Does chronic stress mediate predator-prey interactions in wild fish? An experimental approach using exogenous cortisol implants.

By

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Abstract

The hypothalamic-pituitary-interrenal (HPI) axis serves as one of the central neuroendocrine systems in mediating the stress response in teleosts. However, HPI axis stimulation, particularly over a prolonged activation, can result in fitness-related consequences. Previous work has shown that stressed teleosts exhibit higher rates of predation when compared to unstressed conspecifics. However, the mechanism(s) underlying this effect, at the physiological and behavioural levels, are currently unknown. My overarching hypothesis is that because of higher metabolic expenditures under chronic cortisol treatment, an individual fish should exhibit riskier behavioural phenotypes leading to higher predator vulnerability. I tested this hypothesis using sunfish (*Leopmis sp.*) that were implanted with cocoa butter containing either cortisol or as a sham control. Treated animals were subject to a thorough metabolic and physiological profiling. Fish were then assessed for a suite of behavioural traits related to anti-predator and risk-taking behaviours and were accompanied by a field assessment of predator mortality. Cortisol-treatment did result in a significant alteration to the animal's metabolic physiology which included a higher standard metabolic rate, ammonia excretion rates, hepatic [glycogen], and blood [glucose], against respective controls. Despite an altered metabolic state, I observed no alteration to behavioural phenotypes associated with anti-predator or risk-taking behaviours under cortisol-treatment. Similarly, predation rates were comparable across all of my treatment groups. These data indicate that the HPI axis, in this context, is not the driving mechanism underlying higher predation rates in stressed teleosts. It could be that another physiological system associated with the stress response or in conjunction with the HPI axis may be driving

this effect. Additionally, metabolism-behavioural interactions are often highly context specific and may not have been manifested under the current environmental context. Lastly, the timescale used in my study may have been too acute with fish being behaviourally resilient. Together, this work provides a foundational assessment looking at the mechanistic role of the stress response in dictating predator-prey interactions. While further work investigating metabolic and behavioural interactions is required, particularly at the individual level, these data could prove useful in better understanding anthropogenic impacts on aquatic ecosystems; a highly relevant consideration in the contemporary Anthropocene.

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Thesis Format and Co-authorship

The following thesis is arranged in the "sandwich" format in accordance with the recommendations and approval of my supervisory committee. This thesis consists of five chapters written in manuscript format. Chapter 1 outlines a general introduction and background of information relevant to my thesis work including thesis objectives and hypotheses. Chapter 2 characterizes the physiological effects of chronic cortisol elevations on sunfish metabolism. Chapters 3 and 4 investigate the behavioural modifications associated with cortisol-treatment. Chapter 5 sought to quantify predator-induced mortality. Chapter 6 summarizes my general conclusions, the relevance of my research, and propose future experiments that may conducted to support this thesis. This thesis contains all of my own research but was conducted in collaboration with a number of other researchers to which a summary of their contributions is listed below.

Chapter 1: General introduction, thesis objectives and hypotheses.

Chapter 2: Cortisol modulates metabolism and energy mobilization in wild-caught pumpkinseed (*Lepomis gibbosus*)

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All authors contributed to the design of the experiment. The experimental series were conducted by M.J.L. and A.J.Z. Assays and data analyses were performed by M.J.L. with help by E.J.E., D.L., C.B., and K.M.G. The manuscript was written by M.J.L. with all

authors contributing to revisions.

Chapter 3: Does experimental cortisol elevation mediate risk-taking and antipredator behaviour in a wild-caught teleost fish?

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Chapter 4: Chronic plasma cortisol elevation does not promote riskier behaviour in a teleost fish: A test of the behavioural resiliency hypothesis

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Chapter 5: Cortisol does not increase risk of mortality to predation in juvenile bluegill sunfish: a manipulative experimental field study

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Chapter 6: General conclusions and future directions

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Chapter 1: General Introduction

This thesis will attempt to address how chronic stress influences predator-prey dynamics in teleost fishes. More specifically, it will investigate the effects of a homeostatic overload state on the metabolism, behaviour and ecology of centrarchid fishes. This general introduction provides all of the necessary and relevant information for understanding the development of the ideas, hypotheses and predictions that I present in this thesis. The first section addresses the evolutionary and functional significance of the hypothalamic-pituitary-interrenal axis in the teleostean stress response (Section 1.1) and its role in affecting the fish's metabolism (Section 1.2). I then discuss the reactive scope model and its implications for understanding stressors in addition to providing my definition of what chronic stress is for the purposes of this thesis (Section 1.3). Next, predation risk and its implications for fish behaviour (Section 1.4) and the prior knowledge of its interactive effects with the hypothalamic-pituitary-interrenal axis (Section 1.5) is outlined. Lastly, this introduction presents my objectives, hypotheses, and predictive framework for this thesis (Section 1.6) while also discussing the validity of using centrarchids as a model taxonomic group in this setting (Section 1.7).

1.1. The role of the hypothalamic-pituitary-interrenal axis in mediating the stress response and its physiological actions

In nature, consistency in the environment is a rather uncommon phenomenon. While often tending around some average value, conditions (e.g. temperature, oxygen, light, etc.) in natural systems tend to fluctuate through time and may enter a state which is acutely unfavourable to its inhabitants. Furthermore, increasing anthropogenic activities in natural systems can act to accelerate and galvanize alterations in the environment

while also potentially introducing novel and unforeseen perturbations in local conditions (Sondergaard and Jeppesen 2007; Sih et al. 2011). These challenges, referred to henceforth as stressors, have the capacity to perturb internal physiological state in an organism which, if left unmitigated, could potentially result in decreased organismal performance and, in extreme cases, compromised survival. Thus, through evolutionary time, organisms have evolved a suite of physiological systems that function to buffer and mitigate the deleterious effects of stressors (Overli et al. 2007; Romero and Butler 2007; Denver 2009; Boonstra 2013a). Collectively, the host of adaptive physiological responses to a stressor is referred to as the stress response (Schreck and Tort 2016) and it allows the animal to retain a high degree of fitness despite being confronted with sub-optimal environmental and/or physiological conditions.

In vertebrates, the stress response is largely under the regulatory control of hypothalamic coordination. Here, a stressor is perceived by the organism in some manner (e.g. olfaction, ion sensing, visual cues, etc.) which stimulates the release of host of neuroendocrine factors responsible for a suite of physiological adjustments on downstream regulatory targets. The vertebrate stress response generally includes the coordinated actions of two central axes: the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis (reviewed in Romero and Butler 2007 & Schreck and Tort 2016). These two axes are responsible for the production of catecholamines and glucocorticoids, respectively, and work in tandem to facilitate homeostatic re-adjustment following stressor exposure. Although, they are generally considered to be temporally distinct with the actions of the SNS occurring over rather acute timeframes (i.e. seconds to minutes) while the HPA axis occurs over more

prolonged durations (i.e. minutes to hours; reviewed in Wendelaar Bonga 1997 & Reeder and Kramer 2005). For the purposes of this thesis, further discussion will be limited to the functionality of the HPA axis.

The adaptive significance of the HPA axis in stressor mitigation has resulted in this neuroendocrine system being largely conserved amongst most vertebrate clades. In teleost fishes specifically, the hypothalamic-pituitary-interrenal (HPI) axis is homologous to the HPA system found in birds and mammals (Figure 1.1; Wendelaar Bonga 1997; Lohr and Hammerschmidt 2011; Schreck and Tort 2016). Here, the response to a stressor is integrated and coordinated by the hypothalamus ultimately resulting in the secretion of the peptide, corticotropin-releasing factor (CRF). CRF is believed to be produced by the cells of the hypothalamic nucleus preopticus (NPO) to which neurons from the NPO directly innervate into the pituitary gland resulting in CRF paracrine signaling (Reviewed in Flik et al. 2006 & Bernier et al. 2008; 2009). Agonism of CRF receptors in the anterior pituitary gland prompts the biosynthesis and release of adrenocorticotropic hormone (ACTH) into circulation via corticotropes (Reviewed in Wendelaar Bonga 1997 & Alsop and Vijayan 2009). In teleosts, circulating ACTH will bind to the melanocortin receptors (MCR; MC2R specifically) of the inter renal tissue prompting an activation of glucocorticoid biosynthesis (reviewed in Bernier et al. 2009), specifically cortisol (Mommsen et al. 1999; Schreck and Tort 2016). Across a wide range of species, contexts and stressor types, cortisol titres in the blood often increase following stressor exposure (Reviewed in Barton and Iwama 1991, Pottinger 2008, & Sopinka et al. 2016). Consequently, measurement of cortisol in the plasma is often used as an index of the stress status of fishes (Barton and Iwama 1991; Sneddon et al. 2016; Sopinka et al. 2016).

Cortisol has a number of physiological roles that are integral to mounting a successful stress response. Homeostatic re-adjustment following stressor exposure is considered to be an energetically demanding process (Barton and Iwama 1991; Sokolova 2013; Schreck and Tort 2016). This is evident in both stressed (Barton and Schreck 1987; Sloman et al. 2000; Lankford et al. 2005) and cortisol-treated (Chan and Woo 1978; Morgan and Iwama 1996; De Boeck et al. 2001; O'Connor et al. 2011) fish wherein oxygen consumption rates (MO_2) are well above baselines values. As such, cortisol's primary functional role in the stress response is to facilitate allocation of metabolic resources towards stressor mitigation. More specifically, cortisol aids in mobilizing energetic reserves and by re-prioritizing energetic resources towards survival-based processes (Mommsen et al. 1999; Schreck and Tort 2016). In this way, cortisol provides the organism with sufficient resources in order to overcome the physiological perturbations associated with stressor exposure.

Cortisol's role in energy mobilization primarily results from alterations in gluconeogenic activity. Gluconeogenesis, the biosynthesis of *de novo* glucose from non-carbohydrate-based substrates (e.g. amino acids & lipids), is often higher in stressed and cortisol-treated fishes (reviewed in Wendelaar Bonga 1997 & Mommsen et al. 1999). Stimulation of hepatic glucocorticoid receptors (GR) by cortisol elevates both the activity and expression of enzymes mediating gluconeogenic pathways including phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase and glucose 6-phosphatase (Chan and Woo 1978; Vijayan et al. 1996; Aluru and Vijayan 2007; Momoda et al. 2007; Wiseman et al. 2007; reviewed in Mommsen et al. 1999). Consequently, hyperglycemia is often observed following stressor exposure in teleosts

(Morgan and Iwama 1996; Vijayan et al. 1997; Wells and Pankhurst 1999; Grutter and Pankhurst 2000) as well as in cortisol-treated fish (Chan and Woo 1978; Morgan and Iwama 1996; McConnachie et al. 2012; Jerez-Capa et al. 2019). This gluconeogenic activity provides the much-needed metabolic substrates for fueling stressor mitigation.

In sustaining continued gluconeogenesis under a stressor, cortisol also acts to increase the pool of available gluconeogenic substrates (i.e. amino acids, pyruvate, glycerol, etc.) through an upregulation of both proteolytic and lipolytic pathways (reviewed in Mommsen et al. 1999 & Aluru and Vijayan 2009). Consequently, elevations in blood cortisol levels often correspond with an increase in the concentrations of circulating free amino acids (Andersen et al. 1991; Milligan 1997; Vijayan et al. 1997; Costas et al. 2011) and lipids (Butler 1968; Jerez-Capa et al. 2019). Additionally, enhanced proteolytic activities under cortisol's regulatory control would conceivably increase nitrogenous waste production, namely ammonia (Wright 1995). Indeed, cortisol does appear to have a role in not only enhancing production of nitrogenous wastes in teleosts (Storer, 1967; Hopkins et al., 1995; Wood et al., 1999; McDonald and Wood, 2004; Liew et al., 2013; Lawrence et al., 2015; Jerez-Capa et al. 2019) but in also facilitating an upregulation of excretory transport mechanisms (Nawata and Wood, 2009; Tsui et al., 2009; Lawrence et al., 2015).

Endogenous energy reserves also seem to be under the regulatory control of the HPI axis. Liver glycogen content, an important endogenous energy store useful in stressor mitigation, appears to be stress responsive to a certain extent. However, the direction of the response appears to be quite variable with stressors increasing (Narasimhan and Sundararaj 1971; Leach and Taylor 1980), decreasing (Vijayan et al.

1997; Costas et al. 2011) or having no effect (Vijayan and Moon 1992; Vijayan et al. 1994) on hepatic glycogen reserves. At this level, these responses are likely context specific and are further confounded by the actions of the SNS to which catecholamines often enhance glycogenolytic activities in the liver (Perry and Randall 1992; Perry and Capaldo 2011). Cortisol's direct influence, through cortisol-treatment in fish, on hepatic glycogen content appears to also have a variable effect resulting in a null effect (Storer 1967; Leatherland 1987; Vijayan et al. 1994; Jerez-Capa et al. 2019), and higher (Butler 1968; Inui and Yokote 1975; Chan and Woo 1978; De Boeck et al. 2001; Laiz Carrion et al. 2002, 2003; Vijayan et al. 2003) and lower (Whiting and Wiggs 1977; Foster and Moon 1986; Andersen et al. 1991) liver glycogen concentrations against controls. This effect is likely tied to cortisol's differential regulatory actions on enzymes mediating glycogen formation, namely glycogen phosphorylase (Laiz Carrion et al. 2002, 2003; Milligan 2003; Baltzegar et al. 2014; Jerez-Capa et al. 2019) and glycogen synthase (Milligan, 2003; Leung and Woo, 2010). These discrepancies highlight that further work is needed to ascertain cortisol's role in regulating the various catabolic and anabolic processes that underscore net glycogen formation in the teleost liver.

Cortisol also has a role in the allocation of metabolic resources under periods of stress. Metabolic energy allocation towards various life history traits is typically considered to be finite and is generally constrained within the animal's metabolic scope (see Section 1.2). However, as stressor mitigation is generally energetically costly, the accompanying increase in metabolic load can interfere with and constrain the energetic budgeting of the fish as energetic demands may not necessarily match with supply (Schreck 1982; Sokolova 2013; Schreck and Tort 2016; Figure 1.2). As such, one of

cortisol's primary roles during the stress response includes the re-allocation of energetic inputs away from non-essential processes and directing it towards physiological systems involved with stressor mediation (Wendelaar Bonga 1997; Schreck and Tort 2016). Consequently, stressed teleosts often exemplify reduced inputs into growth (reviewed in Sadoul and Vijayan 2016), reproduction (reviewed in Pankhurst 2016), and immune function (reviewed in Yada and Tort 2016), particularly over prolonged stressor durations. These effects are well characterized in the literature in a number of stressor and cortisol-treatment scenarios (reviewed in Barton and Iwama 1991 & Wendelaar Bonga 1997). Thus, cortisol's regulatory actions ensure that metabolic resources are being properly allocated towards necessary survival-based processes rather than being squandered on non-essential functions.

Combined, the actions of the HPI axis, through cortisol's regulatory input on energy mobilization and re-allocation, ensures that the animal has sufficient energy at its disposal to mitigate the effects of the stressor and to re-establish internal homeostasis (Wendelaar Bonga 1997; Schreck and Tort 2016). Thus, especially under acute durations, the HPI axis represents a highly adaptive physiological mechanism in dealing with the various challenges that a fish may encounter in its environment helping to ensure a high degree of fitness.

1.2. Sustained HPI axis stimulation and its consequences for metabolic scope

The HPI axis and the accompanying effects of cortisol are generally considered to be an adaptive mechanism in maintaining internal homeostasis over an acute stressor duration through its management of the fish's energy metabolism (see Section 1.1). However, over more chronic or sustained durations, the actions of cortisol can be

problematic with respect to its influence on the fish's overall fitness and metabolic energy availability. As highlighted earlier, the stress response is an energetically demanding process which is reflected in the increase in both the animal's routine (Barton and Schreck 1987; Chan and Woo 1978; Morgan and Iwama 1996; DeBoeck et al. 2001) and standard metabolic rates (SMR; Sloman et al. 2000; De Boeck et al. 2001; O'Connor et al. 2011) during times of cortisol elevation. In such instances, it may be expected that the animal's aerobic scope, the difference between the fish's maximal (MMR) and standard metabolic rates (SMR; Fry 1947), may be reduced provided that MMR remains unchanged. Aerobic scope represents the amount of aerobic, metabolic power that can be allocated towards non-maintenance related activities (e.g. swimming, reproduction, growth, etc.) and is considered to be the primary determinant of an animal's energy budget (Fry 1947; Farrell et al. 2008; Figure 1.2). Consequently, the reduced aerobic scope of a stressed fish may act to constrain available metabolic power amongst competing life history traits resulting in impaired organismal performance and reduced fitness (Portner and Farrell 2008; Guderley and Portner 2010; Sokolova et al. 2012; Sokolova 2013; Clark et al. 2013; Figure 1.2). Thus, from a metabolic perspective, chronic induction of the HPI axis or components thereof could represent a significant challenge to the organism in maintaining a suitable degree of fitness.

Despite the importance of aerobic scope in dictating performance in the environment, there has been little investigated into its relationship with the HPI axis and the resulting fitness impacts in the literature. To a certain extent, fish experiencing either a sustained elevation in blood cortisol or a chronic stressor have been observed to have an elevated SMR with respect to control animals (Lankford et al. 2005; O'Connor et al.

2011). While not a teleost, green sturgeon (*Acipenser medirostris*) experiencing a non-habituating, chronic stress regime (chasing, netting, air exposure) demonstrated an elevated SMR that corresponded with a hyperglycemia, a reduction in liver glycogen content and a reduced aerobic scope (Lankford et al. 2005). This clearly illustrates that chronic stress can influence the flux of metabolic power in the organism which may result in impaired performance. Similarly, using bioenergetics modeling, cortisol-implanted largemouth bass (*Micropterus salmoides*) were shown to have a significantly higher SMR which corresponded with a reduced growth performance over time (O'Connor et al. 2011). Thus, there appears to be a basis by which stress, at the level of cortisol, can influence the metabolism of a fish in a negative manner. Although, the effects of cortisol in regulating SMR and aerobic scope remain poorly characterized in the literature.

While aerobic scope-stress interactions are limited, the long-term consequences of HPI axis activation are well represented in the literature. In many instances where either cortisol has been chronically elevated in the blood or where the animal is exposed to a sustained stressor, the growth rates of many teleosts are often depressed (Davis et al. 1985; Barton et al. 1987; Abbott and Dill 1989; McCormick et al. 1998; Gregory and Wood 1999; reviewed in Van Weerd and Koman 1998). Furthermore, the body condition of these fish, reflected in various indices (i.e. condition factor, hepatosomatic index [HSI], etc.), were consistently lower than unstressed conspecifics (Fagerlund et al. 1981; Davis et al. 1985; Barton et al. 1987; McCormick et al. 1998; Gregory and Wood 1999). Similar effects have also been noted in both the reproductive (reviewed in Pankhurst 2016) and immune systems (reviewed in Yada and Tort 2016) of teleost fishes whereby

sustained HPI axis stimulation and/or cortisol elevations correspond with a reduced energetic input into these physiological systems. This culminates in a poor reproductive performance and an increased susceptibility to diseases, respectively (reviewed in Wendelaar Bonga 1997). Combined, the impacts of stress-induced energetic diversions in production input (e.g. growth, swimming, reproduction, etc.) can confer significant performance impairments in the fish's various life history traits and fitness-relevant processes including predator avoidance and fecundity (Portner and Farrell 2008; Guderley and Portner 2010; Sokolova 2013). Furthermore, these findings suggest that differential metabolic power allocation (i.e. diversion from production) under a continued stressor could have significant impacts on a number of life history attributes in fish with the HPI axis acting in a maladaptive fashion.

1.3. The reactive scope model

Classically, the influences of the stress response on a fish's physiology, with respect to fitness related consequences, has often been delineated into maladaptive and adaptive actions resulting from chronic and acute stimulation, respectively (Barton and Iwama 1991; Korte et al. 2005). This results in a sort of paradox whereby the HPI axis can act in both a beneficial and detrimental capacity that is entirely dependent on the timescale by which it is activated. Furthermore, this also makes defining what chronic/acute stress actually is difficult as the lines between the two can often be blurred. For the purposes of my thesis work, I will define chronic stress as a sustained elevation in plasma cortisol in excess of 24 hours whereby the effects of "wear and tear" on the animal become evident thus representing a homeostatic overload condition (Romero et al. 2009). The logic for this definition is rooted in the argument presented in the reactive

scope model as proposed by Romero et al. (2009; see below). Instead of using a classical notion of chronic exposure (e.g. 96 h), I elected to conduct my experimental series at a time period that is outside of the animal's reactive scope entering a range of homeostatic overload. Furthermore, as found in Chapter 2, the use of 24-48 h post-implant for behavioural assays ensured that organisms still demonstrated high circulating levels of cortisol reflective of a "stressed" state.

