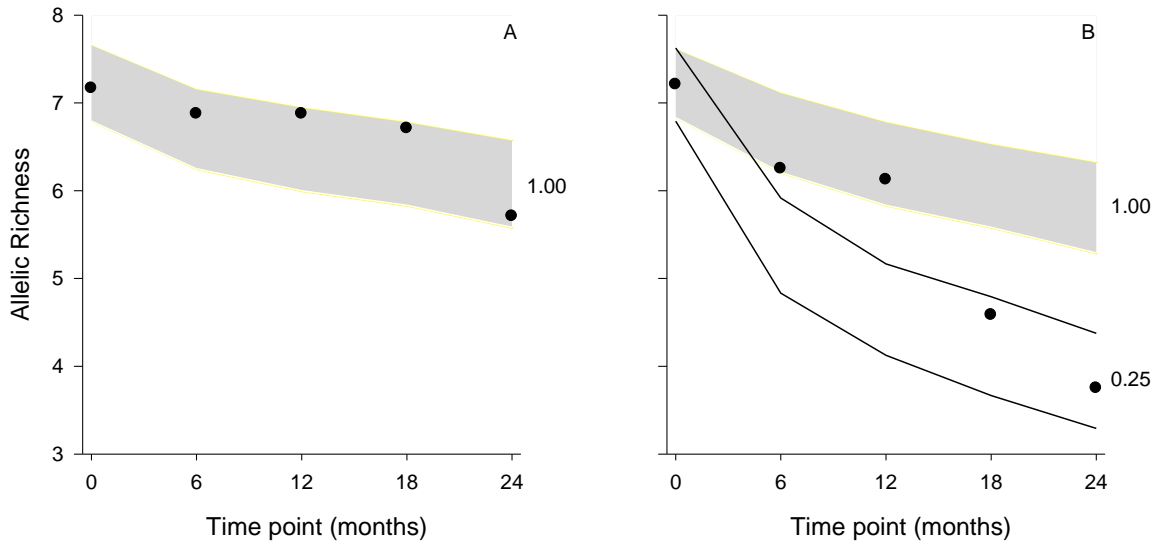


Figure 5.3 Allelic richness in experimental populations of guppies (*Poecilia reticulata*). Shown are populations at 25°C (A) and 28°C (B).

The black dots indicate the average observed allelic richness (mean number of alleles) at each time point. The shaded section denotes the 99% confidence intervals from a simulation that modelled declines in allelic richness based solely on genetic drift. The solid lines in panel B denote the 99% confidence intervals for the simulated population size that best matched the observed declines in allelic richness, with the number to the right of the graph indicating the proportional size (effective size) of the simulated population relative to the observed experimental population.

Table 5.3 Pair-wise population comparisons of Nei's standard genetic distance after 24 months in experimental populations of guppies (*Poecilia reticulata*).

		25°C			28°C	
		1	2	3	4	5
25°C	2	0.100				
	3	0.139	0.139			
	4	0.114	0.140	0.136		
28°C	5	0.189	0.162	0.192	0.220	
	6	0.181	0.166	0.182	0.168	0.183

N.B. Experimental populations 1-3 and 4-6 were held at a constant temperature of 25°C and 28°C, respectively. Shaded boxes represent pair-wise comparisons between experimental populations at the same temperature.

with the reduced effective population size in the 28°C experimental populations and consequently increased genetic drift relative to the 25°C experimental populations.

5.4 Discussion

Global warming is predicted to have a negative impact on population viability in many species (e.g. Karayücel et al 2008; Zeh et al 2012). Previous research conducted using guppies acclimated to various temperatures from birth documented reduced offspring survival in water temperatures equal to or higher than 29°C (Karayücel et al 2008). As well, Dzikowski et al (2001) found differential survival between the sexes at higher temperatures in guppies, resulting in a male biased sex ratio. However, we found no difference in population size, sex ratio, or brood survivorship between our control temperature (25°C) and the elevated temperature predicted by global warming (28°C), at any of our three sampling time points. Guppies may be able to tolerate 28°C because they periodically experience temperatures that high in their natural environment (Alkins-Koo 2000). We did find, however, that the performance of offspring from the 28°C experimental populations were compromised when reared at the control temperature; these offspring were significantly shorter than fish from the control populations after 6 and 18 months. The F₂ fish did not display this effect which implies that it is not a genetic effect but perhaps explained by maternal or developmental effects. Interestingly, after 24 months, there was no longer any evidence of this compromised performance suggesting that fish from the 28°C populations had become better adapted to the higher temperature. Taken together, these results suggest that, although temperatures up to 28°C have a

limited effect on demographic parameters in guppies, temperatures at or above 29°C are associated with a significant decrease in survival, particularly for females.

