The determination of behavioural plasticity in yellow perch, Perca flavescens

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The determination of behavioural plasticity in yellow perch, *Perca flavescens*

By

Meagan Patricia McCloskey

A Thesis
Submitted to the Faculty of Graduate Studies through the Great Lakes Institute for Environmental Research in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

2016

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The determination of behavioural plasticity in yellow perch, *Perca flavescens*

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January 22, 2016
DECLARATION OF CO-AUTHORSHIP

I hereby declare that this thesis incorporates material that is result of joint research, as follows: all chapters were co-authored with my advisor, Dr. Christina Semeniuk. In all cases, my co-author provided advice on experimental design and statistical analyses, in addition to feedback during the writing of this thesis. The Fisk lab conducted stable isotope sample analysis and provided input for Chapter 3 and Appendix A. Appendix A was co-authored with my advisor, Dr. Semeniuk, and Dave Yurkowski, who provided input during the writing of the manuscript. However, the primary contributions for this thesis have been by the author. Appendix A has been prepared as a manuscript and has been submitted to Journal of Fish Biology for publication.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from the co-authors to include the above materials in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work completed during my registration as a graduate student at the University of Windsor.

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ABSTRACT

Behaviour is often an individual’s first line of response to a stimulus, allowing them to adapt to changes and stressors in their environment. An individual’s behaviour is a product of environmental conditions, including local adaptation, rearing experience, and internal processes (e.g. metabolic rate). I examined the effects of early rearing experience, population differences (i.e. local adaptation/selection) and ontogeny on the behavioural repertoire of young yellow perch (*Perca flavescens*). They were tested in behavioural assays at three time points to quantify activity, exploration, neophilia and antipredator responses over ontogeny. Fitness correlates were used to explain behavioural differences, and survival was quantified to examine the fitness consequences of various behavioural types. Yellow perch show behavioural flexibility for activity and consistency for antipredator responses; their overall behavioural phenotype was characterized by coping styles, with some individuals showing relatively fixed phenotypes and others showing increases in activity, exploration and neophilia over time. An individual’s level of neophilia, degree of behavioural flexibility and their morphology were predictive of mortality.
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Chapter 1: General Introduction

Species around the globe are increasingly affected by rapid environmental change and stressors, both from naturally-occurring and anthropogenic sources, including habitat fragmentation, overexploitation, climate change-induced warming and the spread of non-native species. This environmental change can, in effect, create new “environments”, with different sets of conditions and challenges that species will have to cope with. In addition, individuals of a species could be displaced to a new habitat, which may also present a different set of conditions that the species may not be accustomed to. These new “environments” can increase risk of population extinction and even species extirpation if reproduction and survival are diminished under the new conditions.

The ability to persist in new or rapidly-changing environments depends on a species’ capacity, at the individual-level, to produce an appropriate phenotype which enables the organism to survive and reproduce in the new environment (Ghalambor et al. 2007). Aspects of individual phenotype that can respond to changes in the environment include physiology, morphology, development, phenology, and behaviour. A population with **phenotypic plasticity** can produce a range of phenotypes across different environments or conditions; this plasticity can be adaptive, maladaptive or neutral for an individual (Ghalambor et al. 2007). **Adaptive** phenotypic plasticity produces a phenotype that is optimal for the new set of conditions, maintaining or increasing fitness in the new environment (West-Eberhard, 2003, book). However, if these new environments produce conditions that are outside of the range of conditions that the species has experienced during its evolutionary history, then the species may not have the phenotypic plasticity necessary to survive in the new environment (Ghalambor et al. 2007). Given that a behavioural change is often the first line of phenotypic response when an individual’s environment is altered, both individual- and population-level behaviour should be considered when studying conservation-related issues and designing conservation programs for species threatened by rapid environmental change.

The role of animal behaviour in the conservation of a species has long been considered (Sutherland, 1998). Only more recently has there been an interest in meaningful individual variation in behaviour, specifically animal personality, and
variation in behaviour between different populations of a species (Bell, 2005; Dingemanse et al. 2012; Reale et al. 2010); this variation was previously thought of as random “noise” around an optimal behavioural mean. Personality, often synonymous with temperament or behavioural type, is defined as consistent individual differences in a single behaviour or a suite of behaviours that persist across situations and over time (Reale et al. 2010). Consistent individual variation in behaviour within populations has been documented for many taxa, including birds (Dingemanse et al. 2002), insects (Sih & Watters, 2005), reptiles (McEvoy et al. 2015) and fish (Bell & Stamps, 2004; Budaev et al. 1999). Animal personality is generally studied in terms of 5 ecologically-relevant axes: 1) boldness-shyness, reaction to a risky (but not novel) situation, such as antipredator responses, 2) exploration-avoidance, reaction to new situation or object (e.g. response to a novel food item), 3) activity, an individual’s general activity level in a safe and/or familiar environment, 4) aggression, how agonistic an individual is towards others; and 5) sociability, how an individual interacts with others in a non-aggressive context (Reale et al. 2007).

Populations of species that have the potential to respond to stressors with optimal or nearly optimal behaviours (i.e. behaviourally plastic) will possess some resilience to environmental change (Sih et al. 2012). Behavioural plasticity can be present in a population as: (1) “individual flexibility”, where individuals can alter their level of a behaviour from one situation to the next or (2) “behavioural type diversity”, where there is between-individual variation in behavioural types present in the population. If multiple behaviours are correlated across contexts at the population-level, then the population may have a behavioural syndrome (Sih et al, 2004), a phenomenon that could be detrimental or beneficial, depending on the environmental change.

*Individual behavioural flexibility* – Individuals of a species exhibit behavioural flexibility if they can change their level of a behaviour from one situation or context to the next. Behavioural flexibility can produce adaptive responses to a changing environment, in some cases increasing fitness of individuals. For example, a behaviourally flexible individual might be highly active when foraging in a predator-free environment, but would show reduced activity under predation risk. Flexible behaviour is seen in many species, including cod (*Gadus morhua*) reared in a hatchery environment,
where individuals exposed to variable spatial and foraging cues more rapidly explored novel environments, exploited novel prey and recovered more quickly after a stressor than cod reared in a homogeneous environment (Braithwaite and Salvanes, 2005). Vehanen (2003) showed that juvenile Atlantic salmon (*Salmo salar*) exhibit behavioural flexibility by assessing predation cues and food availability in their environment, with hungrier fish spending less time in a refuge in the presence of a predator than satiated fish. Individual flexible behaviour can be adaptive and is likely a product of living in a variable environment (Braithwaite and Salvanes, 2005), however this flexibility can be costly and limited within and between species. There is a trade-off between the need to produce the appropriate behaviour for a given environment and the costs of maintaining flexible behaviour. Costs of flexibility can include: maintenance of the necessary sensory/regulatory mechanisms; production of the structures needed for flexible behaviour; costs (i.e. risk and time) of acquiring information from the environment, and genetic costs due to gene linkage and pleiotropy (DeWitt et al. 1998). Behavioural flexibility can be limited by existing morphological and sensory capabilities, as well as learning abilities and how different behaviours interact at the cognitive level (Hazlett, 1995). Individuals, however, will be flexible in their behaviour if the cost of behavioural consistency outweighs the cost of flexibility. For example, Brown et al. (2013) found that guppies (*Poecilia reticulata*) from a high-predation environment exhibited a greater change in anti-predator behaviour than guppies from low-predation environments when exposed to a novel predator cue, as the cost of not modulating responses would be greater (i.e. predation). A population of behaviourally flexible individuals may be better able to survive under environmental stressors if individuals can appropriately modulate their behaviour to be optimal for the “new” environment.

*Behavioural type diversity* – Individuals with a behavioural type, or personality, show *repeatability* or *consistency* in behaviour over time and/or across situation and contexts, maintaining rank order with other individuals (Bell et al. 2009; Reale et al. 2007; Sih et al. 2004a, b). A behavioural type does not necessarily imply that an individual cannot change its behaviour but simply that the individual changes its behaviour in a consistent manner (e.g., a shy individual will remain shy in comparison to a bold individual, but to differing degrees). Behaviours in a single context, such as exploratory behaviour in novel
environments, have been found to be repeatable across individuals in great tits (*Parus major*; Dingemanse et al. 2002) and in the bluegill sunfish (*Lepomis macrochirus*; Wilson & Godin, 2009). Repeatability of behaviour across functional contexts (i.e. territory maintenance, feeding, aggression) has also been found, in species such as cichlids (*Neolamprologus pulcher*; Witsenburg et al. 2010). Certain behaviours are more repeatable than others, a difference which could be due to the ecological importance of the behaviour (i.e. how it affects reproduction and survival), how sensitive the behaviour is to changes in the environment, or how costly it may be to maintain flexibility of the behaviour (Bell et al. 2009). For example, in a species of lizard (*Egernia whitii*), McEvoy et al. (2015) found that aggression, boldness and exploration behaviours were repeatable over time (i.e. 1-12 days), but activity and sociability were not. Exploration and aggression, in addition to mating and habitat selection, were found to be highly repeatable classes of behaviour in a meta-analysis of behavioural repeatability; activity was found to be one of the least repeatable classes of behaviour (Bell et al. 2009). Consistency of individual behaviour could arise from living in a homogeneous environment that has changed little over evolutionary time, where the costs of behavioural flexibility outweigh the cost of being consistent. This behavioural consistency could have survival consequences for a population if individuals cannot produce the appropriate behaviour in the face of an environmental stressor. However, if a population has a diversity of behavioural responses (i.e. multiple different behavioural types) to a novel stressor, then it should be able to better cope if at least some of the behavioural types can respond appropriately to the changing environment (Sih et al. 2004b, 2012)

**Behavioural Syndromes** – In some cases, individuals may display a behavioural syndrome, where multiple behaviours are correlated from one situation/context to the next. For example, individuals who are more highly bold and aggressive than others in one situation will be relatively more bold and aggressive in a different situation/context. Boldness behavioural syndromes have been found for fishing spiders (*Dolomedes triton*), where the normally boldest individuals are also the boldest in foraging and mating contexts (Johnson and Sih, 2007), as well in activity and risk-taking contexts in bluegill sunfish (*Lepomis macrochirus*; Wilson and Godin, 2009). The occurrence of behavioural
syndromes can be adaptive, increasing fitness, or maladaptive, decreasing fitness, depending on the situation, context or life-history stage and can have negative consequences for reproduction and survival of a species if behaviours inappropriately carry over between contexts (Sih et al. 2003). Salamander larvae (*Ambystoma barbouri*) found in streams with fish predators show an inappropriate carryover of behaviour that leads to higher predation rates. These larvae leave refuges to forage in the absence of fish predators; however, longer exposure times carry over in the presence of fish predators, both during the night, when exposure would be less costly, and during the day, when visual predators more successfully prey upon the larvae (Sih et al. 2003).

**Knowledge Gaps in Behavioural Ecology**

Current behavioural ecology research presents some knowledge gaps that are critical to effectively conserving animal populations in the face of rapid environmental change. Although many studies quantify individual differences in behaviour, fewer attempt to understand the proximate and ultimate causes of individual variation in behaviour. These knowledge gaps, which I will address in this thesis, include: 1) how rearing environment and genetics affect behaviour, 2) how behaviour changes over ontogeny and 3) the relationship between individual behaviour and fitness-linked traits, including survival.

1) **G (genetic, or individual) and E (environment) contributions to behavioural phenotype** – Genetic causes and environmental experience need to be considered as potential drivers of behavioural variation between individuals and populations of a species. Individuals or populations can find themselves in new environments either directly through displacement to another area or if their present environment changes. Local adaptation of these populations can occur over many generations as natural selection acts against certain genotypes, favouring other genotypes that are better suited and have higher fitness in the new environment (Williams, 1966). New environments can present novel selective pressures that can drive local adaptation of populations, including predation regime, competition, habitat heterogeneity and anthropogenic disturbances. One of the ways in which populations can adapt to new environments or novel stressors is through selection
on behavioural responses that are under genetic control. Populations of the same species can show vastly different behavioural responses to similar contexts due to selection that has acted upon behavioural phenotypes. Predation regime is one of the most commonly studied aspects of the behavioural ecology of a species. Populations originating from high-predation environments often have different behavioural responses to predation threat than those originating from low-predation environments, even when early rearing environment is controlled for. In a common garden experiment, Brown et al. (2007) found that lab-reared first generation tropical fish (*Brachyraphis episcopi*) with parents from a high-predation environment behaved more boldly than lab-reared first generation individuals whose parents originated from low-predation environments, indicating strong support for a genetic component of boldness and adaptation to local conditions. Locally-adapted populations of a species can also show differences in levels of behavioural flexibility to a novel context or situation. Great tits (*Parus major*) from four populations across Europe showed differences in their level of behavioural flexibility when tested for exploration of a novel environment over multiple tests spanning about half a year (Dingemanse et al. 2012). Local behavioural adaptation needs to be studied in the context of rapid environmental change and responses to novel stressors as behaviour can be tightly linked to life history and fitness in some species (Reale et al. 2010b).

The conditions under which an individual or population is reared can additionally affect behaviour at all stages of life. Experiences that can play a role in shaping behaviour include predation risk (Bell and Stamps, 2004), parental care (McGhee & Bell, 2014), food resource use (Heynen et al. 2011), rearing population density and other environmental abiotic variables, such as temperature (Biro et al. 2010) and hypoxia (Frost et al. 2013). For example, Eurasian perch (*Perca fluviatilis*) originating from a high cannibalism-risk lake were less bold than perch from a low cannibalism-risk lake, when tested for foraging behaviour in a risky situation (i.e. in view of a cannibalistic perch; Magnhagen et al. 2012). Bell (2005) found that wild threespined sticklebacks (*Gasterosteus aculeatus*) from a high predation environment were more aggressive and shyer under risk than those from a low predation environment. These high predation regime fish also showed a behavioural syndrome for boldness and aggression, which low predation regime fish did not. Bell & Sih (2007) found that stickleback from the same
low predation environment studied in Bell (2005) developed a boldness-aggression behavioural syndrome following predation by trout, both through predator selection and behavioural flexibility. These studies demonstrate how behaviour is highly dependent on rearing environment due to the trade-offs animal face in differing environments. It is also possible that individual behaviour is not shaped solely by an individual’s genotype or by environment, but is instead determined by how the environment affects a certain genotype or individual (i.e. genotype by environment (G × E) or individual by environment (I × E) interaction).

Studying how environmental conditions, such as predation regime, can affect behaviour could assist in species conservation efforts, for example determining how a native species will cope if a non-native predatory species invades the environment. For example, following an experimental introduction of non-native goldfish (*Carassius auratus*), foraging activity of newts (*Lissotriton helveticus*) decreased, especially in the presence of more aggressive goldfish (Winandy & Denoël, 2015). A native species’ ability to cope with species introductions can have consequences for its fitness, such as reduced foraging (Winandy & Denoël, 2015), disruption of dominance hierarchies (Blanchet et al. 2007), or loss of preferred habitat through agonistic interactions (Chucholl et al. 2008). In addition, knowledge of individual behaviour could provide valuable insight into designing reintroduction programs to supplement declining wild populations. Specifically, this research could help to determine how to rear animals in captivity in order to promote survival in the wild upon reintroduction, as captive environments are typically more homogeneous and have little or no predation compared to wild environments. Braithwaite & Salvanes (2005) were able to “train” cod (*Gadus morhua*) to show behaviours that would encourage survival in the wild by rearing them in structurally complex tanks and by varying their feeding schedule since typical captive environments can decrease anti-predator responses of animals. Anti-predator responses, such as latency to enter a refuge following simulated predator attack and vigilance, decreased as time spent in captivity increased in a study of oldfield mice (*Peromyscus polionotus subgrisues*; McPhee, 2003). Captive reintroduction programs would benefit greatly from knowledge of how behaviour is affected by both experience and genetics, by
allowing conservation managers to breed individuals for certain types of behaviour or train them to increase survival in the wild.

2) Ontogeny of Behaviour — Ontogeny can influence an individual’s behaviour through reversible and irreversible factors that occur throughout different life stages, such as migration, reproduction, parental care, growth and morphological changes (Senner et al. 2015). These different life stages can present unique challenges to individuals in the form of fitness trade-offs (e.g. growth-mortality) where individuals must balance the needs of growth, reproduction and survival in order to maximize their fitness (reviewed in Mittlebach et al. 2014). Juvenile animals are usually presented with a different set of challenges than adult animals, for example, increasing growth vs. allocating energy to reproduction (Groothius & Trillmich, 2011). Biro & Stamps (2008) argue that individual differences in behaviour may be maintained by differences in life-history strategies of individuals (e.g. growth rates, early vs. late fecundity). However, few studies in the behavioural literature examine an individual’s behaviour over a period of their life (e.g. Francis, 1990; Shurch & Heg, 2010). Some of these studies have shown individual consistency of boldness (Niemela et al. 2012), aggression (Schurch & Heg, 2010), and exploratory behaviours (Schurch & Heg, 2010; Wilson & Krause, 2012) throughout a period of an individual animal’s life, whereas other studies have shown that behavioural types change over ontogeny (Bell & Stamps, 2004; Edenbrow & Croft, 2011). Individual boldness, measured as latency to become active and latency to emerge from a shelter, was repeatable across ontogeny from juvenile stage until after maturity in field crickets (Gryllus integer; Niemela et al. 2012). Individual cichlid fish (Neolamprologus pulcher) were highly repeatable in their exploratory behaviour over an ontogenetic switch point (i.e. sexual maturation; Schurch & Heg, 2010). Another study on that cichlid species demonstrated that individual repeatability of boldness, aggression and exploration was high early in life, but also showed that repeatability of those behaviours declined significantly over the total lifespan of individuals (Chervet et al. 2011). Knowledge of the developmental trajectory of individual behaviour could point to life stages that are critical to behavioural development and therefore, to species conservation. This knowledge would allow conservation managers to 1) more effectively preserve habitat critical to certain life stages of a species, 2) remove or reduce anthropogenic disturbances during
those life stages, and 3) design more effective reintroduction programs that account for behavioural changes over time.

3) **Fitness-Behaviour Links** – Individual differences in behaviour can have consequences for reproduction and survival of an individual. However, proximate causes (i.e. structural/physiological mechanisms) are rarely considered in understanding behavioural variation between individuals. Few studies in the behavioural ecology literature have examined how these individual differences in behaviour ultimately relate to fitness-linked traits, such as metabolic rate (Huntingford et al. 2010), morphology (i.e. size and condition can indicate an individual’s energetic reserves)(Brown et al. 2007), diet, mate selection and parental care (Stein & Bell, 2014). An individual’s diet can be a proxy for its foraging abilities and its energetic intake, both of which can have fitness consequences. These fitness-linked traits can be mediated by behaviour of an individual, or vice versa, with this interaction being caused by the trade-offs between growth, reproduction, and survival, which all individuals face during their lifetime. For example, studies on the ubiquitous trade-off between growth (i.e. foraging) and predator avoidance have shown contrasting results. Smaller cichlid fish (*Brachyraphis episcopi*) emerged from a refuge sooner (Brown & Braithwaite, 2004) and had a greater tendency to approach a novel object (Brown et al. 2007) than larger fish, independent of the predation risk in their environment, which is consistent with the metabolic hypothesis; smaller fish are more motivated to forage than larger fish, even under predation risk, due to their relatively higher energetic needs (i.e. higher metabolic rate per mass unit) than larger-bodied fish (Huntingford et al. 2010). Along the same lines, individuals with higher intrinsic growth rates are willing to accept more risk in foraging activities, regardless of predation risk, and this can have negative effects on fitness in environments with high predator abundance (Biro et al. 2004). Individuals more likely to exploit resources than others will also demonstrate differential diet selection, growth and survival (Sih et al. 2004b). For example, bolder individuals may be more likely to exploit a novel food resource than shy individuals. These bolder individuals may be better able to survive in changing environments, where preferred resources may no longer be available and survival may depend on willingness to feed on novel resources. In the case of non-native
species invasions, birds that were more successful invaders had greater behavioural flexibility in terms of exhibiting foraging innovations (Sol et al. 2002).

In this thesis, I have investigated the presence of behavioural plasticity by looking at the structure and repeatability of individual behaviours over time, which included looking at the contributions of genetic background and rearing environment to behaviour in an important Great Lakes fish species, the yellow perch (*Perca flavescens*) (Chapter 2). In this chapter, I tested for flexibility and/or consistency of ecologically-relevant behaviours (i.e. activity, boldness, exploration) of individual juvenile (young of the year; YOY) yellow perch using an open field test, a novel object test, and a test of the trade-off of foraging under predation risk to determine if perch display consistent behavioural types or if they exhibit flexible behaviour within and between contexts/situations throughout ontogeny (YOY to cusp of adulthood). To investigate the contributions of local adaptation and rearing environment, I examined behavioural differences between three treatment groups of perch: one derived from multiple generations in semi-natural ponds with both inter- and intra-specific competitors/predators (captive, high complexity environment: ‘CH’), one treatment of wild-collected fertilized eggs reared in a semi-natural pond with only intra-specific competitors/cannibals of same cohort (wild-captive, low complexity environment: ‘W-CL’) and one wild-caught treatment (‘wild, high complexity environment: ‘WH’) treatment (see Chapter 2, Table 2.1). In Chapter 3, I have attempted to sum up behaviours of yellow perch into an overall individual behavioural phenotype and examine whether this behavioural phenotype (or changes in the phenotype over time) relates to fitness-linked traits, specifically growth rates, diet and morphology. In this chapter, I also investigate reasons for differential mortality, in terms of behaviours and fitness-linked traits. Yellow perch was chosen as the study species due to its importance for the Great Lakes, in terms of both fisheries and ecosystem functioning. In Chapter 4, I discussed the findings of this thesis, offering insights and recommendations for the conservation of yellow perch in the face of multiple environmental stressors, and suggesting next steps for research with yellow perch.

The overall goal of this thesis is to contribute behavioural knowledge to the conservation and management of yellow perch. To date, there are no studies that have
investigated the behavioural repertoire of *P. flavescens*, representing a knowledge gap that is critical to effective species management. The importance of this species to Great Lakes fisheries (Baldwin et al. 2009; Smith, 1968) and ecosystem dynamics (Sanderson et al. 1999), combined with the lack of knowledge of its behavioural ecology, make yellow perch an ideal study species. Yellow perch face a suite of multiple stressors in the Great Lakes, including invasive species, water pollution, habitat degradation, exploitation, and climate change. This species specifically is threatened by climate change (i.e. shorter, warmer winters reduce larvae/egg quality and juvenile abundance; Farmer et al. 2015), diseases (Viral hemorrhagic septicemia virus die-offs; Kane-Sutton et al. 2010) and chemical water pollution (e.g. heavy metals; Mirza et al. 2009). Ultimately, the baseline behavioural data collected in this study lays the groundwork for future studies that will take a multiple stressors approach to predict how yellow perch will fare under changing environmental conditions.
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Chapter 2: Interaction of early rearing experience, genetic divergence, and ontogeny on the behavioural repertoire of yellow perch, *Perca flavescens*

**Introduction**

Integrating behaviour with ecological knowledge and species management is critical for current and future environments undergoing rapid change, as behaviour is often the first line of response to environmental change. Freshwater aquatic environments are extremely susceptible to rapid change and the species that live in them are exposed to multiple stressors including pollution, habitat destruction, non-native species introductions, climate change and other anthropogenic disturbances, such as commercial fishing (Stendera et al. 2012). Studies on the behavioural repertoire of a species or its populations are a critical first step in determining how a species may respond to changes and/or stressors in its environment.

Numerous studies have found that individuals consistently differ in certain behaviours, such as boldness (Magnhagen et al. 2014), aggression (Huntingford et al. 2010), exploration (Dingemanse et al. 2002) and sociability (Magnhagen & Bunnefeld, 2009). These consistent individual differences are often termed personality, behavioural type, or coping styles and they can confer fitness advantages under different environmental contexts. The underlying proximate mechanisms and ultimate causes of these individual differences is less clear, representing a critical knowledge gap in behavioural ecology (Bell, 2007). Disentangling these underlying factors is a necessary step towards anticipating the ecology of a species and how populations will fare in fluctuating environments.