The reactive scope model is an attempt to quantify the effects and impacts of a physiological mediator during varying magnitudes and durations of the stress response. This model is built extensively off of the allostatic load model (reviewed in Korte et al. 2005) whereby an organism entering an "emergency life history stage" reduces or suspends non-maintenance activities thereby reducing allostatic load (i.e. energy needed to maintain allostasis/internal balance; high load = high demand; McEwan and Wingfield 2003; 2010). Thus, remaining in this phase for extended durations incurs a significant cost to the organism in terms of lost fitness-related opportunities (Wingfield et al. 1998; Wingfield 2005). However, because of issues with the association between physiological mediators (e.g. cortisol, catecholamines, etc.) and energy regulation, this model is considered sub-optimal. Briefly, issues associated with the allostatic load model can include a disconnect between metabolic expenditures and glucocorticoids, minimal metabolic costs associated with stress responses in particular contexts (e.g. behavioural responses), and the variable and contextual nature of an organism's metabolic expenditures (reviewed in Romero et al. 2009). Unlike allostatic load, the reactive scope model considers all of the impacts of the stress response purely through the physiological

mediators that respond to the stressor thus removing the issues surrounding energy-mediator interactions (Romero et al. 2009).

The reactive scope model is as follows summarizing Romero et al. (2009; Figure 1.3). In all organisms, a basal level of the physiological mediator (e.g. cortisol) is required to finely regulate the basal metabolic processes of the animal. Reducing the concentrations of that mediator below this basal threshold results in a condition of "homeostatic failure" where the animal cannot sustain itself metabolically resulting in its death. Above the baseline level, the concentrations of the mediator can fluctuate throughout the course of the day and/or season accounting for predictable changes in functions that are essential to the organism's daily life (e.g. feeding, locomotion, circadian rhythms, etc.). This is referred to as the "predictive homeostasis". Crossing the upper threshold of predictive homeostasis, the mediator reaches concentrations that are considered within the "reactive homeostasis" range (Figure 1.3). This encompasses the concentrations of the mediator that are used to deal with physiological perturbations that may occur as a result of unforeseen challenges in the environment and is considered to be analogous to an acute stress response. The concentrations of the mediator that lie between the lower end of predictive homeostasis and the upper limits of reactive homeostasis constitute the "reactive scope" of the organism (Figure 1.3). Here, the physiological mediator is beneficial to the metabolic functioning of the organism permitting regulation of basal processes as well as coping with environmental challenges in a manner that optimises the organism's fitness and survival. Upon the concentration of the mediator extending beyond the upper threshold of reactive homeostasis, it enters a zone referred to as "homeostatic overload" (Figure 1.3) Here, the mediators themselves start to cause

physiological damage and disruption to the metabolic operation of the organism and generally represents what is referred to as a chronic stressor. Over sustained durations in the homeostatic overload state, the physiological condition of the organism will continue to degrade producing noticeable physiological impairments associated with the stressor (e.g. reduced growth, fecundity, immunocompetence, etc.). Furthermore, continued homeostatic overload can also result in a decreased reactive homeostatic threshold as a result of the "wear and tear" associated with the overload's actions making the animal less likely to cope with a future/continued stressor (e.g. depletion of energy reserves). As such, rather than depending on an absolute time scale of chronic vs acute, the reactive scope model provides a prediction of the effects of stress response mediators based on the dynamics of the mediator itself thereby simplifying the acute/chronic debate.

1.4. Predation risk and its effects on fish behaviour

Predation has been a significant force shaping the evolution of both predator and prey. In teleosts, the threat of predation has resulted in the evolution of a vast array of deterrent mechanisms, behavioural avoidance strategies, and alterations in life history traits in order to minimize predator-induced mortality on the part of the prey animal (reviewed in Smith 1997 & Godin 1997). Indeed, the threat of predation can have a significant impact on the behavioural dynamics of a fish which can include excluding prey fish from non-refuge environments (Werner et al. 1983; Gilliam and Fraser 1987; Krause et al. 1998; 2000), reductions in the prey's activity patterns (Lawrence and Smith 1989; Bean and Winfield 1995; Pettersson et al. 2001; Laurel and Brown 2006; Dunlop-Hayden and Rehage 2011), higher shoal association (Rehnberg and Smith 1988; Sogard and Olla 1997; Brown and Dreier 2002; Orpwood et al. 2008), and compromised prey

foraging (Werner et al. 1983; Milinski 1985; 1993; Mikheev et al. 2006), amongst others. All of these actions serve to minimize the risk of predation which, as defined by Lima and Dill (1990), represents the probability of a fish succumbing to its predator in the environment. Often, predation risk (i.e. P(death)) is represented mathematically as the product of three main variables including the encounter rate with the predator (α), the odds that individual prey item survives the predation event (d), and duration that the prey animal is vulnerable to the predator (T), such that $P(death) = 1-\exp(-\alpha dT)$ (Lima and Dill 1990). As individual prey animals can elect to modify their conspicuousness and vulnerability to a predator in a number of ways (see Godin 1997 & Smith 1997), predation risk then becomes a product of decisions made by the individual prey item (Ydenberg and Dill 1986; Godin 1997). Indeed, risk-taking behaviours in teleosts appear to be governed by state dependent decision making in which the internal energetic/nutritional state of the animal deems the level of predation risk and the corresponding behaviours, that is acceptable under the current context (reviewed in Godin 1997). Furthermore, these decisions are often made in manner that balances the risk of predation (i.e. predation mortality) against a net gain in some fitness-related aspect (e.g. foraging & mating opportunities) such that overall fitness is optimized (e.g. μ/g rule; Gilliam 1982; Werner and Gilliam 1984; Gilliam and Fraser 1987; Lima and Dill 1990).

In teleost fish, state dependent decision making appears to dictate a large proportion of the risk-taking behaviours in this taxon. The model predicts that animals that are energetically compromised are more likely to spend a greater proportion of their time seeking foraging opportunities to improve nutritional acquisition at a cost of having a greater degree of predation risk (Millinski and Heller 1978; Dill and Fraser 1984;

reviewed in Millinski 1993). For example, guppies (*Poecilia reticulate*) that had been starved for an extended duration (24 h) incurred a higher predator-induced mortality rate than fed and 1 h fasted fish suggesting that hunger status was a strong mediator of predation risk (Godin and Smith 1988). Additionally, fasted Atlantic salmon (*Salmo salar*) showed a reduced foraging latency period after being exposed to a model predator suggesting a greater degree of risk-taking behaviour in these fish (Gotceitas and Godin 1991). Hunger state was also found to increase the number of predator inspections, a potentially risky behaviour, in threespine stickleback (*Gasterosteus aculeatus*) conferring a greater degree of predation risk alongside an increased foraging gain (Godin and Crossman 1994). As well, Iowa darters (*Etheostoma exile*) that had been starved for 48 h did not change their foraging activity levels when presented with a predator suggesting that energy acquisition rather than predation risk was more important in maintaining a high level of fitness (Smith 1981). It should be noted that, in support of state dependent decision making under energetic duress, as a fish fulfills its energetic requirements, the relative 'cost' associated with feeding, with respect to predation, is increased and as such, foraging activity decreases (reviewed in Ydenberg and Dill 1986 & Godin 1997). This has been seen in coho salmon (*Oncorhynchus kisutch*) where the attack distance of the fish (i.e. how far the fish would strike a food item from its current position) decreased with an elevation in satiation levels (Dill and Fraser 1984). Together, these works, and others provide significant evidence that risk-taking behaviours under a predation threat are highly dependent on the energetic status of the animal (reviewed in Milinski 1993 & Lima 1998).

Refuging activity under predation threat is also paired to an organism's energetic status. Refuging is a method employed by fish to reduce the relative risk of predation through occupying some environment or habitat whereby predator access is limited or restricted in some manner. This can include structurally complex physical features such as dense weed beds or sunken timber (Godin 1997) as well as associating with a shoal where the individual risk of predation is diluted by the number of shoal members (i.e. risk = 1/N; Pitcher and Parrish 1993). However, there is a cost associated with refuging behaviour in that fish may either be missing out on potential foraging opportunities and/or that the resources found in the refuge are sub-optimal when compared to areas where predation risk is higher (reviewed in Godin 1997 & Cooper and Frederik 2007). As with foraging activity, refuging behaviour is dependent on a number of internal and external factors. In a model developed by Sih (1992), energetic stressors, including body condition and hunger state, were found to correspond with a reduced time spent amongst a refuge environment conferring a greater degree of predation risk. This suggests that animals under energetic distress should spend a reduced proportion of their time refuging to maximize their fitness from a metabolic perspective (i.e. nutrient acquisition). The consequence of this is an elevated predation risk from occupying areas where predator access of the prey fish is higher (reviewed in Godin 1997). This has been supported empirically in that the time that stickleback remain in a refuge (i.e. low risk environment) corresponds tightly with the relative metabolic rate of the fish (i.e. body length) and its hunger level suggesting that energetic status is an important mediator of predation risk in fish (Kraus et al. 1998). Similar refuge use patterns have been noted in other species as well, with respect to body size (i.e. mass specific metabolic rate; Krause et al. 1998;

Dowling and Godin 2002; Brown and Braithwaite 2004; Polverino et al. 2016), hunger levels (Krause et al. 2000; Petrie and Ryer 2006; Killen et al. 2011) and parasitism (i.e. higher metabolic loading; Jakobsen et al. 1988; Bean and Winfield 1989; Krause and Godin 1994), indicating that energetic expenditure and demand could dictate risk taking behaviours in teleost fishes (Dowling and Godin 2002; Vehanen 2003; Petrie and Ryer 2006). Thus, the reduction of refuge use by an energetically stressed fish confers the benefit of optimal resource acquisition at the expense of an elevated predation risk coefficient (reviewed in Godin 1997).

1.5. The effects of the HPI axis on predator-prey interactions

The actions of the stress response appear to have a significant role in influencing predator-prey interactions in teleosts. In a number of studies and contexts, teleosts exposed to a prior stressor (e.g. handling, toxicants, disease, etc.) demonstrated higher rates of predation when compared to unstressed conspecifics (Jarvi 1989; Olla and Davis 1989; Olla et al. 1992; Marine and Cech 2004; Ryer et al. 2004; Danylchuk et al. 2007; reviewed in Mesa et al. 1994 & Raby et al. 2014). While the underlying mechanism(s) for this effect are unknown, it is currently believed that the poor body condition developed during the stressor limits the ability to sustain anti-predator behaviours resulting in a greater vulnerability to predation (Mesa et al. 1994; Schreck et al. 1997). In support of this notion, stressed fish have often been shown having reduced escape distances (Handeland et al. 1996; Ryder et al. 2004; Allan et al. 2015), impaired swimming performance (Brown et al. 1985; Kruzynski et al. 1994; Mesa et al. 1994; Danylchuk et al. 2007; Allan et al. 2015) and disrupted shoaling behaviour (Sullivan et al. 1978; Brown et al. 1985; Kruzynski et al. 1994; Mesa et al. 1994; Handeland et al. 1996; Ryder et al.

2004), which may make the animal more prone to predation events. This suggests, in part, that predator avoidance capacity and risk, at the physiological level, may be the result of a metabolic effect.

Stress-induced changes in the fish's available aerobic scope may be an explanatory factor in predation. As discussed earlier, teleosts experiencing elevated circulating cortisol levels show an increased rate of metabolic expenditure (see Section 1.2) that could correspond with a reduction in the organism's aerobic scope (Lankford et al. 2005; Sokolova 2013). This could be quite problematic as anti-predator tactics are generally considered to be energetically costly with respect to metabolic investment. For example, the metabolic rate of Atlantic salmon that had no access to a refuge or cover was found to be 30% higher than those who could enter a spatial refuge environment (Millidine et al. 2006). Additionally, largemouth bass maintained tachycardia for a number of hours post-predator encounter representing a significant metabolic cost of sustaining anti-predator responses (Cooke et al. 2003). As such, under a cortisol-induced reduction in aerobic scope, we might expect higher predator vulnerability and risk-taking behaviours resulting from metabolic constraints decreasing the flux of energy inputs into anti-predator behaviours (Guderley and Portner 2010; Sokolova et al. 2012; Sokolova 2013). In this instance, inputs into predator vigilance and other fitness enhancing activities (e.g. foraging, mating, etc.) may result in ecologically relevant trade-offs that could act to modulate the animal's risk of predation (Hawlena and Schmitz 2010; Guderley and Portner 2010).

While the metabolism of stress may be an important regulator of predator prey interactions, studies investigating the relationships between cortisol, aerobic scope and

predator vulnerability are rather limited in the literature. A few examples do exist though that illustrate the influences of metabolic scope in mediating predator-prey interactions. In Atlantic silversides (*Menidia menidia*), differential investment into growth and swimming performance led to altered predator susceptibility whereby higher growth input corresponded with an elevated predation rate suggesting a role for energy budgeting in mediating predation risk (Lankford et al. 2001; Munch and Conover 2003; Arnott et al. 2006). Additionally, in golden grey mullet (*Liza aurata)*, the magnitude of aerobic scope was found to be directly proportionally to the animal's ability to sustain anti-predator activities. Interestingly, following an acute stressor, high investment into anti-predator activities corresponded with a reduced ability to recover from the physiological effects of the acute stressor representing an energetic trade-off between these two life history traits (i.e. finite scope; Killen et al. 2015b). Similarly, capture avoidance performance in common minnows (*Phoxinus phoxinus*) was found to be directly proportional to the animal's aerobic scope suggesting a role for metabolic scope in mediating predator-prey interactions (Killen et al. 2015a). Together, these effects demonstrate the importance of aerobic scope and trade-offs relating to life history traits in maintaining a low degree of predator vulnerability (Guderley and Portner 2010). As sustained activation of the stress response (i.e. homeostatic overload) can have the potential to alter the energy dynamics of a teleost fish (Sokolova et al. 2012; Sokolova 2013), it would be expected that the HPI axis, through the metabolic regulatory actions of cortisol, would likely play a significant role in predator-prey interactions presumably through a constrained ability to maintain anti-predator behaviours. This would likely manifest itself through a reduced input into vigilance-related activities as the fish cannot afford to maintain a high degree of this

behaviour in conjunction with other life history traits (Milinski 1993; Guderley and Portner 2010; Killen et al. 2013, 2015b). In an analogous situation, feeding on high densities of prey items where the fish's focus is directed towards feeding activities, ignorance of a predator is higher and, in some instances, predation rate is elevated suggesting a trade-off between foraging and anti-predator behaviours (Milinski 1984; Godin and Smith 1988; reviewed in Milinski 1993). Consequently, this could have implications for risk mediation whereby the ability to detect and/or avoid a predator is diminished thereby enhancing predation risk (reviewed in Lima and Dill 1990 & Milinski 1993).

The metabolic effects of sustained cortisol elevation (i.e. homeostatic overload) are also likely to play an important role in affecting the fish's risk-taking behaviours. As discussed earlier (see Section 1.2), sustained cortisol elevations are likely to increase the metabolic rate of the fish with a concurrent deterioration of the animal's body condition and energetic status (i.e. depleted reserves). As such, the state-dependent decision-making model (reviewed in Lima 1998) would suggest that fish experiencing a sustained homeostatic overload should spend (1) a greater proportion of their time foraging, (2) a reduction in refuge usage, and (3) an increased predation risk which should correspond with a greater predation rate. Together, these effects could allow the fish to offset the metabolic effects associated with chronic stress (i.e. higher SMR) at the cost of increasing its predation risk (Lima 1998). This effect may also help to explain the observed increase in predator-induced mortality observed in stressed fishes (Mesa et al. 1994; Raby et al. 2014). At this time though, the influences of the stress response and cortisol in mediating predation risk, with respect to spatial use patterns under a high

perceived predation risk environment, have yet to be investigated to any great extent. Currently, some of the only information pertaining to stress-induced changes in predation risk comes from observations on the effects of parasitism on threespine stickleback. Increasing parasite load, which could act as chronic stressor, was found to increase the overall activity of the animal as well as reduce the time by which the fish recovered from a predation attack (i.e. behavioural latency; Giles 1983, 1987; Godin and Sproul 1988). As well, fish with higher parasite loads were found to retreat shorter distances from a predation threat and forage for longer (Godin and Sproul 1988) and in closer proximity to a model predator against respective controls (Milinski 1985). Together, these results are consistent with what has been observed in energetically stressed fishes with poor condition acting to enhance risk-taking behaviours which generally includes reduced refuge use, shorter behavioural latencies and greater bouts of activity, among others (Gotceitas and Godin 1991; Krause et al. 1998; 2000; Dowling and Godin 2002; Petrie and Ryer 2006; Killen et al. 2011). Furthermore, under certain contexts and stressors, risk-taking behaviours can correlate with resting metabolic rates suggesting an interplay between metabolism and behaviour dynamics (Killen et al. 2011; 2012; reviewed in Biro and Stamps 2010). Presumably, as cortisol mediates energy metabolism, we may expect similar occurrences with respect to risk-taking behaviours in cortisol-treated fish. Although, this has not been investigated to any great extent.

Cortisol's direct role in mediating predator-prey interactions has not been characterized to any great extent and is severely lacking in the literature. Despite this, a handful of studies have provided some insight into cortisol-predation relationships. In schoolmaster snapper (*Lutjanus apodus*), cortisol-treatment over a 24 h period did not

alter any metrics associated with predation risk or anti-predator behaviours with predator mortality being comparable to controls (Lawrence et al. 2017; 2018a). Similarly, predator defense mechanisms in checkered pufferfish (*Sphoeroides testudineus*) as well as swimming performance was not impaired by cortisol-treatment (Cull et al. 2015; Pleizier et al. 2015). While these studies could suggest that cortisol has little role in mediating predator-prey interactions, they represent a rather limited contextual scope and did not address more fine-scale behavioural parameters keeping the analyses used at very coarse levels of scale. This highlights the need to further investigate the interactions between the HPI axis, cortisol and behavioural traits relevant to mediating predator-prey interactions.

1.6. Hypotheses and objectives

This dissertation will focus its attention on how chronic stress, mimicked through cortisol manipulations, can influence the interactions between predator and prey in wild centrarchid fishes. Specifically, the primary objective of this thesis will be to address how chronic stress influences the energy metabolism of centrarchid fishes and relate those changes to alterations in risk-taking and anti-predator behaviours, and predation mortality. Increasing anthropogenic activities in many of the world's aquatic systems threatens to elevate both the frequency and magnitude of stressors that a fish may encounter (Sondergaard and Jeppesen 2007; Sih et al. 2011). As centrarchid fishes are of substantial ecological, cultural and economic value (Cooke and Philipp 2009), understanding the interplay between physiological, behavioural and ecological levels of scale is of importance in addressing the potential fitness consequences and life-history trade-offs associated with chronic stress. As mentioned earlier, the physiological and behavioural mechanism(s) by which chronic stress influences predator-prey dynamics are

poorly understood in the current body of literature. Thus, this thesis will look to enhance our understanding of how perturbations in an individual's physiological processes can have population and ecosystem level impacts addressing a poorly understood area of fish biology. From a fisheries management context, this information could be relevant in potentially assessing the impacts of a particular stressor on centrarchid population viability and ecosystem stability. Specifically, the methodological framework as well as the conclusions derived in this thesis could provide fisheries managers with a more integrative and diverse toolset to address fitness impacts associated with stressor exposure.

My overarching hypothesis is that the metabolic alterations associated with a homeostatic overload state (i.e. chronic stress) promote increased risk-taking behaviours and/or compromised anti-predator behaviours resulting in higher predator-induced mortality. I test this hypothesis using two main species of interest, pumpkinseed (*Lepomis gibbosus*) and bluegill (*Lepomis macrochirus*) sunfishes, across four experimental studies. This thesis is arranged in a logical pattern whereby I first address the physiological effects of long-term cortisol elevations (Ch 2), I next address the behavioural level changes associated with chronic cortisol administration (Ch 3 & 4), and, lastly, I quantify HPI axis modulation of predator mortality (Ch 5). In Chapter 2, I explored the hypothesis that prolonged cortisol elevations would increase SMR resulting in a reduced aerobic scope with no effect on MMR. Once I had characterized the basic physiological impacts of chronic cortisol administration, I tested the hypothesis that, because of elevated metabolic expenditures, sunfish would tend to exhibit riskier behavioural phenotypes in isolated behavioural tests (Ch 3) and in a microcosm setting

(Ch 4). In both experimental series, I tested a number of specific behavioural parameters related to risk-taking and anti-predator behaviours that would be relevant in a predator-prey interaction context. Finally, I sought to address the hypothesis that, because of behavioural dysfunction under cortisol treatment, that predator mortality would be higher in cortisol exposed fish than sham or GR antagonised fish (i.e. treated with RU486; Ch 5).