On the other hand, we also found that there were fewer successful pregnancies at 28°C than at 25°C and the brood size of the 28°C females was half that of 25°C females at the 24 month time period. Zeh et al (2012) have argued that, in a warming climate, reproduction is likely to be particularly vulnerable for tropical species, and indeed, many studies have documented effects on reproductive traits in response to increases in temperature (e.g. Karayücel et al 2008; Zeh et al 2012; Breckels and Neff 2013; Lahnsteiner and Leitner 2013). In our case, the reduced reproductive success could have been the result of dysfunctional sperm as we have previously shown that multiple sperm traits in guppies are negatively affected by increasing temperature (Breckels and Neff 2013). The reduced reproductive success also could be a product of a change in female investment in reproduction (e.g. Zeh et al 2012), or perhaps a sign of inbreeding depression, as there was a sharp reduction in genetic diversity in the 28°C experimental populations. Although this reduction in reproductive success did not yet translate into lower population sizes, our results suggest that even if other demographic parameters are unaffected by an increased temperature of 28°C, reproduction in guppies is compromised. Thus, as suggested by Zeh et al (2012), reproduction may indeed be the “Achilles’ heel” for tropical ectotherms.

According to the temperature-size rule, global warming should result in earlier maturation at a smaller size for ectotherms (Atkinson 1994; Angilletta et al 2004). Numerous other studies on ectotherms have found that exposure to increased temperature

results in a younger age at maturity (e.g. Dhillon and Fox 2004; Zeh *et al.* 2012). Our results partially support the temperature-size rule in guppies; males showed a plastic response of maturing at a younger age when reared at a higher water temperature. Indeed, this earlier maturation may reduce generation time in the 28°C experimental populations and maintain population viability despite reduced reproductive performance. Increased temperature should also result in smaller size at maturity (Angilletta *et al.* 2004; e.g. Dhillon and Fox 2004; Zeh *et al.* 2012). However, our results did not support this latter prediction as length at maturity did not differ across the two rearing temperatures, suggesting that this trait is canalised. It is possible that guppies have compensating mechanisms to counteract the relationship between higher growth rate and decreased size at maturity, which is likely driven by strong size-dependent predation that favours reaching a threshold size before allocating resources towards reproduction (Reznick and Endler 1982). Overall, guppies exposed to warmer temperatures matured at a younger age as predicted by the temperature-size rule, although their size at maturity was not affected by the increased temperature.

Selection on favourable traits can result in the loss of genetic diversity within experimental populations even if demographics are unaffected (e.g. Santos *et al.* 2005; Athrey *et al.* 2007; reviewed in Hoffman and Willi, 2008; Pauls *et al.* 2013). Here, we found that the multi-generational exposure to an elevated temperature (28°C) significantly reduced allelic richness compared to the control temperature (25°C) despite no reduction in population size. As well, our simulation model suggested that the loss of allelic richness was far greater than could be explained by genetic drift alone; relative to the control experimental populations, only about one quarter as many fish from the 28°C

experimental populations were likely contributing their genes to the next generation. The initial deviation observed in allelic richness compared to our simulation model in the 28°C experimental populations may simply reflect an increased effective population size due to the initial females used to seed the experimental population being pregnant. Regardless, we did not find any evidence of a genetic response in any life history traits that we measured, which may reflect an absence of additive genetic variance in these traits (see Crnokrak and Roff 1995; Roff and Emerson 2006). We also found no evidence that the microsatellites we used consistently deviated from Hardy-Weinberg equilibrium, indicating that these loci were not linked to genes under selection to the thermal environment. Instead, this signature of selection could be driven by a gene for thermal tolerance, at least one of which may reside on the X chromosome in guppies (Fujio et al 1990; Nakajima et al 2009), or possibly selection acting on sperm traits as we have previously documented (Chapter 4; Breckels and Neff *In review*). Nevertheless, our data clearly show a signature of selection in response to increased temperature, mediated by increased variance in reproductive success among individuals.

Despite showing a clear signature of selection to increased temperature, the future adaptive potential of guppies in the 28°C experimental populations may nevertheless be compromised. There is mounting evidence that the adaptive potential of populations is hampered by small effective population sizes and reduced genetic diversity after exposure to a stressor (e.g. Athrey et al 2007; Nowak et al 2009). Although the experimental populations exposed to elevated temperature in our study maintained similar population sizes as the control experimental populations, they displayed significantly less genetic diversity and consequently lower effective population sizes. The 28°C experimental

populations were also significantly more diverged from each other after 24 months than were the control experimental populations, which was likely a product of increased genetic drift acting on the 28°C experimental populations in the latter time points. This reduction in diversity may have led to increased inbreeding (e.g. Kristensen et al 2003; reviewed in Keller and Waller 2002; Frankham et al 2005), which can result in inbreeding depression and reduced population viability (Charlesworth and Charlesworth 1987). Inbreeding depression might explain the reduced fertility of the female guppies in the elevated temperature populations at the latter time points (see Kristensen et al 2003; Pitcher et al 2008). Importantly, the loss of genetic diversity and lower effective population sizes will decrease the chance for further adaptation to other stressors (e.g. Meyer and Di Giulio 2003; Vogt et al 2010). Thus, although the demographic and life history traits appear unaffected by increased temperature, there was an underlying erosion of genetic variation which will reduce the adaptive capacity of the populations. Given that climate change is predicted to result in multiple stressors, populations may become too genetically impoverished to adapt to all environmental or ecological changes. Certainly more studies examining multiple stressors are needed to more fully understand the adaptive capacity of populations to climate change.