In addition to individual variation in behaviour, populations of a species can show variation in their behavioural phenotype, also known as behavioural plasticity. Behavioural plasticity can be manifested in the form of individual behavioural flexibility, behavioural types (personality or coping styles) and/or behavioural syndromes, where multiple behaviours are correlated with each other (Sih et al. 2004a). Behavioural plasticity (or behavioural variation) can be viewed as a form of biodiversity that should
be studied and maintained in order for populations to survive under rapid environmental change (Smith & Blumstein, 2013). Even if individuals in a population show limited behavioural flexibility, the population itself can still be “plastic” and cope with environmental change if behavioural types exist, with some responding appropriately (Sih et al. 2004a).

A behaviourally flexible individual can modulate its behaviour across an environmental gradient. For example, a flexible individual showing high activity in a foraging context would show an appropriate decrease in activity under the risk of predation. Individuals and populations may differ in their degree of behavioural flexibility in response to many types of environmental change. For example, individual guppies (*Poecilia reticulate*) from high-predation risk sites showed an anti-predator response to a novel predator cue, whereas individuals from low-predation risk sites did not show a response to the novel cue above and beyond their response to a known risk cue (Brown et al. 2013). Coral reef fish (*Pomacentrus moluccensis*) showed variation in their levels of behavioural flexibility with increasing temperature: some individuals increased activity, while others remained constant in their activity levels (Biro et al. 2010). Marine crabs (*Ozius truncatus*) showed differences in their latency to feed with changes in temperature, with some individuals showing little change in latency, while others decreased their latency to feed with increasing temperatures (Biro et al. 2013). However, Biro et al. (2013) also showed consistent individual differences within temperature (i.e. personality) and demonstrated that response to a change in temperature was related to an individual’s personality within temperature. Behavioural flexibility may be favoured in environments where fluctuations and stochasticity, for example, in terms of predation risk levels or temperature changes, are common and environmental cues are reliable (Reed et al. 2010). However, individuals do not always show optimal behaviour in any given environment. Behavioural flexibility can be more costly (e.g. energetic/maintenance costs, risk of information acquisition) and limited (e.g. by reliability of environmental cues or lag time in producing flexible phenotype) in some cases than consistency of behaviour (Dewitt et al. 1998; Hazlett, 1995).
The presence of a behavioural type or personality indicates that individuals show consistency in their behaviour, maintaining their rank order across different behavioural contexts (Sih et al. 2004a, b). The presence of a behavioural type does not imply that individuals have purely fixed behaviour, but that if individuals change their level of a behaviour, they maintain their rank order with one another. For instance, the boldness of individual two-spotted gobies (Gobiusculus flavescens) when tested in shelter emergence and exploration tests increased and decreased, respectively, over repeated runs for each assay; however, individuals maintained their rank-order with others between runs (Magnhagen et al. 2014), indicating that individuals show behavioural types or personality for boldness. The level of behavioural consistency (or flexibility) can sometimes be related to an individual’s underlying personality, termed a coping style. For example, in response to predation risk in the field cricket (Gryllus integer), bold personality individuals became shyer and shy personality individuals became bolder; the shyer, reactive coping style individuals were able to assess the level of predation risk and adjust their behaviour according to the environment (Niemela et al. 2012). Similarly, in rainbow trout (Oncorhynchus mykiss), bold individuals became shyer and shy individuals became bolder in their latency to approach a novel object, and this change was greater in individuals exposed to higher predation risk and hypoxic conditions (Frost et al. 2013); the bold, proactive individuals may lack the necessary sensory input to assess predation risk, and thus rely on routines, whereas reactive individuals can assess the risk and environmental conditions more accurately (Koolhaas et al. 1999). Coping styles within a population can represent different ways of dealing with local environmental conditions.

Behavioural variation within a species may also be a product of differential experience within a generation (i.e. “E, environmental effects”), local adaptation through natural selection on heritable behaviours over generations (i.e. “G, genetic effects”), or a combination of both (i.e. “G × E, genotype by environment”; Dingemanse et al. 2010). Individuals can show differences in behaviour and in their response to changes in their environment (i.e. flexibility) that may not be caused by underlying genetic variation (i.e. “I × E, individual by environment”; Brommer & Class, 2015). Specifically, an individual’s behaviour can be shaped by multiple environmental factors that can include predation experience, competition, population density, habitat structure and
anthropogenic disturbances. Early experience of the environment can foster behaviours that maximize individual fitness under later conditions. For example, early life experience of unpredictable food availability increased bold and exploratory behaviour of individual guppies (*Poecilia reticulata*) in terms of refuge use and during a novel maze task (Chapman et al. 2010). Individual cod (*Gadus morhua*) showed greater behavioural flexibility, in terms of prey choice and stress recovery, when they were reared in an environment with more variability in terms of spatial cues (i.e. cobble and kelp) and foraging cues (i.e. fluctuating feeding schedule; Braithwaite & Salvanes, 2005).

Hellstrom & Magnhagen (2011) found that wild Eurasian perch (*Perca fluviatilis*) from low-predation risk lakes were bolder than wild perch from high-predation risk lakes, but this behavioural difference was nonexistent when individuals from both lakes were reared in a common environment, suggesting that early experience (not genetic predisposition) determines bold behaviour in this fish species.

Populations of a species often live in different environments that can vary spatially, temporally, and in the communities of species that occur. Populations, or individuals, can also find themselves in novel environments through direct displacement, non-native introductions, or through anthropogenic alteration or destruction of native habitat. Survival in these different environments is partially dependent upon the ability of a population to show appropriate behavioural adaptations to local conditions, maximizing fitness in the short term, and passing on heritable behavioural traits to offspring. Three-spined sticklebacks (*Gasterosteus aculeatus*) originating from two rivers differing in predation pressure showed contrasting behavioural repertoires when reared in the laboratory: the population from high predation risk environment showed a behavioural correlation (i.e. syndrome) for boldness and aggression, whereas the population from low predation risk environment did not (Bell & Stamps, 2004). In another common garden experiment, populations of nine-spined stickleback (*Pungitius pungitius*) from small, isolated, predator-free pond populations were more aggressive, bold, and were more driven to feed than marine, predator-sympatric populations when reared in the laboratory (Herczeg et al. 2009). These studies suggest that adaptation to local environments over many generations may drive differences in behaviour between populations of a species.
The study of behaviour over ontogeny is an often neglected area of behavioural ecology as personality is often considered to be a relatively rank-order stable aspect of an individual’s phenotype (Groothius & Trillmich, 2011). Some studies have shown rank-order behavioural consistency with age (e.g. Niemela et al. 2012; Wilson & Krause, 2012). However, given the changes in selective pressures most species experience over the course of their lives, it would not be unexpected to see phenotypic, and behavioural, changes accompanying that. For instance, cichlids (Steatocranus casuarius) were inconsistent in activity, inspection behaviour and aggression early in life, but became highly consistent after 1 year of age (Budaev et al. 1999). In another study, Adriaennsens & Johnsson (2013) showed that a behavioural syndrome between activity, aggression and neophilia emerged during the early critical months of life following hatching in brown trout (Salmo trutta). The early emergence of a behavioural syndrome between exploration and boldness also occurred in mangrove killifish (Kryptolebias marmoratus), likely linked to the beginning of sexual maturity (Edenbrow & Croft, 2011). Ontogenetic changes in behaviour need to be considered due to the fact that different developmental stages and life history events can bring changing selective pressures; organisms may show different behaviours between early life and adulthood in order to maintain fitness and survive at these life stages.

Yellow perch (Perca flavescens) is an ideal study species in which to investigate behavioural variation, as it is a widespread North American freshwater fish species that inhabits a range of habitats from small ponds to large lakes; it coexists with a variety of predators and competitors, and experiences habitat and environmental changes throughout its development (Pyle & Couture, 2015). This species is also of tremendous importance to the Great Lakes, in terms of both commercial and recreational fisheries (Baldwin et al. 2009), and ecosystem dynamics (Sanderson et al. 1999). Previous behavioural studies have been done on the closely-related European perch (Perca fluviatilis), but to date, no studies have examined the behaviour of P. flavescens. The majority of the studies done on P. fluviatilis investigated behaviour of individuals in a social context, such as in small shoals, and tend to examine trade-offs between foraging and predator avoidance (Magnhagen, 2012). Magnhagen & Bunnefeld (2009) and Kekalainen et al. (2014) demonstrated that behaviour of an individual when alone was
related with an individual’s behaviour when in a shoal. Individual behaviour in a group is also affected, to some extent, by the group’s composition of behavioural types (Magnhagen & Staffan, 2005) and to overall group behaviour (Magnhagen, 2007). Foraging of Eurasian perch is strongly influenced by the presence of a predator, with individuals choosing their foraging patches more so based on predation risk rather than food availability (Utne et al. 1997). Additionally, Eurasian perch from lakes with higher cannibalistic predation were shyer than perch from lakes with low cannibalistic predation, spending less time in the open and attacking fewer prey in the presence of a predator (Magnhagen, 2006). Behaviour of the perch from these lakes that differ in predation regime is apparently linked to year-specific cannibalistic predation levels, with individuals showing an overall bolder phenotype in years of lower predation pressure (Magnhagen et al. 2012). There is strong evidence that risk-taking behaviour in the presence of a predator is under environmental, rather than genetic, control in this species. In a common garden experiment, wild perch originating from low predation lakes were bolder than wild perch from high predation lakes, but this difference did not persist when individuals from both lakes were reared in a common environment (Hellstrom & Magnhagen, 2011). The major finding of these studies is that individual differences in behaviour are consistent, supporting the presence of behavioural types and even behavioural syndromes in Perca species. My study will be the first to look at the presence of behavioural flexibility and/or behavioural types in Perca flavescens, as well as the effects of genetic background, early rearing experience, and ontogeny on behaviour.

In this chapter, we investigate the structure of the behavioural phenotype of yellow perch, and attempt to disentangle the effects of early rearing environment, population differences (i.e. through local adaptation/selection) and ontogeny on its behavioural phenotype. To accomplish this, individual yellow perch from “treatment” groups differing in early rearing experience (i.e. variation in habitat complexity) and time since removed from the wild environment (i.e. genetic divergence) were subjected to a range of behavioural assays at multiple points during ontogeny (i.e. young of the year (YOY) to cusp of adulthood) to test activity, boldness, exploration and antipredator responses. Using this study system, we investigated the following objectives: whether 1) yellow perch show behavioural consistency and/or flexibility across ontogeny, 2a)
behaviours are correlated with one another within a time period (i.e. behavioural syndrome), 2b) behavioural syndromes/types are present and stable though ontogeny, and 3) early rearing experience and/or genetic background (i.e. time removed from wild, local adaptation) affects behavioural phenotype. We hypothesize that 1) early rearing experience (in terms of habitat complexity and competition/predation) would influence behaviour more than genetic background, and 2) individuals reared in more complex, stochastic environments would show greater behavioural flexibility or a more bold, exploratory behavioural type.

Methods

Study Species

Yellow perch (*Perca flavescens*) is a member of the Percidae family, consisting of about 200 species, characterized by the separation of the two dorsal fins (Brown et al. 2009). *P. flavescens* is very closely related to the European perch (*Perca fluviatilis*), due to highly similar morphology and ability to interbreed (Brown et al. 2009; Thorpe, 1977), suggesting the possibility that they show other similar phenotypic attributes. Yellow perch typically grow up to approximately 25 cm and spawn once a year in the spring when temperatures rise above 7°C (DFO, 2011). Yellow perch are native to northern North America, east of the Rockies; however, the species has spread to western regions of Canada and the United States, mainly because of authorized and unauthorized introductions (Brown et al. 2009). Spawning occurs in shallow areas of lakes, and after hatching, larvae move into the near-shore pelagic zone for 30-40 days (Brown et al. 2009). Juveniles and adults are typically found in shallower waters, amongst vegetation or near man-made structures (Brown et al. 2009). Juvenile yellow perch feed on small zooplankton, and as they increase in age and size, tend to switch to larger prey items such as macroinvertebrates and small fishes (Fullhart et al. 2002; Graeb et al. 2006; Scott and Crossman, 1973; Whiteside et al. 1985). Cannibalism of younger age classes by larger individuals has been documented for *P. flavescens* (Sanderson et al. 1999) and *P. fluviatilis* (Persson et al. 2000; Urbatzka et al. 2008), and this phenomenon has the potential to strongly affect year-class recruitment in natural lakes, with younger age
classes serving as important prey for older yellow perch and other fish species (Sanderson et al. 1999). Yellow perch are an important species for both commercial fisheries and recreational angling in the Great Lakes (Baldwin et al. 2009; Smith, 1968).

**Sampling**

Individual YOY yellow perch were sampled from three treatment groups (WH, W-CL, CH) that differed in early rearing environment and population differences (through local adaptation or time since removed from wild; Fig. 2.1), and were then measured for behavioural responses to standard behavioural assays. Because this study focuses on the behavioural variation of YOY individuals, we selected individuals who were ≤ 110 mm in total length. This size threshold was selected as a conservative estimate based on YOY size distributions used in other studies of *Perca* sp. (Borcherding et al. 2007; Urbatzka et al. 2008).

1) **Wild, Highly Complex Environment (WH)**

A total of 102 YOY yellow perch were sampled by seine net directly from Balsam Lake (44.58044, -78.84165) in early fall of 2013. Balsam Lake, located in the Kawartha Lakes region of central Ontario, has an area of 4665 ha and a mean depth of 5.0 m (OMNR, 2008). This lake is characterized by a changing fish community structure; relative abundance of walleye (*Sander vitreus*), a predator of yellow perch, has decreased since the early 1980s, with the piscivore community structure shifting towards higher relative abundances of other yellow perch predators, namely smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*Micropterus salmoides*; Robillard and Fox, 2006). In addition, lake-wide surveys from 1998-2005 showed a higher proportion of larger size categories of yellow perch (140 mm+) than the smaller stock fish (< 140 mm) compared to many other lakes in the Kawartha region (OMNR, 2008), indicating the potential for cannibalism of YOY yellow perch by larger, older yellow perch.

2) **Wild-Captive, Low Complexity Environment (W-CL)**

A total of 73 YOY yellow perch were sampled from 8-30 October 2013 from a small nursery pond (~0.1 ha, ~8 feet deep) at Leadley Environmental Inc., Essex, Ontario.
using minnow traps baited with either food or light. Seining was not possible due to the depth and substrate consistency of the ponds. Minnow traps were baited with a combination of food or light to attempt to minimize sampling bias towards certain behavioural types. Light has been shown to attract YOY yellow perch and other fish species < 50mm total length into traps, due to their photosensitivity at younger age classes (Manci et al. 1983). The yellow perch from this treatment group were the first-generation removed from their wild environment (in Balsam Lake, ON) and reared from birth in the captive, nursery environment. They emerged in the nursery pond from egg strands that were fertilized in Balsam Lake, ON during the spring of 2013. This nursery pond was newly dredged and filled and contained no fish other than the first-generation yellow perch, therefore this rearing environment was considered to be less complex because it contained a relatively lower abundance of predators and competitors and represented a stable, semi-natural pond environment. However, the potential for cannibalism within the treatment cannot be ruled out given the phenomenon of bimodal size cohorts for YOY in *Perca* sp. (Urbatzka et al. 2008; Braband, 1995), with YOY yellow perch shifting towards a diet of fish prey as early as 80 mm total length (Graeb et al. 2006). We also cannot rule out the possibility of avian predation, as cormorants and herons were spotted near the pond (MM, personal observation). They were fed floating trout pellets in addition to what live food they could find in the pond.

3) Transgenerational Captive, Highly Complex Environment (CH)

A total of 75 YOY yellow perch were sampled over a 1-month period during the fall of 2013 from two holding ponds (~0.25 ha each, ~13-15 feet deep) at Leadley Environmental Inc. using minnow traps baited with either food or light. The yellow perch collected from this pond were a trans-generational sample derived from multiple generations (a minimum of 7) removed from wild environments (including Balsam Lake, ON as the predominant, main source). Yellow perch from this treatment were significantly genetically divergent from WH and W-CL (determined using the fixation index which was based on 6 non-coding microsatellite loci, results not shown). Other fish species were present in these holding ponds, in addition to the potential for cannibalism from other perch, such as smallmouth bass (*Micropterus dolomieu*) and bluegill (*Lepomis*
These ponds had been established for many years (~20 years), are much deeper than the nursery pond that held W-CL, and had a higher density of predators/competitors than the W-CL ponds, therefore this environment was considered highly complex. Again, we cannot rule out the possibility of avian predation, as cormorants and herons were spotted near the pond. Perch in these ponds were fed floating trout food, in addition to what live food they could find in the pond.

All YOY perch collected from Balsam Lake, ON (WH) or Leadley Environmental Inc. (W-CL, CH) were immediately transported in aerated haulers to the Great Lakes Institute for Environmental Research (GLIER) Aquatic Facility at the University of Windsor in Windsor, Ontario, Canada.

**Fish Husbandry**

The collected YOY perch ranged in size from 57-106 mm, 78.5 ± 11.1 mm for CH, 49-96 mm, 75.2 ± 11.7 mm for W-CL, and 51-79 mm, 62.4 ± 6.8 mm for WH, with treatment size distributions overlapping. They were kept at the GLIER aquatic facility in recirculating tanks under constant conditions (low intensity red light conditions to reduce stress and facilitate acclimation to captive conditions (Calbert and Huh, 1976; Malison and Held, 1992); 12L: 12D photoperiod; temperature: 17-19°C; pH: 7-8; dissolved oxygen: 6-9 mg/L) from the time of capture until the end of the study. The perch were fed live blackworms (*Lumbriculus variegatus*) ad lib every 48 hours from the time of capture until December 2013 (to facilitate transition from live feed), when they were transitioned to a diet of frozen bloodworms and freeze-dried krill ad lib every 48 hours after that. After at least two weeks of acclimation to tank conditions following capture and transport, the 250 yellow perch were individually marked with passive integrated transponder (PIT) tags (Biomark FDX-B Mini HPT8: 8.4mm) and photographed for length/weight measurements. Caudal fins were clipped and stored in vials with 95% ethanol for future genetic- and diet-related analyses.
Behavioural Experimental Design

A series of behavioural trials were conducted on 25 individual YP from each treatment group (WH, W-CL and CH) at multiple time points during their first year to assess individual- and treatment-level consistency and/or flexibility of activity, boldness, neophobia and anti-predator behaviour. Each trial consisted of three different assays performed in the same order: an open field test, a novel object test and a stimulus response test to examine the trade-off of foraging under predation risk. Assays were performed in this particular order, with the open field test first, in order to minimize disturbance from the novel object or the alarm stimulus, as this disturbance might have biased activity and exploration of the open field. Three trials were performed on the same individuals, barring early mortality, at intervals spaced 2 months apart: Trial 1 from 23-27 January 2014, Trial 2 from 20-23 March 2014 and Trial 3 from 26-30 May 2014 (Ch. 3, Fig. 3.1). Individuals were randomized over all trial days for every set of trials. Trials were conducted over consecutive days, taking no more than 5 days total to complete each set of trials, always commencing in the morning.

All trials were performed under dim red light conditions (similar to conditions they were held in) in opaque flow-through 33-litre experimental tanks (45cm × 27cm; 27cm water height) in which temperature (17 ± 2°C; but usually did not fluctuate more than 1°C during a trial), pH (7.2-8.4) and dissolved oxygen (5-8 mg/l) were maintained during the trial. Individual perch were not fed within 48 hours of the behavioural trials.

Open Field Test

Perch were caught from their holding tanks using a dip net and immediately placed individually into an experimental tank where they were allowed to explore the novel empty tank, undisturbed for 30 minutes. Scoring of behavioural variables started 1 minute after the experimenter exited the room to allow the fish to calm following the disturbance. The open field test is designed to quantify an individual’s level of activity, boldness and exploration of a novel environment (Burns, 2008; Walsh and Cummins, 1976). During the analysis phase, each experimental tank was divided into 4 equal quadrants, along with a zone (23cm × 13cm) directly in the middle of the tank (“centre
zone”). The variables measured were latency to first movement, number of times crossing into a tank quadrant, amount of time spent in the centre, number of times in the centre and average duration spent in the centre zone (i.e. total time in centre divided by number of times in centre). Individuals that did not move during the test were assigned a latency to first movement of 1800 seconds (the duration of this assay).

Novel Object Test

Immediately following the open field test, a novel object was placed into the centre of each experimental tank. The novel object used was a structure made of Lego blocks of various colours, all of approximately the same dimensions (7cm×6cm×20cm high). The novel object test is designed to measure an individual’s level of neophobia, or fear of new objects/situations. Scoring of behavioural variables started 1 minute after the experimenter exited the room. The fish were allowed to swim freely and enter the “novel object zone” (box drawn around novel object extending 5cm on each side of the novel object in the analysis phase) for a duration of 30 minutes undisturbed. Previous studies have used similar distance thresholds to score boldness and neophilia (Frost et al. 2007, 2013). The variables measured were latency to enter the novel object zone, number of times entering the zone, total time spent in the zone, average duration spent in the novel object zone (i.e. total time in novel object zone divided by number of times in novel object zone) and time spent inspecting or oriented towards the novel object (i.e. novel object within 90° field of view). Individuals that did not enter the novel object zone were assigned a latency to enter zone of 1800 seconds. After 30 minutes, the novel object was removed from the experimental tanks, and water flow to the tanks was turned off. Individuals were acclimated to the zero flow conditions for 15 minutes prior to the start of the final test.

Stimulus Response Test

The last test of the behavioural trials measured an individual’s foraging activity under the risk of simulated predation. This test consisted of 3 segments: distilled water control, food stimulus, and alarm cue. Following the 15-minute acclimation to no flow conditions, each experimental tank was injected with 5 ml of distilled water to act as a
stimulus introduction control, used to determine if an individual fish’s behavioural change was due to the addition of a liquid to the tank, rather than the type of stimulus introduced. After 8 minutes, 5 ml of the familiar food stimulus (see below for preparation) was injected into each tank to stimulate foraging and swimming activity (as in Mirza et al. 2009). After 8 minutes, 5 ml of yellow perch alarm cue (see below for preparation) was injected into each experimental tank, simulating a predation event. The trial ended 5 minutes later. All stimulus liquids were measured out into a syringe, with separate syringes for each type of stimulus, and then injected into air tubing connected separately to each experimental tank. The stimuli in the air tubing was then delivered into each tank using a Cole-Palmer peristaltic pump hooked up to a manifold, so that each tank received the stimuli at approximately the same rate. Each segment of this test (i.e. control, food stimulus, and alarm cue) was scored for individual activity and tank area use for 5 minutes following the 3-minute stimulus introduction period. Activity was scored by assessing whether the individual was mobile or immobile every 20 s during the 5 minute period (% mobility). Tank area use was scored by measuring the number of times the individual crossed into a different quadrant in the tank (as in the open field test). Number of times the individual moved into the quadrant closest to the stimulus introduction point was also recorded.

**Food Stimulus Preparation**

A familiar food stimulus was prepared by mixing two types of fish food which the yellow perch were fed on a regular basis in their home tanks: freeze-dried krill and frozen bloodworms. 0.5 g of ground freeze-dried krill and 0.5 g of frozen bloodworms were mixed into 100 ml of distilled water, giving a concentration of 0.01 g food/ml water. The food stimulus solution was allowed to sit refrigerated at 4°C overnight for use in the behavioural trial on the following day. Immediately prior to the start of the test of foraging under predation risk, the food stimulus solution was poured through a funnel lined with a coffee filter to remove food particles. The remaining liquid extract, containing odours of familiar food items, was used as the food stimulus to stimulate foraging activity of the yellow perch (Mirza et al. 2009).
**Alarm Stimulus Preparation**

Yellow perch skin extract stimulus was prepared from the skin of juvenile yellow perch sampled from the ponds at Leadley Environmental Inc. This extract, found in the skin of yellow perch, is a damage-released alarm cue that elicits an antipredator response in YOY yellow perch and a foraging response in 1+ yellow perch (Harvey and Brown, 2004; Mirza et al. 2009; Mirza et al. 2003). The alarm cue stimulus was prepared in two separate batches on January 21 and February 11, 2014. Fish were euthanized with a blow to the head for both batches. Skin was collected from a total of 30 juvenile yellow perch that ranged in size from 51.8-108.4 mm. Skin was removed from both sides of each fish and then placed into a beaker filled with chilled distilled water. The skin-water mixture was homogenized and then filtered through a poly-cotton filter floss to remove the pieces of skin. The remaining extract was then diluted with distilled water to a final volume of 640 ml, giving a final concentration of 0.3 cm² skin/ml, similar to the concentration used by Mirza et al. (2009) and Harvey and Brown (2004). The alarm cue was frozen at -20°C in 50 ml falcon tubes for up to two months until use in behavioural trials.