1.7. Centrarchid fishes as a model

The centrarchid fishes represents a diverse taxonomic group that is native to freshwater systems throughout North America and found globally disturbed through anthropogenic introductions (Holm et al. 2009). Included in this taxonomic group are several notable sportfish species including largemouth and smallmouth bass (*Micropterus dolomieu*), pumpkinseed sunfish (*Lepomis gibbosus*) and crappie (*Pomoxis sp.*), amongst others. The centrarchid fishes as a whole represents an extremely important family with respect to its cultural and economic relevance. Recent surveys conducted by Southwick Associates (2018) placed largemouth bass, smallmouth bass, and panfish (which includes several centrarchid species) as three of the most targeted recreational sportfish groups in North America. Furthermore, recreational fisheries contribute more than $100 billion to the US economy with anglers spending in excess of $16 billion for costs associated with the angling of largemouth bass (Southwick Associates 2012). For example, a largemouth bass fishery at a single lake in the state of Texas (Lake Fork, TX) was estimated in generating an annual influx in excess of $27 million to the local economy (Chen et al. 2003). Given the high value associated with centrarchid fishes, effective management

strategies are impetrative in maintaining the sustainability of fisheries populations and, consequently, continued economic success.

Centrarchids are also important from a biological perspective. In many watersheds throughout North America, centrarchids occupy a number of ecologically diverse niche spaces and environments. As well, they are functionally diverse being found in an array of trophic levels including top predators (i.e. adult black basses) and secondary consumers (i.e. sunfish, crappie, juvenile black basses etc.) thereby acting to facilitate energy flow between producers and higher-level consumers (i.e. prey for top predators such as bass/pike/birds; Aday et al. 2009). Because of the evolutionary success of this family, centrarchids often form a significant proportion of the biomass and species richness of many of the watersheds in which they inhabit (Spotte 2007; Aday et al. 2009). As such, centrarchids represent an important component to many freshwater systems and are crucial for proper ecological functioning.

From a scientific perspective, centrarchids are an ideal model species. In most instances, the fish are in relatively high abundances and have a large reproductive capacity facilitating the use of high sample sizes in an experimental design with minimal impact on the environment. Secondly, centrarchids have been used for decades in countless physiological, behavioural and ecological experiments whereby the basic biology of these animals is well-known (reviewed in Cooke and Philipp 2009). Thus, as the framework for many of their underlying biological processes and life history traits have been established in the literature, centrarchid fishes serve as excellent model species in addressing complex biological questions.

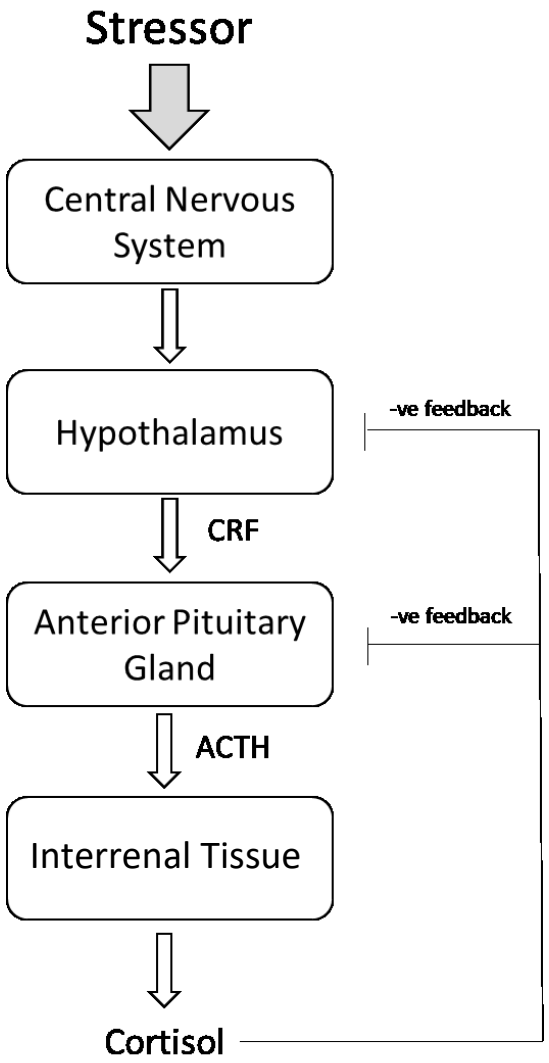


Figure 1.1: Schematic representation of the hypothalamic pituitary interrenal (HPI) axis in a teleost fish. Stressor exposure prompts the secretion of corticotropin-releasing factor (CRF) from the hypothalamus. CRF stimulates the secretion adrenocorticotropic hormone (ACTH) from the anterior pituitary gland which prompts an upregulation in cortisol biosynthesis in the interrenal tissues. Cortisol then acts on a number of downstream regulatory targets and also acts as a negative feedback mechanism for attenuating HPI axis activity. Adapted from Wendelaar Bonga (1997).

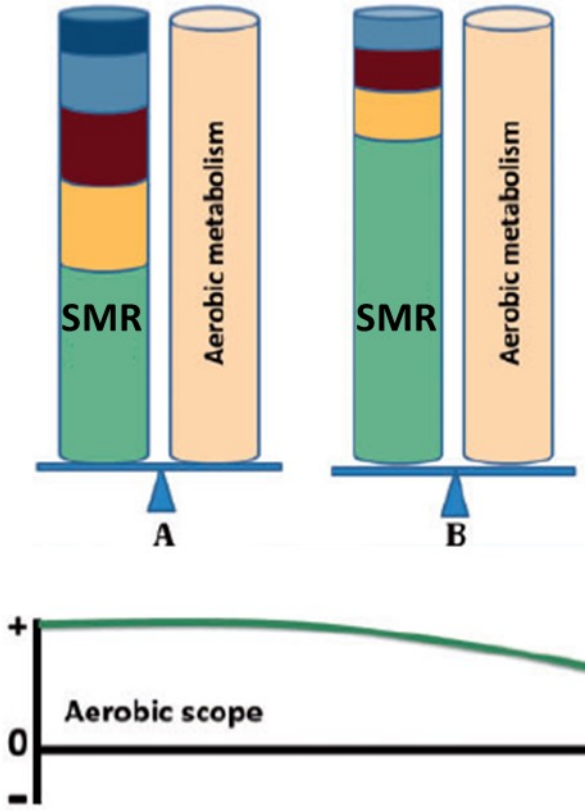


Figure 1.2: Theoretical influence of a sustained stressor on the metabolic operation of a teleost fish with respect to standard metabolic rate (SMR; green cylinder) and aerobic scope (green line below the cylinders). The coloured cylinders represent the various life history traits (e.g. growth, reproduction, predator evasion, etc.) partitioned amongst the animal's aerobic scope. Under resting conditions (**A**), aerobic metabolism (tan coloured cylinders) is allocated toward maintenance metabolism (SMR) and the animal's various life history traits (blue, red, yellow) where production is optimized. During a chronic stressor (**B**), the SMR of the fish is higher resulting in a reduced aerobic scope and a

reduced/constrained energetic input into production activities (e.g. growth, reproduction, swimming, etc.). Adapted from Sokolova (2013).

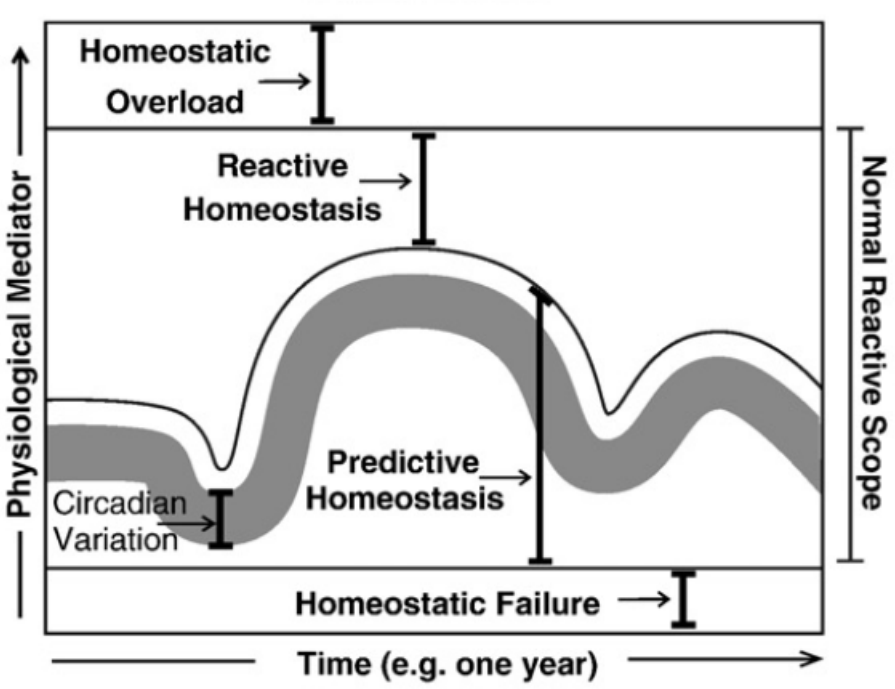


Figure 1.3: Conceptual diagram representing the basic reactive scope model as proposed by Romero et al. (2009). For the sake of illustration, cortisol represents the physiological mediator in this particular context. Seasonality in the physiological mediators are represented by the change in the line curvature of the predictive homeostasis. Adapted from Romero et al. (2009).

Chapter 2: Cortisol modulates metabolism and energy mobilization in wild-caught pumpkinseed (*Lepomis gibbosus*)

2.1. Abstract

Acute elevation of cortisol via activation of the HPI axis aids the fish in dealing with a stressor. However, chronic elevation of cortisol has detrimental effects and has been studied extensively in lab settings. Although, data pertaining to wild teleosts is lacking. Here, I characterized the metabolic consequences of prolonged cortisol elevation (96 h) in wild-caught pumpkinseed (*Lepomis gibbosus*). Pumpkinseed were implanted with cocoa butter alone (sham) or containing cortisol (25 mg kg^{-1} body weight), and at 24, 48, 72 and 96 h, tissue samples were collected, whole body ammonia excretion was determined in pumpkinseed and whole organism metabolism was assessed using intermittent flow respirometry. Cortisol-treated pumpkinseed exhibited the highest plasma [cortisol] at 24 h post-implantation, with levels decreasing over the subsequent time points although remaining higher than in sham-treated fish. Cortisol-treated fish exhibited higher standard and maximal metabolic rates than sham-treated fish, but the effect of cortisol treatment on aerobic scope was negligible. Indices of energy synthesis/mobilization, including blood [glucose], hepatosomatic index, hepatic [glycogen], and ammonia excretion rates, were higher in cortisol-treated fish compared to controls. My work suggests that although aerobic scope was not diminished by prolonged

elevation of cortisol levels, higher metabolic expenditures may be of detriment to the animal's performance in the longer term.

2.2. Introduction

One of the central aims of the stress axis in vertebrates is to provide the necessary physiological adjustments to facilitate the re-establishment of internal homeostasis in response to a stressor. These adjustments include shifts in energy mobilization/allocation and/or the induction of relevant ion/acid-base regulatory systems (Sapolsky et al. 2000; Romero et al. 2009; Schreck and Tort 2016). Over the last century, much research has focused on how stress influences the metabolism of vertebrates. Yet, the current literature relies heavily upon lab-based models with limited assessment of wild vertebrates (Hawlena and Schmitz 2010; Boonstra 2013a; Breuner et al. 2013), particularly wild-caught teleost fishes. In a large number of studies on wild fishes, cortisol's actions on energy metabolism are restricted to measurements of secondary responses (e.g. blood [glucose], plasma ions, hepatosomatic index, etc.; Cook et al. 2012; McConnachie et al. 2012; Zolderdo et al. 2016), with little investigation of the specific physiological pathways mediating these changes (Sopinka et al. 2016). In particular, the literature is deficient in descriptions of the specific metabolic pathways regulated by cortisol in wild teleosts as well as how various aspects of metabolic rate (e.g. standard and maximal metabolic rates, aerobic scope) are modulated by cortisol.

It has been well established that exposure to numerous stressors elicits an increase in circulating cortisol titres in a diversity of teleosts (Wood et al. 1999; Jentoft et al. 2005; Cook et al. 2012; Lawrence et al. 2018b; reviewed in Barton and Iwama 1991 & Barton 2002), although, the magnitude and timing of the cortisol response are often

context- and species-specific (Barton 2002; Cook et al. 2012; Winberg et al. 2016; Lawrence et al. 2018b). The rise in circulating cortisol, which typically occurs over minutes to hours following a stressor, generally assists in the *de novo* synthesis of high energy substrates (i.e. gluconeogenesis), the re-allocation of energetic reserves towards essential processes involved in stressor mitigation (e.g. suppression of growth and reproduction), and the re-establishment of hydromineral balance (reviewed in Mommsen et al. 1999, Aluru and Vijayan 2009 & Schreck and Tort, 2016) which generally includes increased plasma glucose levels (Pickering et al. 1982; Vijayan et al. 2003; McConnachie et al. 2012; Lawrence et al. 2017) as well as increased activity/expression of enzymes associated with gluconeogenesis (Aluru and Vijayan 2009; Momoda et al. 2007; Wiseman et al. 2007; reviewed in Mommsen et al. 1999). Additionally, cortisol is believed to have a role in the regulation of nitrogenous waste excretion in teleost fishes, possibly reflecting a role of cortisol in mediating proteolysis (Mommsen et al. 1999). Cortisol thus exerts considerable influence over the regulation of the organism's energy metabolism. As stressor mitigation is generally considered to be energetically expensive (Barton and Schreck, 1987; O'Connor et al. 2011; Schreck and Tort 2016), the actions of cortisol provide the animal with ample access to energetic reserves to facilitate homeostatic adjustments.

Acute activation of the glucocorticoid stress axis is generally considered to be beneficial to the organism in responding to a stressor (Schreck and Tort 2016). However, under the reactive scope model (Romero et al. 2009), sustained release of glucocorticoids can have a deleterious impact on the organism's physiological status because resources are diverted away from basic physiological requirements. This 'homeostatic overload'

(Romero et al. 2009) is often considered to be synonymous with the classical notion of 'chronic' stress. In teleosts, the effects of homeostatic overload or chronic stress typically manifest as reduced growth (Sadoul and Vijayan 2016) and reproduction (Pankhurst 2016), as well as impaired immune function (Yada and Tort 2016). Homeostatic overload can also have consequences in a metabolic context. Aerobic scope (AS), or scope for activity, represents the energy available for non-maintenance related activities and is the difference between the maximum metabolic rate (MMR) and the standard metabolic rate (SMR; i.e. aerobic scope= MMR-SMR; Fry 1947). Typically, available energy is allocated towards fitness-enhancing activities including growth, reproduction and predator avoidance (reviewed in Guderley and Portner 2010 & Sokolova 2013). Given that cortisol elevation in teleosts generally results in increased routine (Barton and Schreck 1987; Chan and Woo 1978; Morgan and Iwama 1996; De Boeck et al. 2001) and standard (O'Connor et al. 2011) metabolic rates, it may also reduce AS and therefore lower the allocation of energy towards fitness-enhancing activities. In an ecological context, this may have implications for predator evasion capacity (Mesa et al. 1994; Lawrence et al. 2017), growth dynamics (O'Connor et al. 2011) and reproductive output (Algera et al. 2017a,b). Despite the potential role of cortisol/stress in modulating metabolic rate in fishes, to date there has been little research in this area. Indeed, the current literature lacks work addressing the influence of cortisol on maximal metabolic rate, aerobic scope and recovery dynamics in teleost fishes despite these parameters being determinants of organismal performance (Guderley and Portner 2010; Eliason and Farrell 2016; Brownscombe et al. 2017). Furthermore, only a handful of studies have ascertained the impacts of cortisol on standard metabolic rates (O'Connor et al. 2011). To my

knowledge, no studies have addressed the role of cortisol in affecting maximal metabolic rate. Maximal metabolic rate is typically set by cardiovascular performance (Norin and Clark 2016). Although, some evidence suggests that cortisol has little bearing on cardiac performance in adult fish (Farrell et al. 1988). Because cortisol-metabolism interactions have the potential to modulate an animal's fitness, understanding the underlying mechanisms is of critical importance in determining organismal responses to stressors; a significant consideration in the Anthropocene where human activity is having significant impacts on the environment (Madliger et al. 2017).

The present study examined the role of cortisol in modulating standard and maximal metabolic rates, aerobic scope and recovery dynamics in wild-caught pumpkinseed (*Lepomis gibbosus*; Linnaeus 1758). To complement these whole-body metrics, a suite of tissue and blood energy metabolites was characterized, together with the relevant enzymes. I hypothesized that prolonged cortisol elevation would increase standard metabolic rate, with no effect on maximal metabolic rate, thereby reducing the animal's aerobic scope. To test this hypothesis, pumpkinseed were given cortisol-treated cocoa butter (25 mg kg^{-1} body weight; BW) implants, and monitored for oxygen consumption rates over a 96 h period. In addition, liver and blood tissue samples were collected for enzymatic/metabolite profiling.

2.3. Materials and methods

2.3.1. Animal collection, care and implantation

Juvenile pumpkinseed (N=285; mass=24.3±0.7 g; total length=110.6±0.4 mm) were captured in Lake Opinicon (44.5590° N, 76.3280° W) in the months of July and

August, 2016. Animals were collected (OMNRF permit #1082340) in shallow weedy bays using a seine net and were transported to the Queen's University Biological Station (Chaffey's Lock, ON, Canada). Fish were held in flow-through tanks (~435 L) supplied with natural lake water (T=25.2±0.1°C; NH_4^+ <0.25 mg L^{-1}; pH =7.5) under a natural photoperiod. During holding, fish were not fed. Pumpkinseed were allowed to acclimate to these holding conditions for 48 h prior to experimentation. Experimental protocols were approved by the Carleton University Animal Care Committee (AUP #104262) in compliance with the guidelines of the Canadian Council for Animal Care.

Pumpkinseed were randomly selected and given an intraperitoneal implant of cocoa butter (5 ml kg^{-1} body weight [BW]) either alone as a control (sham) or containing cortisol (hydrocortisone 21-hemiscuccinate; 25 mg kg^{-1} BW). This method of elevating circulating cortisol has been validated for use in teleost fishes (Gamperl et al. 1994). Cocoa butter containing cortisol was prepared as described in Hoogenboom et al. (2011). Following implantation, fish all of a single treatment group (25 individuals), were transferred to a holding tank (~211 L) under holding conditions as described above. However, pumpkinseed used in 24 h respirometry and metabolite flux experiments (see below) were transferred immediately to respirometry chambers following implantation. All fish were maintained in a fasted state throughout the experiments.

2.3.2. Experiment 1: Tissue level effects of exogenous cortisol elevation

Shoals of cortisol- or sham-treated pumpkinseed were maintained in holding tanks for 96 h. Starting at 24 h post-implantation, 4 fish per treatment group per day were selected haphazardly from the shoal and blood and liver tissues were collected for

analysis. Individual fish were captured with a small dip net, taking great care to minimize disturbance of conspecifics. A blood sample (~300 μL) was collected immediately via caudal venipuncture using a 23 G needle and a chilled, heparinized (Na^+ heparin, 10,000 USP units ml^{-1}; Sandoz Canada Inc., Boucherville, QC, Canada) 1-ml syringe. Blood samples were collected within three minutes as per the recommendation of Lawrence et al. (2018). Blood [glucose] was immediately determined using a portable, medical grade glucose metre (Accu-Chek Compact Plus, Hoffman-La Roche Limited, Mississauga, ON, Canada) that was previously validated for use with teleost fishes (Wells and Pankhurst, 1999; Serra-Llinares and Tveiten, 2012; Stoot et al. 2014). The remaining blood was centrifuged for 2 minutes (2,000 *g*; Mandel Scientific, Guelph, ON, Canada). Plasma was decanted, flash frozen in liquid nitrogen and stored at -80°C for subsequent analysis of concentrations of total ammonia (T_{amm}) and cortisol. Following blood sampling, the fish was quickly euthanized via cerebral percussion and wet mass and total length were measured. The liver was excised, weighed for determination of hepatosomatic index (HSI; see below), freeze clamped in liquid nitrogen and stored at -80°C for later determination of hepatic [glycogen] and [ammonia] (see below). This process was repeated every 24 h to 96 h, the final time at which samples were collected. This was repeated on 5 separate sets of fish (total N=20/day/treatment) with the final sample size for blood/tissues selected based on a median approach (see below for details).