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Chapter 6

6 General discussion

Anthropogenic stressors are altering ecosystems, both locally and globally, at unprecedented levels and there is a distinct lack of knowledge regarding how species' may respond to different stressors. The overarching goal of my thesis was to conduct research that would provide insight into the plastic and evolutionary responses of species to these anthropogenic stressors, specifically pollution and global warming. While numerous studies have documented the short-term plastic effects of species to anthropogenic stressors (e.g. Allin and Wilson 2000; Milne et al. 2000; Robinson and Davison 2008; Muñoz et al. 2012; Zeh et al. 2012), comparably little research has focussed on the evolutionary responses of organisms to these stressors. I document both the plastic and evolutionary responses of key fitness traits in two fish species to anthropogenic stressors. Taken together, these chapters provide valuable insight as to how species can, and are, responding to different stressors.

6.1 Plastic responses to anthropogenic stressors

Phenotypic plasticity is the first response to anthropogenic stressors for species that cannot disperse (Bradshaw and Holzapfel 2006; Fuller et al. 2010). I found plastic responses in brown bullhead (*Ameiurus nebulosus*) behaviour in response to pollution exposure (Chapter 2; Breckels and Neff 2010), and age at maturity and sperm performance in guppies (*Poecilia reticulata*) in response to increased temperatures (Chapters 3-5; Breckels and Neff 2013; *In review*; Breckels et al. 2013). These plastic

responses are generally indicative of a stress response and could have negative impacts on long-term population viability.

Stress responses can lead to reduced growth, impaired reproduction, and increased susceptibility to parasites and disease (Adams et al. 1989 and reference therein). If this stress is expanded to the population level, it could result in reduced recruitment, and then further to the community level, it could also affect species richness (Adams et al. 1989). Thus, the plastic responses to pollution and increased temperature can be detrimental to individuals, populations, species, and communities. Indeed, many studies have documented short-term decreases in survival and alterations in sex ratios, due to sex-specific selection pressures, with exposure to anthropogenic stressors, such as pollution and global warming (e.g. Milne et al. 2000; Cardinali et al. 2004; Karayücel et al. 2008; Tian et al. 2012; Zeh et al. 2012). However, I found no differences in guppies among temperature treatments in survivorship, sex ratio, and size-at-age (Chapters 3-5; Breckels and Neff 2013; *In review*; Breckels et al. 2013). Another factor that might affect survivorship at increased temperatures is the immune response, yet I also found no difference in immune response among temperature treatments (Chapter 3; Breckels and Neff 2013). These results suggest that guppies can cope with short-term exposure to increased temperature, perhaps because they periodically experience elevated temperatures in the wild (Alkins-Koo 2000).

In Chapter 5 (Breckels et al. 2013), I did find that the plastic response to increased temperature was a decreased male age at maturity. However, the potential consequences of males maturing at a younger age remain unclear (van der Have and de

Jong 1996). As there was no apparent difference in body size between temperatures, this earlier maturation could result in an earlier onset of reproduction, which could be beneficial to the fish, in terms of fitness. Of all the measured life history traits after one generation of exposure to increased temperatures, the only trait that responded via phenotypic plasticity was age at maturity, implying that life history traits in guppies have a large performance breadth so the increased temperature predicted for the end of the century may not impact guppies.

On the other hand, while I found no effect of temperature on measures of ornamentation important in female mate choice (see Karino et al., 2010), I did find that the plastic response in males to increased temperatures was a decrease in sperm length, velocity, and path linearity (Chapter 3; Breckels and Neff 2013). Similarly, other studies have shown short-term increases in temperature to have negative impacts on sperm traits (e.g. Adriaenssens et al. 2012; Zeh et al. 2012). Taken together, these results suggest that reproduction may be compromised at higher temperatures as sperm traits, particularly velocity in guppies (e.g. Boschetto et al. 2011), have been linked to fertilisation success (Snook 2005; Simmons and Fitzpatrick 2012).

In Chapter 2 (Breckels and Neff 2010), I found that the plastic response of wild brown bullheads to polluted sediments was a threefold increase in their locomotion and a reduction in their aggression relative to baseline levels. Decreased aggression typically results in reduced access to resources, such as food, shelter, and mates (e.g. Fero et al. 2007), and locomotion is involved in such activities as feeding, predator avoidance, and reproduction (Baatrup and Bayley 1993; Collar and Wainwright 2009). I did not find an

effect of pollution on the measured aspects of escape response, yet with reduced access to shelter and increased locomotion, individuals would indirectly become more vulnerable to predation. As such, exposure to multiple pollutants simultaneously could potentially reduce an individual's food intake, reproductive output, and predator avoidance. The plasticity displayed by bullheads in response to pollution and guppies in response to elevated temperatures are indicative of stress responses (Kime et al. 1996; Allin and Wilson 2000). Thus, evolutionary responses may be critical in order to maintain population viability.