All behavioural trials were video recorded from above the tanks with a monochrome GigE camera with a 4-8 mm F1.4 megapixels lens (Basler, Germany) attached to a laptop using Media Recorder software (Noldus). Following each trial, test individuals were photographed for measurement of length and mass. Housing tanks were monitored regularly for mortalities. All videos were analyzed using JWatcher (Blumstein et al. 2010). All experimental protocol followed the Canadian Council on Animal Care guidelines (AUPP #13-04).

**Statistical Analysis**

**Behavioural Analysis**

We conducted a principal component analyses (PCA) with varimax rotation on all of the behavioural data collected from the open field test, the novel object test and the stimulus response test in an attempt to reduce the dimensionality of the behavioural dataset and produce a behaviour “score” for each individual for each test. Variables
included in the open field test PCA were: 1) latency to first movement, 2) number of quadrants transitions, 3) number of times crossed into centre zone and 4) total time spent in centre zone and 5) average centre zone duration. Variables included in the novel object test PCA were: 1) latency to enter novel object zone, 2) number of times crossed into novel object zone, 3) total time spent in novel object zone, 4) average novel object zone duration 5) number of times oriented towards novel object and 6) total time spent oriented towards the novel object. Components with eigenvalues greater than 1 were retained (according to the Kaiser-Guttman stopping rule; Guttman, 1954). The PCA was conducted for each test, with data from all three trials pooled together in the same analysis. We included these repeated measures for individuals because we were interested in examining relative changes in individual behaviour over time; however, this violates assumptions of independence. We therefore ran a PCA on each trial separately, that resulted in similar components, thus confirming the validity of this analysis (Adriaenssens & Johnsson, 2011; Dingemanse et al. 2007). Percent mobility in the stimulus response tests (% mobility food, % mobility alarm) was the variable retained for further analyses as it was highly correlated with all other variables collected for the test (Spearman’s rank correlation tests, all \( p < 0.0001 \)). We used Wilcoxon rank sums to compare the responses of individuals from the alarm and food stimulus tests to the control test in order to confirm whether or not individuals responded to the food and alarm stimuli, above their response to the control. We calculated an alarm stimulus response as % mobility alarm - % mobility food and a food stimulus response as % mobility food - % mobility control.

**Behavioural Correlation Analysis**

We used Spearman’s rank correlation to test for 1) behavioural correlations within a trial (i.e. behavioural syndrome) by treatment group and 2) individual behavioural consistency over time by treatment group. The data was highly skewed towards zero and/or maximum values (e.g. individuals that did not move in the open field or approach novel object) therefore it was necessary to perform a non-parametric rank order test. Using sequential Bonferroni correction to control for multiple comparisons, results were
significant at p < 0.01 and marginally significant at p < 0.02. All of the above statistical analyses were conducted using JMP Version 12 (SAS Institute Inc., Cary, NC, USA).

Linear Mixed Effects Modelling

We used general linear mixed modelling (GLMM) to investigate differences in behaviour: 1) within and between treatment groups, 2) within and between individuals, and 3) over time. Using this type of modelling, we could include repeated measures for individuals by fitting them as random effects in the model. Significant random slopes for individuals would indicate behavioural flexibility (i.e., differences in behavioural scores between individuals over time), and estimates of flexibility were obtained from the best linear unbiased predictors (BLUPs) when significant random slopes were found for individuals (Nussey et al. 2007). We used R version 3.2.2 (R Core Team, 2015) with the lme4 package (Bates et al. 2015) to conduct the GLMMs.

Model Selection and Model Fit

We used an information theoretic (IT) approach to evaluate model likelihood using the Akaike Information Criterion corrected for small samples sizes (AICc), the relative differences in AICc (ΔAICc) in a model set, and the Akaike weight (\(w_i\)). A ΔAICc was calculated for each model as the relative difference from the lowest AICc model from a set of models. Lower AICc (and lower ΔAICc) indicated a better model relative to the other models tested for each behaviour-score response variable. Models with ΔAICc < 2 were considered likely models, and models with ΔAICc > 10 were considered very unlikely (Burnham & Anderson, 2002). Akaike weight, which ranges in value from 0 to 1, is the probability that a given model is the most appropriate model in a model set. The fit of the best model was then described using marginal (\(r^2_m\)) and conditional (\(r^2_c\)) r squared. Marginal \(r^2\) is the amount of variance explained by the fixed effects only, and the conditional \(r^2\) is the variance explained by both fixed and random effects (Nakagawa & Schielzeth, 2013).

In the GLMM, the behaviour scores (i.e. PCAs for activity, neophilia, exploration) and alarm stimulus response were fitted individually as the response variables. Time (each trial, 3-factor categorical) and treatment group (3-factor
categorical) were kept as fixed effects in all of the models. Time was additionally modelled as a linear covariate with a second-order polynomial as its fit in all the models was better than time modelled as a linear covariate only (results not shown). Individual and treatment were fitted as random intercepts – (1|ID) and (1|Treatment), and slopes – (Time|ID) and (Time|Treatment) to allow for 1) personality within individuals and replication within treatments (i.e. random intercept) and 2) trajectory/flexibility in behaviour estimated for individuals and treatments (i.e. random slope) over time.

We started by fitting a global model (model 1) which contained all fixed and random effect covariates for each behavioural response variable (see Table 2.4 for model structures). We then fitted reduced models by removing each of the random effects separately and examining model AICc and Akaike weights to determine the most likely model in a model set (e.g. Edenbrow & Croft, 2011). In the second model, we refitted the global model without the random slope effect for treatment (Time|Treatment) and compared model results to determine whether treatment groups differed in their trajectories of behaviour over time. In model 3, we removed the effect of individual random slope to investigate whether individuals show behavioural flexibility over time (i.e. vary in their trajectories). In model 4, we fitted the model without any individual (ID) random effects (i.e. slope and intercept) to determine whether the inclusion of individual personality (i.e. random intercept) and individual flexibility (i.e. random slope) improves the model. We further minimized the next three models (5, 6 and 7) by eliminating terms that did not improve model likelihood or including terms that appeared to improve the model based on ΔAICc. We fitted model 5 with a random intercept for treatment, and no random effects for individual. Model 6 was fitted with random effects (i.e. slope and intercept) for individual. In model 7, we fitted the model with only a random intercept for individual. We also constructed linear models to investigate if including only fixed effects improved the model likelihood (shown as Model 8 if likelihood was improved). This model selection procedure was followed for the activity, neophilia and exploration models. For the model predicting alarm stimulus response, we followed similar steps to find the best model but included an additional interaction term (food response × time) in the model.
Results

Survivorship across Trials

From the 70 individuals tested in behavioural trials at Trial 1, 42 individuals survived to be tested at Trial 2, and 32 of those individuals survived until Trial 3 (Table 2.2). There was a marginally significant difference in survivorship between treatment groups up until trial 2 ($\chi^2 (2, N=70) = 5.84, p = 0.054$) and up until trial 3 ($\chi^2 (2, N=70) = 5.85, p = 0.054$; see Fig. 3.7 Ch. 3). The W-CL treatment group had a survival rate of 65%, compared to 42% for WH and 30% for CH at the end of Trial 3.

Open Field Test PCA

The PCA conducted on the open field test revealed two factors that explained 57% and 23% of the variation in the dataset, respectively, with components being consistent across the three trials (Table 2.3). The first component (PC1) explained variation in the number of times entering the centre zone, total time spent in the centre zone and average duration in the centre zone. A high score for PC1 indicated that an individual spent more time in the centre zone, went into the centre with greater frequency, and had an overall longer average duration spent in the centre. PC1 was therefore interpreted as an individual’s exploration tendency in a risky environment (hereafter “Exploration”), as higher scoring individuals were more likely to calmly explore the risky centre area of the novel environment, as opposed to lower scoring individuals who spent more time around the peripheral or quickly swam through the centre. The second component (PC2) was made up of a negative loading for zone transitions and a positive loading for latency to first movement. An individual scoring high for PC2 was characterized by a longer latency to first movement and fewer zone transitions. This component was interpreted as an individual’s activity level (hereafter “Activity”). A high score for PC2 indicated a low activity level, so we reversed the sign of the PC2 scores for ease of interpretation, with higher scores meaning higher activity.
**Novel Object Test PCA**

A PCA conducted on the novel object (NO) test dataset revealed a single component (PC1) that explained the majority (~62%) of the variation in the data across the three trials (Table 2.3). A second component (PC2) had an eigenvalue of 1, but it explained much less variation (~17%) than PC1 and interpretation was unclear, so we chose to leave it out of further analyses. A high score for the principal component (PC1) was characterized by a shorter latency to enter NO zone, a greater number of times entering NO zone, greater total time spent in the NO zone and longer average duration in the NO zone. We interpreted PC1 as an individual’s degree of neophilia towards a novel object (hereafter “Neophilia”).

**Stimulus Response Test**

When compared to the control, individuals did not significantly change their mobility when exposed to the food stimulus ($Z=1.60$, $p=0.11$), but they did respond with increased mobility to the alarm stimulus ($Z=2.55$, $p=0.011$) at trial 1. At trial 2, individuals responded to the food stimulus with increased mobility ($Z=3.09$, $p=0.002$), but did not respond to the alarm stimulus ($Z=1.03$, $p=0.303$). At trial 3, individuals again responded to the food stimulus with increased mobility ($Z=2.04$, $p=0.041$), but not to the alarm stimulus ($Z=0.298$, $p=0.766$).

**Behavioural Correlations within Trials**

Treatment group W-CL showed a marginally significant correlation between Activity and Neophilia at trial 1 (Spearman’s rank: $r_s(4)=0.52$, $p=0.0116$; Table 2.5). We observed a significant negative relationship between Exploration and Neophilia score at trial 2 for the CH treatment group (Spearman’s rank: $r_s(4)=-0.88$, $p=0.0008$; Table 2.6). In general, we observed few weak, non-significant correlations between behaviours within a trial (Tables 2.5-2.7). There appeared to be a trend for a behavioural correlation between Activity and Neophilia (for CH and W-CL), however, most of these trends were not significant after Bonferroni adjustments for multiple comparisons.
The global models were not the best models to explain treatment- and individual-level changes in any of the behaviour scores over time (Table 2.4). Stepwise removal of random effects from the global model for Exploration revealed that the best model did not include any random effects ($w_i = 0.98; r^2 = 0.14$). Exploration generally decreased slightly from trial 1 to trial 2, levelling off from trial 2 to trial 3 (Fig. 2.1). The CH treatment group had the lowest Exploration score, and W-CL had the highest score at each time point. For Activity, the most likely model included random effects (i.e. slope and intercept) for individuals ($w_i = 0.74; r^2_m = 0.29, r^2_c = 0.72$). Individual random intercept accounted for the majority of the variance (>78%) of the random effects in the most likely model for Activity, with individual random slope and within-individual effects (i.e. residual variance) accounting for 8.8% and 12.8% of the variance, respectively. For Activity, individual intercept and slope were correlated: individuals scoring either high or low for Activity at trial 1 showed a higher degree of behavioural flexibility over time, either in a negative or positive direction, respectively, than individuals with an intermediate level of activity (Fig. 2.2). For all treatments, Activity increased from trial 1 until trial 2 and then levelled off after trial 2, until trial 3 (Fig. 2.3). The CH treatment group was the most active and the WH treatment group was the least active at all time points. For Neophilia, the most likely model included only fixed effect covariates with no random effects ($w_i = 0.99; r^2 = 0.18$). Neophilia increased up until trial 2 and then levelled off to trial 3 (Fig. 2.4). CH was the most neophilic group, with W-CL and WH showing very similar levels of neophilia throughout the trials. Finally, the most likely model for alarm stimulus response score contained the fixed effects for time and treatment, an interaction between food response and time, and an individual random intercept effect ($w_i = 0.37; r^2_m = 0.36, r^2_c = 0.40$). Individual random intercept (i.e. between-individual differences) accounted for 7.1% of the variance of the random effects and within-individual effects accounted for 92.9% of the variance, indicating that individuals differ in their average response to an alarm stimulus. The presence of an interaction between food stimulus response and time showed that the relationship between an individual’s alarm response and food response changed over the course of the
trials. This was illustrated by the increase in the slope of the relationship between food response and alarm response at each trial (Fig. 2.5), however the negative relationship between alarm stimulus response and food stimulus response stayed consistent throughout the trials: Individuals who were more responsive to the alarm stimulus were less responsive to the food stimulus, and vice versa. CH had the highest responses (i.e. most mobile under alarm stimulus), and WH had the lowest responses.

Discussion

Behaviour of juvenile yellow perch appears to be mediated by both early rearing experience and local adaptation to environment of origin over multiple generations. Yellow perch appear to show some consistent individual differences in activity level and in antipredator response; our results also suggest that yellow perch show flexibility for activity over time (Time|ID). We did not find evidence for the presence of behavioural flexibility (Time|ID/Treatment) for neophilia, exploration or for antipredator response (to an alarm stimulus). Exploration trajectories showed a higher degree of exploration during the first exposure to the novel environment, followed by a decline and attenuation over time, indicating that yellow perch may have experienced relaxation of selection in the tank environment over time. Treatment effects were minimal; however, CH and WH showed more similar levels of exploration over time, with W-CL being the most exploratory, indicating a possible effect of rearing environment and early experience on exploration tendency. A higher exploration tendency in W-CL could be linked to the lower intensity predation experienced by that treatment. In the closely related Eurasian perch, bold, exploratory and risk-taking behaviours appear to be affected by recent predation experience more so than local adaptation. Populations from lower predation risk environments showed a higher degree of boldness and risk-taking than populations from higher predation risk environments (Magnhagen, 2006), however, this difference did not persist when reared in a common environment free from predation (Hellstrom & Magnhagen, 2011). In that same study system, levels of exploration and boldness were also modulated by recent predation levels (within ~ 1 year), with Eurasian perch from high predation risk environments becoming bolder under years of relatively lower predation threat (Magnhagen et al. 2012). The behavioural differences we found may
arise to some extent from directional selection against bold phenotypes by predators in CH and WH; however, the findings of Hellstrom & Magnhagen (2011) suggest that individuals can adjust their behaviour in response to recent environmental conditions. Our findings correspond with the patterns observed in these studies conducted on a close relative of the yellow perch: yellow perch recently removed from an environment with relatively lower predation risk were more exploratory in a risky, open environment than perch from higher risk environments.

Activity was measured in terms of movement in an open, novel environment, resulting in variation occurring at the individual- and treatment-level. Individuals appeared to show not only consistent differences (i.e. intercept or personality) in activity levels at each time point, but they also varied in the trajectories of their activity levels over time (i.e. behavioural flexibility; I × E). The degree of flexibility appeared to be related to the behavioural type of the individual, such that high activity types decreased their activity levels over time, low activity types increased their activity levels, and intermediate activity types changed their activity level very little over time. These divergent “coping styles”, showing behavioural flexibility that is dependent on behavioural type, could represent alternative strategies to dealing with the environment. Proactive individuals are characterized by low flexibility and rely more on routines when reacting to changes in their environment. Reactive individuals rely on cues from their environment and adjust their behaviour accordingly when reacting to change, suggesting that they possess greater behavioural flexibility (Coppens et al. 2010). Frost et al. (2013) observed coping styles in rainbow trout, with bold type individuals becoming shyer and shy type individuals becoming bolder after experiencing an environmental stressor (i.e. increased temperature, hypoxia, predation risk). Similar results were found for field crickets, where bold type individuals took longer to resume activity following exposure to predation and shy type individuals took less time to resume activity (Niemela et al. 2012). The yellow perch from this study system appear to show an individual by environment (I × E, or in this case, I × A (age)) and divergent coping strategies, with individuals at the extremes of activity phenotype showing a higher degree of behavioural flexibility than individuals with an intermediate activity level, regardless of rearing environment.
Activity levels differed between treatments, with CH individuals being the most active and WH individuals being the least active throughout the trials. Activity levels of an individual can be linked to internal states (e.g. metabolism; Careau et al. 2008) or external stimuli, such as predation threat, competition and even resource levels in the environment (Olsson et al. 2007). The relatively higher density of competitors with which CH was reared may have contributed to the higher activity levels of those individuals. Given the difficulty of competing for limited resources in a small area, individuals may show high foraging rates (and therefore high activity) in order to grow and survive gape-limited predation. Populations of a species may differ in activity and foraging rates, depending on site-specific environmental factors. Spotted salamander (Ambystoma maculatum) populations differed in their foraging rates along an environmental gradient: foraging rates of individuals at some sites were explained by gape-limited predation risk, while at other sites, foraging was explained by competition for limited resources due to high intraspecific density (Urban & Richardson, 2015). Our yellow perch treatment groups differed in these types of environmental factors which may have led to the observed differences in activity. However, we cannot rule out potential effects of an individual’s internal state (e.g. metabolic rate) on its activity levels (Careau et al. 2008), which is beyond the scope of this study.

Neophilia differed between the three treatment groups, with the CH treatment showing the most neophilic behaviour and W-CL and WH showing very similar levels of neophilia, however the trajectory neophilia over time was similar for all treatments. The treatment group CH was genetically divergent from the other two treatments, W-CL and WH, which were very genetically similar to one another. CH was descended from multiple generations in a captive pond environment, a heterogeneous habitat characterized by high complexity due to high density of hetero/conspecifics and the potential for natural food sources and habitat structure to be present (given the length of time the pond had been established). Neophilic individuals in these high-density ponds may be suited to that type of environment if they have to compete with many other individuals for food. Individuals more likely to exploit novel resources (i.e. more neophilic) would be better able to survive in these high density, high competition environments. In addition, the different levels of neophilia exhibited by the treatments
may have been influenced by an interaction of competition, predation pressure and relative stability of the environments from which they originated. Previous studies have shown the effect of adaptation to local conditions on fish boldness; individual sticklebacks from high predation pond environments were bolder than individuals from high predation river and low predation pond environments when tested for latency to begin a foraging trial and shelter emergence time (Brydges et al. 2008), although this study cannot rule out effects of early rearing environment. In a true example of locally-adapted populations, Herczeg & Valimaki (2011) showed that individual nine-spined sticklebacks (*Pungitius pungitius*) originating from pond environments were quicker to feed, were more risk-taking (i.e. quicker to initiate feeding after a predation threat) and more aggressive than individuals originating from marine environments, even when reared in a common garden environment. Our results suggest that yellow perch show neophilic behaviour that is dependent on local environment conditions over many generations, but further studies will be needed (e.g. common garden experiment) to elucidate the exact causes.

When tested in a common trade-off of foraging under the risk of predation, individuals consistently differed from one another in their level of response to an alarm stimulus. Individuals did not vary in the trajectories of their responses over time (no I × E/A), instead their responses changed over time at similar rates. In other words, individual perch apparently showed differing behavioural types in response to predation threat, but did not show much flexibility in their responses over time. Consistency of anti-predator behaviour across contexts is a common phenomenon in behavioural ecology (Briffa et al. 2008; Brodie & Russell, 1999). For example, hermit crabs show consistency of startle response to a perceived predation threat both in the presence and absence of a chemical predator cue (Briffa et al. 2008). Antipredator behaviours of individual garter snakes were consistent across different thermal environments (Brodie & Russell, 1999). Ontogeny can be considered a context across which behaviour can be measured because individuals go through stages of development, under which selection pressures may change as they age (Dingemanse et al. 2010). In this study system, yellow perch appear to show consistency in antipredator behaviour over ontogeny.
In terms of treatment-level differences in antipredator response, individuals from CH showed the highest responses overall to the alarm stimulus (i.e. higher mobility under predation threat), with WH showing the lowest responses (i.e. lower mobility under predation threat). Both CH and WH were reared in environments with conspecifics and heterospecifics and were likely exposed to a higher level of predation threat than W-CL. However, CH was reared in captivity in a holding pond with a much smaller volume, as opposed to the wild rearing environment (i.e. Balsam Lake) from which WH was sampled. Individuals from CH were potentially exposed to a higher density of predators and competitors (although not directly measured), where the trade-off between growth and predator avoidance was potentially more intense and costly. Under high competition for resources, individuals would need to forage at a higher rate, even under the risk of predation, in order to avoid starvation. CH was the most mobile under predation risk, potentially due to effects of early rearing environment, where competition was intense, and the need to forage was greater. Another possibility is that CH increased mobility when exposed to the alarm stimulus as a foraging response. The alarm stimulus we used has been shown to elicit anti-predator responses in YOY yellow perch (Mirza et al. 2003), with those responses shifting to a foraging response as yellow perch undergo an ontogenetic diet shift from zooplankton and macroinvertebrates to piscivory and cannibalism (Fullhart et al. 2002; Harvey & Brown, 2004). Yellow perch generally switch to piscivory around 100-170 mm total length (Mittelbach & Persson, 1998), starting as early as 80 mm (Graeb et al. 2006). Based on the length measurements, some of the perch used in this study were likely on the cusp of switching to piscivory. In addition to treatment- and individual-level differences in anti-predator responses, there was an overall effect of the level of response to a food stimulus on the alarm stimulus response, and the degree of this effect varied over time (i.e. interaction effect). Regardless of treatment, there was a consistent negative relationship between alarm stimulus response and food stimulus response. Individuals who were more stimulated by the food stimulus were less responsive to the alarm stimulus, and vice versa, providing additional support for the idea that some of these yellow perch were shifting to piscivory. The single behaviours measured in this study were generally not correlated to one another, both within and between trials, indicating that yellow perch likely do not exhibit
syndromes for the observed behaviours – at least during their first year of life. Behavioural syndromes can be the result of underlying genetic constraints and/or adaptation to local conditions (Bell & Stamps, 2004; Sih et al. 2004a, b). Behavioural syndromes may be present if the costs of modulating responses to current environmental conditions (i.e. flexibility) is too high or if the environment does not produce reliable cues (Reed et al. 2010). Some studies have shown that species exhibit behavioural syndromes consistently throughout ontogeny (Brodin, 2008; Schurch & Heg, 2010) whereas others have shown that syndromes may break apart or form at certain stages throughout ontogeny (Adriaenssens & Johnsson, 2013). Environmental conditions may change, or organisms may move to different environments as they age, transitioning through certain life history events, and experiencing changes in predation risk; this may result in coupling or uncoupling of behavioural traits over time. We cannot rule out the possibility that yellow perch exhibit a behavioural syndrome at some earlier or later point in their lives, as selection pressures change. The weak correlations observed between activity and neophilia warrant further study of behavioural syndromes in yellow perch over longer time periods. However, results from this study suggest that yellow perch possess behavioural types for some of the behaviours studied, but do not show behavioural syndromes, indicating that behaviours are not coupled by underlying constraint, but are likely the result of local adaptation.