Plasma [T_{amm}] was assessed using a commercially-available enzyme-linked assay kit (Raichem, Cliniqa, San Marcos, CA, USA) and microplate reader as in Lawrence et al. (2015). A commercial radioimunnoassay kit (ImmuChem Cortisol Coated Tube RIA Kit, MP Biomedicals, Solon, OH, USA) was used to measure plasma [cortisol]. This

assay was previously validated for use in teleost fishes (Gamperl et al. 1994). Intra- and inter-assay coefficients of variation were 8.7 and 4.7%, respectively.

Hepatic glycogen content was determined as described by Keppler and Decker (1974). Liver tissue was sonicated (~20s on an ice-water slurry) in a perchloric acid (PCA; 6%) solution and centrifuged (5 min @ 10,000 g). The resulting supernatant was pH balanced (pH=5.0) with K_2HCO_3 and then incubated with a 1% amyloglucosidase solution for 2 h at 37°C. The reaction was terminated using 25 µl of 70% PCA. Hydrolyzed glycogen samples were then assessed for total glucose content using the hexokinase-linked glucose assay described by Bergmeyer (1974). Hepatic [ammonia] was determined using a commercially-available enzyme-linked assay kit (Raichem, Cliniqa, San Marcos, CA, USA) as described in Lawrence et al. (2015).

2.3.3. Experiment 2: Effects of exogenous cortisol elevation on ammonia excretion

To investigate the effect of exogenous cortisol elevation on whole body ammonia excretion and, consequently, metabolic functioning, total ammonia excretion was determined using a simple flux chamber. As in previous studies (Wilson et al. 1994; Zimmer et al. 2010; Lawrence et al. 2015), flux chambers consisted of small (~1.5 L), blacked-out flow-through boxes supplied with aerated, natural lake water. Immediately following injection of the cocoa butter implant, fish (N=25 total; 14 cortisol-treated and 11 sham) were transferred to individual flux chambers; this point constituted time t=0 h. Unlike the respirometry trials (see Experiment 2), fish were allowed to freely move within the container. Fish were assessed over a 96-h period with fluxes carried out every 24 h to match blood/tissue (Experiment 1) and respirometry trials (Experiment 3). To

carry out a flux, water flow to the chamber was stopped, while still on independent aeration, for the 5 h period leading up to each experimental time point (i.e. 19-24 h, 43-48 h, 67-72 h, 91-96 h post implant). Water samples (720 µL) were collected at the beginning and end of the 5 h flux period and were immediately frozen and stored at -20°C for later analysis of [T_{amm}]. At 96 h, fish were euthanized and weighed. Assessments of control (sham) and cortisol-treated fish were conducted simultaneously. Water total ammonia concentrations were determined using the colourimetric salicylate assay of Verdouw et al. (1978).

2.3.4. Experiment 3: Characterization of metabolic rate under elevated cortisol

Oxygen uptake rates ($\dot{M}O_2$) were assessed at either 24 h or 96 h post-implant. For each time point, three fish from each treatment group (N=6 fish in total) were assessed simultaneously, and this procedure was repeated four times for both time points (for a total of N=12 per treatment per time point). This approach was adopted because pilot studies revealed that continuous confinement of pumpkinseed in a respirometer for 96 h resulted in mortality and confinement stress. For each assessment of oxygen uptake, three fish from the same treatment group were placed into individual respirometry chambers held in a reservoir; separate reservoirs were used for the different treatment groups. Treatment groups were assigned to reservoirs in a systematic randomized fashion to avoid reservoir-induced biases. After transfer into the respirometry chambers, the animals were left undisturbed for 29-35 h during which $\dot{M}O_2$ was continuously assessed by intermittent flow respirometry. Maximum metabolic rate (MMR) was then assessed for each fish by removing an individual from its chamber, manually chasing it for 3 minutes

and then exposing it to air for 1 min as per the recommendations of Norin and Clark (2016). Animals were then returned to their chamber and $\dot{M}O_2$ measurements resumed immediately to capture MMR. Fish were allowed to recover for ~15 h. At the end of the trial, fish were euthanized via cerebral percussion, weighed, and measured for total length.

Intermittent flow respirometry as described in Norin et al. (2014) and Clark et al. (2011) was used to measure $\dot{M}O_2$. Respirometers were held in a reservoir (~435 L) supplied with fresh lake water that was well aerated and thermostated to 25°C using submersible aquarium heaters. The water was replaced after every experimental series. Each respirometer consisted of a rectangular, polypropylene plastic box (~1.3 L) fitted with a recirculation loop that had its own independent flow (~5 L min^{-1}; Eheim Universal 300 model 1046, Germany). A set of baffles on either end smoothed flow through the respirometer and restricted the movement of the fish. The oxygen concentration of the water in the recirculation loop was measured continuously (0.5 Hz), under the control of Pyro Oxygen Logger software (V2.312; Pyroscience, Germany), using an oxygen sensor spot (Pyroscience, Aachen, Germany) coupled to an optical oxygen meter (Firesting O_2, Pyroscience). The flush loop of each chamber consisted of a port attached to a large water pump (flush pump; ~40 L min^{-1}; Atman PH 2000, China) that was under the control of an automatic timer. Based on previous research (Crans et al. 2015) and pilot work, the cycle used for these experiments consisted of a 7-min flush period (i.e. flush pump is running) followed by a 3-min closed (i.e. flush pump is off), measurement period. The slope of the decline in oxygen concentration during the measurement period was used to determine $\dot{M}O_2$. This analysis was conducted in Labchart (V 7.0.2; ADInstruments, Dunedin, New

Zealand). Background rates of oxygen consumption (i.e. no fish in chamber) were determined before and after each experimental trial and were subtracted from the fish's $\dot{M}O_2$. Chambers were cleaned routinely to avoid elevated background $\dot{M}O_2$.

Measurements of $\dot{M}O_2$ were derived during closed respirometry phases where the relationship between water [O2] and time had $r^2 > 0.9$. Standard metabolic rate was calculated as the average of the lowest 10^{th} percentile of all $\dot{M}O_2$ values for an individual fish (Chabot et al. 2016). Routine metabolic rate (RMR) was considered to be the average of all $\dot{M}O_2$ values over the 12 h period from midnight to noon (6 h D: 6 h L) prior to measurement of MMR. Maximum metabolic rate was calculated as the single highest value following the chase/air exposure event (Norin and Clark, 2016). Aerobic scope was calculated as both absolute scope (AS_a= MMR-SMR) and as factorial scope (AS_f=MMR*SMR^{-1}). Recovery time was taken to be the period from chasing/air exposure to the time when a fish's $\dot{M}O_2$ returned to within 10% of its RMR (RMR_{10}; Lee et al. 2003) over three consecutive time points. Excess post exercise oxygen consumption (EPOC) was determined by integrating the area under the $\dot{M}O_2$ curve and subtracting RMR_{10} from it (Lee et al. 2003).

2.3.5. Calculations and statistical analyses

Hepatosomatic index (HSI) was calculated as in Busacker et al. (1990) using HSI=(m_L/m_f)*100% where m_L is the wet mass of the liver (g) and m_f corresponds to the total mass of the fish (g). Ammonia flux (J_{amm}) was calculated as the difference in water ammonia concentrations between the initial and final water samples (M_{diff}), taking

chamber volume (V), fish mass (m) and flux time (t) into account such that $J_{amm}= (M_{diff}*V)/(t*m)$.

Manipulation of glucocorticoid levels using cocoa butter implants often results in variable circulating cortisol concentrations (see Sopinka et al. 2015; Crossin et al. 2016). To avoid confounding effects associated with fish in which the implant was ineffective (plasma [cortisol] not elevated at the sampling time) or too effective (plasma [cortisol] elevated beyond the desired maximum of 140 ng ml^{-1}), individuals that exhibited plasma [cortisol] outside of the desired range of 36 to 140 ng ml^{-1} were excluded from analyses of blood or tissue variables. This approach was not possible for individuals used in respirometry or ammonia excretion trials because plasma samples could only be collected post-experiment, when circulating cortisol levels would have been vulnerable to stress associated with the experiment and handling, and the implants would have been past their effective period.

All statistical analyses were conducted using SigmaPlot v11.0 (Systat Software Inc., San Jose, CA, USA). Unless otherwise noted, all data are presented as mean±1 s.e.m. (N) with statistical significance being accepted at $\alpha=0.05$. A two-way analysis of variance (ANOVA) was employed to compare treatment and time effects for all blood, tissue and metabolic parameters. Ammonia excretion was analyzed using a two-way repeated measures ANOVA with time and treatment group as the two factors. When statistical significance was detected, Tukey's HSD post-hoc test was used.

2.4. Results

2.4.1. Plasma cortisol

Plasma [cortisol] was higher in cortisol-treated fish across all sampling times, relative to sham-treated individuals (Figure 2.1). The time x treatment interaction was found to be significant (F=6.097; df=3; P=0.001). In cortisol-treated fish, plasma [cortisol] decreased by over 70% from 24 to 48 h, remaining relatively constant thereafter.

2.4.2. Metabolic rate parameters

Cortisol-treated fish exhibited significantly higher SMR relative to shams (F=21.678; df=1; P<0.001; Figure 2.2A). In cortisol-treated fish, SMR was ~20 and 12% greater than values for the corresponding shams for pumpkinseed assessed at 24 h and 96 h, respectively. Cortisol treatment also resulted in a significant elevation of RMR relative to sham-treated fish (F=18.536; df=1; P<0.001; Figure 2.2B). For both SMR and RMR, neither the effect of time (*SMR*: F=2.340; P=0.134; df=1; *RMR*: F=1.457; P=0.234; DF=1) nor the interaction of time and treatment group (*SMR*: F=1.368; P=0.249; df=1; *RMR*: F=2.034; P=0.162; DF=1) was found to be significant.

MMR was also significantly higher in cortisol–treated pumpkinseed relative to sham-treated fish (F=7.240; df=1; P=0.010; Figure 2.2C). Measurement time was also found to have a significant influence on MMR (P= 0.017), with fish measured at 24 h post-implant having significantly higher MMR than fish measured at 96 h (Figure 2.2C). However, the interaction of time and treatment group was not significant (P=0.182). The similar rises in both SMR and MMR with cortisol treatment resulted in comparable AS_A (F=2.159; df=1; P=0.150; Figure 2.2D) and AS_F (F=1.901; df=1; P=0.176; Table 2.1) between sham- and cortisol-treated fish. Time was found to have a significant influence on AS_A (P=0.048; Figure 2.2D), with neither AS_A nor AS_F displaying a significant interaction term (P>0.05).

Recovery dynamics were generally unaffected by cortisol treatment. Both recovery time (P=0.404; Table 2.1) and effort (i.e. EPOC; P=0.506; Table 2.1) were similar between treatment groups, with fish taking approximately 4.8 h to return to its RMR_{10} level.

2.4.3. Blood and tissue metabolites

Blood [glucose] was significantly higher in cortisol-treated fish compared to sham-treated fish (F=52.836; df=1; P<0.001; Figure 2.3A). Although there was a significant influence of time on blood glucose concentrations (F=3.376; df=3; P=0.024), no significant interaction was detected between time and treatment group (F=2.480; df=3; P=0.069). Plasma total [ammonia] was not significantly affected by either measurement time (F=0.474; df=3; P=0.702) or treatment group (F=0.887; df=1; P=0.351) (Figure 2.3B).

Cortisol treatment in pumpkinseed resulted in elevation of both HSI (F=20.994; df=1; P<0.001; Figure 2.4A) and liver glycogen content (F=120.163; df=1; P<0.001; Figure 2.4B). However, unlike hepatic [glycogen] (F=0.504; df=3; P=0.681), HSI was found to decrease across sampling times (F=3.559; df=3; P=0.019; Figure 2.4B); in neither case was the interaction of treatment group and sampling time significant. Hepatic [ammonia] was unaffected by sampling time (F=0.763; df=3; P=0.524), treatment (F=1.783; df=1; P=0.192; Figure 2.4C) or the interaction of these two factors (F=1.443; df=3; P=0.251).

2.4.4. Ammonia excretion

An interaction between effects of treatment group and flux time ($F=7.249$; $df= 3$; $P<0.001$; Figure 2.6) was detected for ammonia excretion. Cortisol-treated fish exhibited a higher ammonia excretion rate over the first 48 h, relative to sham-treated fish, returning to sham levels by 72 h post-implant (Figure 2.6). The ammonia excretion rate peaked at 24 h in cortisol-treated fish and was ~1.9x higher than the corresponding sham value.

Table 2.1: The effect of cortisol implantation (25 mg kg^{-1} BW) on factorial scope (AS_f=MMR*SMR^{-1}), excess post-exercise oxygen consumption (EPOC) and recovery time in pumpkinseed (*Lepomis gibbosus*) 24 h and 96 h post-implantation. No significant differences were observed between treatment groups or sampling time points (two-way ANOVA, P>0.05). Values are shown as means±1 SEM.

	Sham		**Cortisol**	
Parameter	*24 h*	*96 h*	*24 h*	*96 h*
Factorial Scope	3.9±0.1 (N=11)	3.8±0.2 (N=11)	3.7±0.2 (N=11)	3.6±0.2 (N=11)
EPOC (mg O_2 kg^{-1})	277±16 (N=11)	327±42 (N=11)	300±55 (N=11)	271±19 (N=11)
Recovery Time (h)	5.0±0.5 (N=11)	5.2±0.5 (N=11)	4.8±0.7 (N=11)	4.8±0.7 (N=11)

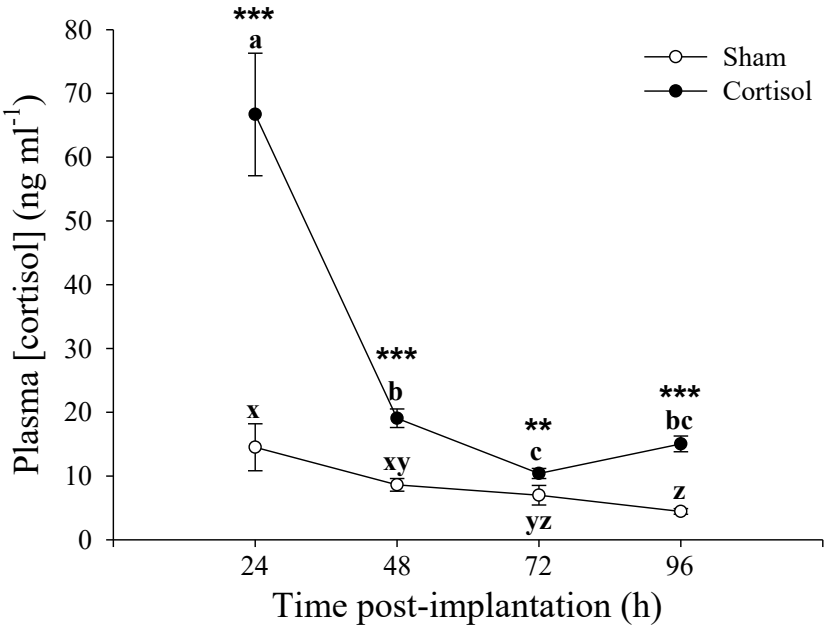


Figure 2.1: Plasma [cortisol] of sham- (5 ml kg-1 BW cocoa butter) and cortisol-treated (25 mg kg-1 BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) over a 96-h sampling period for only those fish used in tissue assays (N=9). Values are shown as means±1 SEM. Statistical significance was accepted at $\alpha=0.05$ with differences between treatment groups represented by an asterisk (** $P\leq0.01$, *** $P\leq0.001$) whereas different letters designate differences within a treatment group.

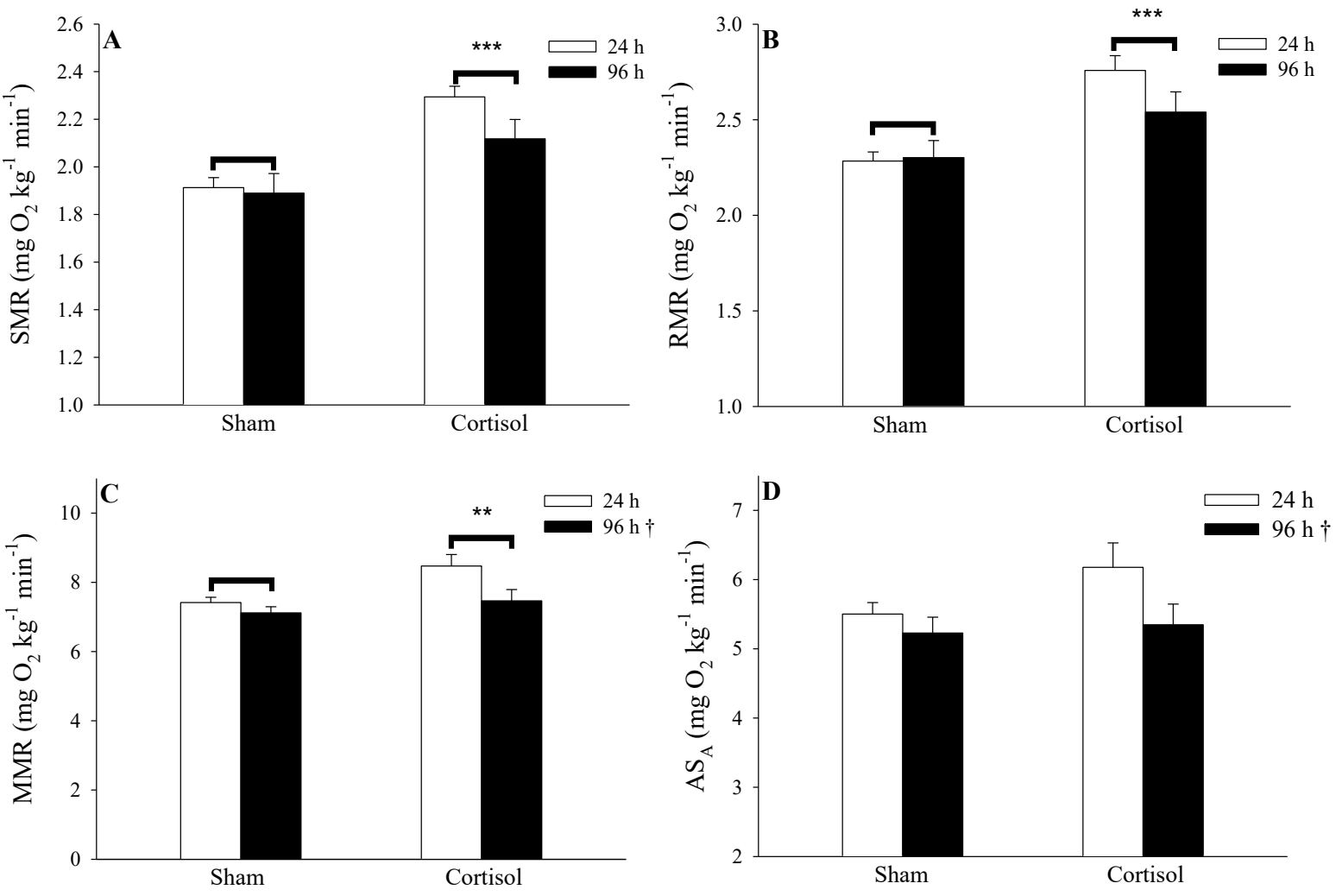


Figure 2.2: The influence of sham- (5 ml kg^{-1} BW cocoa butter) and cortisol-treatment (25 mg kg^{-1} BW cortisol in cocoa butter) on pumpkinseed (*Lepomis gibbosus*) metabolic parameters over a 96-h sampling period. The metabolic parameters measured included (**A**) standard metabolic rate (SMR; N=11), (**B**) routine metabolic rate (RMR; N=11), (**C**) maximal metabolic rate (MMR; N=11) and (**D**) absolute aerobic scope (AS_A; N=11). Values are shown as means±1 SEM. Statistical significance was accepted at α=0.05 with differences between treatment groups represented by an asterisk (** $P \leq 0.01$, *** $P \leq 0.001$). A dagger (†) denotes a statistically significant effect of time ($P < 0.05$).