6.2 Evolutionary responses to anthropogenic stressors

The findings of Deutsch et al. (2008) that tropical ectotherms are most at risk due to global warming have been recently challenged. In particular, Walters et al. (2012) predict that the risk of extinction of tropical ectotherms is no greater than temperate species because, generally, tropical ectotherms: (1) are smaller (see Atkinson 1994); (2) are more fecund; (3) have larger population sizes; and (4) have shorter generation times than temperate species. Thus, such species should possess an evolutionary advantage because adaptation can occur more rapidly in larger populations possessing higher levels of standing genetic variation, larger growth rates, and shorter generation times (Walters et al. 2012). However, many temperate ectotherms, such as brown bullheads, also possess many of the same biological characteristics and thus may be capable of rapid evolutionary responses. Providing Darwin's (1859) four postulates are true for a given trait and the selection pressure generated by anthropogenic stressors is strong enough (Reznick and

Ghalambor 2001), both tropical and temperate populations with the above biological characteristics have the potential to rapidly evolve to anthropogenic stressors. Indeed, I found evidence for rapid evolutionary responses in brown bullhead behaviour and guppy sperm length to pollution and increased temperature, respectively.

Exposure to anthropogenic stressors, such as pollution and increased temperature, often results in reduced genetic diversity as the population responds to the selection pressures imposed upon them (e.g. Silbiger et al. 2001; Santos et al. 2005; Athrey et al. 2007; reviewed in Pauls et al. 2013). For example, brown bullheads from Lake Erie, US, had lower levels of genetic diversity relative to controls (Silbiger et al. 2001). In Chapter 5 (Breckels et al. 2013), I also found a reduction in genetic diversity and effective population size in guppies from the increased temperature populations as compared to the control populations. These results clearly infer a signature of selection on the populations at increased temperature (see discussion in Chapter 5; Breckels et al. 2013). Perhaps selection was also acting on tolerance to the corresponding stressor (e.g. Athrey et al. 2007; Nakajima et al. 2009), yet survival was unaffected by short-term exposure to increased temperature (Chapter 3; Breckels and Neff 2013). Furthermore, despite showing an apparent adaptive genetic response to increased temperature, the population's potential to adapt to further stressors could now be hampered by this genetic impoverishment (e.g. Meyer and Di Giulio 2003; Athrey et al. 2007; Nowak et al. 2009; Vogt et al. 2010; reviewed in Pauls et al. 2013).

Anthropogenic stressors, such as global warming, have already triggered many species' extinctions, and currently threaten the viability of many others (Thomas et al.

2004; reviewed in Parmesan 2006). As well, contemporary levels of global warming are causing sex-specific mortality, resulting in altered sex ratios (e.g. Leonardos et al. 2009; Wedekind et al. 2013). However, I found that guppy census population size, survivorship, and sex ratios were unaffected by multi-generational exposure to increased temperatures (Chapter 5; Breckels et al. 2013). Warming temperatures are predicted to lead to an increase in the growth rate, transmission, and virulence of both pathogens and parasites (reviewed in Marcogliese, 2008). However, a more thorough analysis is needed as I did not test the evolutionary response of the immune system. As well, I did not test the evolutionary response of parasites or pathogens to increased temperatures, which could also affect the immune response by adding increased pressure on the immune system. Thus, an objective for potential future work is to detail the evolutionary responses of both pathogen or parasite and its host to anthropogenic stressors (see future work detailed below).

Non-reproductive life history traits, such as age and size at maturity, typically have low levels of additive genetic variance because they have been eroded by thousands of generations of selection in order to optimize traits (reviewed in Crnokrak and Roff 1995; Roff and Emerson 2006). Accordingly, I found no evidence of a genetic response in age at maturity, suggesting that cryptic genetic variation does not exist for this trait (e.g. Runcie et al. 2012; reviewed in Gibson and Dworkin 2004) or that selection pressures were not high enough to result in an evolutionary response. In many taxa, body size is positively correlated to reproductive success (Andersson 1994). The fact that size does not seem to be affected by the temperature increase in this study means that maturing at a younger age may not be detrimental to guppies as reproduction can

commence earlier in life. As such, selection on this trait would not be high. The results of the life history traits suggest that guppies may have the capacity to survive in a warming environment.

A recent study by Zeh et al. (2012) claimed reproduction to be the “Achilles’ heel” in the face of global warming. However, sperm traits typically have high levels of additive genetic variance (e.g. Simmons and Moore 2009) and therefore can evolve rapidly if selection acting on them is great enough. Indeed, in Chapter 4 (Breckels and Neff 2013; *In review*), I found that sperm length evolved complete compensation (i.e. returned to baseline levels) after just 6 months (approximately 2-3 generations) in elevated temperatures. Sperm velocity has previously been shown to be important in guppy reproduction (Boschetto et al. 2011) and has high levels of additive genetic variance (e.g. Simmons and Moore 2009), yet showed no sign of an evolved response after 24 months (approximately 8-12 generations). Perhaps the sperm kinematics or cell composition has been altered by exposure to increased temperatures (e.g. Labbé et al. 1995), which, in turn, would affect sperm velocity (Beirão et al. 2012). Nevertheless, the fact that sperm length evolved, but there was no evidence of evolution in the other two sperm traits examined, suggests that sperm length may be playing an important role in reproduction in guppies, at least in an increasingly thermal environment.