Behavioural repertoire of yellow perch appears to be influenced by both early rearing experiences and by genetic background (resulting from local adaptation), given the patterns of similarities and differences between the treatments under study. Treatment differences in exploration and activity appeared to be linked to rearing experience. The treatment reared in an environment with a relatively lower predation risk (W-CL) showed the highest exploration tendency in an open, risky environment, compared to the treatments reared under relatively higher predation risk (CH and WH). This result is not unexpected given the previous studies showing the effect of recent predation threat on exploration and risk-taking behaviours in perch species (Hellstrom & Magnhagen, 2011; Magnhagen, 2006; Magnhagen et al. 2012). The treatments CH and W-CL showed similar levels of activity, with CH showing the highest activity over the course of the study. These two treatments were reared in captive, pond environments, where density of
conspecifics (and heterospecifics in the case of CH) was relatively high and the habitat was relatively stable, compared to a wild environment. Pond environments seem to foster individuals with higher activity, neophilia, exploration and risk-taking than larger lake or marine environments (Brydges et al. 2008; Herczeg & Valimaki, 2011). Greater competition for resources in the limited space of the pond, which changes little over time, would necessitate these types of behaviours in order to gain access to limited resources to grow and survive.

Treatment-level differences in the other two behaviours measured in this study, neophilia and response to a predation threat, appeared to be related to genetic background of the treatment groups. W-CL and WH, which were found to be very genetically similar (compared to CH), also showed similarities in terms of neophilia and response to predation threat (i.e. alarm stimulus). CH was most neophilic and had a higher response to the alarm stimulus (i.e. increased mobility) than W-CL and WH. This evidence suggests that response to novelty and antipredator behaviour may be influenced by adaptation to local conditions over many generations. Neophobic and anti-predator responses in yellow perch may be driven by predation risk levels in the environments from which they originated, not necessarily their rearing environments. Neophobia and predation responses in WH and W-CL, being more recently removed from Balsam Lake, may be an adaptation to risk-levels in the wild environment, and not due to recent predation experience. If risk level is higher in the wild, than individuals from these treatment groups may show a generalized neophobic response towards predators. Other studies have documented generalized neophobia responses in predator-naïve prey from environments with high background risk (Brown et al. 2013; Ferrari et al. 2015). Another explanation for the higher response to the alarm stimulus that was observed in the CH group, may be that CH was undergoing an ontogenetic diet shift towards piscivory, so their increase in mobility was a foraging response, rather than an antipredator response.

An additional noteworthy finding was that behaviour of yellow perch appeared to attenuate over time for all of the behaviours studied, regardless of the treatment. Moreover, activity, neophilia and stimulus responses increased overall, and exploration decreased during the fishes’ time in their common captive environment. Our models
indicated that individual yellow perch show behavioural flexibility (i.e. I × E/A) for Activity, and consistency in antipredator responses (i.e. individual differences, but rank-order consistency over time in Stimulus response, no I × E/A). Due to the similar attenuation and patterns experienced by all treatment groups (i.e. no random treatment effects), these responses are likely a product of ontogeny, rather than habituation to the common tank environment over time. If habituation were the cause of the observed attenuation, it would be expected that treatments would show larger behavioural differences when first brought into the tanks (i.e. behaviour as a product of rearing environment), but treatment behavioural responses would converge with longer time spent in the common tank environment. In other words, treatments would have shown random slope effects, with behavioural responses becoming more similar over time. Behaviour can change as individuals near critical life stages, such as sexual maturity and reproduction (Edenbrow & Croft, 2011), however yellow perch typically do not reach sexual maturity until they are 2-4 years old (Scott & Crossman, 1973), so this explanation is unlikely for the YOY yellow perch in this study. An additional possible explanation would be that this behavioural attenuation comes at a time when the yellow perch are undergoing an ontogenetic diet shift to piscivory, resulting in behavioural changes as well. Yellow perch are known to begin this ontogenetic diet shift to piscivory or cannibalism as early as 80 mm total length (Graeb et al. 2006). Another explanation would be that this attenuation is a response to relaxation of selection pressures in the common tank environment, as captive environments are typically associated with reduction of predation risk, reduced stochasticity and constant food sources provided (McPhee, 2003).

This study is the first to document the behavioural repertoire of *P. flavescens*, and one of the first to simultaneously account for the influence of rearing experience, genetic architecture and ontogeny on behaviour. This behavioural data could be used to investigate the connections between behaviour and fitness, to see if certain behaviours lead to increased or decreased fitness for individuals. Future studies will also use this baseline behavioural data to research the effect of ecologically-relevant multiple stressors (e.g. chemical pollution, increasing temperatures) on the behaviour of yellow perch.
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Figure 2.1 Treatment-level reaction norms for Exploration over time (i.e. across behavioural trials) for three treatment groups: CH (solid line; dots), W-CL (dashed line; crosses), and WH (dotted line; diamonds)
Figure 2.2 Predicted trajectories of activity for individuals over time for each treatment group (a) CH, (b) W-CL and (c) WH. Trajectories are based on best linear unbiased predictors for each individual generated from mixed model with individual random slope and random intercept terms.
Figure 2.3 Treatment-level reaction norms of Activity over time (i.e. across behavioural trials) for three treatment groups: CH (solid line; dots), W-CL (dashed line; crosses), and WH (dotted line; diamonds)
Figure 2.4 Treatment-level reaction norms of Neophilia over time (i.e. across behavioural trials) for three treatment groups: CH (solid line; dots), W-CL (dashed line; crosses), and WH (dotted line; diamonds)
Figure 2.5 Interaction between response to food stimulus and time in alarm stimulus response test at trial 1 (solid line; circles), trial 2 (dashed line; crosses) and trial 3 (dotted line; diamonds)
Table 2.1 Differences between the three treatment groups in terms of evolutionary time scales (i.e. local adaptation) and ontogenetic time scales (i.e. rearing environment and environment during behavioural trials)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Evolutionary Time Scale/Environment of Origin</th>
<th>Ontogenetic Time Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early Rearing Environment</td>
</tr>
<tr>
<td>CH</td>
<td>Pond - Predominantly Balsam Lake, ON</td>
<td>Pond</td>
</tr>
<tr>
<td>Captive, Highly Complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W-CL</td>
<td>Wild – Balsam Lake, ON</td>
<td>Wild</td>
</tr>
<tr>
<td>Wild, Captive, Less Complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WH</td>
<td>Wild, Highly Complex</td>
<td></td>
</tr>
<tr>
<td>Wild, Highly Complex</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2 Sample sizes for each treatment group for Trials 1-3

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild, High Predation (WH)</td>
<td>24</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Wild, Captive Low Predation (W-CL)</td>
<td>23</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Captive, High Predation (CH)</td>
<td>23</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>70</strong></td>
<td><strong>42</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>
Table 2.3 Principal component analysis loadings for each behavioural variable for the Open Field test and the Novel Object test for trials 1-3 pooled together

<table>
<thead>
<tr>
<th>Measurements</th>
<th>PCA Loadings</th>
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<tr>
<td></td>
<td>PC1</td>
</tr>
<tr>
<td><strong>Open Field Test</strong></td>
<td></td>
</tr>
<tr>
<td>Latency to first movement (s)</td>
<td>0.01</td>
</tr>
<tr>
<td>Times entering centre zone</td>
<td>0.89</td>
</tr>
<tr>
<td>Total time in centre zone (s)</td>
<td>0.97</td>
</tr>
<tr>
<td>Average duration in centre zone (s)</td>
<td>0.83</td>
</tr>
<tr>
<td>Number of quadrant transitions</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Variance Explained (%)</strong></td>
<td>57.20</td>
</tr>
<tr>
<td><strong>Eigenvalue</strong></td>
<td>2.86</td>
</tr>
<tr>
<td><strong>Novel Object (NO) Test</strong></td>
<td></td>
</tr>
<tr>
<td>Latency to enter NO zone (s)</td>
<td>-0.78</td>
</tr>
<tr>
<td>Times entering NO zone</td>
<td>0.69</td>
</tr>
<tr>
<td>Total time in NO zone (s)</td>
<td>0.92</td>
</tr>
<tr>
<td>Average duration in NO zone (s)</td>
<td>0.89</td>
</tr>
<tr>
<td>Times inspecting NO</td>
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</tr>
<tr>
<td>Total time inspecting NO (s)</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Variance Explained (%)</strong></td>
<td>61.70</td>
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<tr>
<td><strong>Eigenvalue</strong></td>
<td>3.70</td>
</tr>
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</table>
Table 2.4 Linear mixed effect model structures and model selection criteria for Exploration, Activity, Neophilia, and Stimulus Response behaviour scores

<table>
<thead>
<tr>
<th>Model Type/Number</th>
<th>Model Structure</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>wi</th>
<th>r²_m</th>
<th>r²_c</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exploration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (global)</td>
<td>Exploration ~ Time + Time² + Treatment + (Time</td>
<td>Treatment) + (Time</td>
<td>ID)</td>
<td>427.7</td>
<td>19.2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Exploration ~ Time + Time² + Treatment + (Time</td>
<td>ID) + (1</td>
<td>Treatment)</td>
<td>423.0</td>
<td>14.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Exploration ~ Time + Time² + Treatment + (Time</td>
<td>Treatment) + (1</td>
<td>ID)</td>
<td>424.3</td>
<td>15.8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Exploration ~ Time + Time² + Treatment + (Time</td>
<td>Treatment)</td>
<td>426.2</td>
<td>17.7</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>Exploration ~ Time + Time² + Treatment + (1</td>
<td>Treatment)</td>
<td>421.7</td>
<td>13.2</td>
<td>0</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>Exploration ~ Time + Time² + Treatment + (Time</td>
<td>ID)</td>
<td>420.7</td>
<td>12.2</td>
<td>0</td>
<td>0.13</td>
</tr>
<tr>
<td>7</td>
<td>Exploration ~ Time + Time² + Treatment + (1</td>
<td>ID)</td>
<td>417.5</td>
<td>9.0</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>8</td>
<td>Exploration ~ Time + Time² + Treatment</td>
<td>408.5</td>
<td>0</td>
<td>0.98</td>
<td>0.14</td>
<td>-</td>
</tr>
<tr>
<td><strong>Activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (global)</td>
<td>Activity ~ Time + Time² + Treatment + (Time</td>
<td>Treatment) + (Time</td>
<td>ID)</td>
<td>370.2</td>
<td>7.0</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>Activity ~ Time + Time² + Treatment + (Time</td>
<td>ID) + (1</td>
<td>Treatment)</td>
<td>365.5</td>
<td>2.3</td>
<td>0.24</td>
</tr>
<tr>
<td>3</td>
<td>Activity ~ Time + Time² + Treatment + (Time</td>
<td>Treatment) + (1</td>
<td>ID)</td>
<td>393.0</td>
<td>29.8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Activity ~ Time + Time² + Treatment + (Time</td>
<td>Treatment)</td>
<td>395.2</td>
<td>32.0</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td>Activity ~ Time + Time² + Treatment + (1</td>
<td>Treatment)</td>
<td>388.0</td>
<td>24.8</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>Activity ~ Time + Time² + Treatment + (Time</td>
<td>ID)</td>
<td>363.2</td>
<td>0</td>
<td>0.74</td>
<td>0.29</td>
</tr>
<tr>
<td>7</td>
<td>Activity ~ Time + Time² + Treatment + (1</td>
<td>ID)</td>
<td>386.2</td>
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<td>0</td>
<td>0.30</td>
</tr>
<tr>
<td>Model Type/Number</td>
<td>Model Structure</td>
<td>AICc</td>
<td>ΔAICc</td>
<td>wi</td>
<td>$r^2_m$</td>
<td>$r^2_c$</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-------</td>
<td>-------</td>
<td>-----</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Neophilia</td>
<td>Neophilia ~ Time + Time$^2$ + Treatment + (Time</td>
<td>Treatment) + (Time</td>
<td>ID)</td>
<td>421.6</td>
<td>22.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Neophilia ~ Time + Time$^2$ + Treatment + (Time</td>
<td>ID) + (1</td>
<td>Treatment)</td>
<td>416.9</td>
<td>17.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Neophilia ~ Time + Time$^2$ + Treatment + (Time</td>
<td>Treatment) + (1</td>
<td>ID)</td>
<td>417.1</td>
<td>18.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Neophilia ~ Time + Time$^2$ + Treatment + (1</td>
<td>Treatment)</td>
<td>412.6</td>
<td>13.6</td>
<td>0</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Neophilia ~ Time + Time$^2$ + Treatment + (Time</td>
<td>ID)</td>
<td>414.6</td>
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<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Neophilia ~ Time + Time$^2$ + Treatment + (1</td>
<td>ID)</td>
<td>410.9</td>
<td>11.9</td>
<td>0</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Neophilia ~ Time + Time$^2$ + Treatment</td>
<td>399.0</td>
<td>0</td>
<td>0.99</td>
<td>0.18</td>
<td>-</td>
</tr>
<tr>
<td>Stimulus Response</td>
<td>Alarm Response ~ Food Response + Time + Treatment + (Time</td>
<td>Treatment) + (Time</td>
<td>ID)</td>
<td>1245.8</td>
<td>13.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Alarm Response ~ Food Response + Time + Treatment + (1</td>
<td>Treatment)</td>
<td>1235.0</td>
<td>2.4</td>
<td>0.11</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Alarm Response ~ Food Response + Time + Treatment + (1</td>
<td>ID)</td>
<td>1235.0</td>
<td>2.4</td>
<td>0.11</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Alarm Response ~ Food Response × Time + Treatment + (1</td>
<td>ID)</td>
<td>1234.7</td>
<td>2.1</td>
<td>0.13</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Alarm Response ~ Food Response × Time + Treatment + (1</td>
<td>Treatment) + (1</td>
<td>ID)</td>
<td>1235.2</td>
<td>2.6</td>
<td>0.10</td>
</tr>
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<td></td>
<td>Alarm Response ~ Food Response × Time + Treatment + (1</td>
<td>Treatment) + (1</td>
<td>ID)</td>
<td>1236.9</td>
<td>4.3</td>
<td>0.04</td>
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<td></td>
<td>Alarm Response ~ Food Response × Time + Time$^2$ + Treatment + (1</td>
<td>ID)</td>
<td>1232.6</td>
<td>0</td>
<td>0.37</td>
<td>0.36</td>
</tr>
</tbody>
</table>

The most likely model (based on wi, Akaike weight) is shown in bold. AICc = Akaike information criterion corrected for small sample sizes. $r^2_m$ = marginal r squared, $r^2_c$ = conditional r squared.
Table 2.5 Behavioural correlations within trial 1 for three treatment groups

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Treatment Group</th>
<th>Spearman’s $r_s$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity-Neophilia</td>
<td>CH</td>
<td>0.20</td>
<td>0.3619</td>
</tr>
<tr>
<td></td>
<td>W-CL</td>
<td><strong>0.52</strong></td>
<td><strong>0.0116</strong></td>
</tr>
<tr>
<td></td>
<td>WH</td>
<td>-0.02</td>
<td>0.9133</td>
</tr>
<tr>
<td>Exploration-Neophilia</td>
<td>CH</td>
<td>0.47</td>
<td>0.0230</td>
</tr>
<tr>
<td></td>
<td>W-CL</td>
<td>0.22</td>
<td>0.3213</td>
</tr>
<tr>
<td></td>
<td>WH</td>
<td>0.15</td>
<td>0.4726</td>
</tr>
<tr>
<td>Stimulus Response-Activity</td>
<td>CH</td>
<td>0.23</td>
<td>0.2988</td>
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<tr>
<td></td>
<td>W-CL</td>
<td>0.30</td>
<td>0.1609</td>
</tr>
<tr>
<td></td>
<td>WH</td>
<td>-0.06</td>
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<td>Stimulus Response-Exploration</td>
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<td>WH</td>
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<td>0.0799</td>
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<tr>
<td>Stimulus Response-Neophilia</td>
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<td>0.06</td>
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</tr>
<tr>
<td></td>
<td>W-CL</td>
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<td>0.0459</td>
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<tr>
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<td>WH</td>
<td>-0.06</td>
<td>0.7695</td>
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</tbody>
</table>

Marginally significant (at 10% level after Bonferroni adjustments) correlations are shown in bold. Significant correlations are bold with an *
Table 2.6 Behavioural correlations within trial 2 for three treatment groups

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Treatment Group</th>
<th>Spearman’s $r_s$</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Activity-Neophilia</td>
<td>CH</td>
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<tr>
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<td>WH</td>
<td>-0.30</td>
<td>0.2841</td>
</tr>
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<td>Exploration-Neophilia</td>
<td>CH</td>
<td>-0.88</td>
<td>0.0008*</td>
</tr>
<tr>
<td></td>
<td>W-CL</td>
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<td>0.6390</td>
</tr>
<tr>
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<td>WH</td>
<td>0.27</td>
<td>0.3356</td>
</tr>
<tr>
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<td>0.02</td>
<td>0.9602</td>
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<td>W-CL</td>
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<td>WH</td>
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<tr>
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<td>W-CL</td>
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</tr>
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<td>WH</td>
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</tr>
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<td>0.7770</td>
</tr>
<tr>
<td></td>
<td>W-CL</td>
<td>0.00</td>
<td>0.9899</td>
</tr>
<tr>
<td></td>
<td>WH</td>
<td>-0.24</td>
<td>0.3960</td>
</tr>
</tbody>
</table>

Marginally significant (at 10% level after Bonferroni adjustments) correlations are shown in bold. Significant correlations are bold with an *
Table 2.7 Behavioural correlations within trial 3 for three treatment groups

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Treatment Group</th>
<th>Spearman’s $r_s$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH</td>
<td>0.50</td>
<td>0.2532</td>
</tr>
<tr>
<td></td>
<td>W-CL</td>
<td>0.52</td>
<td>0.0462</td>
</tr>
<tr>
<td></td>
<td>WH</td>
<td>0.53</td>
<td>0.0947</td>
</tr>
<tr>
<td>Activity-Neophilia</td>
<td>CH</td>
<td>0.54</td>
<td>0.2152</td>
</tr>
<tr>
<td></td>
<td>W-CL</td>
<td>0.14</td>
<td>0.6296</td>
</tr>
<tr>
<td></td>
<td>WH</td>
<td>0.28</td>
<td>0.4080</td>
</tr>
<tr>
<td>Exploration-Neophilia</td>
<td>CH</td>
<td>0.32</td>
<td>0.4821</td>
</tr>
<tr>
<td></td>
<td>W-CL</td>
<td>-0.45</td>
<td>0.0924</td>
</tr>
<tr>
<td></td>
<td>WH</td>
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<td>0.8734</td>
</tr>
<tr>
<td>Stimulus Response-Activity</td>
<td>CH</td>
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<td>0.4821</td>
</tr>
<tr>
<td></td>
<td>W-CL</td>
<td>-0.41</td>
<td>0.1320</td>
</tr>
<tr>
<td></td>
<td>WH</td>
<td>0.21</td>
<td>0.5372</td>
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<td>Stimulus Response-Exploration</td>
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<tr>
<td></td>
<td>WH</td>
<td>0.42</td>
<td>0.1994</td>
</tr>
</tbody>
</table>

Marginally significant (at 10% level after Bonferroni adjustments) correlations are shown in bold. Significant correlations are bold with an *. Values in grey are suspect due to low sample size. CH results should be interpreted with caution due to low sample size (n=7)
Chapter 3: The relationship between behavioural phenotype, fitness and survival of yellow perch, *Perca flavescens*

Introduction

Animal personalities, behavioural types, or consistent individual differences in behaviour, are now recognized as meaningful, rather than random, variation that can be a product of experience or underlying genetic architecture. Individuals consistently differ in behavioural traits, such as boldness (Magnhagen et al. 2014), aggression (Huntingford et al. 2010), exploration (Dingemanse et al. 2002, 2004), and sociability (Magnhagen & Bunnefeld, 2009), across contexts and over time (e.g. ontogeny; Brodin, 2009). These consistent differences in behaviour can have ecological and evolutionary implications for individual fitness, especially under rapid environmental change (Biro & Stamps, 2008; Smith & Blumstein, 2008). Determining the proximate mechanisms underlying this inter-individual variation and its fitness consequences, represents a critical next step in behavioural ecology. This knowledge will shed light on ecological processes and adaptive evolution, as well as have applied benefits in assisting species conservation efforts in the wild under human-induced rapid environmental change and for captive breeding and reintroduction programs.

Behaviour has fitness consequences, in terms of both reproduction and survival, for an individual (Smith & Blumstein, 2008). Personality-related fitness and survival have indeed also been documented in many taxa, including fish (Smith & Blumstein, 2010), insects (Niemela et al. 2015), mammals (Boon et al. 2008) and birds (Dingemanse et al. 2004). Bolder mosquitofish (*Gambusia holbrooki*) tended to be smaller and less fecund (i.e. number of eggs) than shyer mosquitofish; this variation was probably driven by the trade-off between short- and long-term reproductive investment, with bolder individuals investing more energy into short-term fitness gains and shyer individuals investing more in long-term fitness by avoiding predation risk (Wilson et al. 2010). In another study, number of offspring recruited to the next generation was related to maternal exploration tendency in great tits (*Parus major*), likely due to higher offspring condition from exploratory parents (Dingemanse et al. 2004). In addition to reproductive
fitness, behaviour can also have consequences for how well an individual survives in a given environment. Individuals that survive longer may have higher reproductive output and therefore, higher fitness; if certain personality types survive better than others, then natural selection may act on those personality traits if they are heritable (Reale & Festa-Bianchet, 2003). Adult survival was related to individual exploration tendency in great tits, with the relationship between exploration rate and survival fluctuating between years for the sexes, depending on winter food availability (Dingemanse et al. 2004). In juvenile damselfish species (*Pomacentrus wardi* and *P. moluccensis*), bolder and more exploratory individuals (i.e. low shelter use, high foraging rate, high activity in a novel environment) survived longer than shyer individuals during a critical life-history bottleneck (i.e. reef settlement; McCormick & Meekan, 2010; White et al. 2013).

Although bold individuals generally tend to expose themselves more to risk, they may also inspect predators more than shy individuals do which may help them to ward off predators and increase survival (Godin & Davis, 1995). Behavioural types or personalities may trade-off short-term and long-term fitness to different degrees as well. In red squirrels (*Tamiasciurus hudsonicus*), more active behavioural types were less likely to survive the winter, but were more likely to bequeath territory to their offspring, increasing overwinter survival of offspring (Boon et al. 2008), trading off long term survival for short term reproductive output. In addition to natural selection, organisms are faced with anthropogenic stressors which can differentially affect fitness of personality types. Commercial gillnet fisheries can indirectly select for faster-growing individuals because their more active, bold personalities make them more vulnerable to capture, reducing fitness of those personality types and artificially selecting for slower-growing individuals (Biro & Post, 2008); this phenomenon is in line with other studies linking growth rates to trappability of animals through underlying behavioural traits (Biro & Sampson, 2015).

Correlated behaviours across contexts (i.e. syndromes), can have significant implications for survival as well. When behaviours were measured together as a syndrome, more active, bolder and more exploratory guppies (*Poecilia reticulata*) survived longer under direct predation threat than less active, shyer and less exploratory individuals; this is likely due to their increased need to assess risk given their high
activity and exploration levels (Smith & Blumstein, 2010). Behavioural syndromes can cause isolated traits to appear maladaptive for fitness when observed in certain contexts. For example, female fishing spiders (*Dolomedes triton*) show a spillover of aggressive behaviour in the form of pre-copulatory sexual cannibalism that appears maladaptive for fitness; however, the benefits of high aggression in other contexts may outweigh the costs of aggression in a courtship context (Johnson & Sih, 2005). Consequently, given the difficulty of directly measuring fitness consequences of behaviour of individuals in laboratory and wild conditions, ecologically-relevant fitness correlates could be used in place of direct measures to assess links between fitness and personality and/or syndromes.