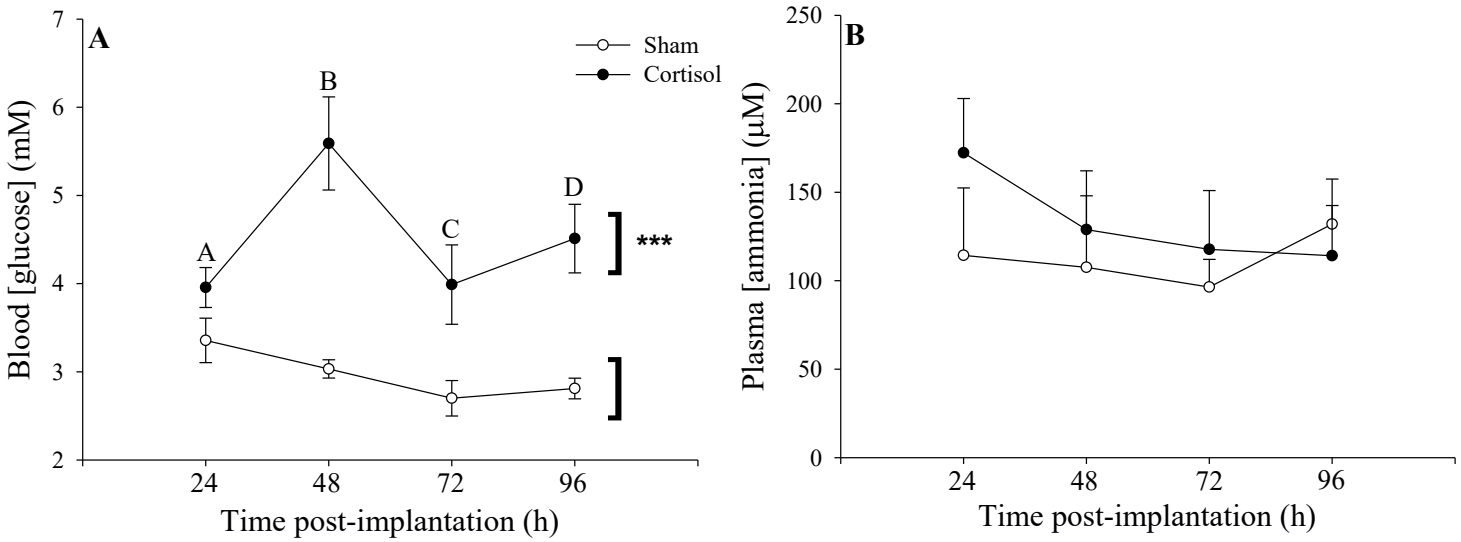


Figure 2.3: Blood [glucose] (**A**; N=9) and plasma [ammonia] (**B**; N≤9) for sham- (5 ml kg^{-1} BW cocoa butter) and cortisol-treated (25 mg kg^{-1} BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) over a 96-h sampling period. Values are shown as means±1 SEM. Statistical significance was accepted at $\alpha=0.05$ with differences between treatment groups represented by an asterisk (*** $P\leq0.001$). Capital letters denote a statistically significant effect of time ($P<0.05$).

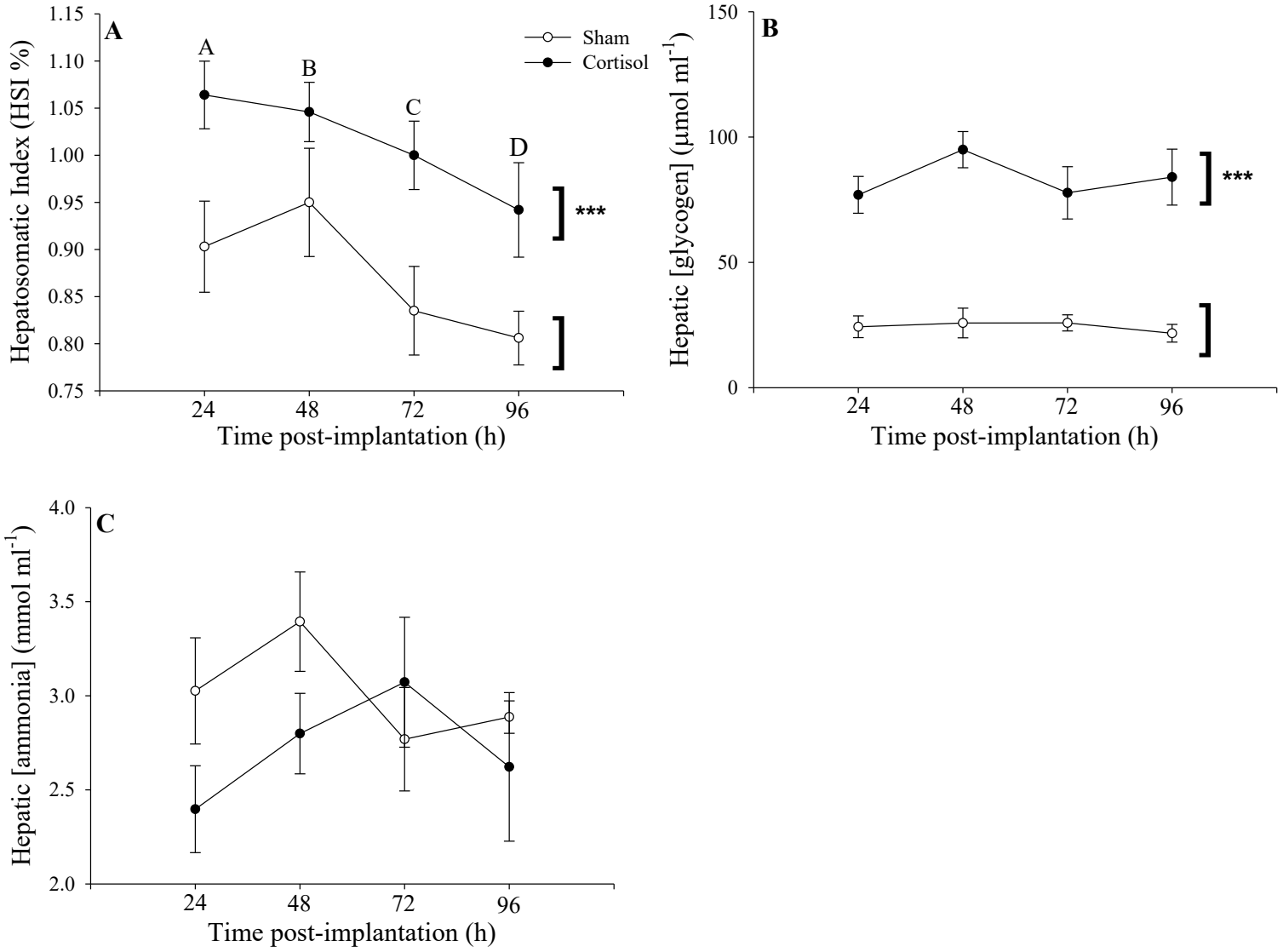


Figure 2.4: Hepatosomatic index (**A**; N≤9), hepatic [glycogen] (**B**; N≤9), and total hepatic [ammonia] (T_{amm}; **C**; N≤6) for sham- (5 ml kg^{-1} BW cocoa butter) and cortisol-treated (25 mg kg^{-1} BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) over a 96-h sampling period. Values are shown as means±1 SEM. Statistical significance was accepted at α=0.05 with differences between treatment groups represented by an asterisk (*** P≤0.001). Capital letters denote a statistically significant effect of time (P<0.05).

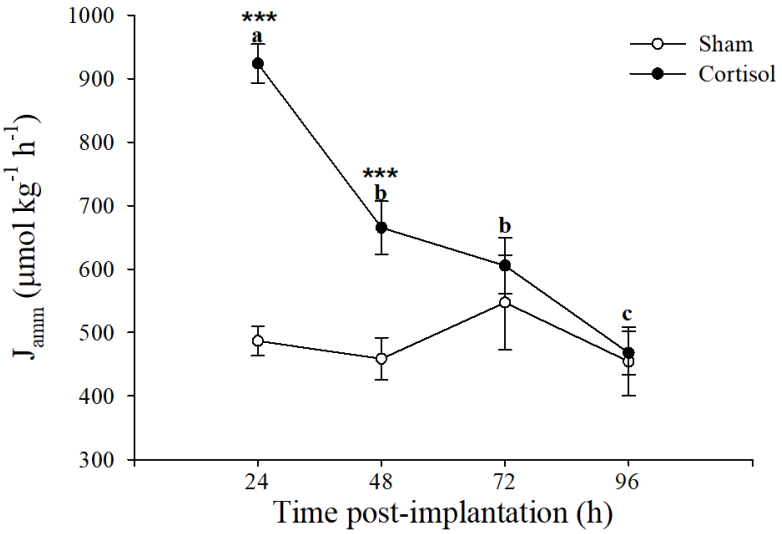


Figure 2.5: Whole body ammonia excretion rate for sham- (5 ml kg^{-1} BW cocoa butter) and cortisol-treated (25 mg kg^{-1} BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) in individual pumpkinseed monitored over a 96 h period. Values are shown as means±1 SEM. Statistical significance was accepted at α=0.05 with differences between treatment groups represented by an asterisk (*** P≤0.001), whereas different letters designate differences within a treatment group.

2.5. Discussion

2.5.1. Overview:

Cortisol treatment of wild-caught pumpkinseed using intraperitoneal implants revealed effects of prolonged cortisol elevation on both SMR and MMR. Higher SMR likely reflects increased maintenance costs under cortisol elevation whereas higher MMR could be the result of several factors, including adrenergic sensitization, enhanced mitochondrial capacity and/or increased branchial/cutaneous O_2 uptake. Neither AS_A nor recovery dynamics was affected by prolonged cortisol elevation, suggesting that physiological performance would not be impaired. Cortisol treatment appeared to modulate carbohydrate metabolism by increasing blood glucose levels and hepatic glycogen content. Despite the absence of changes in plasma and hepatic ammonia concentrations, cortisol treatment elicited higher rates of ammonia excretion in pumpkinseed, which was likely a result of either increased turnover of proteins or enhanced transport activity. This work contributes to our knowledge of cortisol effects on metabolism by focusing on SMR, MMR and AS_A, and the specific metabolic pathways that are impacted by prolonged cortisol elevation, in a wild-caught teleost fish, a group that is under-represented in the literature.

2.5.2. Validation of cortisol implants

Cortisol implants have been widely used as a mechanism of elevating plasma cortisol levels over sustained durations in a number of teleost species (Basu et al. 2001; DiBattista et al. 2005; Lawrence et al. 2017; reviewed in Gamperl et al. 1994 & Sopinka et al. 2015), including wild centrarchids (O'Connor et al. 2009; McConachie et al. 2012;

Zolderdo et al. 2016; Algera et al. 2017b). This method attempts to mimic sustained cortisol titres in the blood that would be reflective of a semi-chronically stressed state (Carmichael et al. 1984; Pickering and Pottinger, 1989; Sloman et al. 2001; Lankford et al. 2005). Although, it is important to realize that cortisol represents one small component of a much larger stress response which includes a number of neuroendocrine inputs which were not represented in this work (Sopinka et al. 2015; 2016). Cortisol titres in pumpkinseed plasma never exceeded those that would be observed under a natural, acute stressor for a centrarchid fish (e.g. 66 ng ml^{-1} peak here; 115-546 ng ml^{-1} in other centrarchids; Davis and Parker 1986; Cook et al. 2012). The sharp decrease in plasma cortisol concentrations from 24 h to 48 h post implant is typical of this methodology, with plasma cortisol titres stabilizing at an elevated level over more chronic timeframes (Vijayan et al. 1991; Gamperl et al. 1994; McConnachie et al. 2012; i.e. to 96 h in the present study).

2.5.3. Cortisol's influence on whole-body metabolism

As predicted, both standard and routine metabolic rates were higher in cortisol-treated wild-caught pumpkinseed relative to shams. Similarly, previous studies in teleost fishes reported that cortisol treatment can increase routine (Barton and Schreck 1987; Chan and Woo 1978; Morgan and Iwama 1996; De Boeck et al. 2001; Liew et al. 2013) and standard metabolic rates (O'Connor et al. 2011). As well, chronic stress can also increase standard metabolic rates (24 h social stress; Sloman et al. 2000). Furthermore, even longer exposure to stress, 28 days, elevated SMR to approximately 30% higher than the control value in green sturgeon (*Acipenser medirostris*; Lankford et al. 2005). The higher SMR observed here likely stems from increased maintenance costs associated with

cortisol's regulatory actions (e.g. impacts on protein synthesis, energy substrate formation, ionoregulation, etc.; reviewed in Wendelaar Bonga 1997 & Schreck and Tort 2016). Supporting this notion are the higher blood glucose and hepatic glycogen concentrations, as well as the higher whole-body ammonia excretion rate observed in cortisol-treated pumpkinseed. Interestingly, prolonged elevation of circulating cortisol titres has been shown to reduce locomotory activity in teleosts (Overli et al. 2002; Algera et al. 2017a), suggesting that the changes in routine metabolism observed here likely reflected alterations in internal metabolic processes rather than behaviour.

Cortisol treatment also resulted in higher MMR relative to sham-treated pumpkinseed. Information pertaining to the influence of cortisol on MMR in fishes appears to be lacking in the literature. In green sturgeon, exposure to a randomized, chronic stressor had no influence on MMR but elevated SMR and lowered AS (Lankford et al. 2005). In common carp (*Cyprinus carpio*), cortisol treatment via an implant resulted in higher $\dot{M}O_2$ during active swimming when compared to sham-implanted and control fish (Liew et al. 2013), but MMR was not assessed. The present work appears to be the first to directly investigate the role of cortisol on MMR. In teleosts, MMR is dictated primarily through the animal's ability to deliver oxygen to its tissues (i.e. cardiac performance) and oxygen extraction by the tissues (Fry and Hart 1948; Clark et al. 2011; Eliason et al. 2011; reviewed in Farrell et al. 2009 & Norin and Clark 2016). Cortisol may sensitize adrenergic responsiveness of the cardiovascular system, thereby enhancing oxygen delivery to tissues (e.g. Reid et al. 1992; Perry and Reid 1993; Reid et al. 1996; reviewed in Perry and Capaldo 2011). Alternatively, citrate synthase activity, has been found to be higher under elevated blood cortisol in teleosts (Foster and Moon 1986;

Tripathi and Verma 2003), and this enzyme is considered to be a proxy for mitochondrial density and aerobic capacity (Johnson 1981; Torres and Somero 1988). Another possibility is that cutaneous and gill oxygen uptake may increase in fish with elevated plasma cortisol levels, which would increase MMR (Farrell et al. 2014) but this idea remains highly speculative and unproven. Clearly, the mechanisms underlying changes in MMR with cortisol treatment require further study.

In teleost fishes, AS represents the available aerobic energy that can be allocated towards fitness-related activities (e.g. growth, reproduction, swimming, etc.), and is calculated as the difference between SMR and MMR (Fry 1947; Guderley and Portner 2010; Sokolova 2013). My results contrast with my prediction that cortisol would constrain AS of pumpkinseed primarily through an increase in SMR. To my knowledge, the present study is the first pertaining to a direct role of cortisol in modulating AS. However, using repeated stress in green sturgeon over 28 days, Lankford et al. (2005) reported increased SMR and reduced AS_A relative to control fish. Because organismal performance is thought to reflect AS (e.g. swimming capacity, reproduction, growth, etc.; Fry 1947; Guderley and Portner 2010; Sokolova 2013), my results suggest that performance, at the physiological level, should not be impacted by semi-chronic (i.e. 24-96 h) cortisol elevation in pumpkinseed. However, I remain cautious in this interpretation because the greater SMR under cortisol treatment likely would require increased food intake (Brett and Grooves 1979; Metcalfe 1986; Gregory and Wood 1999). At the same time, cortisol reduces food conversion efficiency (Gregory and Wood 1999; Bernier et al. 2004) and acts as an anorexigenic agent at high doses (Gregory and Wood 1999; Bernier et al. 2004; Madison et al. 2015). Thus, if pumpkinseed are unable to meet their basic

nutritional demands, then there may be physiological consequences of elevated SMR such as reduced growth. Furthermore, increased time spent foraging to meet dietary requirements could have implications for predator avoidance, thereby impacting organismal fitness (reviewed in Lima and Dill 1990 & Godin 1997).

2.5.4. Regulation of carbohydrate metabolism by cortisol

In teleost fishes, cortisol acts as the primary glucocorticoid promoting increased gluconeogenic capacity and the diversion of energy resources away from non-essential processes (reviewed in Wendelaar Bonga 1997 & Schrek and Tort 2016). As such, elevation of circulating cortisol increases blood glucose levels in a variety of teleost fishes under various environmental settings (e.g. Soivio and Oikari 1976; Pickering et al. 1982; Vijayan et al. 1997; Suski et al. 2007; McConnachie et al. 2012; Lawrence et al. 2017; reviewed in Mommsen et al. 1999). In centrarchids, this effect has been well characterized following exposure to stressors (Carmichael et al. 1984; Gustaveson et al. 1991; Suski et al. 2003; Cook et al. 2012; Lawrence et al. 2018b) or cortisol implants (McConnachie et al. 2012; Zolderdo et al. 2016). In line with the literature, my cortisol-treated pumpkinseed demonstrated a significantly higher blood glucose concentration.

Although cortisol can have variable effects on hepatic glycogen content in teleost fishes (Storer 1967; Foster and Moon 1986), in the present study, as in other works (Butler 1968; Inui and Yokote 1975; Chan and Woo 1978; De Boeck et al. 2001; Laiz Carrion et al. 2002, 2003; Vijayan et al. 2003), cortisol elicited higher hepatic glycogen content. As in mammals, teleost hepatic glycogen content is the net product of simultaneous glycogen synthesis (i.e. glycogen synthase) and catabolism (i.e. glycogen phosphorylase; reviewed in Faught and Vijayan 2016). Cortisol treatment in teleosts has

been found to lower glycogen phosphorylase activity (Laiz Carrion et al. 2002, 2003; Milligan 2003) and increase glycogen synthase transcripts/activity (Milligan, 2003; Leung and Woo, 2010), effects that could account for the increase in glycogen content noted in cortisol-treated pumpkinseed. In contrast, however, cortisol has also been shown to increase the expression of glycogen phosphorylase (Baltzegar et al. 2014), highlighting the variation within the literature (Mommsen et al. 1999).

2.5.5. Nitrogenous waste metabolism

Whole body ammonia excretion was higher under cortisol treatment, suggesting that amino acid turnover was likely elevated (Wood et al. 1999; Lawrence et al. 2015). In teleosts, ammonia is the primary nitrogenous waste product and is formed from the transdeamination of amino acids (reviewed in Wright 1995 & Wright and Wood 2009). Thus, heightened protein turnover is often associated with elevated ammonia production and excretion (Smith, 1929; Wood et al. 1999; Lim et al. 2001; Zimmer et al. 2010; Lawrence et al. 2015). Because cortisol regulates protein turnover and amino acid metabolism (reviewed in Mommsen et al. 1999), a rise in cortisol is expected to be accompanied by increased ammonia production/excretion (Storer 1967; Hopkins et al. 1995; Wood et al. 1999; McDonald and Wood 2004; Liew et al. 2013; Lawrence et al. 2015), which is consistent with the pumpkinseed data presented here. Cortisol could also enhance ammonia excretion through upregulation of transport mechanisms, namely Rhesus (Rh) glycoproteins, as proposed in previous studies (Nawata and Wood 2009; Tsui et al. 2009; Lawrence et al. 2015). Comparable plasma ammonia concentrations among treatment groups is likely the result of elevated ammonia excretion to prevent

accumulation in the blood/tissue. Together, my data suggests that cortisol is an important mediator of protein metabolism in pumpkinseed.

2.5.6. Conclusions

Despite higher SMR with cortisol treatment, AS was unaffected in pumpkinseed, owing to concomitantly higher MMR in cortisol-treated fish. Thus, aerobic performance is unlikely to be impaired under prolonged cortisol elevation. Additionally, cortisol treatment resulted in hyperglycemia paired with increased stores of glycogen in the liver and increased whole body ammonia excretion rates. Thus, energy mobilization and storage appeared to be enhanced under cortisol treatment. Together, my results are some of the first characterizations of the direct role of cortisol and the HPI axis in mediating metabolic dynamics in a wild-caught teleost fish. While providing insight into the specific physiological mechanisms by which cortisol exerts an effect on the metabolic operation of a teleost, this work is also relevant to understanding the "ecology of stress" (Boonstra 2013a) in wild animals - an important consideration in the ever-changing Anthropocene (Madliger et al. 2017).

Chapter 3: Does experimental cortisol elevation mediate risk-taking and antipredator behaviour in a wild-caught teleost fish?

3.1. Abstract

The hypothalamic-pituitary-interrenal (HPI) axis is centrally implicated in stressor mitigation in teleost fishes. Sustained HPI axis activation can be detrimental to the physiological functioning of an organism and can result in fitness-related trade-offs.