Future work is needed to better understand the reproductive success of males at elevated temperature (see future research) and the effects of anthropogenic stressors on the evolutionary response of other sperm traits, such as kinematics (e.g. beat frequency) and cell composition (e.g. levels of adenosine triphosphate; ATP). As well, sperm

velocity and path linearity measurements were performed in solutions held at room temperature (i.e. approximately 25°C), thus the control males (25°C) may have had an advantage over the increased temperature males (28°C) as the solutions were closer to control temperatures.

Many studies of different taxa have documented reduced successful parturition in response to increases in temperature (e.g. Dzikowski et al. 2001; Karayücel et al. 2008; Zeh et al. 2012). In Chapter 5 (Breckels et al. 2013), I found that females from elevated temperatures produced significantly fewer, smaller broods than control females. This reduced reproductive output could be explained by younger males with poor quality sperm courting females (Chapters 3-5; Breckels and Neff 2013; *In review*; Breckels et al. 2013); lower successful parturition rates occur when females are courted by lower quality mates (e.g. Sato et al. 2011). Maturing at an earlier age could potentially shorten the generation time of guppies, explaining the similar census population sizes yet reduced reproductive output, as there were more reproductive episodes in a given time. Hence, future research could look into generation time in guppies at elevated temperature as compared to controls. The lower successful parturition could also be explained by reduced female investment in reproduction (e.g. Zeh et al. 2012), although the females role in reproduction has not been as extensively studied to date. Hence, future work should detail the effect of anthropogenic stressors on the female investment in reproduction. Nevertheless, despite sperm length displaying an evolutionary response, the lack of an evolved response in other male and female reproductive traits provides support to the suggestion that reproduction will likely be highly vulnerable in a warming environment (Zeh et al. 2012).

Reduced effective population sizes, genetic diversity, and reproductive output, as seen in the increase temperature populations of guppies, are often signs that a population is inbred (reviewed in Charlesworth and Charlesworth 1987). Perhaps inbreeding could explain the lack of an evolved response in some sperm traits as inbreeding reduces sperm quality (Gage et al. 2006). Inbreeding could also explain the reduced reproductive output as mating with kin reduces sperm competitiveness (Michalczyk et al. 2010; Gasparini and Pilastro 2011), resulting in reduced brood sizes and reduced fertilization success (e.g. Pitcher et al. 2008; Zajitschek et al. 2009). Inbreeding depression could have negative effects on fitness, such as depressing the immune system (e.g. Herber et al. 2013), and consequently population viability.

Evolutionary changes in behavioural traits precede most other traits as they tend to be more labile (Wcislo 1989; West-Eberhard 2003). Consequently, adaptive changes in behavioural traits should become established more rapidly than other traits. Indeed, I found an evolutionary response in aspects of behaviour of brown bullheads in response to multi-generational exposure to pollution (Chapter 2; Breckels and Neff 2010). Specifically, their locomotion and aggression behaviours likely evolved in response to pollution. This seemingly rapid evolutionary response occurred within approximately 100 years which represents at most 33 generations in brown bullheads. However, temporal constraints meant that I could not distinguish between an evolutionary response and maternal environmental effects or epigenetics. Nevertheless, the polluted fish would have an advantage over control fish in a polluted environment even if this response was not genetic, in that they would not have to spend as much energy on a plastic response and could channel this 'extra' energy towards other somatic processes, such as growth and

reproduction. Future research could examine an F₂ generation and determine if my results, in fact, represent an evolutionary response.

6.3 Additional future research directions

6.3.1 Parasite resistance

In Chapter 3 (Breckels and Neff 2013), I showed that exposure to a range of temperatures did not affect the immune response to phytohaemagglutinin (PHA) in guppies. As well, I found that the PHA response after 6 months of exposure to 28°C did not differ from the control (25°C; results not presented). While PHA injections provide a simple test of immune response, they do not account for the potential increased transmission, growth rate, and virulence of parasites that increases in temperature are predicted to cause (reviewed in Marcogliese 2008). Increases in parasites can affect individual survival, leading to population or species declines (Marcogliese 2008). As well, increased levels of inbreeding, as suggested in Chapter 5 (Breckels et al. 2013), have been shown to depress the immune response (e.g. Reid et al. 2003). To that end, future research could explore the effect of anthropogenic stressors, especially increased temperature, on parasite resistance. For example, the effect of temperature on guppy susceptibility to gyrodactylus infections could be studied (see Fraser and Neff 2010). Also, the ability of parasites to respond to anthropogenic stressors should be detailed in order to get a comprehensive view of the effects of these stressors on both parasite and host.

6.3.2 Measuring thermal tolerance

Guppies have the ability to acclimate their thermal tolerance rapidly to increased temperature (e.g. Chung 2001). Chung (2001) showed that guppies acclimated to higher temperatures for only a few days had higher critical thermal maxima (the temperature at which organized locomotion ceases; CT_{\max}) and death points than guppies acclimated to lower temperatures. However, it is not known how quickly their thermal optimum (T_{opt}) and CT_{\max} evolve. Using insects as a model system, Deutsch et al. (2008) estimated that the ‘warming tolerance’, the difference between CT_{\max} and the mean habitat temperature (T_{hab}), and the thermal safety margin, the difference between T_{opt} and T_{hab} , of temperate species is three and five times that of tropical species, respectively. These estimations suggest that tropical species are far more vulnerable to the slightest temperature rise than temperate species. Currently, the T_{hab} of tropical species is increasing (IPCC 2007), and they are residing closer to their thermal limits (Deutsch et al. 2008; Angilletta 2009; Dillon et al. 2010). As a result, tropical species have less scope for plasticity (e.g. Stillman 2003) and thus genetic adaptation, whereby they evolve their reaction norms (the phenotypic expression of a given trait over a range of environments) may be crucial for survival.