Personality is commonly related to correlates, or indicators, of fitness, such as growth rate (Adriaenssens & Johnsson, 2010), morphology (Vrtelova et al. 2015), diet and foraging rate (Nannini et al. 2012) and disease/parasite infection (Bajer et al. 2015). Contrary to predictions from laboratory studies, fast-exploring individual brown trout (*Salmo trutta*) had slower growth rates than slow-exploring individuals under natural conditions because individual growth-mortality trade-offs can fluctuate with environmental changes (e.g. resource availability, predation risk) and over time (Adriaenssens & Johnsson, 2010). Individual boldness has been shown to be positively correlated with morphology, including body mass (Brown et al. 2007) and body depth (Vrtelova et al. 2015). In the common carp (*Cyprinus carpio*), individuals who were consistently more active and showed less shelter use developed deeper bodies compared to less active, shelter-using fish, demonstrating a link between behaviour and morphology in antipredator defenses (Vrtelova et al. 2015). Bolder individuals are often more successful foragers than shy individuals in terms of feeding rate or number of prey captured (Brodin, 2009); however, shy individuals may reduce predation risk exposure by foraging less, but exploit more profitable prey items when they do forage, probably resulting in relatively equal fitness outcomes (Nannini et al. 2012). During times of limited resources, a neophilic individual who is more likely to feed on a novel resource may have a better chance of survival and therefore, higher fitness, than a neophobic individual who is fearful of novelty. For instance, bold personality individuals in the fallow deer (*Dama dama*) were more likely to feed on familiar foods in the presence of
novel objects and to feed on novel foods (Bergvall et al. 2011). Exploration tendency was linked to body length and parasite infection in European green lizards (*Lacerta viridis*): faster explorers tended to be larger and were uninfected by parasites compared to the slower explorers who were smaller and infected by parasites, the cause of which is currently unknown (Bajer et al. 2015).

In this chapter, we further investigated the behavioural phenotype of yellow perch (*Perca flavescens*) by exploring the association of ecological correlates of fitness with divergent behavioural phenotypes, and the subsequent impact on survival. Yellow perch (*Perca flavescens*) are an important Great Lakes fish species, in terms of commercial and recreational fisheries (Baldwin et al. 2009) and for the stability of the ecosystem food webs (Sanderson et al. 1999). Relatively little is known about the behavioural ecology of the species, with the exception of work done on a close relative, the Eurasian perch (*Perca fluviatilis*; Magnhagen, 2006, 2007; Magnhagen & Bunnefeld, 2009; Magnhagen et al. 2012), and even less is known about how behavioural variation differentially affects fitness of yellow perch. Our previous work demonstrated that yellow perch vary in their behavioural repertoire and responsiveness, which in turn is influenced by their early rearing environment as well as their underlying population differences (i.e. local adaptation to wild vs. captive environments or time since removed from wild).

The objectives in this chapter were to 1) investigate the presence of ecologically-relevant behavioural phenotypes in yellow perch over ontogeny, 2) explore the influence of size, condition, growth rate and diet (using the stable isotopes $\delta^{15}N$ and $\delta^{13}C$) on this overall behavioural phenotype and 3) predict individual survivorship, for both the short-term (~1 year) and long-term (~2 years, after release and overwintering in ponds), using behaviours from an earlier time point (activity, neophilia, exploration and antipredator responses) and body morphology (i.e. size and condition). We expect that 1) individuals with a more active, bold and exploratory phenotype will be larger, in better condition, faster-growing and will switch their diet sooner (indicated by a change in isotopic value) than individuals with a less active, shy, and less exploratory phenotype. Bolder, more active individuals will forage more than shyer individuals, even in the presence of predation risk, increasing their size, improving body condition and changing their diet
sooner (i.e. may switch to tank feed or even cannibalism sooner). We also expect that 2) changes in an individual’s behavioural phenotype over time will be correlated with changes in fitness correlates. Finally, we expect 3) survivorship will be predicted by an individual’s activity level and boldness (i.e. neophilia), as well as body condition (i.e. body depth), as an individual’s ability to escape gape-limited predation by other perch will be dependent on its body depth. Knowledge of the behavioural repertoire of yellow perch and how it is linked to fitness during a vulnerable life history stage (juvenile) will provide crucial information to understand the ecology of the species, how to manage it under human-induced rapid environmental change and potentially, could contribute to the future of captive breeding and aquaculture production of yellow perch.

**Methods**

**Study System**

Three treatment groups of yellow perch were tested in behavioural trials at three time points (2 months apart, spanning 6 months; Fig. 3.1) to examine the relative contributions of early rearing experience and genetic (local) adaptation (Chapter 2, Table 2.1). The treatment groups, which all originated from Balsam Lake, ON (44.58044,-78.84165) were as follows:

1) **Wild, Highly Complex, Stochastic Environment (WH)** – Yellow perch were sampled directly from their wild environment (Balsam Lake, ON) by seine net. This rearing environment was considered relatively more stochastic and complex than the pond environments; compared to small ponds, wild environments would typically be more spatially and temporally heterogeneous, and they may be more exposed to changes in weather, wave action, disease, and invasive species.

2) **Wild-Captive, Low Complexity Environment (W-CL)** – The yellow perch from this treatment group were the first-generation removed from their wild environment (in Balsam Lake, ON) and reared from birth in the captive, nursery environment. They emerged in the nursery pond from egg strands that were fertilized in Balsam Lake, ON during the spring of 2013. This nursery pond was newly dredged and filled and contained
no fish other than the first-generation yellow perch, therefore this rearing environment was considered to be less complex because it contained a relatively lower abundance of predators and competitors and represented a stable, semi-wild, captive pond environment. However, the potential for cannibalism within the treatment cannot be ruled out given that YOY yellow perch undergo a shift towards piscivory and cannibalism early in life (Fullhart et al. 2002; Graeb et al. 2006).

3) Transgenerational Captive, Highly Complex Environment (CH) – Yellow perch from this treatment group were sampled from two holding ponds at Leadley Environmental Inc. using minnow traps baited with either food or light. The yellow perch collected from this pond were a trans-generational sample derived from multiple generations (i.e. a minimum of 7) removed from wild environments (including Balsam Lake, ON as the predominant source). Other fish species known to be predators of yellow perch were present in these holding ponds, in addition to the potential for cannibalism from other perch (Fullhart et al. 2002; Graeb et al. 2006). These ponds had been established for many years (~20) and had a higher density of predators/competitors than the W-CL ponds, therefore this environment was considered highly complex, but fairly stable due to the nature of pond environments.

See Chapter 2 Methods for specific details for each treatment sampling site. All yellow perch sampled were ≤ 110 mm total length. All yellow perch were collected during September-November 2013. After collection, they were immediately transported in aerated haulers back to the Great Lakes Institute for Environmental Research (GLIER) Aquatic Facility at the University of Windsor in Windsor, Ontario, Canada. The perch were fed live blackworms (*Lumbriculus variegatus*) ad lib every 48 hours from the time of capture until December 2013 (to facilitate transition from live feed), when they were transitioned to a diet of frozen bloodworms and freeze-dried krill ad lib every 48 hours after that. For details of fish husbandry see Chapter 2 Methods.

**Behavioural Trials**

Behavioural trials consisted of 3 assays conducted in the following order: an open field test, a novel object test and a test of the trade-off of foraging under predation risk.
See Methods section of Chapter 2 for details of behavioural assays. These tests were designed to quantify individual activity, exploration, neophilia and antipredator responses.

**Morphological Measurements**

Immediately following the first and last behavioural trials (1 and 3; Fig 3.1), individuals were placed on their side on a ruler set on an electronic scale and photographed from above using a Nikon P5500 digital camera attached to a tripod. Fish measurements were taken to the nearest 0.1 mm using Image J image analysis software (Schneider et al. 2012). Individual fish were measured for fork length and body depth. Fish mass was recorded to the nearest 0.1 g.

To examine differences in fish growth over the course of the trials, we calculated specific growth rate (SGR) using the following formula: 

\[ SGR = \left( \frac{\ln W_2 - \ln W_1}{t} \right) \times 100, \]

where \( W_2 \) is the weight at the end of the trials, \( W_1 \) is the weight at trial 1 and \( t \) is the number of days in between measurements (\( t=123 \) days between trials 1 and 3).

**Stable Isotope Sample Collection and Analysis**

Fish fin tissue can be used as a reliable indicator of the stable isotopes \( \delta^{15}N \) and \( \delta^{13}C \) in internal tissues, such as liver and muscle (Sanderson et al. 2009), and has the additional benefit of being a non-lethal alternative to sampling internal organs. However, it is recommended that researchers first determine the species-specific relationship between fin and organ \( \delta^{15}N/\delta^{13}C \) before using and interpreting fin values exclusively for that species in stable isotope studies. Therefore, before using caudal fin for evaluating \( \delta^{15}N \) and \( \delta^{13}C \) in the yellow perch from our behavioural study, we conducted a short study to examine the validity of using caudal fin as a non-lethal alternative to liver and muscle when measuring \( \delta^{15}N \) and \( \delta^{13}C \) in yellow perch (see Appendix A for full study). This was done in order to ensure that \( \delta^{15}N \) and \( \delta^{13}C \) in caudal fin could accurately predict \( \delta^{15}N \) and \( \delta^{13}C \) in liver and muscle tissue, using correction factors where necessary. Values of \( \delta^{15}N \) can be used to assess relative trophic position of individuals in a food web.
(Vander Zanden et al. 1997) and δ\textsuperscript{13}C can be used to determine dietary organic matter sources (Finlay et al. 2002).

Individuals were sampled for caudal fin tissue at the time of PIT tagging ("pre-trial 1") and at approximately five weeks following Trial 3 ("post-trial 3"; Fig. 3.1). The sample taken at pre-trial 1 was meant to represent their early rearing environment diet, and the sample at post-trial 3 was meant to be representative of their diet in the captive tank environment. We felt these sampling times were adequate given that turnover rates of stable isotopes in tissues, especially slow-growing structural tissues like fin, is suggested to be somewhat long. The few studies that have looked at turnover in structural tissues found that it can take on the order of ~3-4 weeks (Suzuki et al. 2005) or as long as a ~3-4 months (Xia et al. 2013), depending on the tissue type. A small (2-3mm) section was removed from the dorsal section of the caudal fin using fine scissors. The fin clips were stored individually in vials in 95% ethanol for up to 5 months until stable isotope analysis. By analyzing the stable isotopes, δ\textsuperscript{15}N and δ\textsuperscript{13}C, in these caudal fin samples, we could coarsely determine an individual’s position, relative to others, in terms of diet, and potentially, indicate the degree to which they had shifted their diet (i.e. from their early rearing environment to the food provided in the tanks, including cannibalism). All experimental protocol followed the Canadian Council on Animal Care guidelines (AUPP #13-04).

Fin tissues preserved in 95% ethanol were drained, oven dried at 40°C for 24 hours and then rinsed with distilled water. At this point, all samples were freeze-dried at -44°C for 40-48 hours. The fin tissues were cut into smaller pieces before weighing. From each tissue sample, 0.4-0.6 mg was weighed out and placed into a tin capsule for SIA. Stable nitrogen δ\textsuperscript{15}N and carbon δ\textsuperscript{13}C isotopic values were determined using a Thermo Electron Delta V Advantage mass spectrometer interfaced to a Thermo Fisher Scientific Conflo IV elemental analyzer. The δ\textsuperscript{15}N and δ\textsuperscript{13}C values in the samples were determined using atmospheric nitrogen (N\textsubscript{2}) and PeeDee Belemnite Limestone Formation as reference standards, respectively. Isotopic ratios were expressed in parts per thousand (‰) units with δ notation using the following formula: \( \delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3 \),
where \( X \) is \(^{15}\text{N}\) or \(^{13}\text{C}\), with \( R_{\text{sample}} \) and \( R_{\text{standard}} \) being the ratios of \(^{15}\text{N}\) to \(^{14}\text{N}\) or \(^{13}\text{C}\) to \(^{12}\text{C}\) of the sample and the international standard, respectively.

During the analysis, every 10\(^{th}\) sample was run in triplicate to ensure analytical precision, in addition to analyzing standard reference materials (internal standard tilapia, \( n=50 \); bovine liver, \( n=50 \)) after every 12 samples. National Institute of Standards and Technology (NIST) standard reference materials (L-glutamic acid, ammonium sulfate for \( \delta^{15}\text{N} \); sucrose, L-glutamic acid for \( \delta^{13}\text{C} \)) were used to ensure accuracy over the course of the study. Analytical precision (SD) was 0.1 ‰ (tilapia) and 0.12 ‰ (bovine liver) for \( \delta^{15}\text{N} \) and 0.08 ‰ (tilapia) and 0.07 ‰ (bovine liver) for \( \delta^{13}\text{C} \). Standard deviations of replicate samples (\( n=6 \) samples run in triplicate) were kept to less than 0.2 ‰. Analytical accuracy of \( \delta^{15}\text{N} \) NIST standards was -4.41 ± 0.18 ‰ (L-glutamic acid) and 20.49 ± 0.18 ‰ (ammonium sulfate); accuracy of \( \delta^{13}\text{C} \) NIST standards was -10.55 ± 0.12 ‰ (sucrose) and -26.35 ± 0.07 ‰ (L-glutamic acid). See Appendix A Methods for details of handling lipids.

**Short- and Long-Term Survivorship**

Tanks were monitored every other day throughout the course of the behavioural trials and all mortalities were recorded up until the end of trial 3 (hereafter “short-term survivorship”). Following completion of trials and collection of fin clips “post-trial 3”, individuals were released into a small holding pond (which held no other fish) at Leadley Environmental Inc. in Essex, Ontario in Fall 2014. The holding pond was revisited and sampled by seine net to record survivors in April of 2015 (hereafter “long-term survivorship”; Fig. 3.1). Given our high sampling effort and our ability to completely cover the area of the holding pond with the seine net, we feel confident that our survivorship records are due to actual survivors/mortalities and not to sampling bias.

**Statistical Analysis**

**Morphology and Diet**

Using the morphological measurements for each individual at Trials 1 and 3, we calculated relative body depth (RBD):
We used RBD as a measure of fish depth that controls for fork length of an individual. RBD has been shown to be highly correlated with other condition factors, such as Fulton’s condition K, in this study and other studies of Perca species (Mustamaki et al. 2014). We conducted a principal component analysis (PCA) with varimax rotation on fork length, mass, and relative body depth for trials 1 and 3 pooled in order to generate a score for overall body size (including length and weight) and a score for condition (i.e. body depth with size removed) at trials 1 and 3 (as in Cote et al. 2010). A PCA conducted on morphological variables can be used to remove the effect of size from shape when examining morphology of animals (Somers, 1986). A PCA conducted individually on each trial produced very similar components as a PCA conducted on the trials pooled, therefore we felt confident in using this method (as in Adriaenssens & Johnsson, 2010; Dingemanse et al. 2007). From the PCA components, we calculated a change in size (Δsize) and a change in condition (Δcondition) for each surviving individual by subtracting the final score (at trial 3) from the initial score (at trial 1) for size and condition, respectively.

In order to score individuals in terms of their diet, we used the values of caudal fin δ¹⁵N and δ¹³C and the relative change of those values over time. We conducted an unrotated PCA on the values of δ¹⁵N and δ¹³C to combine them for each individual, with trials 1 and 3 pooled together, to generate a “diet score” for each individual for each time point. We followed the Kaiser-Guttman stopping rule for retaining principal components (Guttman, 1954). We calculated a change in diet score (Δ diet score) for individuals for which there was stable isotope data from both trials 1 and 3 by subtracting final diet score (at trial 3) from initial diet score (at trial 1). This scoring was used to indicate whether differences in stable isotope values (as a proxy of foraging ecology) are associated with an individual’s behavioural repertoire and survival. Treatment-level differences in diet score, size and condition were analyzed using a Kruskal-Wallis test because parametric assumptions were not met.
Behavioural Phenotype Score

To generate relevant behavioural scores, we used principal component analysis (PCA) to condense the measured variables from each behavioural assay into interpretable individual scores of exploration, activity, neophilia, and antipredator response (“stimulus response”), for which we scored each fish at each time point (barring early mortality; see Chapter 2 Methods and Results for specifics of PCA behaviour scores for exploration, activity, and neophilia). To generate the score for stimulus response, we conducted a PCA on % mobility with food stimulus, food stimulus response (% mobility food - % mobility control), % mobility alarm stimulus and, alarm stimulus response (% mobility alarm - % mobility food).

To further simplify this dataset, we conducted a second-order unrotated PCA on all four of these behavioural PCA scores (i.e. exploration, activity, neophilia, stimulus response) in order to condense the scores into a single “behavioural phenotype score” for each individual at each time point. We pooled together both trials 1 and 3, so that each individual had a behavioural phenotype score for the beginning and ending of trials. Pooling the two trials together would allow us to examine relative behavioural changes within and between individuals over time. In order to validate this method, we conducted a PCA on trials 1 and 3 separately; this produced very similar component loadings as the pooled PCA (as in Adriaenssens & Johnsson, 2010; Dingemanse et al. 2007). This confirmed that the structure of the behavioural phenotype score did not change between trials 1 and 3; however, individuals did not necessarily maintain their phenotype score over time (see Results). We calculated a change in behavioural phenotype score ($\Delta$BP) for the individuals who survived up to the end of trial 3. The $\Delta$BP was calculated as the difference between an individual’s score at trial 3 and their initial, trial 1 score. We used the absolute value of $\Delta$BP in our models, along with the direction of phenotypic change (increase or decrease from trial 1 to trial 3) in our behavioural models.

Behavioural Modelling with Ecological Correlates

Linear models incorporating ecological correlates of performance were used to explain individual behavioural variation at the beginning (trial 1) and end (trial 3) of the
trials. For trials 1 and 3 separately, individual behavioural phenotype (BP) score was fitted as the response variable. Behavioural phenotype scores at trial 1 and trial 3 were transformed using $\sqrt{y + 0.95}$ to satisfy parametric assumptions. Initial size (at trial 1) was controlled for as a fixed effect in all models. The fixed effect covariates investigated were: treatment (3-factor categorical), condition score (continuous), diet score (continuous) and all two-way interactions: treatment $\times$ size, treatment $\times$ condition, and treatment $\times$ diet score for both BP1 and BP3 (see Tables 3.3, 3.4 for model structures).

We also fitted change in behavioural phenotype score ($\Delta$BP) from trial 1 to trial 3 as a response variable in third linear model with the following fixed factors: initial size (continuous), $\Delta$ size (continuous), initial condition (continuous), $\Delta$ condition (continuous), $\Delta$ diet score (continuous), SGR (continuous), initial behavioural phenotype score (BP1; continuous) and an interaction between BP1 and direction of phenotypic change (2-factor categorical, increase or decrease)(see Table 3.5 for model structure).

Treatment was not included in the model for $\Delta$BP due to the small sample sizes for each group.

In Chapter 2, individual yellow perch were found to be additionally flexible in their activity levels over time. We therefore extracted these response coefficients per individual (i.e. BLUPs, best linear unbiased predictors) and used them as a response variable in an additional linear model to explain this individual flexibility. We interpreted the absolute value of the coefficients to be the degree of behavioural flexibility (BFA). The sign of coefficients (+/-) was interpreted as the direction of behavioural flexibility over time. The degree of behavioural flexibility was transformed to meet parametric assumptions. Degree of behavioural flexibility was fit as the response variable in a linear model with the following fixed effects: direction of behavioural flexibility (2-factor categorical), treatment, condition (at trial 1), SGR, change in size from trial 1 to trial 3 ($\Delta$ size; continuous), change in condition from trial 1 to trial 3 ($\Delta$ condition), change in diet score from trial 1 to trial 3 ($\Delta$ diet score) and initial activity score (at trial 1; continuous), controlling for size (at trial 1) in all models. Interactions term included were: treatment $\times$ size at trial 1, treatment $\times$ condition at trial 1 and treatment $\times$ SGR (see Table 3.6 for model structure).
**Modelling Short-term and Long-term Survivorship**

To investigate whether early behavioural phenotypes and ecological correlates of performance can predict differences in survivorship, we modelled short-term survivorship (i.e. individuals who died before the start of trial 3 vs. individuals who survived up until the end of trial 3) and long-term survivorship (i.e. individuals who died before April 2015 vs. individuals who survived to up to April 2015, ~1 year following behavioural trials; see Fig. 3.1) as response variables in generalized linear binomial models with logit link function using the glm2 package in R version 3.2.2 (R Core Team, 2015). The factors included in the model were: treatment, initial condition (at trial 1), initial size (at trial 1), and the trial 1 behavioural scores for Activity, Neophilia, Exploration, and stimulus response, controlling for individual size at trial 1 in both models. Degree of behavioural flexibility was also fit as a continuous predictor in the models. Interactions terms treatment × initial size and treatment × initial condition were included in the models (see Tables 3.7, 3.8 for model structures).

**Model Selection and Model Fit**

For all models, we used an information theoretic (IT) approach to evaluate model likelihood and select the relative best model using the Akaike Information Criterion corrected for small samples sizes (AICc), the relative differences in AICc (ΔAICc) in a model set, and the Akaike weight (w). A ΔAICc was calculated for each model as the relative difference from the lowest AICc model from a set of models. Lower AICc (and lower ΔAICc) indicated a better model relative to the other models in a set. Models with ΔAICc < 2 were considered likely models, and models with ΔAICc > 10 were considered very unlikely (Burnham & Anderson, 2002). Akaike weight, which ranges in value from 0 to 1, is the probability that a given model is the most appropriate model within a model set. Model fit was assessed for the likely models using r² for the behavioural phenotype models and McFadden’s pseudo-r² (McFadden, 1974) for the survivorship models.
Results

Morphology Scores

We retained one principal component (with eigenvalue >1) in the analysis that explained 83% of the variation in the morphology data (Table 3.1). Individuals scoring high for this PC had a longer fork length and were heavier than individuals with low scores, therefore this PC represents a score for individual “size”. The majority of the remaining variation left in the data, after accounting for an individual’s length and weight, could be explained by an individual’s relative body depth. In further analyses, we used an individual’s relative body depth as a condition score. Relative body depth was correlated with another condition index in this study (with K condition factor, Spearman’s rank, p < 0.001).

At trial 1, there were treatment group-level differences found for size score (KW: H = 23.16, d.f.=2, p<0.0001) and condition score (KW: H = 27.15, d.f.=2, p<0.0001), with perch from the WH treatment scoring lower for size and condition compared to W-CL and CH perch. At trial 3, the same treatment-level differences persisted for size (KW: H = 14.25, d.f.=2, p = 0.0008) and condition (KW: H = 9.12, d.f.=2, p = 0.0105). There was no difference in specific growth rate (from trial 1 to trial 3) between the treatment groups (KW: H = 4.13, d.f.=2, p = 0.1271).

Stable Isotopes and Diet

Fin tissue proved to be a moderate to excellent predictor of muscle and liver δ¹⁵N and δ¹³C (Appendix A). Results from Appendix A suggest that fin tissue δ¹⁵N and δ¹³C reflects recent to intermediate dietary uptake, although isotopic turnover rate were not directly tested in this study.

There were significant differences in δ¹⁵N (KW: H = 27.15, d.f.=2, p<0.0001) between treatment groups pre-trial 1, with W-CL having higher δ¹⁵N than CH and WH.
There were also differences in $\delta^{13}C$ (KW: $H = 27.15$, d.f.=2, $p<0.0001$) pre-trial 1, with WH having the lower $\delta^{13}C$ than CH and W-CL. In some cases, $\delta^{15}N$ values changed over the course of the study (i.e. from pre-trial 1 to post-trial 3), but $\delta^{13}C$ values did not. Over the course of the study $\delta^{15}N$ decreased by an average of 0.52 ‰ for W-CL and increased by an average of 1.19 ‰ for WH.

The unrotated PCA of $\delta^{15}N$ and $\delta^{13}C$ values at pre-trial 1 and post-trial 3 had similar loadings for both stable isotopes at both time points. Individuals scoring high along this PC had higher values of both $\delta^{15}N$ and $\delta^{13}C$. This PC was interpreted as an individual’s position, relative to others, in terms of diet, and may indicate the degree to which they shifted their diet (i.e. from their early rearing environment to the food provided in the tanks, including cannibalism) by comparing trial 1 to trial 3 values.

**Behavioural Phenotype Scores**

The PCA conducted on the stimulus response test variables revealed a component that explained ~57% of the variation in the data. This PC had positive loadings for % mobility food and $\Delta$ mobility food, and a negative loading for $\Delta$ mobility alarm. Individuals scoring high for this PC1 became highly mobile when the food stimulus was introduced to the tank, but became relatively less mobile when the alarm stimulus was introduced to the tank. Lower scoring individuals did not respond to the introduction of the food stimulus, and became more mobile when the alarm stimulus was introduced to the tank. We interpreted PC1 as the trade-off an individual makes when foraging under the risk of predation. Behavioural scores generated from the other assays are presented in Chapter 2.