Predator-induced mortality is known to be higher in stressed fish than in unstressed conspecifics, suggesting a role for the HPI axis in mediating fish behaviour. However, the underlying specific mechanism(s) for this phenomenon is(are) unknown. The purpose of the current study was to address how the HPI axis influences risk-taking, and antipredator behaviours in a wild-caught teleost, the pumpkinseed sunfish (*Lepomis gibbosus*). Here, individual juvenile pumpkinseed were implanted either with cocoa butter as a sham control or with a biologically-relevant concentration of cortisol. Forty-eight hours post-implantation, fish were assessed for behavioural metrics associated with boldness and risk taking in three sequential behavioural tests: (i) a predation-risk test, (ii) an exploration tendency test, and (iii) a shoaling tendency test, with test order randomized between different trials. Cortisol treatment had no influence on antipredator, exploratory, or shoaling behaviours. However, post-attack swimming duration (in predation-risk test) and exploratory activity (in Z-maze exploration test) were significantly affected by body mass. Collectively, my results indicate that cortisol may not have a role in mediating sociability, boldness, and risk-taking behaviours in pumpkinseed sunfish, at least under the current laboratory conditions. However, cortisol may nonetheless play a role in mediating predator-prey interactions in fishes in more natural environmental settings that were not considered here.

3.2. Introduction

In teleost fishes, the hypothalamic-pituitary-interrenal (HPI) axis represents one of primary axes involved in the stress response. Briefly, under hypothalamic coordination, the HPI axis regulates the biosynthesis of cortisol, the primary glucocorticoid hormone in teleosts, which is upregulated in response to stressors

(reviewed in Barton and Iwama 1991; Mommsen et al. 1999; Barton 2002). Stressor mitigation is typically considered to be an energetically expensive process (Davis and Schreck 1997; Schreck 2010; Schreck and Tort 2016). As such, cortisol's functional role facilitates the prioritization of metabolic energy towards homeostatic readjustment while simultaneously increasing energy substrate mobilization via gluconeogenesis (reviewed in Mommsen et al. 1999; Schreck and Tort 2016). In this manner, cortisol's actions enable the animal to cope with the stressor-induced physiological challenge, ensuring continued survival during stressor exposure (Romero et al. 2009).

Chronic HPI axis stimulation can be detrimental to optimal physiological performance. Under sustained cortisol elevation, such as in a chronically-stressed state, metabolic power is diverted away from non-essential activities resulting in divestment from fitness-enhancing processes (reviewed in Pankhurst 2016, Sadoul and Vijayan 2016; Yada and Tort 2016). Furthermore, basal metabolic expenditures are typically elevated under chronic elevations of cortisol (De Boeck et al. 2001; O'Connor et al. 2011), which may constrain available energy budgeting (Sokolova 2013). Sustained cortisol elevation is also associated with glucocorticoid receptor downregulation (Sathiyaa and Vijayan 2003; Aluru and Vijayan 2007), which could conceivably impair the animal's response to subsequent stressors resulting in a decreased ability to cope with environmental challenges (Sneddon et al. 2016). Thus, there is considerable evidence suggesting that continued HPI axis stimulation can be costly to an animal in certain contexts, especially during chronic stimulation (i.e. homeostatic overload; Romero et al. 2009).

Currently, our understanding of how a homeostatic overload, specifically cortisol, influences organismal performance/fitness and its effects on higher orders of biological scale (e.g. behaviour and population dynamics) in wild teleosts is relatively poor. This is especially true for how the HPI axis regulates predator-prey interactions in nature, wherein stressed teleosts tend to exhibit riskier behaviour (Brown et al. 1985; Handelhand et al. 1996; Piato et al. 2011) and suffer higher rates of predation (reviewed in Mesa et al. 1994; Raby et al. 2014), relative to unstressed conspecifics. However, no apparent mechanism(s) underlying these observations has been characterized (Schreck et al. 1997). Behavioural decision making, in the context of predator-prey interactions, in prey fish is considered state dependent and reflects a trade-off between individual risk of mortality to predation and fitness-enhancing activities such as foraging and reproduction (reviewed in Lima and Dill 1990; Lima 1998). Indeed, fish experiencing energetic distress, such as starvation or parasitism, are more likely to accept a greater degree of predation risk (i.e. the vulnerability to a predation event; Lima and Dill 1990) as exemplified in reduced post-attack behavioural latencies (Giles 1983; Godin and Sproul 1988; Gotceitas and Godin 1991), a greater proportion of their time foraging in open environments (Magnhagen 1988; Godin and Smith 1988) and reduced refuge use (Skajaa et al. 2003; Vehanen 2003; Petrie and Ryer 2006; Killen et al. 2011). Additionally, the ability to sustain vigilance behaviours is believed to be directly tied to a fish's available metabolic scope (Milidine et al. 2006; Killen et al. 2015), with metabolism playing a role in mediating risk-taking behavioural phenotypes (i.e. represented as a suite of consistent behavioural metrics (reviewed in Conrad et al. 2011; Godin and Sproul 1988; Krause et al. 1998; Killen et al. 2011). Collectively, these findings suggest that the metabolic

changes associated with chronic cortisol elevations may be an important mediator of predator-prey interactions in wild fish; an effect tested only in a limited number of settings to date (Cull et al. 2015; Pleizier et al. 2015; Lawrence et al. 2017, 2018a).

The objective of the current study was therefore to further our understanding of the role of the HPI axis, specifically cortisol, in mediating risk-taking and antipredator behaviours in a wild-caught teleost fish, the pumpkinseed sunfish (*Lepomis gibbosus*, Linnaeus 1758). Previous work has shown that externally-administered cortisol in this species elicits an increased standard metabolic rate (Chapter 2). As such, I hypothesized that animals subject to sustained cortisol elevations should exhibit riskier behavioural phenotypes as a product of elevated metabolic expenditures. To test this hypothesis, wild-caught sunfish were treated with either a sham- or cortisol-containing implant and assessed 48 h post-implantation for a variety of behavioural indices of risk-taking and antipredator activities (see Chapman et al. 2010).

3.3. Methods

3.3.1. Fish collection and holding conditions

Juvenile pumpkinseed sunfish (mass = 8.4 ± 0.2 g; total length = 81.4 ± 7.5 mm) were haphazardly collected using a hand seine in the shallow nearshore reaches of Lake Opinicon, Ontario, Canada (44.5590° N, 76.3280° W) during June and July 2017 (under Ontario Ministry of Natural Resources and Forestry permit #1086180). Capture sites were always of the same habitat type, which consisted of a muddy bottom with short vegetation interspersed with woody debris. This was done to avoid potential confounds with specific behavioural phenotypes being associated with habitat type (e.g. Kobler et al.

2011; Wolf and Weissing 2012). Seine netting was the preferred capture method to avoid any potential biases in the selection of specific personality types (i.e. angling; see Wilson et al. 2011; Gutowsky et al. 2017). Collected fish were transferred to an indoor holding tank (~212 L) at the nearby Queen's University Biological Station (Chaffey's Lock, ON, Canada) and held overnight prior to receiving a cocoa butter implant. Here, fish were maintained on a flow-through circulation (23.82 ± 0.3°C; O_2 >90% saturation) with independent aeration under a seasonally-appropriate illumination cycle (15 h L: 9 h D). A subset of the captured fish were retained in a large, free-floating net pen (1.3 x 1.3 x 1.1 m) situated in the lake. These fish were used solely as stimulus conspecifics in the shoaling tendency test (see below). All experimental procedures received prior approval of the Carleton University Animal Care Committee (AUP's #104262 & #104281) and therefore are consistent with the guidelines for the care and use of research animals of the Canadian Council on Animal Care and the laws of Canada.

3.3.2. Experimental treatments

Hunger state commonly influences foraging and risk-taking decisions in teleost fishes (e.g. Godin and Smith 1988; Gotceitas and Godin 1991; Godin and Crossman 1994). Therefore, fish were not fed during the holding period and experimental trials. Test fish were subjected to the implantation of either cocoa butter (5 ml kg^{-1} body weight [BW]) containing suspended cortisol (hydrocortisone 21-hemisuccinate; 25 mg kg^{-1} BW) or a sham implant (i.e. no cortisol). Cocoa butter-containing implants are a common and validated means by which cortisol can be elevated in the circulatory system of teleost fishes over semi-chronic durations (Gamperl et al. 1994; Sopinka et al. 2015). I selected the aforementioned dosage based on previous validation work with this species (see

Chapter 2). Here, cortisol levels in experimental fish were on average higher over the first 48 h following implantation (~67 ng ml^{-1} and 19 ng ml^{-1} for 24 h and 48 h cortisol-treated fish, respectively) than in sham-control fish (~14 ng ml^{-1} and 8 ng ml^{-1}, respectively). Preparation of the cortisol-treated cocoa butter followed the methods of Hoogenboom et al. (2011). Fish were selected haphazardly from a pool of available fish and assigned to a treatment group. The order of which fish were implanted with the cortisol or sham treatment was alternated on a daily basis to avoid possible biases in fish selection. Cocoa butter implants were injected intraperitoneally in the fish's abdomen, at a site just posterior to the pelvic fins, using a 1 ml syringe tipped with a 16 G needle. Following implantation, individual fish were immediately transferred to a blacked-out holding chamber (~2.6 L) that was maintained on a flow-through of fresh, aerated lake water (McConnachie et al. 2012). Animals were held in these individual blacked-out (darkened) chambers for 48 h prior to behavioural testing to allow the administered cortisol to reach biologically active concentrations in their blood (McConnachie et al. 2012). Sham and cortisol-treated fish had comparable mean body masses (sham = 8.8±0.3 g, cortisol = 8.1±0.3; t = 1.608, DF = 50, P = 0.114) and total lengths (sham = 82.2±9.9 mm, cortisol = 86.7±11.0 mm; t = 1.029, DF = 50, P = 0.308). Water conditions in the experimental arenas were maintained at >90% O_2 saturation and 23.59 ± 0.1°C.

3.3.3. Behavioural trials

Individual implanted pumpkinseed (n = 28 sham-treated fish, n = 29 cortisol-treated fish) were subjected to three sequential behavioural tests: (i) a predation-fright test, (ii) an exploration tendency test, and (iii) a shoaling tendency test, with 2 – 3.7 h elapsed between consecutive tests. Testing occurred on fish that had been held for ~72 h

post-capture. The order of the tests was randomized for each individual fish using a random number generator to minimize any potential effects of trial time and handling stress on their behaviour in the three tests. A maximum of 10 fish (5 from each treatment group) were run through the experimental series on any given day. Following a behavioural trial, the test fish was removed from the experimental arena and returned to its original holding chamber, where it was allowed to recover for at least 2 h before being used in the next test in sequence. Every effort was made to minimize air exposure times and handling stressors throughout the experimental series to minimize the effects of these stressors in modifying the focal fish's behavioural dynamics. Systematic randomization was used to determine the order of the treatment groups (i.e. cortisol, sham, cortisol, sham, etc.) and was alternated on a daily basis. Fish behaviour in each of the tests was recorded using an overhead Go Pro camera (Go Pro Hero 3; Struthers et al. 2015) and water temperature was recorded at the end of each trial. Behavioural datum were subsequently extracted from the video films. Fish wet body mass was recorded to the nearest half gram using a Valor 2000W balance (Ohaus, Parsippany, NJ, USA) following the end of the experimental series.

3.3.4. Predation-fright test

The experimental arena (Figure 3.1A) consisted of a fibreglass raceway style tank (156 cm x 27.7 cm, L x W), with a water depth of 24.8 cm and devoid of any sort of cover or substrate. A realistically painted model of the head and neck of a great blue heron (*Ardea herodias*, Linnaeus 1758), designed and constructed by Godin and Sproul (1988), was placed near the tank's rim in the horizontal centre of the tank. In the wild, great blue herons are natural predators of sunfish and are perceived as a significant

predation threat to them (Forbes 1987; Coleman and Wilson 1996). As described in Godin and Sproul (1988), the heron model was hinged on a frame external to the experimental tank, allowing it to fall forward (when triggered) and its bill to penetrate the water's surface (to ~10 cm depth) thereby simulating an overhead strike event by the bird. Post-strike, the model was immediately returned to its previous upright position by an overhead spring and braided fishing line suspension system anchored to the external frame. The model was present above the water surface near the rim of the experimental arena, presumably within the test fish's visual field, throughout the experimental test including during the acclimation phase.

For each experimental trial, a focal test fish was transferred from its holding cell to the centre of the aforementioned test arena and allowed to swim freely. Care was taken to minimize handling and air exposure times during the transfer. The fish was then left undisturbed for a 5-min acclimation period. Following this period, an avian predator attack was simulated by gently tipping the heron model forward to strike the water surface near the fish. The test fish's behavioural response to the simulated attack and thereafter was recorded over 5 min (i.e. the total duration of the trial). Behavioural variables were the type of immediate antipredator response (i.e. immobility/freezing vs. escape/flight) and the time spent swimming or total time spent immobile following the attack. Time spent swimming constituted the time from when the fish started swimming following the attack until it ceased activity for more than 5 s. At the end of the trial, the test fish was returned to its holding cell until the onset of the next test, as described above.

3.3.5. Exploration tendency test

To assess the potential influence of cortisol on exploratory activity, individual focal fish were introduced into a novel environment that constituted a Z maze (Figure 3.1B), following the methodology of Chapman et al. (2010). The maze comprised an arena (40 cm x 50 cm) that contained a shaded and gated refuge (10 cm x 20.3 cm) in one corner and three staggered opaque partitions arranged so as to form a Z-pattern. Black plastic marker lines on the bottom of the arena delineated eighteen equal squares (10 x 10 cm), used to record fish location and activity. Water depth was 6.6 cm. Prior to the onset of a trial, a test fish was introduced into the refuge (with gate down) and allowed to acclimate undisturbed for 5 min. Following this period, the refuge gate was remotely raised using a pulley system and the fish allowed to explore the maze for 10 min. The experiment was filmed from above using a Go Pro Hero 3 camera. I recorded (i) latency time to exit the refuge, (ii) the number of lines crossed (= 'exploration' of the novel environment), (iii) total time spent inside the refuge and (iv) the square in the maze (out of 18) furthest from the refuge entered by the fish. At the end of the trial, the test fish was returned to its holding cell until the onset of the next test, as described above, and the maze was completely drained and re-filled with fresh lake water in preparation for the next test fish.

3.3.6. Shoaling tendency

To assess the potential influence of cortisol on sociability, I quantified the tendency of individual test fish to socially associate (i.e. 'shoal') with a stimulus group of conspecifics in choice apparatus (Figure 3.1C). Shoaling is a common response to perceived predation threats, and as such reduces individual risk of mortality to predation, in teleost fishes (reviewed in Godin 1986). Following Chapman et al. (2010), I used a

raceway style tank arranged into three compartments separated by clear Plexiglas partitions that were perforated with small holes to permit water flow between the compartments (Figure 3.1C). Two smaller compartments (20.0 x 27.7 cm; either of which would contain a conspecific stimulus shoal) flanked a large central experimental arena (112 x 27.7 cm), wherein the test fish could freely swim. Associated with each end compartment was a 20-cm wide social association zone used to assess the test fish's preference for either end compartment. Water depth was maintained at 26.8 cm. Consistent with Chapman et al. (2010), I used a stimulus shoal of three pumpkinseed sunfish of similar body size to the test fish. The stimulus fish were not implanted with cocoa butter and were taken from the floating net pen in the lake, previously described above. As such, they were presumably socially unfamiliar with the test fish. Individual stimulus fish were only used once per day but were randomly reused on subsequent test days throughout the experiment. The lake net-pen shoal contained approximately 40 individuals at any given time, with any mortalities being compensated for with the addition of new fish.

Prior to the onset of a behavioural trial, the stimulus shoal was placed in one of the two end compartments, determined pseudo-randomly (with a coin toss). The other end compartment remained empty. The test fish was then introduced into the central arena and allowed to swim freely. Both the test fish and the stimulus shoal were left undisturbed to acclimate to the experimental tank for 5 min. Following this period, I observed the behaviour of the test fish using a Go Pro camera mounted above the experimental arena and I recorded the cumulative time that it spent near either of the end compartments over a 5-min trial. I quantified the test fish's shoaling tendency as a

difference score (S_{DS}), calculated as the amount of time spent in the association zone near the stimulus shoal (t_c) minus the time it spent in the association zone near the empty chamber (t_e), such that $S_{DS} = (t_c - t_e)$.

3.3.7. Behavioural metrics and data analyses

All statistical analyses were conducted in R Studio (Version 1.1.423; R Studio Team 2015). Statistical significance was accepted at $\alpha = 0.05$ and, unless otherwise noted, data are presented as means ± SE. My statistical models included the main effect of treatment (i.e. cortisol vs. sham) and three covariables (mass of test fish, trial time of day, test order [the number of trials prior to the current assessment]). For shoaling tendency only, the statistical model included the location (left or right) of the particular end compartment containing the stimulus shoal as an additional covariable. All models were subjected to model simplification using AICc methodology (Hurvich and Tsai 1989; Burham and Anderson 2002). Data on the type of antipredator response (Predation-fright test) were fitted to a generalized linear model (GLM) with a binomial distribution specified. Data on time spent swimming were fitted to a GLM with a Gaussian distribution. Data on time spent swimming were normalized using a logarithmic transformation. Latency time to emerge from refuge, total refuging time, and furthest square reached (Exploration tendency test) were converted to proportional data (out of total trial duration/maximum count) and analyzed using a beta regression model (package: 'betareg', V3.1-0; Cribari-Neto and Zeileis 2009, 2010). Data on the number of lines crossed were fitted to a GLM with a Poisson distribution. The relationship between lines crossed and emergence latency time was characterized using a linear regression. Shoaling tendency data were transformed to a proportion of the amount of time that the

animal spent with the shoal out of the total time spent in both association end zone and was assessed using a beta regression model, as described above.

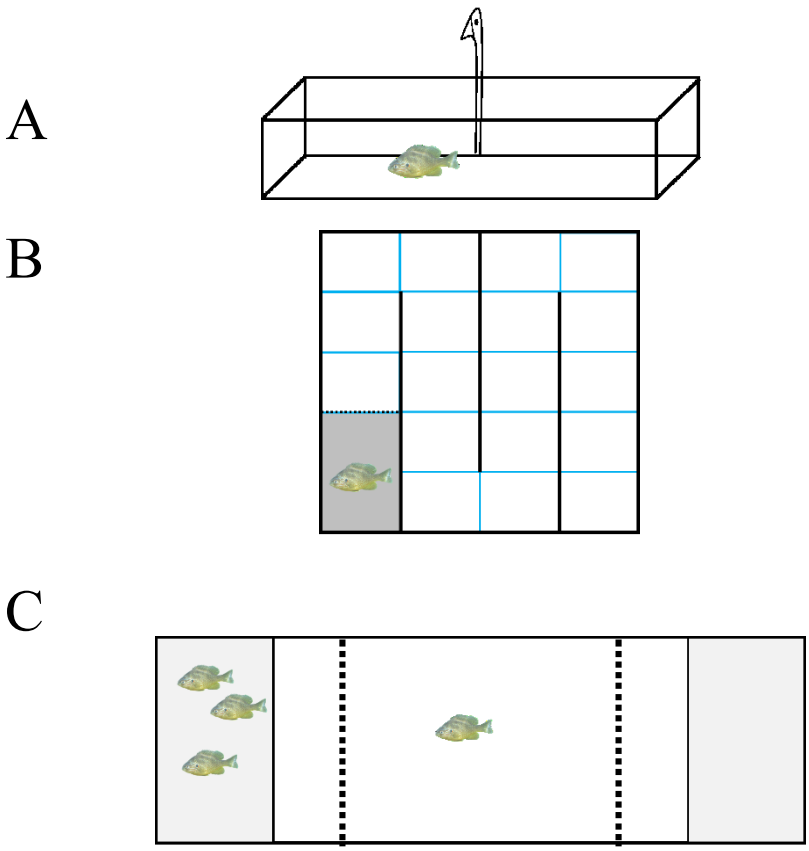


Figure 3.1: Schematic representation of the behaviorual trials used in this experiment including the predation fright trial (**A**), the Z-maze (**B**), and the shoaling trial (**C**). In the *predation fright trial*, a model of a great blue heron (*Ardea herodias*) was centred over the side of the raceway tank. On the attack, the heron's beak penetrated 10 cm below the water surface. In the *Z-maze trial*, the grid pattern consists of 18, 10 cm x 10 cm squares (blue lines) arranged in a Z pattern. The grey rectangular box represents the refuge area where the fish was acclimated in. This was gated (dash line) until the experiment commenced. In the *shoaling trial*, shaded areas represent the two choice compartments, containing 3 conspecifics or nothing (control), which were separated by means of perforated Plexiglas. Dashed lines represent the 20 cm association zone with each choice

compartment. The empty region in the centre of the tank, in between the association zones, represents the “no-man’s land” region of the experiment.