In Chapter 3 (Breckels and Neff 2013), I documented the phenotypic plasticity of various traits in guppies over a range of temperatures. Then, in Chapters 4 and 5 (Breckels and Neff *In review*; Breckels et al. 2013) using the same species, I determined whether some of these traits had evolved after long term exposure to higher temperature. However, it would be informative to document the plasticity of these traits in fish from higher temperatures and the controls over a wider range of temperatures (not just 25°C

and 28°C) as evolution of their reaction norm for these traits may have gone unnoticed. Indeed, from this design you could determine the T_{opt} and CT_{max} of various traits from the two different experimental temperatures to determine the potential evolved response of the reaction norms in guppies. In Chapter 4 (Breckels and Neff *In review*), there is some evidence that the reaction norm of sperm length has evolved as fish from the 28°C experimental populations had similar lengths to the controls and fish from the 28°C experimental populations but reared at 25°C had longer sperm than the controls. However, this would have to be studied over a broader range of temperatures, both higher and lower than the temperatures tested, to fully examine the evolution of reaction norms and determine both the T_{opt} and the CT_{max} . As well, the genetic variance of thermal tolerance has not been studied (Walters et al. 2012), so measuring genetic variance is the focus of the next section.

6.3.3 Quantitative genetics

Many studies have shown additive genetic variance to be high for sperm traits (e.g. Simmons and Moore 2009), while other studies show little additive genetic variance for other traits, such as life history traits (reviewed in Crnokrak and Roff 1995; Roff and Emerson 2006). It would be interesting to know how quickly the additive genetic variance and overall genetic variance of sperm traits, as well as other traits (e.g. thermal tolerance, life history, and ornamentation), decreases as individuals with favourable traits are selected for. This could be measured through simple breeding designs involving stressed and control fish. A virgin female would be mated to a single male and the resulting brood of full-sibling offspring would be split between the two treatments (50% stressed and 50% control). This would enable us to determine how much of each trait

measured is attributable to additive genetic, non-additive genetic, and maternal environmental effects, and determine how the stressed environment compares to the control environment. Even if the stressed population initially shows signs of an evolved response, if the genetic variance decreases too much due to selection, this genetic impoverishment will lead to reduced population viability (i.e. little standing genetic variation for selection to act upon; reviewed in Pauls et al. 2013).

6.3.4 The effects of other anthropogenic stressors

Throughout my thesis I concentrated on two anthropogenic stressors, pollution and the temperature increase associated with climate change. While these two stressors are perhaps the most significant anthropogenic stressors impacting biodiversity today, particularly aquatic species, there exist other important threats, including habitat degradation and fragmentation, and the introduction of invasive species, which also have the potential to pose significant selection pressures on species. As well, the temperature increase associated with climate change will cause additional environmental changes (reviewed in Ficke et al. 2007; IPCC 2007), which all have the potential to add extra selection pressures to organisms. For example, increases in evaporation, and altered precipitation and hydrological regimes due to climate change, may put added selection pressure on morphological traits involved in such things as locomotion and foraging in aquatic species as flow rates are altered (e.g. Mauget 2003; Colborne et al. 2011 and references therein). As well, different flow rates may also alter fish behaviour, such as foraging and reproduction as they may be exposed to different foraging opportunities and different mating tactics.

Perhaps the most important indirect effect of global warming for fish is that increases in temperature will decrease the levels of dissolved oxygen in the water while simultaneously increasing the biological oxygen demand for ectotherms (Kalff 2000). This decreased oxygen will be most problematic for organisms that reside in large water bodies where there is less surface area for oxygen to dissolve (e.g. Pörtner and Knust 2007). Although, decreased oxygen may also become a problem for many river and stream fish. In the dry season it is not uncommon for sections of streams to dry out (Alkins-Koo 2000), and this will likely become more frequent as altered hydrologic regimes could result in decreased flow (Mauget 2003; Ficke et al. 2007). This will result in pools of stagnant water where more fish will have to reside due to smaller stream areas with less dissolved oxygen due to a lower or no flow rate. As well, the temperature in the pools will likely rise, which, in turn, will result in lower dissolved oxygen levels. This will create an “oxygen squeeze” whereby the demand for oxygen will exceed the supply (Ficke et al. 2007), potentially resulting in decreased individual growth or even decreased survival (e.g. Pörtner and Knust 2007). Thus, it is essential that we better understand the evolutionary responses of fish to decreased oxygen levels as this is likely to be an emerging stressor for aquatic organisms in the future.