The unrotated PCA constrained the behaviour scores into a single PC that explained ~38% of the variation in the data (Table 3.2). At both trials, individuals scoring high for this PC were more active, more neophilic, more exploratory (in terms of calmly exploring the exposed centre area of a novel environment) and were considered to have a relatively more responsive antipredator behaviour (i.e. freezing behaviour). Individuals scoring low for this PC were relatively more inactive, more neophobic, less exploratory (i.e. quickly swam through the exposed centre area or remained in the periphery of novel
environment) and displayed weaker antipredator responses toward the predator stimulus. Despite the fact that the structure of the phenotype score (i.e. mean) remained the same over time, individual yellow perch changed their overall behavioural phenotype score to varying degrees over time. Nine individuals increased their score over time, while 5 decreased their score over time. Most individuals showed a relatively small change in behavioural phenotype score over time (absolute value, range, mean ± SD: 0.12 – 0.90, 0.38 ± 0.25; Fig. 3.2).

**Behavioural Modelling with Ecological Correlates**

At trial 1, behavioural phenotype score (BP1) was explained best by a model that included treatment, diet score, size, condition and interactions of treatment with diet score and condition (\( w_i = 0.88, r^2 = 0.30 \); Model 2; see Table 3.3 for model structures). Smaller individuals generally had higher phenotypes scores than larger individuals at trial 1 (Fig. 3.3a). W-CL and WH individuals in better condition had lower phenotype scores (i.e., inactive, neophobic, non-exploratory, predator-reactive), whereas CH individuals in better condition had higher phenotype scores (Fig. 3.3b). W-CL and WH individuals with a higher diet score (i.e. higher \( \delta^{15}N \) and \( \delta^{13}C \)) had lower phenotype scores, whereas CH individuals with a higher diet score had a higher phenotype score (Fig. 3.3c). This interaction suggests that neophilic and active CH individuals and neophobic and inactive W-CL/WH individuals had been feeding on relatively higher trophic level foods in their rearing environments, potentially even feeding on small fish. Treatment differences in phenotype score at trial 1 were minimal (Fig. 3.3d).

At trial 3, variation in behavioural phenotype score (BP3) was best explained by fewer fixed effects: diet score, condition and an interaction between treatment and size (\( w_i = 0.47, r^2 = 0.43 \); Model 4; Table 3.4), controlling for size at trial 3. Individuals in better condition and with a higher diet score generally had a lower behavioural phenotype score at trial 3 (Fig. 3.4a, b), suggesting that these better condition individuals had switched to higher trophic level foods or even cannibalism in their common tank environment. The addition of an interaction term between treatment and size improved the model: size had a minimal effect on BP3 for CH and W-CL, however, larger
individuals from WH tended to have a higher phenotype score at trial 3 (Fig. 3.4c). A main effect of treatment did not necessarily improve the model, however there was a trend for CH to have a higher phenotype score than W-CL and WH (Fig. 3.4d).

When looking at whether individuals themselves changed phenotype score over time (ΔBP), and if so, what factors could explain it, the model that best described the change included the main effects condition, Δ diet score and direction of phenotypic change (DPC)(Model 5; Table 3.5), controlling for initial size (Fig. 3.5a). Individuals in better condition showed the greatest change in behavioural phenotype score over time (Fig. 3.5b). Individuals who increased their diet score (i.e. higher δ¹⁵N and δ¹³C) over time showed little change in behavioural phenotype score over time (Fig. 3.5c). Individuals who increased their behavioural phenotype over time (becoming more active, exploratory and neophilic) tended to show a larger change over time than individuals who decreased in behavioural phenotype (Fig. 3.5d).

The best model to explain individual behavioural flexibility in activity (BFA) contained the fixed effects for treatment, Δ diet score, size at trial 1, and specific growth rate (wᵢ = 0.99, r² = 0.50; Model 8; Table 3.6). Individuals who were initially smaller at (at trial 1) showed higher behavioural flexibility than initially larger individuals (Fig. 3.6a). Individuals with greater behavioural flexibility for activity also showed a greater change in diet score from trials 1 to 3 (Fig. 3.6b). Higher growth-rate individuals tended to show higher behavioural flexibility between trials 1 and 3 (Fig. 3.6c). Treatment-level differences were not apparent, however, inclusion of treatment as a main effect improved the model likelihood (Fig. 3.6d).

Survivorship Models

The best model to explain short-term survivorship (i.e. up until trial 3) contained the fixed effects: neophilia score at trial 1, BFA, and condition at trial 1 and an interaction between treatment and size (wᵢ = 0.25, r² = 0.282; Model 5; Table 3.7). Treatment group W-CL had the highest survivorship, with CH suffering the highest mortality up until trial 3 (Fig. 3.7). Individuals surviving up until trial 3 tended to be less neophilic and showed less flexibility for activity than those individuals that did not
survive (Fig. 3.8a, b). Survivors also initially had higher condition at trial 1 than mortalities (Fig. 3.8c).

Long-term survivorship (i.e. 1 year following behavioural trials) was modelled best with the fixed effects neophilia score at trial 1, BFA, treatment and condition at trial 1 ($w_i = 0.73$, $r^2 = 0.362$; Model 6; Table 3.8), controlling for size at trial 1. Treatment differences in long-term survival were apparent: 22% of CH, 49% of W-CL and 0% of WH survived up to one year following behavioural trials (Fig. 3.9). Again, the longest-surviving individuals were generally less neophilic and showed less behavioural flexibility for activity (Fig. 3.10a, b). With regards to morphology, the surviving individuals were the ones that were initially in better condition and were larger (Fig. 3.10c, d).

Discussion

Few studies to date have attempted to relate individual behaviour to fitness correlates and most of those studies are concerned with how behaviour is correlated with reproductive success (Smith & Blumstein, 2008). In this study, we have demonstrated that behavioural phenotype (or behavioural type) and behavioural flexibility appear to be related to fitness-linked traits, specifically diet, condition, size and growth rates, in juvenile yellow perch. In addition, individual behaviour and morphology seemed to be predictive of short- and long-term survivorship. Genetic architecture (i.e. local adaptation) and early rearing environment appeared to have an influence on behavioural phenotype and its change over time, in addition to its effects on survival.

Although treatments did not seem to differ overall behavioural phenotype at trial 1 and 3 (i.e. minimal treatment effects), they did differ in the relationship between fitness-linked traits and behaviour. At trial 1, individuals in lower body condition and lower diet score (i.e. eating relatively lower trophic level foods) generally were more active, neophilic and more exploratory (i.e. higher BP scores), but this relationship was reversed for the CH treatment group, with CH having slightly higher behavioural phenotype score overall than W-CL and WH. Assuming that our diet score is indicative of diet in the early rearing environment (before being sampled and brought into tanks).
and represents relative differences in diet between individuals, our results indicate bolder, more active, more exploratory and higher-condition individuals from CH may have already begun an ontogenetic diet shift towards cannibalism in their early rearing environment. Yellow perch are known to undergo an ontogenetic diet shift towards piscivory and even cannibalism early in life (Fullhart et al. 2002), with the shift happening when individuals are as small as 80 mm total length (Graeb et al. 2006). The fact that lower condition, lower diet score individuals from W-CL and WH exhibited high activity, high neophilia and were more exploratory suggests a growth-mortality trade-off, with individuals prioritizing foraging activity above predator avoidance. This observation is in line with the “metabolic hypothesis”, which suggests that smaller or poorer condition individuals who have higher relative metabolic needs and/or less energy reserves will be bolder (e.g. emerge from a refuge sooner) in order to forage and increase energetic gains (e.g. Brown et al. 2005; Krause et al. 1998).

At trial 3, four months after the beginning of the behavioural trials, and despite all being moved to a common environment, behavioural phenotype was again best explained by condition, diet and, this time, by size. Individuals in lower condition and with a lower diet score generally had a higher phenotype score, consistent with trial 1. The loss of the interactions between treatment and diet/condition were likely a result of either perch adjusting all in the same manner to the common environment despite rearing/genetic background, or reduction in power to detect interaction effects due to mortality between trial times. For this same reason, the interaction between treatment and size should be interpreted with caution. The consistent relationship observed between condition, diet and behavioural phenotype over the course of the trials, and despite early mortalities, suggests an ecologically-meaningful phenomenon. As discussed above, these results at trial 3 are consistent with the metabolic hypothesis, linking behavioural repertoire to an individual’s underlying state through a fitness correlate such as body condition. For example, in zebra finches (Taeniopygia guttata), proactive individuals and individuals in poorer body condition were more motivated to feed following food deprivation than reactive individuals in better condition (David et al. 2012). Similar results, linking smaller size to riskier behaviour (e.g. shorter shelter emergence times), have been observed in tropical poeciliids (Brachyrphis episcopi; Brown et al. 2005; Brown & Braithwaite, 2004), and
in banded killifish (*Fundulus diaphanous*), even under predation threat (Dowling & Godin, 2002). Behaviour of yellow perch, measured as boldness and exploration in novel and risky contexts, seemed to be related to an individual’s body condition and diet throughout the study.

Many of the individual yellow perch changed their phenotype scores over time, with the majority increasing from trial 1 to trial 3 (i.e., becoming more active, bolder, and antipredator responsive). There was no evidence, from Chapter 2 or 3, that yellow perch exhibit behavioural syndromes, as they change their behaviour over time and to different degrees. This change in phenotype was largely explained by condition, change in diet score and whether or not individuals increased or decreased their behavioural phenotype over time. Individuals who were initially in better condition showed a greater change in phenotype score over time, along with a relatively fixed (or reduction in) diet score. Few studies have attempted to link individual behaviour to diet, but this study shows that a larger increase in $\delta^{15}$N and $\delta^{13}$C (which we are interpreting as a diet shift towards higher trophic level foods or cannibalism of other perch in their common tanks) was correlated with a more fixed behavioural phenotype. In addition, individuals who became *more* exploratory, active, and neophilic over time showed a larger absolute change in their phenotype score, than individuals who became *less* exploratory, active, and neophilic over time. These findings suggest that the yellow perch in this study exhibit a coping style, with some individuals changing their phenotype relatively little over time and others showing larger increases or decreases over time. Individuals across taxa with proactive and reactive coping styles do indeed show different levels of boldness and behavioural flexibility; proactive individuals are generally bolder and readily form routines, showing minimal flexibility whereas reactive individuals are shyer and react to their environment with flexible behaviour (Koolhaas et al. 1999). Compared to W-CL and WH, the treatment group CH generally showed the highest behavioural phenotype score over the course of time, suggesting that individuals in that treatment group show proactive, bold phenotypes, with relatively fixed diets as a product of adaptation to their local conditions. The relatively small, highly competitive, high predation risk pond (i.e. highly complex) that CH became adapted to over multiple generations may have favoured relatively fixed, active, neophilic, exploratory, antipredator phenotypes due to
the potential costs associated with more flexible behaviour in these types of environments. Behavioural flexibility can be risky and/or costly, in terms of growth, predation risk and ultimately survival, if an individual cannot gain reliable information about the environment to produce an appropriate phenotype (Dewitt et al. 1998). The CH treatment may have adopted a relatively proactive phenotype because the risk of obtaining information about the environment or receiving inaccurate information (e.g. predation risk) may outweigh the benefits and expose individuals to unnecessary risk (Komers, 1997).

Yellow perch in this study showed behavioural flexibility for activity over ontogeny. Individual behavioural flexibility was best explained by a change in diet score and specific growth rate, regardless of direction of the flexibility (i.e. increase or decrease in activity) over time. More actively flexible individuals tended to change their diet score more over time, and had a slightly higher growth rate over the course of the trials, suggesting a link between flexible activity and feeding. Assuming that a change in our “diet score” reflects a true change in an individual’s diet over time (i.e. shifting to other foods or cannibalism of other perch in common tanks), some of the individuals in our study adjusted their diet more than others. Few studies have examined the link between behavioural flexibility and flexibility in diet or foraging (Herborn et al. 2014; Sol et al. 2002). Some individuals in our study may not have made the transition from live food in their early rearing environments to the commercial fish food they were given in captivity as well as others did, and this reflected as a relatively fixed diet. Findings from aquaculture show that Eurasian perch and yellow perch do not always transition well from live feed to formulated feed (Kestemont et al. 2015). In addition, faster-growing individuals were slightly more behaviourally flexible (for activity) than slower-growing individuals. A similar pattern was observed in a study on brown trout (Salmo trutta), where individuals who were more flexible in their foraging activity grew faster than individuals with more consistent foraging behaviour (Adriaenssens & Johnsson, 2010). If individuals who are more behaviourally flexible are also more flexible in terms of their diet, they may be able to use multiple different foraging techniques (Sol et al. 2002) and/or are quicker to exploit more energetically-rich food sources (e.g. live prey vs.
pellets; Braithwaite & Salvanes, 2005), which could allow them to experience higher-growth rates.

Previous studies have attempted to predict individual survival by examining personality traits (Niemela et al. 2015; Smith & Blumstein, 2010). In this study, we found that survival of an individual could be predicted by its initial (early) level of neophilia, behavioural flexibility and its morphology (i.e. size and condition). Individuals who survived the longest (i.e. up until 1 year following the end of behavioural trials; overwintering in a semi-natural pond) were those who were initially less neophilic, less behaviourally flexible (for activity), and were larger and in better condition than individuals who did not survive. The relationship between body condition and survival is well established; however, the link between individual behaviour and survival is less well understood. For example, more active, bolder and more exploratory (or more neophilic) guppies (*Poecilia reticulata*) survived longer when exposed to a predator than less active, shyer and less exploratory (or more neophobic) individuals (Smith & Blumstein, 2010). Less active individuals experienced higher mortality than active individuals in brown trout (*Salmo trutta*) released into the wild (Adriaenssens & Johnsson, 2013). On the other hand, bolder individuals (i.e. shorter flight initiation distance) did not survive as long as shyer individuals in field cricket nymphs (*Gryllus campestris*) in the wild (Niemela et al. 2015). It is likely that growth-mortality trade-offs and environmental factors drive differences in personality-related survival. Bolder individuals expose themselves more to predation risk, but gain more access to resources, whereas shyer individuals reduce their predation risk but may have limited access to the resources necessary to grow and survive (Stamps, 2007). In our study, boldness (measured as willingness to approach a novel object) was negatively related to survival, with shyer (more neophobic) individuals tending to survive more than bolder individuals. This observation may be due to the fact that bolder individuals exposed themselves more to risk, and therefore suffered higher mortality; another more unlikely explanation may be that neophilia is indirectly correlated with another trait that is more relevant for fitness. Our finding that larger, better condition individuals survived more than smaller, poorer condition individuals is consistent with the results found in other studies (McCormick & Meekan, 2010; White et al. 2013). This suggests that larger yellow perch grew more or grew faster to reach a size
refuge and escape gape-limited predation from other cannibalistic perch (Persson et al. 1996). In the long-term, WH had the lowest survival rate with no individuals surviving over winter up to one year following behavioural trials. WH individuals were much smaller and in poorer condition than W-CL and CH when released into the over wintering ponds, therefore they may not have had the energy reserves necessary to survive the winter, or may have exposed themselves to more risk while trying to acquire resources.

It is hypothesized that individual behaviour may affect fitness correlates, or vice versa, however, relatively little is actually known, especially in fish species. This represents an important avenue of research for the Great Lakes as fish provide a significant amount of protein for human consumption (Baldwin et al. 2009), in addition to the food webs that rely on the critical links provided by fish species (Sanderson et al. 1999). Determining how behaviour is linked to fitness outcomes will provide information that is valuable to conservation efforts in the wild and for increasing the success of captive breeding and reintroduction programs (McDougall et al. 2005). For example, cod (**Gadus morhua**) reared in captivity with variable spatial cues (i.e. addition of visual stimuli) and variable foraging cues (i.e. randomized feeding regime) were more behaviourally flexible in terms of their ability to recover from a stressor and exploit novel prey, as opposed to individuals raised in a standard hatchery environment (Braithwaite & Salvanes, 2005). Fish raised in captive conditions have vastly different behavioural repertoires than wild fish, often showing reduced antipredator responses, increased aggression, and rapid growth rates, all of which can contribute to poor post-release survival in reintroduction programs (Huntingford, 2004) or could potentially create problems for native wild fish (e.g. competition, hybridization) due to aquaculture escapees (Biro et al. 2004). Aquaculture operations would benefit from this knowledge as well to optimize production of protein and long-term sustainability. We have shown that less flexible (i.e. more proactive), larger and better condition individuals had higher survival rates up to a year and a half following the start of behavioural trials. This information could be used as an early screening tool to identify robust individuals meant for reintroduction programs. In addition to natural stressors experienced by all organisms in the wild, individuals are faced with multiple anthropogenic stressors in the wild including habitat degradation, climate change, pollution and exploitation by fishing.
These stressors can act to artificially select for certain personality types based on their vulnerability to these stressors. For instance, commercial gillnet fisheries can indirectly select against faster-growing individuals because their more active, bolder personalities make them more vulnerable to capture, reducing fitness of those personality types and artificially selecting for slower-growing individuals (Biro & Post, 2008). Our study shows that behaviour and survival of yellow perch are linked to fitness correlates (morphology, diet and growth rate), rearing environment and potentially, adaptation to local conditions. Some of our results suggest that behaviour, and ultimately survival, are determined by an individual’s internal state (i.e. metabolic hypothesis), however, future studies should directly investigate whether differences in metabolism (i.e. resting metabolic rate) drive variation in boldness and exploration. These findings have implications for yellow perch, and fish species in general, in a changing climate, especially with the projected increases in water temperature. As an increase in water temperature drives up the metabolic needs of fish, individuals (especially of small size) will have to increase activity and forage more to keep up with energetic demands (Ficke et al. 2007), thus exposing themselves more to predation risk and decreasing survival rates (Biro et al. 2007). In the face of multiple interacting stressors, such as fisheries exploitation and competitive invasive species, the effect of behaviour and morphology on survival may be even more pronounced, but further studies are needed to elucidate this effect.
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Figure 3.1 Timeline of components of the study. SIA=stable isotope analysis. PIT=Passive Integrated Transponder.
Figure 3.2 Individual behavioural phenotype scores at trials 1 (January 2014) and 3 (May 2014)
Figure 3.3 Relationship between behavioural phenotype score and the fitness correlates, size (a), interaction of treatment and condition (b), and interaction of treatment and diet score (c) at trial 1 (n=46). For interaction terms: CH = solid line, circle; W-CL = dashed line, cross; WH = dotted line, diamond. Treatment medians and quartiles are shown in (d)
Figure 3.4 Relationship between behavioural phenotype score and the fitness correlates, interaction of treatment and size (a), condition (b), and diet score (c) at trial 3 (n=23). For interaction term: CH = solid line, circle; W-CL = dashed line, cross; WH = dotted line, diamond. Treatment medians and quartiles are shown in (d)
Figure 3.5 Relationship between change in behavioural phenotype score (ΔBP) and the fitness correlates, initial size (a), initial condition (b), change in diet score (c), and direction of phenotypic change (d) between trials 1 and 3 (n=14)
Figure 3.6 Relationship between degree of behavioural flexibility for activity and the fitness correlates, initial size (a), change in diet score (b) and specific growth rate (c) (n=17). Treatment medians and quartiles are shown in (d)
Figure 3.7 Proportion of survivors (grey) and mortalities (black) for each treatment group up until the end of trial 3 (short-term survivorship; May 2014)
Figure 3.8 Relationship between short-term survivorship (to the end of trial 3; May 2014) and (a) initial neophilia score, (b) degree of behavioural flexibility and (c) initial condition. Medians and quartiles are shown.
Figure 3.9 Proportion of survivors (grey) and mortalities (black) for each treatment group up until April 2015 (long-term survivorship; April 2015).
Figure 3.10 Relationship between long-term survivorship (~1 year after end of behavioural trials) and (a) initial neophilia score, (b) degree of behavioural flexibility, (c) initial condition and (d) initial size. Medians and quartiles are shown.
Table 3.1 Principal component analysis loadings of morphological measurements of yellow perch at trials 1 and 3 (pooled together)

<table>
<thead>
<tr>
<th></th>
<th>Median (Range)</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fork Length (mm)</td>
<td>87.6 (61.3-108.7)</td>
<td>0.96</td>
<td>0.26</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>4.4 (1.5-9.5)</td>
<td>0.90</td>
<td>0.41</td>
</tr>
<tr>
<td>Relative Body Depth</td>
<td>0.18 (0.15-0.22)</td>
<td>0.32</td>
<td>0.95</td>
</tr>
<tr>
<td>Variance Explained (%)</td>
<td>82.51</td>
<td>16.32</td>
<td></td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>2.48</td>
<td>0.49</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.2 Principal component analysis loadings from PCA conducted on behaviour scores for trials 1 and 3

<table>
<thead>
<tr>
<th>Behavioural Score</th>
<th>PC Loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>0.72</td>
</tr>
<tr>
<td>Exploration</td>
<td>0.51</td>
</tr>
<tr>
<td>Neophilia</td>
<td>0.70</td>
</tr>
<tr>
<td>Stimulus Response</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Variance Explained (%)</strong></td>
<td><strong>38.06</strong></td>
</tr>
<tr>
<td><strong>Eigenvalue</strong></td>
<td><strong>1.52</strong></td>
</tr>
</tbody>
</table>
Table 3.3 Model structures and selection criteria for models constructed for behavioural phenotype score at trial 1 (BP1; n=46)

<table>
<thead>
<tr>
<th>Model Number</th>
<th>Model Structure</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>wi</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (global)</td>
<td>BP1 = Treatment × Size + Treatment × Condition + Treatment × Diet Score</td>
<td>148.3</td>
<td>11.6</td>
<td>0</td>
<td>0.34</td>
</tr>
<tr>
<td>2</td>
<td>BP1 = Size + Treatment × Condition + Treatment × Diet Score</td>
<td>136.7</td>
<td>0</td>
<td>0.88</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>BP1 = Treatment × Size + Treatment × Diet Score</td>
<td>144.2</td>
<td>7.5</td>
<td>0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>4</td>
<td>BP1 = Size + Condition + Treatment × Diet Score</td>
<td>144.1</td>
<td>7.4</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>BP1 = Treatment × Size + Treatment × Condition</td>
<td>207.9</td>
<td>71.2</td>
<td>0</td>
<td>0.23</td>
</tr>
<tr>
<td>6</td>
<td>BP1 = Size + Treatment × Diet Score</td>
<td>141.6</td>
<td>4.9</td>
<td>0.08</td>
<td>0.06</td>
</tr>
</tbody>
</table>

The presence of an interaction term implies the inclusion of the main effects. Shown for each model are: AICc, relative difference within each set of models (ΔAICc), Akaike weights (wi) and $r^2$ as a measure of model fit. Models are shown in the format: response variable = fixed main effects + interaction terms (indicated by “×”). The most likely model (according to the ΔAICc, Akaike weight) is shown in bold.
Table 3.4 Model structures and selection criteria for models constructed for behavioural phenotype score at trial 3 (BP3; n=23)

<table>
<thead>
<tr>
<th>Model Number</th>
<th>Model Structure</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>wi</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (global)</td>
<td>BP3 = Treatment × Size + Treatment × Condition + Treatment × Diet Score</td>
<td>66.6</td>
<td>39.8</td>
<td>0</td>
<td>0.55</td>
</tr>
<tr>
<td>2</td>
<td>BP3 = Size + Treatment × Condition + Treatment × Diet Score</td>
<td>34.7</td>
<td>7.9</td>
<td>0.01</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>BP3 = Treatment × Size + Treatment × Diet Score</td>
<td>31.4</td>
<td>4.6</td>
<td>0.05</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td>BP3 = Treatment × Size + Condition + Diet Score</td>
<td>26.8</td>
<td>0</td>
<td>0.47</td>
<td>0.43</td>
</tr>
<tr>
<td>5</td>
<td>BP3 = Size + Condition + Treatment × Diet Score</td>
<td>29.1</td>
<td>2.3</td>
<td>0.15</td>
<td>0.37</td>
</tr>
<tr>
<td>6</td>
<td>BP3 = Treatment × Size + Treatment × Condition</td>
<td>31.1</td>
<td>4.3</td>
<td>0.01</td>
<td>0.48</td>
</tr>
<tr>
<td>7</td>
<td>BP3 = Size + Treatment × Diet Score</td>
<td>27.9</td>
<td>1.1</td>
<td>0.27</td>
<td>0.28</td>
</tr>
</tbody>
</table>