3.4. Results

3.4.1. Antipredator behaviour

Pumpkinseed sunfish responded to a simulated heron attack by either immediately fleeing (i.e. rapidly swimming away from the threat) or becoming immobile (‘freezing’). Most (85.4%) fish exhibited a fleeing (escape) response to the perceived predation threat. However, neither the cortisol treatment ($t = -0.189$, $P = 0.850$), fish body mass ($t = -1.498$, $P = 0.134$), trial order ($t = 0.336$, $P = 0.737$) nor time of day ($t = -0.438$, $P = 0.661$) influenced the particular antipredator response tactic adopted by the test fish (Table 3.1). Post-attack swimming duration was also not affected by cortisol treatment ($t = -1.122$, $P = 0.270$, Figure 3.2A, Table 3.1), trial order or time of day, but interestingly was influenced negatively by individual body mass (Table 3.1). The number of animals that froze in response ($n = 7$) was too small to conduct statistical analyses on, with respect to treatment effects, and has thus been omitted from the results.

3.4.2. Exploratory behaviour

In the exploration tendency test, neither latency to emerge from the refuge ($z = -0.543$, $P = 0.587$; Figure 3.3A), exploratory activity, measured as number of lines crossed ($z = 0.426$, $P = 0.670$, Figure 3.3B), total time spent in the refuge ($z = -0.353$, $P = 0.724$, Figure 3.3C) nor the furthest square reached ($z = 0.441$, $P = 0.659$, Figure 3.3D) was affected by the cortisol treatment (Table 3.1). None of the covariates influenced refuge emergence time, total time spent in the refuge and furthest square reached (Table 3.1),

but all covariates significantly affected exploratory activity (Table 3.1), with fish body mass negatively influencing exploratory activity ($z = -5.176$, $P < 0.001$). A strong negative relationship was observed between refuge emergence time and exploratory activity, with individual fish that exited the refuge sooner exhibiting greater exploratory activity in the maze ($r^2 = 0.923$, $F = 550.773$, $P < 0.001$; Figure 3.4).

3.4.3. Shoaling behaviour

Pumpkinseed sunfish exhibited a strong preference to socially associate with a conspecific stimulus shoal over an empty end stimulus compartment, which resulted in positive difference scores for both cortisol- and sham-treatment groups (Figure 3.5). However, fish in the two treatment groups did not differ in their shoaling tendency ($z = -0.455$, $P = 0.649$, Figure 3.5, Table 3.1). Similarly, individual body mass, trial order, time of day, and the position (left or right) of the stimulus shoal did not significantly influence the shoaling tendency of test fish (Table 3.1).

Table 3.1: Summary statistics for all behavioural variables measured in the behavioural trials here relating the main effect of cortisol-treatment alongside a number of covariate parameters (body mass, trial order, time of day, side). Bolded values indicated statistically significant results ($\alpha = 0.05$). Test parameters are specific to the statistical model used (see 3.3.7.). The constant represents the Y intercept of the model.

Trial	Behavioural Metric		Test statistic value	P
			t-value	
Predation Fright	*Fright Response*	Constant	0.877	0.380
		Treatment group	-0.189	0.850
		Body mass	-1.498	0.134
		Trial order	0.336	0.737
		Time of day	-0.438	0.661
			t-value	
	Post-Attack Swimming Duration	Constant	3.320	**0.002**
		Treatment group	-1.122	0.270
		Body mass	-2.235	**0.032**
		Trial order	-1.548	0.131
		Time of day	1.060	0.296
			z-value	
Z-Maze Trial **Trial**	*Emergence Time*	Constant	1.323	0.186
		Treatment group	-0.543	0.587
		Body mass	0.306	0.760
		Trial order	1.175	0.240
		Time of day	-0.376	0.707

			z-value	
	Exploratory Activity	Constant	0.097	0.923
		Treatment group	0.426	0.670
		Body mass	-5.176	**<0.001**
		Trial order	14.611	**<0.001**
		Time of day		**<0.001**
			11.187	
			z-value	
	Total Refuge Time	Constant	1.870	0.062
		Treatment group	-0.353	0.724
		Body mass	0.347	0.728
		Trial order	1.145	0.252
		Time of day	-0.472	0.637
			z-value	
	Furthest Square	Constant	-0.902	0.367
		Treatment group	0.441	0.659
		Body mass	-0.113	0.910
		Trial order	-1.385	0.166
		Time of day	0.753	0.452
Shoaling Tendency Trial	*Difference Score*		*z-value*	
		Constant	-1.196	0.233
		Treatment group	-0.455	0.649
		Body mass	0.927	0.354
		Trial order	0.498	0.619

Time of day	0.778	0.437
Side	-0.125	0.901

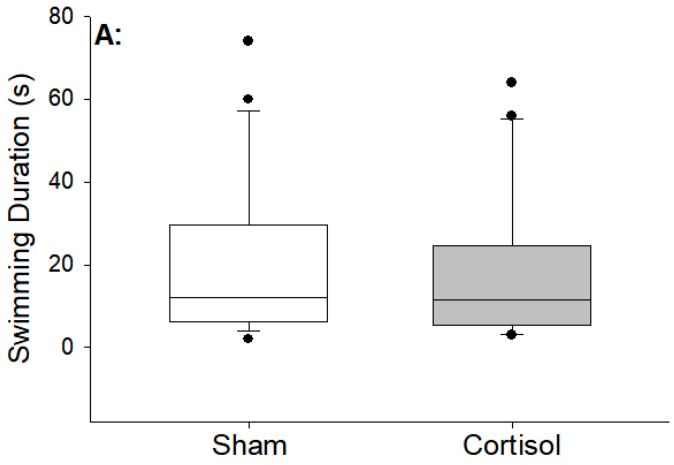


Figure 3.2: Swim duration of pumpkinseed following a mock predator attack for 48-h post-implant sham- (white bars; 5 ml kg^{-1} body weight; n=20) and cortisol-treated (grey bars; 25 mg kg^{-1} body weight; n=21) fish. No significant effects of cortisol, body mass, trial order or time of day were found. Data are presented as a box plot containing the median value delineated by the interquartile range (1^{st} to 3^{rd} quantile) and an accompanying whisker that represents 1.5x beyond this range. Suspected statistical outliers are presented as black circles outside of the interquartile range. Statistical significance was accepted at $\alpha = 0.05$.

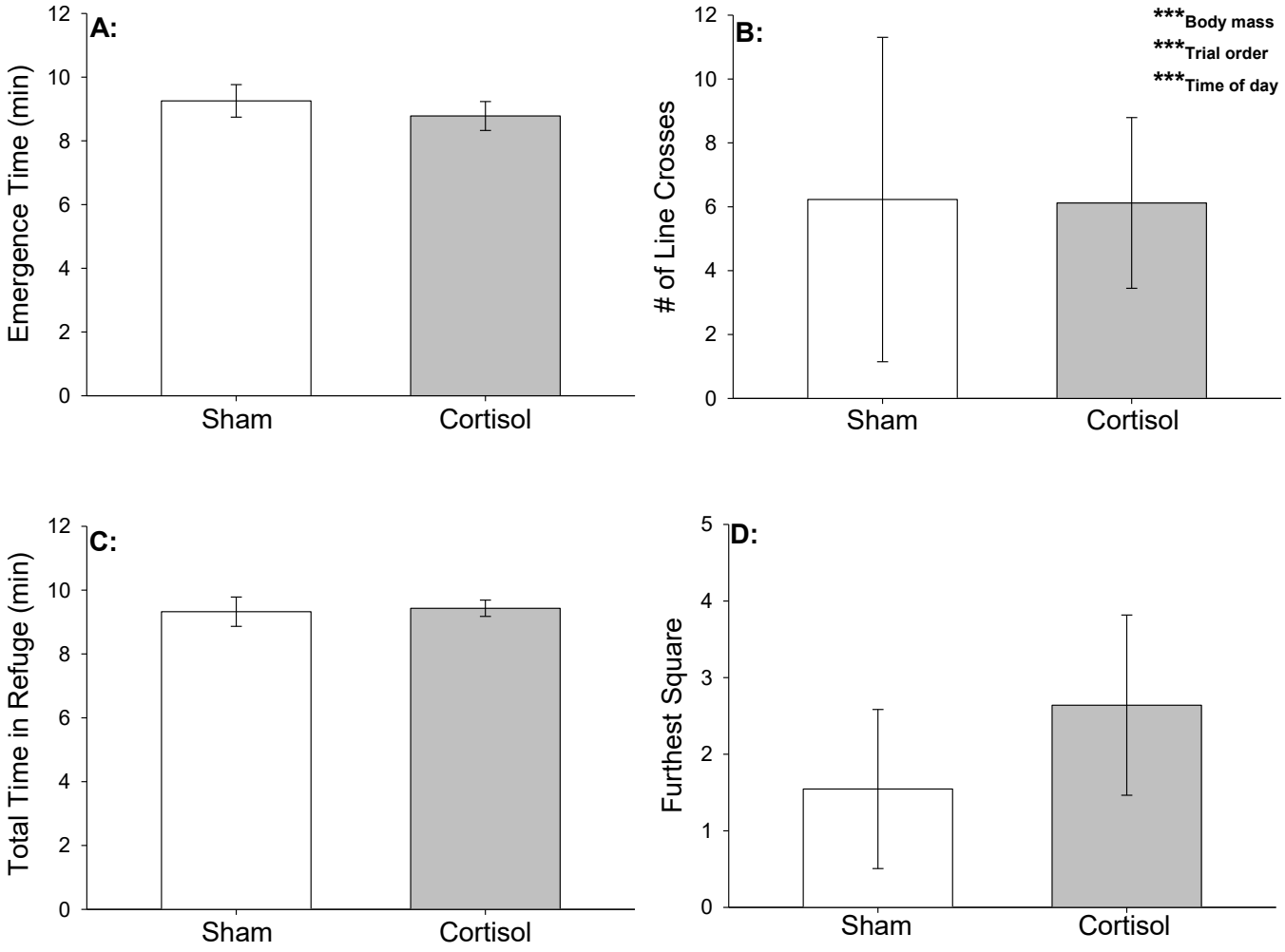


Figure 3.3: Metrics for sham- (white bars; 5 ml kg^{-1} body weight; n = 22) and cortisol-treated (grey bars; 25 mg kg^{-1} body weight; n = 25) pumpkinseed in the Z maze trial displaying refuge emergence time (**A**), the number of lines crossed in the maze (**B**), the total amount of time spent in the refuge area (**C**), and the furthest square reached (**D**). For the number of lines crossed, a statistically significant effect of body mass ($z = -5.176$; $P<0.001$), trial order ($z = 14.611$; $P <0.001$) and time of day ($z = 11.187$; $P <0.001$) was noted. Values are shown as mean±1 SEM. Statistical significance was accepted at $\alpha=0.05$ with differences between treatment groups represented by an asterisk (*** $P < 0.001$).

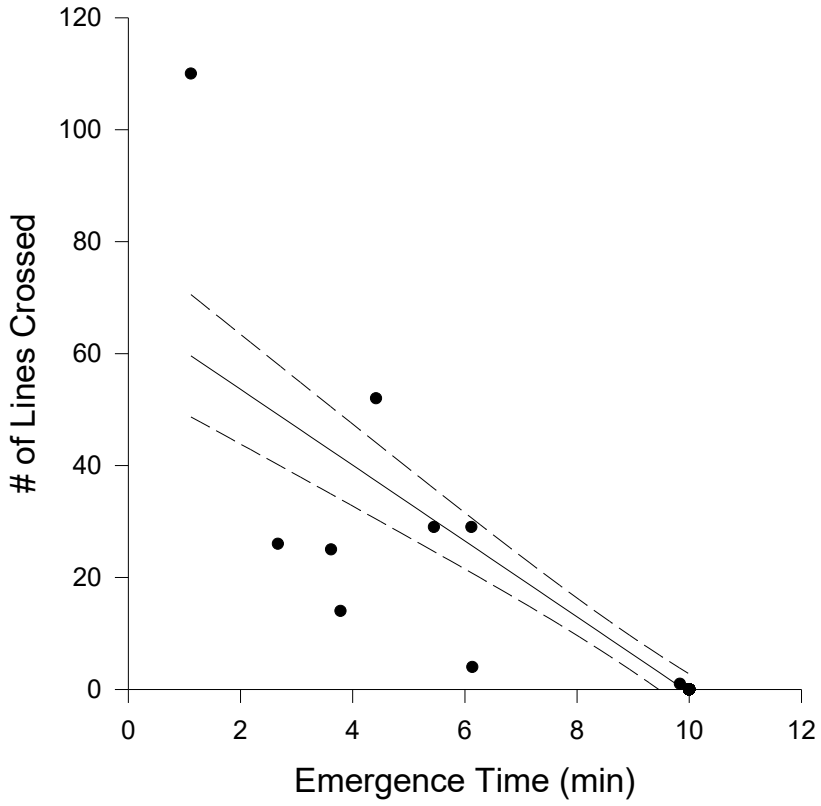


Figure 3.4: Relationship between the emergence time from a refuge environment and the activity, presented as the number of lines crossed, for individual pumpkinseed in the Z maze trial. A significant relationship was detected between the two variables ($F = 550.773$; $P < 0.001$; $r^2 = 0.923$; $n = 47$).

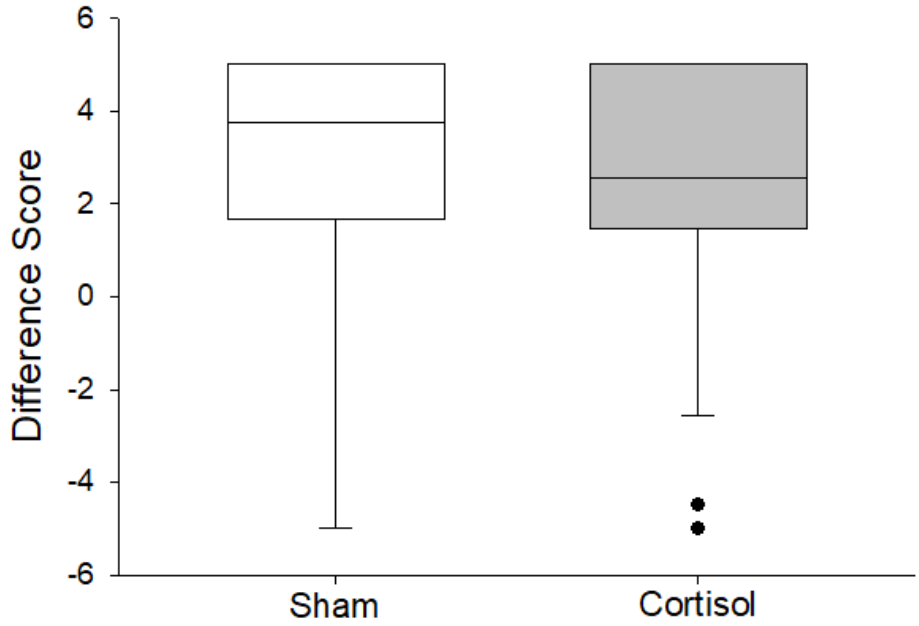


Figure 3.5: Box plot depicting a difference score for the strength of shoaling association for sham- (white bars; N=22) and cortisol-treated (grey bars; N=23) fish in the shoaling tendency trial. Positive values indicate the animal is spending its time associating with the conspecific choice-compartment, a negative value represents the focal fish associating with the empty choice-compartment and a value of zero represents no preference with either compartment. No significant effects of cortisol, body mass, trial order or time of day were found. Data are presented as a box plot containing the median value delineated by the interquartile range (1^{st} to 3^{rd} quantile) and an accompanying whisker that represents 1.5x beyond this range. Suspected statistical outliers are presented as black circles outside of the interquartile range. Statistical significance was accepted at $\alpha=0.05$.

3.5. Discussion

3.5.1. Behavioural responses to a predation threat

In the simulated predation threat test, most test fish chose to engage in a rapid burst fleeing response rather than remain immobile post-attack, which may have reflected a lower cost of fleeing and a relatively high cost of remaining in terms of perceived risk of predation from a sit-and-wait predator such as a great blue heron (Ydenberg and Dill 1986; Godin 1997). This response is a common strategy among teleosts in general (e.g. Faber et al. 1989; Domenici and Blake 1991; Marras et al. 2011; reviewed in Godin 1997; Domenici 2010) and in centrarchid fishes in particular (Moody et al. 1983; Webb 1986; Tytell and Lauder 2008; Chadwell et al. 2012). In contrast to my predictions, cortisol had no influence on the predator avoidance tactic used or on post-attack swimming duration of pumpkinseed. This may reflect a lack of fitness incentive for fish to remain in the area (i.e. foraging opportunities; Godin and Sproul 1988; Ydenberg and Dill 1986; Godin 1997). While data on cortisol-predation responses are lacking, cortisol treatment in checkered pufferfish (*Sphoeroides testudineus*) resulted in post-startle durations comparable to sham controls (Pleizier et al. 2015), which supports my current findings. As it stands, my data suggest that cortisol has little role in mediating antipredator behaviours in juvenile sunfish in this laboratory context.

3.5.2. Boldness and exploration activity

Contrary to my *a priori* prediction, cortisol did not influence refuge emergence time, exploratory activity, or the total time spent refuging. These negative results likely stem from the context-dependent nature of metabolism-behaviour interactions (Dowling

and Godin 2002; Killen et al. 2011, 2012, 2013; Metcalfe et al. 2016). Specifically, metabolism-boldness relationships are usually only apparent under additional stressors such as food deprivation (Killen et al. 2011) and hypoxia (Killen et al. 2012). In other contexts, no such metabolism-boldness relationship exists (Farwell and McLaughlin 2009; Polverino et al. 2016) or the behavioural outcome is highly variable (Biro et al. 2010). As such, perhaps cortisol alone is not sufficient to alter pumpkinseed behavioural phenotypes in this context (reviewed in Schreck et al. 1997, Sopinka et al. 2015, Crossin et al. 2016). Interestingly, I observed a significant relationship between refuge emergence time and lines crosses in the maze, suggesting that individual traits may be important in mediating behavioural phenotypes (Wilson and Godin 2009a,b; Wilson et al. 2011).

3.5.3. Shoaling behaviour

I found that juvenile pumpkinseed sunfish associated strongly with conspecifics (i.e. shoaled) under laboratory experimental conditions, which they also do in their natural habitats (Miller 1963; Brown and Colgan 1982; Golub et al. 2005) as part of an anti-predator defense strategy (Godin 1986; Pitcher and Parrish 1993; McCartt et al. 1997; Marcus and Brown 2003). However, I observed no effect of cortisol treatment on shoaling tendency in pumpkinseed sunfish in the current study, despite previous works showing HPI axis involvement in mediating shoal cohesion (Piato et al. 2011; Pavlidis et al. 2015). The chronic period of HPI axis stimulation in the latter studies (i.e. 12-14 days) was much longer than in my current study (48 h). This suggests that my fish likely had sufficient capacity to maintain 'normal' shoaling behaviour in the face of relatively short-term physiological dysregulation (Romero et al. 2009; Piato et al. 2011; Sopinka et al. 2015; Lawrence et al. 2017).

3.5.4. Body mass and pumpkinseed behaviour:

Body mass influenced post-attack swimming duration in juvenile pumpkinseed sunfish in the current study. In wild bluegill sunfish (*Lepomis macrochirus*), predator-induced mortality is inversely correlated with body size as a result of limitations in predator gape (Werner and Hall 1988; Santucci and Wahl 2003; Hill et al. 2004). Smaller fish in the current study were likely more vulnerable to gape-limited predators, and thus exhibited behavioural traits that were risk adverse, compared with larger conspecifics (Sogard 1997; Metcalfe et al. 1998; Dowling and Godin 2002; Brown and Braithwaite 2004; Ioannou et al. 2008; Porvilino et al. 2016).