6.3.5 Relating performance to fitness: reproductive success

Most research examining the effects of anthropogenic stressors on species detail the effect of said stressors on some aspect of performance (e.g. Kime et al. 1996; Dhillon and Fox 2004), as I have done throughout my four data chapters. However, unless a trait is directly linked to fitness, such as survival, we can only infer the possible effects that the trait’s performance may have on fitness in the stressed environment. For example,

does a decreased age at maturity really matter at higher temperatures if all ectotherms decrease in size in warmer environments? Does decreased sperm velocity due to increasing temperatures affect reproductive success? Perhaps the 'new' stressed environment has new optimal trait values. Thus, more studies are needed which document both particular trait values and the effects of these trait values on fitness in the new environment.

The evolved response of sperm traits to increased temperature (Chapter 4; Breckels and Neff *In review*) clearly follows the hotter-is-better hypothesis (Huey et al. 1999), but I found no evidence of evolution in sperm velocity or path linearity. As well, reproductive output decreased over time. These results open up a plethora of research questions which would add to our knowledge about the role of sperm performance in reproduction. Does sperm length really matter? Does sperm velocity matter at increased temperatures? What role, if any, does the female play in post-copulatory selection (cryptic female choice)? Measures of sperm quality and performance are good indicators of stress in a population, however, they do not convey rates of actual reproductive success. Determining the reproductive success of male guppies from the increased temperature populations, or any stressed population, could be achieved by artificial insemination of a virgin female by two rival males of known sperm phenotype (see Evans and Rutstein 2008) and determining the paternity of the offspring. Alternatively, sperm could be stained with fluorescent labels in order to view them progressing through the female reproductive tract (e.g. Fisher and Hoekstra 2010). Next, determining the proportion of offspring sired by each male would be conducted by parentage analysis. Thus, it can be determined what the most successful sperm phenotype is by comparing

reproductive successes of each treatment. Indeed, there was a large amount of variation in sperm velocity in fish from increased temperature and this could explain the decrease in reproductive output if velocity is still important in increased temperatures.

Future work could also detail sperm competitiveness of each of my four temperature treatments (25-25, 25-28, 28-28, and 28-25), or by using the same framework with species from any stressful environment, using the same methods as above. Using guppies from my experimental set-up would determine whether sperm length is a key fertilising characteristic or whether fish from higher temperature are being hindered in terms of reproductive success by lower velocity sperm.

6.4 Concluding remarks

My thesis research provides considerable evidence that fish can evolve rapidly in response to anthropogenic-induced environmental change. Indeed, I found evolutionary responses to pollution and increased temperature in behaviour and sperm length, respectively. However, even though population demographics and some life history traits appeared to be unaffected, evolutionary responses to such stressors may be offset by a lower reproductive output and a loss of genetic diversity. These latter results could potentially result in fewer individuals with reduced genetic variation which may limit the future adaptive potential of populations to respond to other anthropogenic stressors. Thus, even though I have found evidence of rapid evolutionary responses, the rate and magnitude of anthropogenic induced environmental change may ultimately decide the fate of organisms. Future research focused on examining the effects of multiple stressors

in combination on fish and other organisms will allow us to better understand the consequences of these stressors on the long-term persistence of species.

6.5 References

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Appendix A: Chapters 4 and 5 supplementary material

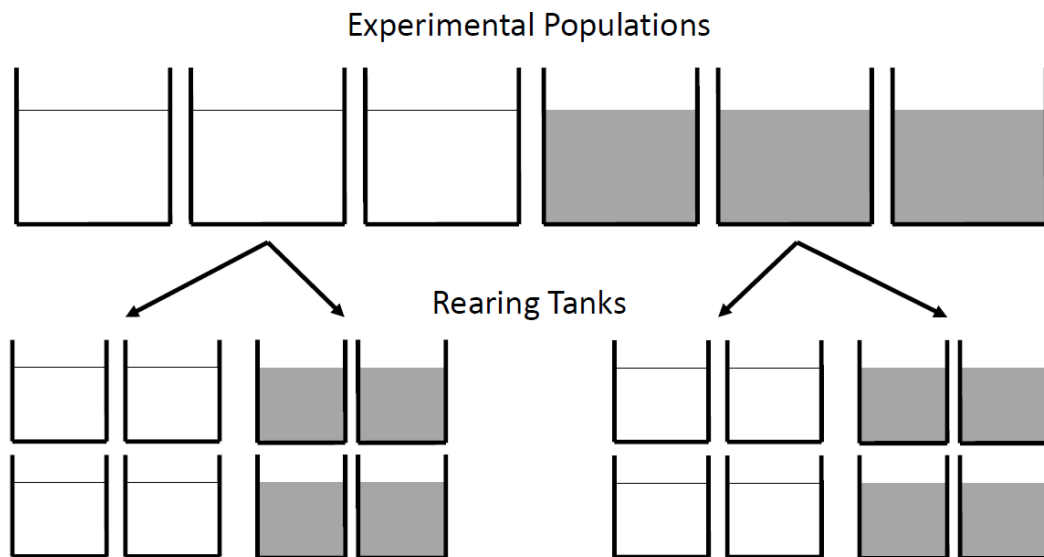


Figure A.1 The 2×2 common garden experimental design used to assess multiple sperm and life history traits in guppies (*Poecilia reticulata*).