The presence of an interaction term implies the inclusion of the main effects. Shown for each model are: AICc, relative difference within each set of models (ΔAICc), Akaike weights (wi) and r² as a measure of model fit. Models are shown in the format: response variable = fixed main effects + interaction terms (indicated by “×”). The most likely model (according to the ΔAICc, Akaike weight) is shown in bold.
**Table 3.5** Model structures and selection criteria for models constructed for change in behavioural phenotype score from trial 1 to trial 3 (ΔBP; $n=14$)

<table>
<thead>
<tr>
<th>Model Number</th>
<th>Model Structure</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>$w_i$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (global)</td>
<td>$\Delta BP = \text{ConditionT1} + \text{SizeT1} + \Delta \text{Diet Score} + \Delta \text{Size} + \Delta \text{Condition} + \text{SGR} + \text{BP1} \times \text{DPC}$</td>
<td>262.5</td>
<td>245.1</td>
<td>0</td>
<td>0.84</td>
</tr>
<tr>
<td>2</td>
<td>$\Delta BP = \text{ConditionT1} + \text{SizeT1} + \Delta \text{Diet Score} + \Delta \text{Size} + \Delta \text{Condition} + \text{BP1} \times \text{DPC}$</td>
<td>106.8</td>
<td>89.4</td>
<td>0</td>
<td>0.84</td>
</tr>
<tr>
<td>3</td>
<td>$\Delta BP = \text{ConditionT1} + \text{SizeT1} + \Delta \text{Diet Score} + \Delta \text{Size} + \text{BP1} \times \text{DPC}$</td>
<td>54.9</td>
<td>37.5</td>
<td>0</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>$\Delta BP = \text{ConditionT1} + \text{SizeT1} + \Delta \text{Diet Score} + \Delta \text{Size} + \text{DPC}$</td>
<td>24.5</td>
<td>7.1</td>
<td>0.03</td>
<td>0.61</td>
</tr>
<tr>
<td>5</td>
<td>$\Delta BP = \text{ConditionT1} + \text{SizeT1} + \Delta \text{Diet Score} + \text{DPC}$</td>
<td>17.4</td>
<td>0</td>
<td>0.97</td>
<td>0.41</td>
</tr>
</tbody>
</table>

The presence of an interaction term implies the inclusion of the main effects. T1 = Trial 1. SGR = Specific growth rate. BP1 = behavioural phenotype score at trial 1. DPC = direction of phenotypic change (+/-). Shown for each model are: AICc, relative difference within each set of models (ΔAICc), Akaike weights ($w_i$) and $r^2$ as a measure of model fit. Models are shown in the format: response variable = fixed main effects + interaction terms (indicated by “×”). The most likely model (according to the ΔAICc, Akaike weight) is shown in bold.
Table 3.6 Model structures and selection criteria for models constructed for degree of behavioural flexibility for activity (BFA; n=17)

<table>
<thead>
<tr>
<th>Model Number</th>
<th>Model Structure</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>$w_i$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (global)</td>
<td>$BFA = \text{Treatment} \times \Delta\text{Condition} + \text{Treatment} \times \Delta\text{Size} + \text{Treatment} \times \text{SGR} + \text{Treatment} \times \text{Condition} + \Delta\text{Size} + \Delta\text{Diet Score} + \text{Activity}T1$</td>
<td>488.5</td>
<td>482.8</td>
<td>0</td>
<td>0.67</td>
</tr>
<tr>
<td>2</td>
<td>$BFA = \text{Treatment} \times \Delta\text{Condition} + \text{Treatment} \times \Delta\text{Size} + \text{Treatment} \times \text{SGR} + \text{Treatment} \times \text{Condition} + \Delta\text{Diet Score} + \text{Activity}T1$</td>
<td>216.5</td>
<td>210.8</td>
<td>0</td>
<td>0.67</td>
</tr>
<tr>
<td>3</td>
<td>$BFA = \text{Treatment} \times \Delta\text{Condition} + \text{Treatment} \times \Delta\text{Size} + \text{Treatment} \times \text{SGR} + \text{Treatment} \times \text{Condition} + \Delta\text{Diet Score} + \text{Activity}T1$</td>
<td>126.0</td>
<td>120.3</td>
<td>0</td>
<td>0.66</td>
</tr>
<tr>
<td>4</td>
<td>$BFA = \text{Treatment} \times \Delta\text{Condition} + \text{Treatment} \times \text{Condition} + \Delta\text{Diet Score} + \text{Activity}T1$</td>
<td>54.1</td>
<td>48.4</td>
<td>0</td>
<td>0.65</td>
</tr>
<tr>
<td>5</td>
<td>$BFA = \text{Treatment} \times \Delta\text{Condition} + \text{Treatment} \times \text{Condition} + \text{Activity}T1$</td>
<td>36.3</td>
<td>30.6</td>
<td>0</td>
<td>0.64</td>
</tr>
<tr>
<td>6</td>
<td>$BFA = \text{Treatment} \times \Delta\text{Condition} + \text{Treatment} \times \text{Condition} + \text{Activity}T1$</td>
<td>23.9</td>
<td>18.2</td>
<td>0.01</td>
<td>0.63</td>
</tr>
<tr>
<td>7</td>
<td>$BFA = \text{Treatment} \times \Delta\text{Condition} + \text{Treatment} \times \text{Condition} + \text{Activity}T1$</td>
<td>11.0</td>
<td>5.3</td>
<td>0.99</td>
<td>0.53</td>
</tr>
<tr>
<td>8 (global)</td>
<td>$BFA = \text{Treatment} \times \Delta\text{Condition} + \Delta\text{Diet Score} + \text{Activity}T1$</td>
<td>5.7</td>
<td>0</td>
<td>0.99</td>
<td>0.50</td>
</tr>
</tbody>
</table>

The presence of an interaction term implies the inclusion of the main effects. T1 = Trial 1. SGR = Specific growth rate. ActivityT1 = Activity score at trial 1. Shown for each model are: AICc, relative difference within each set of models (ΔAICc), Akaike weights ($w_i$) and $r^2$ as a measure of model fit. Models are shown in the format: response variable = fixed main effects + interaction terms (indicated by “×”). The most likely model (according to the ΔAICc, Akaike weight) is shown in bold.
Table 3.7 Model structures and selection criteria for generalized linear survivorship models constructed for survival up until trial 3 (n=70) using initial (trial 1) behaviour scores

<table>
<thead>
<tr>
<th>Model Number</th>
<th>Model Structure</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>(w_i)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (global)</td>
<td>Survival = Activity + Neophilia + Exploration + Stimulus Response + BFA + Treatment (\times) SizeT1 + Treatment (\times) ConditionT1</td>
<td>100.6</td>
<td>10.5</td>
<td>0</td>
<td>0.326</td>
</tr>
<tr>
<td>2</td>
<td>Survival = Neophilia + Exploration + Stimulus Response + BFA + Treatment (\times) SizeT1 + Treatment (\times) ConditionT1</td>
<td>97.8</td>
<td>7.7</td>
<td>0.01</td>
<td>0.324</td>
</tr>
<tr>
<td>3</td>
<td>Survival = Neophilia + Exploration + Stimulus Response + BFA + Treatment (\times) SizeT1 + ConditionT1</td>
<td>93.9</td>
<td>3.8</td>
<td>0.04</td>
<td>0.303</td>
</tr>
<tr>
<td>4</td>
<td>Survival = Neophilia + Stimulus Response + BFA + Treatment (\times) SizeT1 + ConditionT1</td>
<td>91.9</td>
<td>1.8</td>
<td>0.11</td>
<td>0.293</td>
</tr>
<tr>
<td>5</td>
<td>Survival = Neophilia + BFA + Treatment (\times) SizeT1 + ConditionT1</td>
<td>90.3</td>
<td>0.2</td>
<td>0.25</td>
<td>0.282</td>
</tr>
<tr>
<td>6</td>
<td>Survival = Neophilia + Stimulus Response + BFA + Treatment + SizeT1 + ConditionT1</td>
<td>90.4</td>
<td>0.3</td>
<td>0.23</td>
<td>0.254</td>
</tr>
<tr>
<td>7</td>
<td>Survival = Neophilia + Stimulus Response + BFA + SizeT1 + ConditionT1</td>
<td>90.1</td>
<td>0</td>
<td>0.27</td>
<td>0.204</td>
</tr>
</tbody>
</table>

The presence of an interaction term implies the inclusion of the main effects. T1 = Trial 1. BFA = degree of behavioural flexibility for activity. Shown for each model are: AICc, relative difference within each set of models (ΔAICc), Akaike weights \(w_i\) and \(r^2\) as a measure of model fit. Models are shown in the format: response variable = fixed main effects + interaction terms (indicated by “\(\times\)”). The most likely model (according to the ΔAICc, Akaike weight) is shown in bold. \(r^2\) = McFadden’s pseudo \(r^2\)
### Table 3.8 Model structures and selection criteria for generalized linear survivorship models constructed for long-term survival (n=70) using initial (trial 1) behaviour scores

<table>
<thead>
<tr>
<th>Model Number</th>
<th>Model Structure</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>(w_i)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (global)</td>
<td>(\text{Survival} = \text{Activity} + \text{Neophilia} + \text{Exploration} + \text{Stimulus Response} + \text{BFA} + \text{Treatment} \times \text{SizeT1} + \text{Treatment} \times \text{ConditionT1})</td>
<td>83.2</td>
<td>19.5</td>
<td>0</td>
<td>0.367</td>
</tr>
<tr>
<td>2</td>
<td>(\text{Survival} = \text{Activity} + \text{Neophilia} + \text{Exploration} + \text{Stimulus Response} + \text{BFA} + \text{SizeT1} + \text{Treatment} \times \text{ConditionT1})</td>
<td>77</td>
<td>13.3</td>
<td>0</td>
<td>0.367</td>
</tr>
<tr>
<td>3</td>
<td>(\text{Survival} = \text{Activity} + \text{Neophilia} + \text{Exploration} + \text{BFA} + \text{SizeT1} + \text{Treatment} \times \text{ConditionT1})</td>
<td>74.1</td>
<td>10.4</td>
<td>0</td>
<td>0.367</td>
</tr>
<tr>
<td>4</td>
<td>(\text{Survival} = \text{Activity} + \text{Neophilia} + \text{Exploration} + \text{BFA} + \text{Treatment} + \text{SizeT1} + \text{ConditionT1})</td>
<td>68.9</td>
<td>5.2</td>
<td>0.05</td>
<td>0.364</td>
</tr>
<tr>
<td>5</td>
<td>(\text{Survival} = \text{Activity} + \text{Neophilia} + \text{BFA} + \text{Treatment} + \text{SizeT1} + \text{ConditionT1})</td>
<td>66.2</td>
<td>2.5</td>
<td>0.21</td>
<td>0.364</td>
</tr>
<tr>
<td>6</td>
<td>(\text{Survival} = \text{Neophilia} + \text{BFA} + \text{Treatment} + \text{SizeT1} + \text{ConditionT1})</td>
<td>63.7</td>
<td>0</td>
<td>0.73</td>
<td>0.362</td>
</tr>
</tbody>
</table>

The presence of an interaction term implies the inclusion of the main effects. BFA = degree of behavioural flexibility for activity. Shown for each model are: AICc, relative difference within each set of models (ΔAICc), Akaike weights (\(w_i\)) and \(r^2\) as a measure of model fit. Models are shown in the format: response variable = fixed main effects + interaction terms (indicated by “×”). The most likely model (according to the ΔAICc, Akaike weight) is shown in bold. \(r^2\) = McFadden’s pseudo \(r^2\)
Chapter 4: General Discussion

The primary goals of this thesis were to investigate behavioural repertoire and its consequences for fitness and survival in a significant Great Lakes fish species, the yellow perch. The behavioural repertoire of *Perca flavescens*, investigated for the first time in this thesis, appears to be influenced by a combination of early environmental experience, adaptation to conditions over many generations (i.e. genetic architecture), and change over ontogeny. The behavioural phenotype appears to be characterized by plasticity in the form of coping styles that are dependent on behavioural type: individuals showed an $I \times E$ interaction for activity over time (Table 4), with more extreme phenotypes showing greater flexibility. In addition, individuals changed their overall behavioural phenotype over time to different degrees: those individuals who became more active, neophilic, exploratory and antipredator responsive over time showed a greater phenotypic change than individuals who maintained a relatively fixed phenotype or became less active, less neophilic, less exploratory and showed less antipredator responsiveness. Finally, an individual’s behavioural phenotype and fitness correlates, such as size and condition, were predictive of early mortality.

The behavioural repertoire of an individual, which changes over time, appears to be influenced by both early rearing experience and local adaptation (i.e. genetic background). Levels of behaviours which can have a large impact on competitive ability and survival, such as activity, neophilia (e.g. exploiting novel resources), and exploration seem to be related to the intensity of competition and predation in the environment. Individuals from the treatment that was both adapted to and reared in the highly complex captive pond environment (i.e. CH; long time established, high density of competition and predation in small area) were generally the most active, neophilic, exploratory and responsive to predators. Whether these results are due to local adaptation over many generations to conditions in captivity or are a product of early rearing is difficult to determine; however, the fact that W-CL (pond-raised) individuals were more active and exploratory than the closely related WH (wild-raised) individuals, from the same cohort from Balsam Lake, ON, suggests an effect of early rearing experience behaviour (i.e.}
captive pond environments), as W-CL and WH experienced different environments early in their rearing (Chapter 2, Table 2.1). Previous studies on the closely related Eurasian perch (*Perca fluviatilis*) support this idea that early experience affects exploratory and bold behaviours more so than local adaptation (Hellstrom & Magnhagen, 2011; Magnhagen et al. 2012). It seems that pond environments may directionally select for certain types of behaviours, likely due to intensity of competition and availability of food; for example, individual sticklebacks (*Pungitius pungitius*) originating from pond environments were quicker to feed, were more risk-taking and more aggressive than individuals from marine environments, even when reared in a common garden environment (Herczeg & Valimaki, 2011). Threespined sticklebacks (*Gasterosteus aculeatus*) reared in high predation pond environments were generally bolder (in terms of foraging and shelter emergence) and more active than river or low predation ponds populations (Brydges et al. 2008). Our results, and the findings from other studies, suggest that the behavioural repertoire of yellow perch is largely affected by early rearing experiences (e.g. predation regime, intensity of competition) and, to some extent, local adaptation.

The behavioural repertoire of yellow perch in our study changed over time, from approximately 9 months up to 13 months after hatching. Activity, neophilia, exploration and antipredator behaviours seem to attenuate over time to a similar degree for all three treatments. This attenuation may be a response to life in the common tank environment; selection is often relaxed in captive hatchery and tank environments as a result of reduced predation risk and the abundant food provided (McPhee, 2003). If individual yellow perch were in fact responding to life in the common tank environment, than we might expect the three treatments to exhibit different levels of the behaviours initially (as a product of their early rearing environment), with all individuals eventually converging over time as a response to life in captivity (i.e. treatment differences in trajectories of behaviour). However, the fact that the there were no between-treatment differences in behavioural trajectories over time suggests that a common factor, likely age, was affecting all treatments, with treatments showing different levels of a behaviour at each time point. Other studies have reported changes in behaviours over ontogeny (Adriaennsens & Johnsson, 2013; Budaev et al. 1999; Edenbrow & Croft, 2011); our
results, and support from other studies, show that behaviour changes over ontogeny, and this change is likely due to passage through critical developmental stages, or the experience of changes in selection pressures (changes in food types, predator regimes, habitat shifts, etc.), as organisms age.

The data presented in this thesis represents baseline behavioural data, and its extrapolation to wild environments, with multiple, interacting stressors, is challenging. One of the most significant stressors of our time, which affects all organisms, is human-induced climate change. Climate change has the potential to affect species phenology, cause range and distribution shifts, change community structures, and disrupt trophic interactions among organisms (Walther et al. 2002). Organisms may find themselves forced into novel environments, either through climate change-induced range shifts or through stochastic events, where their survival will depend on their phenotypic response to the change; part of this phenotypic response is the behaviour of an organism. Populations that are made up of individuals who are more behaviourally flexible or that have a greater diversity of behavioural types may be able to survive better in the face of environmental change (Smith & Blumstein, 2013). In the case of yellow perch, it appears that they may display coping styles characterized by behavioural types and some flexibility. Individuals at the extremes of activity phenotype (i.e. very inactive vs. very active) show more flexibility in their activity levels than individuals with a more intermediate activity level. Showing flexibility for activity may be advantageous in a changing environment where situations, such as fluctuations in temperature or the introduction of a novel invasive predator species, may necessitate a change in activity level in order to survive. Individual yellow perch did not appear to show flexibility for neophilia, exploration or antipredator response however, they did appear to show behavioural phenotypes. The presence of these behavioural phenotypes, classified as high, intermediate and low for all of the behaviours tested (activity, neophilia, exploration, antipredator) demonstrate that yellow perch populations may possess some behavioural diversity. The presence of this behavioural diversity could allow yellow perch to adapt to current and future stressors if at least some of the behavioural phenotypes can respond appropriately to a stressor (Sih et al. 2004), however the fact that the species will likely be faced with multiple, interacting stressors warrants further study.
Environmental changes and stressors, including pollution, habitat degradation, exploitation, introduction of invasive species and climate change, are occurring at unprecedented rates. For yellow perch and other important fish species, future behavioural studies should incorporate individual stressors into their designs to measure the effects on ecologically-relevant behaviours (e.g. antipredator response, neophilia) and survival. Researchers have already begun to investigate the effects of stressors on behaviour and survival; studies have looked at the effects of chemical pollutants (Mirza et al. 2009), increased water turbidity (Engstrom-Ost & Candolin, 2007), increased lake warming (Biro et al. 2007), fishing (Biro & Post, 2008) and invasive species introductions (Winandy & Denoel, 2015) on animal behaviour and, ultimately, fitness and survival. For example, in a manipulative whole-lake experiment, a warmer temperature year led to decreased survival of juvenile trout, compared to a colder temperature year; this was due to their increased metabolic needs, which resulted in increased activity and thus increased exposure to predation (Biro et al. 2007). Another recommended future avenue of study should look at whether chemical substances that are detected in the environment (e.g. heavy metals, pharmaceuticals) can induce behavioural changes in fish (Montiglio & Royaute, 2014; Scott & Sloman, 2004). In a study done on yellow perch, individuals exposed to environmentally-detectable concentrations of a psycho-therapeutic drug (oxazepam) showed increased activity, increased boldness and feeding rate, and reduced sociality (Brodin et al. 2013). Mirza et al. (2009) found that, compared to cleaner reference lakes, yellow perch from metal-contaminated (Cu, Ni, Zn) lakes showed a reduction in antipredator responses (i.e. freezing, activity) to a chemical predator stimulus (i.e. conspecific skin extract). Increases in water turbidity, especially through runoff of fertilizer and subsequent eutrophication, should be studied as a stressor that has the potential to impact survival of yellow perch in the Great Lakes. Increases in water turbidity due to eutrophication have already been shown to reduce foraging success in young yellow perch (Wellington et al. 2010) and future studies should examine the effects of turbidity on antipredator behaviours.

We found that individual behaviour and fitness correlates seemed to have consequences for survival over the short- and long-term in yellow perch. Understanding the links between behaviour and survival in yellow perch could prove valuable for
captive breeding and reintroduction programs to work effectively. Individuals who were more neophobic and less flexible for activity tended to survive better in a captive tank environment and in semi-natural conditions (i.e. pond) than individuals who were more neophilic and flexible for activity. Bold, neophilic individuals tend to expose themselves more to risk; this risk exposure may be part of a growth-mortality trade-off, where an individual is driven to acquire resources due to energetic demands, even in risky situations (Stamps, 2007). We also found that smaller and poorer condition individuals suffered higher mortality than larger, better condition individuals. These findings suggest that small, poor condition individuals exhibited higher neophilia, exposing themselves more to risk, as a consequence of their higher relative energetic needs, resulting in higher mortality rates likely due to cannibalism, being outcompeted by larger individuals, or simply not having enough energy stores to survive winter in the pond. If yellow perch are to be bred in captivity for reintroduction to the wild, the interactions between morphology (e.g. size and condition) and neophilia need to be considered in order to effectively screen for robust individuals with traits that promote survival in the wild. However, neophilia could actually prove to be an advantageous trait upon reintroduction to the wild, where environmental stochasticity and heterogeneity would necessitate an ability to exploit novel resources or habitats. Selecting for and breeding individuals who are neophilic, but only releasing them into the wild when they are large and in good condition, could prove favourable for reintroduction efforts, should they be required.

Overall, our study incorporated the effects of early rearing experience, local adaptation (or genetic background), and ontogeny on behaviour of yellow perch. We have shown that behaviour is generally a product of early rearing experiences in the form of habitat complexity, competition and predation and, that behaviours change over ontogeny, regardless of early rearing experience (Table 4). Notably, behavioural phenotype was linked to fitness correlates, such as size, condition and diet; and early behaviour and morphology were predictive of long-term survival. Our findings address proximate and ultimate questions, and stimulate further research into the effects of multiple, interacting stressors on the behavioural ecology of this important fish species.
References


Biro PA, Post JR (2008) Rapid depletion of genotypes with fast growth and bold personality traits from harvested fish populations. PNAS 105:2919-2922


Table 4 Summary of behavioural repertoire of yellow perch in this study at the individual-, treatment- and population-level over time (i.e. age, x-axis). Population-level results indicate the presence of behavioural plasticity (i.e. individual behavioural flexibility or diversity of behavioural types).

<table>
<thead>
<tr>
<th>Individual-Level</th>
<th>Treatment-Level</th>
<th>Population-Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploration</td>
<td>Activity</td>
<td>Neophilia</td>
</tr>
<tr>
<td>No Individual Effects</td>
<td>Individual Flexibility</td>
<td>No Individual Effects</td>
</tr>
</tbody>
</table>
Appendix A: Validating fin tissue as a non-lethal tissue for stable isotope analysis of yellow perch (*Perca flavescens*)

**Introduction**

Stable isotope analysis (SIA) is an important tool used in ecological studies examining food web structure, organic matter sources, and trophic relationships among organisms (Peterson & Fry, 1987; Schmidt et al. 2009; West et al. 2006). The isotope ratios most commonly measured are stable nitrogen ($\delta^{15}N$) and stable carbon ($\delta^{13}C$). Values of $\delta^{15}N$ are used to assess relative trophic position of consumers in food webs (Vander Zanden et al. 1997) and $\delta^{13}C$ can be used to trace dietary organic matter sources (Finlay et al. 2002; Peterson, 1999). Stable isotope analysis has also been used in studies tracking animal migration routes by comparing isotope signatures of animal tissues to local food webs (Hobson, 1999), as well as documenting species invasions in aquatic ecosystems (Vander Zanden et al. 1999).