Shown are six experimental populations (top); three of which remained at 25°C (white tanks) throughout the experiment (controls) and three were raised at a rate of 1°C every 45 days up to 28°C (grey tanks). After 6, 18, and 24 months, eight pregnant females were removed from each experimental population and put into separate rearing tanks (bottom) with the temperature set to the same as the experimental population that the female had originated. After the female had given birth, four treatments were established by switching the water temperature in four of the eight rearing tanks to that of the alternate experimental populations. Multiple sperm and life history traits were estimated from the offspring in these tanks.

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Appendix C: Animal care protocol approval documentation



June 1, 2009
*This is the 3rd ReNEWal of this protocol
*A Full Protocol submission will be required in 2010

Dear I

Your Animal Use Protocol form entitled:

Behavioural and Molecular Ecology of Fishes

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from **June 1, 2009 to May 31, 2010**

The protocol number for this project remains as **2006-062**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your Institutional safety officers.

c.c. Approved Protocol
Approval Letter

The University of Western Ontario
Animal Use Subcommittee / University Council on Animal Care



May 26, 2010

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2014

Dear

Your Animal Use Protocol form entitled:
 Behavioural and Molecular Ecology of Fishes
 Funding Agency NSERC Discovery Grant - R3244A04; NSERC Grant - R3244A16

has been approved by the University Council on Animal Care. This approval is valid from **May 26, 2010 to May 31, 2011**. The protocol number for this project is **#2010-214 which replaces #2006-062-05 which has expired.**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.

If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.

4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 4 Years

Species	4 Year Total Numbers Estimated as Required	List All Strain(s)	Age / Weight
Other	800	Sunfish - Bluegill (<i>Lepomis macrochirus</i>); Pumpkinseed (<i>L. gibbosus</i>)	0-11 years
Other	800	Guppies (<i>Poecilia reticulata</i>)	0-2 years
Other	800	Salmonids - Chinook (<i>Oncorhynchus tshawytscha</i>); Atlantic salmon (<i>Salmo salar</i>); Brown trout (<i>S. trutta</i>); Rainbow trout (<i>O. mykiss</i>)	0-6 years

REQUIREMENTS/COMMENTS

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c. Approval

The University of Western Ontario
 Animal Use Subcommittee / University Council on Animal Care

Curriculum Vitae

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Post-secondary Education and Degrees: University of East Anglia
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2013

Dr. Irene Uchida Fellowship in Life Science
2013

Graduate Student Teaching Award nominee
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Environment and Sustainability Program Graduate Student Travel
Award
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Environment and Sustainability Collaborative Research Seminar
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Related Work Experience Teaching Assistant
The University of Western Ontario
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Research Assistant
Nautica Environmental Associates LLC
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Publications:

In Review

Breckels, R.D. and Neff, B.D. *In review*. Rapid evolution in a sperm trait in response to increased temperature in a tropical ectotherm. *Evol. Ecol.*

Published

Breckels, R.D., Garner, S.R. and Neff, B.D. 2013. Rapid evolution in response to increased temperature maintains population viability despite genetic erosion in a tropical ectotherm. *Evol. Ecol.* DOI: 10.1007/s10682-013-9668-5

Breckels, R.D. and Neff, B.D. 2013. The effects of elevated temperature on the sexual traits, immunology, and survivorship of a Tropical ectotherm. *J. Exp. Biol.* **216**, 2658-2664.

Muñoz, J.N., Breckels, R.D. and Neff, B.D. 2012. The metabolic, locomotor, and sex-dependent effects of elevated temperature on Trinidadian guppies: limited capacity for acclimation. *J. Exp. Biol.* **215**, 3436-3441.

Breckels, R.D. and Neff, B.D. 2010. Pollution-induced behavioural effects in the brown bullhead (*Ameiurus nebulosus*). *Ecotoxicology.* **19**, 1337-1346.

Presentations

Breckels, R.D. and Neff, B.D. Evolutionary responses of sperm traits to increased temperature. Earth Day Colloquium. 2013 – oral presentation.

Breckels, R.D. and Neff, B.D. *Pollution-induced behavioural effects on the brown bullhead (Ameiurus nebulosus)*, Biology Graduate Research Forum. 2012 – poster presentation.

Breckels, R.D. and Neff, B.D. *Temperature-induced physiological and adaptive responses in the guppy*. Evolution. 2012 – oral presentation.

Breckels, R.D. and Neff, B.D. *Temperature-induced physiological and adaptive responses in the guppy*. Canadian Society of Zoology. 2012 – oral presentation.

Breckels, R.D. and Neff, B.D., *Pollution-induced behavioural effects on the brown bullhead (Ameiurus nebulosus)*. Biology Graduate Research Forum. 2010 – poster presentation.

Breckels, R.D. and Neff, B.D. *Pollution-induced behavioural evolution in the brown bullhead*. Earth Day Colloquium. 2010 – oral presentation.

Breckels, R.D. and Neff, B.D. *The effects of pollution on the brown bullhead (Ameiurus nebulosus)*. Environment Western Earth Day Colloquium. 2009 – oral presentation.

Breckels, R.D. and Neff, B.D. *Brown bullheads and contamination stress: partitioning behavioural differences to the acute, acclimation, and genetic responses*. Environment Western Earth Day Colloquium. 2008 – oral presentation.