Studies of stable isotope values in fish are usually performed using multiple tissue types, including muscle, liver, gonads (Jardine et al. 2005) and otoliths (Schwarcz et al. 1998), all of which are lethal methods that require sacrificing the animal. In studies involving endangered or rare species, developing non-lethal sampling techniques that do not affect species survival are paramount. Non-lethal sampling for SIA would also be useful in studies examining ontogenetic or life history stage diet shifts, where long-term monitoring of individuals of a species is necessary. Non-lethally sampled tissues that have been used for fish SIA include fins (Sanderson et al. 2009), scales (Kelly et al. 2006) and epidermal mucus (Robbins-Church et al. 2009). These studies of stable isotopes have shown correlations for both $\delta^{15}N$ and $\delta^{13}C$ between lethally- and non-lethally sampled tissue (Andvik et al. 2010; Fincel & VanDeHey, 2012; Sanderson et al. 2009); however, the magnitude of the correlation usually differs between species. These differences therefore necessitate developing species-specific models in order to use non-lethal sampling techniques in place of lethally-sampled tissues.
Preservation method can also significantly affect stable isotope values in animal tissues (Kelly et al. 2006; Olin et al. 2014; Sarakinos et al. 2002). Freezing and ethanol are two commonly used ways to preserve animal tissues when SIA cannot be performed immediately after sampling. Remote field work sites may require the use of ethanol to preserve samples where freezing may not be possible. Studies have shown preservation in ethanol can result in an increase (i.e. enrichment of the heavier isotope) in $\delta^{15}$N and $\delta^{13}$C isotopes in tissue samples relative to frozen or oven-dried samples (Andvik et al. 2010; Kaehler & Pakhmov, 2001; Sarakinos et al. 2002), while other studies have shown minimal effects of the preservative (Barrow et al. 2008; Hobson et al. 1997). Samples that become isotopically altered by a preservation medium could bias interpretation of ecosystem trophic dynamics, but might potentially provide a reliable indication of isotopic values if the magnitude and direction of the alteration is predictable and can be corrected. Furthermore, the magnitude and direction of stable isotope enrichment or depletion due to preservation varies between species and tissue type (Jardine et al. 2005; Kelly et al. 2006). Developing species-specific correction factors for different preservation methods would allow researchers to account for isotopic variation in tissue samples that is not naturally-occurring.

The aim of this study was to compare lethal and non-lethal SIA sampling techniques, as well as compare tissue preservation methods in order to develop species-specific correction factors that can be used for future SIA of yellow perch *Perca flavescens* (Mitchill 1814), a species that is important for both commercial and recreational fisheries in North America (Baldwin et al. 2009). The objectives of this study were to: (1) determine if caudal fin tissue could be used as a reliable non-lethal sampling alternative to muscle and liver tissue when measuring $\delta^{15}$N and $\delta^{13}$C; and (2) evaluate the effect of a preservation method (i.e. ethanol vs. frozen) on $\delta^{15}$N and $\delta^{13}$C isotopic values. Ultimately, the resulting correction factors could be used by future studies to estimate $\delta^{15}$N and $\delta^{13}$C values of fish liver and muscle using fin tissue, when keeping them alive is necessary, such as in long-term behavioural studies.
Materials and Methods

Sampling

Yellow perch were collected using minnow traps baited with food or light from holding ponds at Leadley Environmental Inc. in Essex, Ontario, Canada during the months of October - November 2013. After collection, the perch were immediately transported to the Great Lakes Institute for Environmental Research (GLIER) Aquatic Facility at the University of Windsor, Ontario, Canada and held until processing for SIA in February 2014. The yellow perch were kept in recirculating tanks under constant conditions (temperature: 17-19°C; pH: 7-8; dissolved oxygen: 6-9 mg/L; low intensity red light photoperiod 12L:12D). They were fed live blackworms (*Lumbriculus variegatus*) ad lib every 48 hours for the first month and then transitioned to a diet of frozen bloodworms and freeze-dried krill ad lib every 48 hours after that.

Twenty-four yellow perch ranging in standard length from 51.8 – 108.4 mm (mean ± SD: 90.7 ± 17.7 mm) and weight 2.0 – 15.7 g (mean ± SD: 9.2 ± 3.7 g) were dissected for SIA in February 2014. Yellow perch were measured for standard length (mm) and weight (g) prior to being euthanized by percussive stunning. Each fish was dissected and sampled for white muscle tissue (from the dorsal region) and whole liver which were frozen at -80°C for 2 months before SIA. Two caudal fin samples were obtained from each fish, with one being stored in 95% ethanol (for 5 months) and the other being frozen at -80°C (for 2 months) before analysis. The frozen fin sample was used in both sections of this study: 1) to compare with frozen muscle and liver to validate non-lethal sampling, and 2) to compare with the ethanol-preserved fin sample to correct for preservation technique. Experimental protocol followed the Canadian Council on Animal Care guidelines (AUPP #13-04).

Stable isotope analysis

Fin tissues preserved in 95% ethanol were drained, oven dried at 40°C for 24 hours and then rinsed with distilled water. At this point, all samples (including ethanol and frozen) were freeze-dried at -44°C for 40-48 hours. The muscle and liver tissues were
pulverised into a fine powder before weighing. The fin tissues were cut into smaller pieces before weighing. From each tissue sample, 0.4-0.6 mg was weighed out and placed into a tin capsule for SIA. Stable nitrogen (δ¹⁵N) and carbon (δ¹³C) isotopic values were determined using a Thermo Electron Delta V Advantage mass spectrometer interfaced to a Thermo Fisher Scientific Conflo IV elemental analyzer. The δ¹⁵N and δ¹³C values in the samples were determined using atmospheric nitrogen (N₂) and PeeDee Belemnite Limestone Formation as reference standards, respectively. Isotopic ratios were expressed in parts per thousand (‰) units with δ notation using the following formula: δX = [(R sample / R standard) − 1] × 10³, where X is either ¹⁵N or ¹³C with R sample and R standard being the ratios of ¹⁵N to ¹⁴N or ¹³C to ¹²C of the sample and the international standard, respectively.

The carbon-to-nitrogen ratio (C:N) was also determined for each sample. During the analysis, every 10th sample was run in triplicate to ensure analytical precision, in addition to analyzing standard reference materials (internal standard tilapia, n=50 for δ¹⁵N and δ¹³C; bovine liver, n=50 for δ¹⁵N and δ¹³C) after every 12 samples. National Institute of Standards and Technology (NIST) standard reference materials (L-glutamic acid, ammonium sulfate for δ¹⁵N; sucrose, L-glutamic acid for δ¹³C) were used to ensure accuracy over the course of the study. Analytical precision (SD) was 0.1 ‰ (tilapia) and 0.12 ‰ (bovine liver) for δ¹⁵N and 0.08 ‰ (tilapia) and 0.07 ‰ (bovine liver) for δ¹³C. Standard deviations of replicate samples (n=6 samples run in triplicate) were kept to less than 0.2 ‰ for both δ¹⁵N and δ¹³C.

Analytical accuracy of δ¹⁵N NIST standards was -4.41 ± 0.18 ‰ (L-glutamic acid) and 20.49 ± 0.18 ‰ (ammonium sulfate); accuracy of δ¹³C NIST standards was -10.55 ± 0.12 ‰ (sucrose) and -26.35 ± 0.07 ‰ (L-glutamic acid).

**Lipid Normalization**

Lipids were not extracted via solvents from each sample due to the potential for altering of δ¹⁵N values (Elsdon et al. 2010; Murry et al. 2006; Sotiropoulos et al. 2004). Instead, a mathematical lipid normalization to account for the effect of lipids, as they are typically more depleted in ¹³C relative to ¹²C (DeNiro & Epstein, 1977). The Post et al.
(2007) linear model uses the C:N of a non-lipid-extracted sample. This model relies on the known relationship between the C:N of a sample and the effect of lipids on the $\delta^{13}C$ ($\Delta\delta^{13}C$) of that sample:

$$\Delta\delta^{13}C = -3.32 + 0.99 \times \text{C:N}$$

This relationship was derived from stable isotope analysis of lipid-extracted and non-extracted samples from 16 aquatic organisms. Mathematical lipid correction was not applied to the fin tissue in the analysis of preservation medium as it is currently unknown how preservation in ethanol affects lipids in these tissue types. We compared lipid-uncorrected $\delta^{13}C$ from frozen fin tissue to lipid-uncorrected $\delta^{13}C$ from ethanol-preserved fin tissue.

Data analysis

To test for differences in $\delta^{15}N$ and $\delta^{13}C$ between lethal (muscle, liver) and non-lethal (frozen caudal fin) tissue sampling techniques and between the two preservation methods (freezing vs. 95% ethanol), we used paired t-tests. When differences between the sampling techniques or preservation methods were significant (at $p < 0.05$), a linear regression analysis was used to generate predictive models to estimate liver and muscle $\delta^{15}N$ and $\delta^{13}C$ from caudal fin $\delta^{15}N$ and $\delta^{13}C$ preserved by freezing. We examined lipid-corrected $\delta^{13}C$ stable isotope values for the analysis of lethal vs. non-lethal sampling, but uncorrected $\delta^{13}C$ values for the comparison of preservation method. The regression of isotopic values from samples that were frozen vs. samples preserved in ethanol (showing an effect of preservation method) would give us a model that would allow us to account for possible $^{15}N$ and $^{13}C$ enrichment by ethanol and correct for this effect in future studies where samples are preserved in this medium. Similar to Yurkowski et al. (2015), predictive capacity of each model was assessed using Pred$_{0.1}$ and Pred$_{0.5}$, the proportion (%) of observed values within 0.1 ‰ and 0.5 ‰ of the regression line, respectively. When tissues were not significantly different, Pred$_{0.1}$ and Pred$_{0.5}$ were assessed as the proportion of fin tissue values within 0.1 ‰ and 0.5 ‰, respectively, of muscle and liver tissue values. All data satisfied the assumptions of normality and homoscedasticity.
Results

Lipid normalization

Non-lipid-corrected liver and fin tissues generally had high lipid content, with C:N ratios ranging from 4.1 – 5.9 (mean: 4.4) and 3.2 – 4.6 (mean: 3.8), respectively. Non-lipid corrected muscle tissue lipid content was low (C:N mean: 3.3, range 3.2 – 3.4; Table A1).

Comparison of lethal and non-lethal SIA sampling techniques

Paired t-tests indicated that $\delta^{15}N$ in caudal fin tissue was significantly different from both muscle ($df = 23; t = 3.64; p < 0.0014$) and liver ($df = 23; t = -4.98; p < 0.0001$) tissue (Table A1). The linear regression of muscle $\delta^{15}N$ on fin $\delta^{15}N$ showed a positive highly significant relationship (slope = 0.61, intercept = 3.15, $r^2 = 0.56$, $p < 0.0001$; Fig. A1a). Pred$_{0.1}$ and Pred$_{0.5}$ for the regression of muscle on fin $\delta^{15}N$ were 29% and 71%, respectively. The regression of liver $\delta^{15}N$ on fin $\delta^{15}N$ also gave a positive highly significant relationship (slope = 0.97, intercept = 0.84, $r^2 = 0.78$, $p < 0.0001$; Fig. A1b). For the regression of liver on fin $\delta^{15}N$ the Pred$_{0.1}$ and Pred$_{0.5}$ were 21% and 67%, respectively.

Following lipid normalization, caudal fin $\delta^{13}C$ was found to be significantly different from muscle (paired t-test: $df = 23; t = 6.00; p < 0.0001$) but not from liver (paired t-test: $df = 23; t = -0.58; p = 0.57$) tissue (Table A1). Regression analysis of $\delta^{13}C$ lipid-corrected values yielded highly significant, positive relationships for muscle-fin $\delta^{13}C$ (slope = 1.08, intercept = 0.58, $r^2 = 0.80$, $p < 0.0001$; Fig. A2). Pred$_{0.1}$ and Pred$_{0.5}$ were 8% and 54%, respectively, for the muscle-fin $\delta^{13}C$ regression, and 25% and 63%, respectively, for liver-fin $\delta^{13}C$.

Comparison of preservation methods on $\delta^{15}N$ and $\delta^{13}C$ isotopic values

Values of $\delta^{15}N$ values differed significantly between fins preserved in ethanol and those kept frozen (paired t-test: $df = 23; t = -4.30; p = 0.0003$; Table A2). The linear regression of ethanol-preserved fin $\delta^{15}N$ relative to frozen fin $\delta^{15}N$ values showed a
positive, highly significant relationship (slope = 1.07, intercept = 0.37, \( r^2 = 0.80, p < 0.0001 \); Fig. A3a). Pred\(_{0.1}\) and Pred\(_{0.5}\) were 4% and 63%, respectively.

The uncorrected \( \delta^{13}C \) values differed significantly between ethanol-preserved caudal fins and frozen fins as well (paired t-test: \( df = 23; t = -7.05; p < 0.0001 \); Table A2). There was a positive, highly significant linear relationship between ethanol-preserved fin \( \delta^{13}C \) and frozen fin \( \delta^{13}C \) (slope = 1.02, intercept = 1.53, \( r^2 = 0.85, p < 0.0001 \); Fig. A3b). For this regression, Pred\(_{0.1}\) and Pred\(_{0.5}\) were 17% and 58%, respectively.

**Discussion**

The results of this study indicated that caudal fins could be used as a non-lethal alternative to liver and muscle tissues in studies involving juvenile yellow perch. Specifically, we determined that, although generally the \( \delta^{15}N \) and \( \delta^{13}C \) isotopic values differed between lethally and non-lethally sampled tissue types, a significant, positive relationship with moderate predictive capability could be obtained. Caudal fin tissue proved to be an excellent, uncorrected indicator of \( \delta^{13}C \) in liver tissue (Pred\(_{0.1}\) = 25% and Pred\(_{0.5}\) = 63%). In addition, caudal fin tissue proved to be a moderate to excellent predictor of stable isotopes in muscle tissue (\( ^{15}N \), \( r^2 = 0.56, \) Pred\(_{0.1}\) = 29%, Pred\(_{0.5}\) = 71%; \( ^{13}C \), \( r^2 = 0.80, \) Pred\(_{0.1}\) = 8%, Pred\(_{0.5}\) = 54%) and liver tissue \( ^{15}N \) (\( r^2 = 0.78, \) Pred\(_{0.1}\) = 21%, Pred\(_{0.5}\) = 67%). The correlation of muscle \( \delta^{15}N \) on fin \( \delta^{15}N \) in our study is comparable to that found in previous studies of brown trout *Salmo trutta* L. (\( r^2 = 0.58; \) Graham et al. 2013) and Arctic charr *Salvelinus alpinus* (\( r^2 = 0.56; \) Curry et al. 2014). The correlation of muscle \( \delta^{13}C \) on fin \( \delta^{13}C \) in this study is also similar to those found in previous studies of brown trout *Salmo trutta* L. (\( r^2 = 0.84; \) Graham et al. 2013), Atlantic salmon *Salmo salar* L. (\( r^2 = 0.64; \) Graham et al. 2013), roach *Rutilus rutilus* (\( r^2 = 0.74; \) Cano-Rocabayera et al. 2015), Eurasian minnow *Phoxinus phoxinus* (\( r^2 = 0.79; \) Hette Tronquart et al. 2012), European chub *Squalius cephalus* (\( r^2 = 0.76; \) Hette Tronquart et al. 2012) and freshwater fish species of northern Quebec and Labrador, Canada (Arctic charr *Salvelinus alpinus*, brook charr *Salvelinus fontinalis*; \( r^2 = 0.78; \) Curry et al. 2014). The fact that the strength of our results is consistent with many other studies suggests that fin
tissue is a well-known, proven predictor of muscle tissue δ¹⁵N and δ¹³C. Compared to the relationship between fin and muscle tissue, the relationship between fin and liver tissue δ¹⁵N and δ¹³C is less well studied in stable isotope ecology. In our study, δ¹⁵N was generally higher in liver tissue, compared to fin tissue; for δ¹³C, there was no difference between liver and fin tissue. Despite the strength of our relationships between liver and fin, to our knowledge, there are no studies with which to compare our correlations for liver and fin tissue.

We would use the models for δ¹³C cautiously for predicting muscle tissue δ¹³C due the moderate predictive capacity of the model (Pred₀.₅ = 8%, Pred₀.₅ = 54%). Almost half (46%) of the samples were above 0.5 ‰ of the predicted values, with 13% of the samples greater than ± 1 ‰ δ¹³C of the predicted values. This discrepancy between observed and predicted values could potentially influence results of diet reconstruction studies that use caudal fin tissue for stable isotope mixing model analyses given that the average animal-diet discrimination factor for δ¹³C is 1 ‰ (DeNiro & Epstein, 1978). Given the strength of our results for δ¹⁵N, and the consistency with previous studies, we feel that caudal fin tissue could be used to accurately predict 1) δ¹⁵N in both muscle and liver tissue and, 2) predict δ¹³C in liver. Our correction factors can yield reliable indication of yellow perch trophic position, but we would cautiously use the correction factor for predicting δ¹³C in muscle, depending on the objectives of the study, due to the relatively narrow diet discrimination factor for δ¹³C.

An important consideration in stable isotope studies is how quickly different tissue types turnover, as isotopic values in different tissues can “measure” short or long temporal feeding scales. Liver tissue generally has higher turnover rates than muscle tissue for many taxa and can be used to reflect recent dietary sources (Hobson and Clark, 1992; Logan et al. 2006; Suzuki et al. 2005). Few studies have looked at the turnover of stable isotopes in fish fin tissue, with varying results; some studies indicate that fin tissue may turnover ¹⁵N at the same rate as muscle (Suzuki et al. 2005), faster than muscle and liver tissue (Heady & Moore, 2013; McIntyre and Flecker, 2006) or may have a turnover rate that is intermediate to liver and muscle tissue (Matley et al. 2016). In our study, fin tissue δ¹⁵N and δ¹³C values were in between muscle and liver or closer to liver tissue.
values, suggesting that $\delta^{15}$N and $\delta^{13}$C in caudal fin tissue reflects recent to intermediate dietary uptake, although isotopic turnover was not directly tested in this study.

Isotopic variation among individuals and among tissue types within individuals could be caused by differences in lipid content, which may be attributed to diverse life histories, reproductive investment, different foraging strategies, or seasonality (Arrington et al. 2006; Shultz & Conover, 1997). Lipid extraction techniques have been used in studies comparing stable isotope values between tissue types with differing lipid composition in order to make accurate inferences about dietary sources and timeframe of food consumption. This extraction is sometimes necessary because lipid is depleted in $^{13}$C and high-lipid tissues (e.g. liver) typically have lower $\delta^{13}$C than tissues with lower lipid content (Pinnegar & Polunin, 1999). Lipid synthesis pathways in the lipid fractions of tissues deplete $^{13}$C relative to the lighter $^{12}$C (DeNiro & Epstein, 1977). However, in this study, we did not perform chemical lipid extraction; the procedure can affect $\delta^{15}$N values in tissues, either by depleting or enriching the tissues in $^{15}$N (Elsdon et al. 2010; Murry et al. 2006; Sardenne et al. 2015; Sotiropoulos et al. 2004). Tissue lipid content can also be corrected for using mathematical normalization models as an alternative to chemical extraction. We applied the lipid normalization model (Post et al. 2007), which did not appear to improve the linear regression models, but it did significantly change $\delta^{13}$C values for all three tissue types. We use caution in interpreting the lipid-corrected results as yellow perch was not one of the species used to develop the Post et al. (2007) model; however, our C:N values are in the range of those C:N values used in Post et al. (2007). Nonetheless, it would be useful to develop a species-specific model in order to accurately correct for lipid content in tissues (Logan et al. 2008).

The final objective of this study was to determine if preservation of tissues using 95% ethanol had an effect on $\delta^{15}$N and $\delta^{13}$C values, and to further develop a correction factor to use for ethanol-preserved samples of yellow perch tissue. We found that preservation by ethanol significantly increased both $\delta^{15}$N and $\delta^{13}$C values compared to frozen fin sample values. Previous studies have found that preservation can alter the isotopic values of tissue in various ways, depending on the species (Sarakinos et al. 2002), the type of preservative (Kaehler & Pakhomov, 2001) and the lipid content of the
tissue (Pinnegar & Polunin, 1999). The exact mechanism causing this isotopic change is unknown; however, it could be that the preservative may prevent the loss of the heavier $^{15}$N and $^{13}$C, thus increasing $\delta^{15}$N and $\delta^{13}$C in the tissue (Bosley & Wainright, 1999). Our correction factors generated from the regression of ethanol-preserved fins on frozen fin samples had a $\text{Pred}_{0.5}$ of 63% for $\delta^{15}$N ($r^2 = 0.80$) and 58% for $\delta^{13}$C ($r^2 = 0.85$). For both $\delta^{15}$N and $\delta^{13}$C, 13% of the ethanol-preserved samples were above 1 ‰ from the predicted values. Given the small tissue-diet discrimination for $\delta^{13}$C (~1 ‰; DeNiro & Epstein, 1978), we would use caution when considering ethanol as a preservative in studies of $^{13}$C, where even small changes in $\delta^{13}$C could influence results of diet reconstruction studies. Ethanol-preserved samples could be used reasonably well in studies involving the estimation of trophic position using $\delta^{15}$N in yellow perch tissues, given that tissue-diet discrimination factors are larger (3-4 ‰; DeNiro & Epstein, 1981) for $\delta^{15}$N. Whenever possible, freezing is recommended as the most reliable preservation method, however, if ethanol must be used as a preservation medium for yellow perch tissues, then our correction factor can be applied to account for the effects of ethanol on the tissue.

The development of species-specific correction factors for non-lethal SIA sampling should be of high priority in studies involving threatened species or in studies tracking individual diet over time. Our study has shown moderate predictive relationships between lethally and non-lethally sampled tissues, demonstrating the potential for using non-lethal sampling of fins in fish SIA. However, given the lower predictive capacity of fin tissue for muscle tissue $\delta^{13}$C, and the likelihood of diet misclassification errors for $\delta^{13}$C (within ± 1 ‰), we recommend researchers use non-lethal sampling of $\delta^{13}$C at their discretion, depending on the tissue of interest. We recommend that researchers consider the objectives and resolution of their stable isotope study prior to considering non-lethal tissue sampling; the importance of conducting species-, age- and location-specific studies to validate this technique cannot be emphasized enough.
References


Figure A1 Comparison of nitrogen (δ^{15}N) isotopic values for a) muscle and frozen fin tissue and b) liver and frozen fin tissue. The linear regression is represented by the black line. Dashed grey line denotes a 1:1 relationship.
Figure A2 Comparison of lipid-corrected (Post et al. 2007) carbon ($\delta^{13}C$) isotopic values for muscle and frozen fin tissue. The linear regression is represented by the black line. Dashed grey line denotes a 1:1 relationship
Figure A3 Comparison of ethanol-preserved samples and frozen samples for a) nitrogen ($\delta^{15}$N) and b) carbon ($\delta^{13}$C) isotopic values in yellow perch caudal fins. Dashed grey line denotes a 1:1 relationship.
Table A1 Mean ± SD of $\delta^{15}$N, $\delta^{13}$C (lipid-corrected), $\delta^{13}$C (uncorrected), and C:N values of caudal fin (frozen), muscle and liver tissue from yellow perch

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>N</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{13}$C (corrected)</th>
<th>$\delta^{13}$C (uncorrected)</th>
<th>C:N</th>
</tr>
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<tbody>
<tr>
<td>Caudal Fin</td>
<td>24</td>
<td>9.4 ± 1.0</td>
<td>-23.4 ± 1.9</td>
<td>-23.9 ± 1.7</td>
<td>3.8 ± 0.4</td>
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<td>24</td>
<td>8.9 ± 0.8</td>
<td>-24.6 ± 2.2</td>
<td>-24.6 ± 2.2</td>
<td>3.3 ± 0.1</td>
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<tr>
<td>Liver</td>
<td>24</td>
<td>9.9 ± 1.1</td>
<td>-23.3 ± 1.8</td>
<td>-24.3 ± 1.8</td>
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Table A2 Mean ± SD of $\delta^{15}$N, $\delta^{13}$C (uncorrected), and C:N values of frozen and ethanol-preserved caudal fin tissue from yellow perch

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>N</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{13}$C</th>
<th>C:N</th>
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<tbody>
<tr>
<td>Caudal Fin (Frozen)</td>
<td>24</td>
<td>9.4 ± 1.0</td>
<td>-23.9 ± 1.7</td>
<td>3.8 ± 0.4</td>
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<tr>
<td>Caudal Fin (95% Ethanol)</td>
<td>24</td>
<td>10.0 ± 1.3</td>
<td>-22.8 ± 1.9</td>
<td>3.2 ± 0.2</td>
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</tbody>
</table>
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