3.5.6. Conclusions

Here, I investigated the role of the HPI axis, specifically cortisol, in mediating predator-prey interactions in wild-caught juvenile pumpkinseed sunfish, as previous works indicate a role for an individual's stress state in determining its predator susceptibility (reviewed in Mesa et al. 1994; Raby et al. 2014). Contrary to my predictions, cortisol treatment did not alter risk taking or boldness in this species, suggesting that cortisol has no influence over predator-prey dynamics in agreement with previous research (Cull et al. 2015; Pleizier et al. 2015; Lawrence et al. 2017, 2018a). However, I cannot completely discount a role for cortisol in mediating predator-prey interactions, as this hormone can increase resting/routine metabolic rate (De Boeck et al. 2001; O'Connor et al. 2011; Chapter 2) and, through this effect, can sustain antipredator and foraging activities (Millidine et al. 2006; Killen et al. 2007, 2015b), both important behaviours governing predator-prey interactions (reviewed in Lima and Dill 1990; Milinski 1993; Godin 1997). Moreover, because of the context-dependent nature of

cortisol-behavioural interactions (Crossin et al. 2016; Sopinka et al. 2015), it is possible that cortisol treatment may nonetheless mediate behavioural costs or trade-offs with respect to predator avoidance in other contexts beyond the scope of our current study. As well, I am limited in some of my interpretations because I did not include a pure control group (i.e. no implant treatment) in my experimental design. Consequently, I do not know whether and to what degree the stress related to implantation per se (for both sham- and cortisol-treated fish) may have masked the effects of cortisol on behaviour. As well, stressors associated with handling and moving fish during the experimental series may also have had an effect in altering focal fish behaviour. Future research should address cortisol-behavioural dynamics in more ecologically-relevant settings that includes access to fitness gaining opportunities (e.g. foraging patches) to fully appreciate the potential role of cortisol in mediating predator-prey interactions.

Chapter 4: Chronic plasma cortisol elevation does not promote riskier behaviour in a teleost fish: A test of the behavioural resiliency hypothesis

4.1. Abstract

Stressed fish have been shown to have higher predator-induced mortality than unstressed conspecifics, suggesting a role for the hypothalamic-pituitary-interrenal (HPI) axis in modifying risk-taking behaviours. Yet, there is also evidence of behavioural resiliency in the face of chronic stressors. Here, I tested the behavioural resiliency hypothesis, which posits that animals are able to maintain consistent behavioural phenotypes in the face of significant physiological challenges. I determined whether chronic plasma cortisol

elevation promotes risk-taking behaviours in a model teleost fish, the pumpkinseed sunfish (*Lepomis gibbosus*). Experimental fish were implanted with cocoa butter either as a sham or with cortisol. At 48 h post-implantation, the behaviour of individual focal fish was tested in an experimental arena comprising of a simulated physical refuge, an open zone containing a constrained conspecific shoal, and a compartment containing either a model of a northern pike (*Esox lucius*) paired with corresponding pike olfactory cues or no pike model (control) paired with sham lake water cues only. The fish were assayed individually for their refuge utilization, shoaling tendency and general activity. Neither cortisol treatment nor predation-risk treatment influenced any of these behaviours. This suggests that fish, in the context of my experiment, were behaviourally resilient to the physiological effects of chronic plasma cortisol elevation and in the face of an apparent threat of predation. My results thus provide support for the behavioural resiliency hypothesis in fish under both physiological and ecological stressors. I posit that behavioural resiliency is an evolutionary adaptation ensuring appropriate responses to environmental conditions.

4.2. Introduction

Predation risk, the probability of an organism succumbing to predation (i.e. P(death); Lima and Dill 1990), can have a profound impact on the life history and behaviour of animals. Indeed, in teleost fishes, predators can reduce the foraging effort of prey fish (e.g. Werner et al. 1983; Milinski 1985; Mikheev et al. 2006), force prey to spend a greater amount of time in refuges (Werner et al. 1983; Gilliam and Fraser 1987; Krause et al. 1998), and reduce the general activity of prey (Bean and Winfield 1995; Pettersson et al. 2001; Laurel and Brown 2006). While these behavioural responses can

minimize individual risk of predation, they are also associated with fitness costs in the form of lost opportunities (i.e. poor foraging, mating, etc.; Ydenberg and Dill 1986; Lima and Dill 1990). Thus, prey animals must balance predator-induced mortality risk with fitness-enhancing activities in such a way that the individual's overall fitness is maximized (e.g. μ/g rule; Gilliam 1982; Werner and Gilliam 1984; Gilliam and Fraser 1987; Lima and Dill 1990).

The extent to which an individual accepts predation risk is highly contextual and is considered to be state dependent; a situation where the internal energetic/nutritional status dictates the acceptable level of predation risk and the associated behavioural phenotypes (reviewed in Godin 1997). State dependency has been shown to be an important regulator of risk-taking behaviours in teleost fishes wherein increasing energetic distress, often in the form of increasing hunger or metabolic loading, is associated with riskier behavioural phenotypes. For example, in Atlantic salmon (*Salmo salar*), hungrier fish resumed foraging activities sooner and had a greater foraging range than satiated specifics (Gotceitas and Godin 1991). Indeed, the influence of hunger on risk-taking behaviours appears to be ubiquitous across a number of model teleostean systems (e.g. Smith 1981; Dill and Fraser 1984; Godin and Smith 1988; Godin and Crossman 1994). Furthermore, higher resting metabolic rates (Krause et al. 1998; 2000; Dowling and Godin 2002) and parasitism (Giles 1983, 1987; Milinski 1985; Godin and Sproul 1988) can promote riskier behavioural phenotypes, which generally includes reduced refuge usage, shorter post-attack behavioural latencies and higher activity levels.

The general stress response also appears to be an important mediator of predation risk in teleosts. Under a broad range of contexts, species and settings, stressed teleosts

suffer higher rates of predation relative to unstressed conspecifics (reviewed in Mesa et al. 1994; Raby et al. 2014). Although the specific mechanism(s) underlying this effect are currently unknown, the involvement of stressors in mediating individual susceptibility to predation risk of predation, especially over more chronic stressors, implies the involvement of the hypothalamic-pituitary-interrenal (HPI) axis. Briefly, the HPI axis is one of the primary systems that re-establishes internal homeostasis following a physiological perturbation. HPI axis stimulation results in an increased biosynthesis of cortisol, the primary glucocorticoid hormone in teleosts (reviewed in Gorissen and Flik 2016; Schreck and Tort 2016). Cortisol generally increases energy substrate biosynthesis and availability, the temporary divestment of energetic resources away from fitness enhancing processes, and aids in re-establishing hydromineral balance (Mommsen et al. 1999; Schreck and Tort 2016). Together, this suite of metabolic responses ensures that the animal has sufficient resources to mitigate the effects of stressors, thereby adaptively maintaining internal homeostasis and steady-state conditions (Wendelaar Bonga 1997; Schreck and Tort 2016).

While the use of cortisol as part of the stress response is considered to be beneficial to the organism over an acute or brief duration, more prolonged HPI axis stimulation can result in significant fitness costs. Chronically-stressed teleosts suffer from a number of physiological and behavioural impairments that can negatively affect their organismal performance which, at the whole animal level, are often manifested as reduced energetic investments into growth, reproduction and immune function in addition to maladaptive behaviours (Schreck et al. 1997; Gregory and Wood 1999; Piato et al. 2011; Pavlidis et al. 2015). These effects are believed to be partly mediated through the

prioritization of metabolic resources towards stressor mitigation, which results in performance related trade-offs (Guderley and Portner 2010; Sokolova 2013). Metabolic expenditures in chronically-stressed fish (Lankford et al. 2005) or those experiencing prolonged cortisol elevations (i.e. cortisol implants; De Boeck et al. 2001; O'Connor et al. 2011) are well above basal levels, thus providing support for this notion. Therefore, prolonged activation of the HPI axis can be considered a maladaptive response.

To date, studies addressing the direct role of cortisol in risk-taking and antipredator behaviours are quite limited. The current body of work suggests that, despite significant physiological perturbations, cortisol treatment appears to have little effect on behavioural measures of risk-taking and antipredator behaviours. For example, in schoolmaster snapper (*Lutjanus apodus*; Lawrence et al. 2017, 2018a), checkered pufferfish (*Sphoeroides testudineus*; Cull et al. 2015; Pleizier et al. 2016) and pumpkinseed sunfish (*Lepomis gibbosus*; Ch 3), cortisol treatment failed to affect antipredator and risk-taking behaviours. Furthermore, chronically-stressed zebrafish (*Danio rerio*) maintained a high degree of shoal cohesion for up to 7 days following stressor exposure, with cohesion breaking down thereafter (Piato et al. 2011), and they did not modify their feeding behaviour (Pavlidis et al. 2015). Together, these results suggest that teleosts are able to demonstrate a relatively high degree of behavioural resiliency, that is, the maintenance of steady-state behaviour under chronically-elevated plasma cortisol levels (e.g. Piato et al. 2011; Schmidt et al. 2017a). Behavioural resiliency in the face of physiological perturbations likely serves as an adaptive mechanism ensuring that the animal behaviourally responds to ambient environmental stimuli in an appropriate manner to maximize fitness (Romero et al. 2009; Boonstra

2013a,b; Sørensen et al. 2013). Failure to maintain "normal" behaviour in physiologically stressed animals, in the context of predator-prey interactions, could result in sub-optimal fitness. A loss of behavioural resiliency/coping under stressor exposure may help explain why stressed fish experience higher rates of predation compared with unstressed conspecifics (Mesa et al. 1994; Raby et al. 2014). However, this notion of behavioural resiliency, in the context of cortisol-mediated chronic stress, has not been assessed to any great extent.

The purpose of my current study was to experimentally test the behavioural resiliency hypothesis. I suggest that the behavioural resiliency hypothesis posits that, in the face of physiological perturbations, individuals should be able to maintain a consistent behavioural phenotype that conceivably ensures high fitness. To do so, I evaluated whether wild-caught pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus 1758), used here as a model teleost fish, can exhibit behavioural resiliency with respect to risk-taking behaviours when their plasma cortisol levels are experimentally elevated. Given cortisol's potential role in enhancing metabolic rate (DeBoeck et al. 2001; O'Connor et al. 2011) and the role of energetic state in mediating risk-taking behaviours (e.g. Godin and Smith 1988; Gotceitas and Godin 1991; Skajaa et al. 2003; Killen et al. 2011), I expected that behavioural coping would not be possible in cortisol-treated fish who should therefore exhibit higher risk-taking behaviours than sham-treated conspecifics. Thus, I predicted that cortisol-treated fish should exhibit riskier behaviours, such as earlier emergence from the safety of a refuge, less time spent refuging and shoaling and more time spent in open habitat within an experimental arena (cf. Lima and Dill 1990; Godin 1997), compared with sham-treated controls. If cortisol-treated fish exhibit

behavioural resiliency (cf. Piato et al. 2011; Schmidt et al. 2017a), then there should be no differences in their behaviour compared with the behaviour of sham-control fish in the presence or absence of an apparent threat of predation.

4.3. Methods

4.3.1. Fish collection and implantation procedures

Juvenile pumpkinseed sunfish (mean ± SE mass = 8.7 ± 0.2 g; total length = 81.8 ± 0.5 mm; N=125) were captured hapharzardly using a seine net in the nearshore waters of shallow weedy bays in Lake Opinicon, Ontario, Canada (44º55'90"N, 76º32'80"W) during August 2017 (Ontario Ministry of Natural Resources permit #1086180). Seining was used as the primary collection method to ensure an unbiased sample of behavioural phenotypes in the population (Wilson et al. 2011; Gutowsky et al. 2017). Following capture, fish were immediately transported to the nearby Queen's University Biological Station (QUBS; Chaffey's Lock, Ontario, Canada) in a well aerated cooler and were transferred to a large, indoor flow-through tank containing lake water (~212 L; >90% O_2 saturation, 23.7 ± 0.1°C), where they were held for 24 h prior to experimental manipulation. A subset (N = 40) of the fish captured were retained for use as stimulus conspecifics for the assessment of shoaling tendency in the behavioural experiment (see below) and were not implanted with cocoa butter. These fish were held in a separate tank (~406 L) and were kept under similar holding conditions to the focal test fish. These stimulus fish were released into the lake upon completion of the study.

We captured 8 Northern pike (*Esox lucius* Linnaeus; 549.6 ± 22.4 mm; range 490-650 mm), to generate olfactory cues used in my behavioural experiment (see below),

using rod-and-reel angling techniques including trolling and bait casting (see Lawrence et al. 2018b for more details). Upon capture, pike were transported quickly back to the QUBS and held in large outdoor tanks (~940 L) with flow-through lake water. All pike were eventually live-released back into the lake following this study. Both pumpkinseed and pike were not fed at any time while in captivity. My study conformed to the guidelines for the use and care of experimental animals of the Canadian Council on Animal Care and received prior approval of the Carleton University Animal Care Committee (AUPs #104262 and 104281).

Following a 24-h holding period, focal pumpkinseed fish were given intraperitoneal injections of cocoa butter either containing the vehicle alone as a sham control (5 mL kg^{-1} wet body weight [BW]) or suspended with cortisol (hydrocortisone 21-hemisuccinate; 25 mg kg^{1} BW). Injections were made just posteriorly to the fish's pelvic fin using a 1 mL syringe tipped with a 16 G needle. The use of cocoa butter implants has been employed widely as a means of chronically elevating plasma cortisol titres in teleost fish (Gamperl et al. 1994), are used broadly in behavioural experiments (Crossin et al. 2016; Sopinka et al. 2015) and have been validated for use in my study species (see Lawrence et al. 2018b). Following implantation, fish were transferred to individual blacked-out chambers (McConnachie et al. 2012; see Ch 3) that were maintained on a flow-through of fresh lake water. Thereafter, fish were held for an additional 48 h to ensure that plasma cortisol titres reached biologically relevant levels (McConnachie et al. 2012) prior to the behavioural trials.

4.3.2. Experimental apparatus

Behavioural trials were conducted in a standard glass aquarium (89.3 cm long x 40.6 cm wide, water depth of 28.3 cm; ~102.6 L, Figure 4.1A). The entire bottom of the aquarium was filled with white aquarium gravel (~2 cm deep) to facilitate fish filming from overhead (see below). The experimental arena was illuminated overhead with diffuse fluorescent lighting. Additionally, all but the front side of the aquarium were blacked out to avoid potential external disturbances. The entire apparatus was enclosed within a blind, and all manipulations of the experimental arena were carried out from behind the blind. The behavioural arena was arranged in a conceptually similar manner to those used in prior works assessing risk taking in teleost fish (e.g. Godin and Sproul 1988; Dowling and Godin 2002) and consisted of three compartments: (i) an absolute refuge at one end (14.5 cm long x 40.6 cm wide), (ii) a central open water zone (60.1 cm long x 40.6 cm wide), which contained a constrained shoal of conspecifics, and (iii) a predator compartment at the opposite end (14.8 cm long x 40.6 cm wide), which was either left empty or contained a predator model depending on the treatment (Figure 4.1A). The refuge compartment consisted of a flat piece of plywood (40 cm x 14.8 cm) that had a number of wooden dowels (1.2 cm diameter; ~29 cm height) vertically embedded in it such that they were spaced in an offset grid pattern (3.81 cm in the X plane, 6.35 cm in Y plane; Figure 4.1B). The entire structure was kept submerged through the use of adhered lead weights. This system has been used previously to simulate emergent vegetation and to provide a refuge habitat for prey fish (Mattila 1992; Dowling and Godin 2002; Snickars et al. 2004). The refuge compartment, which represented my lowest-risk habitat in this study, was separated from the rest of the tank by a clear, perforated Plexiglas (Evonik Performance Materials GmbH, Germany)

partition (hereafter termed gate). The gate extended above the water's surface and could be raised or lowered remotely from behind the blind using an overhead string and pulley system.

I placed horizontal gridlines at 10-cm intervals on the bottom (extending ~6 cm long) and across the width of the open zone using small, grey stones (≤1.5 cm diameter) to facilitate the recording of fish activity patterns (Figure 4.1A). A group of three stimulus conspecifics, constrained within a 3.78-L glass jar with a perforated screw-top lid (Figure 4.1A), was placed in the centre of the open zone to assess the sociability of the focal fish. The side of the jar facing the predator compartment was blacked out to prevent the stimulus fish from being startled by the predator model (see below) and thereby influencing the behaviour of the focal fish. On the side of the jar facing the refuge compartment, a semi-circle ring of stones (as above) was placed 10 cm from the jar. Since shoaling reduces individual risk of predation in fishes (Godin 1986; Lima and Dill 1990), I considered this semi-circle zone near the shoal as relatively safe space, but less safe than the refuge. I recorded the shoaling tendency of a focal fish as the time it spent affiliating with the stimulus shoal (i.e. within the semi-circle zone). I regarded the open zone (excluding the latter semi-circle shoal association zone) as the most risky section of the experimental arena because animals are most exposed and vulnerable to predation in open, unstructured habitats (cf. Lima and Dill 1990; Godin 1997).

The predator compartment was separated from the adjacent open zone by a clear Plexiglas partition (hereafter gate) that was blacked out and connected to an overhead string and pully system similar to that of the refuge gate. Depending on the treatment, this compartment either was left empty (control) or contained a realistically painted model of

a northern pike (310 mm TL; Figure 4.1C), which was suspended in the water column ~4 cm off of the substratum by clear monofilament fishing lines attached to an overhead anchoring point. In both treatments and just prior to the onset of the focal fish's acclimation period, I remotely delivered an olfactory cue into the predator compartment using a 50 mL syringe and an 80-cm long piece of aquarium tubing (Figure 4.1A). For the predator present treatment, the olfactory cue consisted of 50 mL of water obtained from a cooler (~340 L) containing a single live northern pike (490-650 mm) that was allowed to sit undisturbed for 30-40 min. Presumably, the excrements and metabolites given off by the pike would generate olfactory cue(s) that would, when paired with the visual stimulus of the pike model, simulate an apparent local threat of predation to the focal fish (cf. Kats and Dill 1998; Brown 2003). This pike olfactory cue(s) was made fresh daily and stored on ice throughout the experimental day. For the predator absent (control) treatment, the olfactory cue introduced into the predator compartment was 50 mL of lake water to serve as a control.

4.3.3. Behavioural tests

My experiment consisted of a 2 x 2 factorial design with cortisol treatment and predation risk treatment being the two main effects. As described above, focal fish received either a cocoa butter implant laced with cortisol (25 mg kg^{-1} BW) or the vehicle alone (i.e. sham implant). The apparent predation risk treatment consisted of two levels, relatively high (predator model present paired with pike olfactory cues) or relatively low (control: predator model absent, lake water cues present). On any given experimental day, a maximum of 8 focal fish were tested in the experimental arena in a balanced combination of the two main effects (i.e. 2 cortisol/2sham fish, 2 predator-present/2

predator-absent control fish). The order of the cortisol treatments was determined through systematic randomization (i.e. cortisol, sham, cortisol, sham, etc.) that was alternated on a daily basis. Individual fish were then assigned to the predation-risk treatment pseudo-randomly using a coin toss, such that there was a maximum of 2 focal fish in each predation-risk treatment (i.e. 2 predator-present trials and 2 predator-absent control trials). Both of these processes were in place to avoid potential treatment order biases within the study's design.

Prior to the onset of a given experimental trial, the gates to both the refuge and predator compartments were closed. Three conspecific fish were selected haphazardly from their holding tank and transferred into the glass jar in the centre of the open zone to form a stimulus shoal. These fish were used only once per day. A focal fish was transferred from its holding tank and to the refuge compartment of the experimental arena. Great care was taken to minimize handling times and air exposure to avoid any acute stress-induced effects on fish behaviour during the trial (Schreck and Tort 2016). The appropriate olfactory cue (i.e. 50 mL of pike odours or lake water) was then remotely delivered into the predator compartment, and the focal fish allowed to acclimate within the refuge compartment for 30 min. During this period, the focal fish and the shoal were within sight of one another. Following the acclimation period, we started a behavioural trial by remotely raising the gate of the refuge compartment, thus providing the focal fish a choice to emerge from the refuge and enter the open zone, affiliate with the stimulus shoal, or remain in the refuge. The trial comprised a 10-min "pre-predator exposure" phase (with the predator compartment closed), followed by a 10-min "predator exposure" phase (with the predator compartment open). During the pre-exposure phase, I recorded

the latency time for the focal fish to initially emerge from the physical refuge (= "refuge emergence time"), and thereafter the total times that it spent in the refuge (= "refuging time"), affiliating with the stimulus shoal (= "shoaling time"), and in the open zone (= "open-zone time") using an overhead Go Pro Hero 3 camera (Go Pro, San Mateo, CA, USA; Struthers et al. 2016). Fish that did not emerge from the refuge for the entire 10-min phase were assigned a maximal emergence latency time of 10 min. General "activity" was scored as the number of grid lines crossed by the fish in the open zone. These behavioural measures provided a baseline level of focal fish behaviour prior to its exposure to the predator compartment. At the end of this first 10-min period, I remotely raised the gate to the predator compartment, thus allowing the focal fish to view (and smell) either the predator model or an empty predator compartment. During the experimental series, the pike remained motionless as the combined visual and olfactory cues should have proved enough to induce a fright response in the focal fish (e.g. Cantalupo et al. 1995; Vilhunen and Hirvonen 2003). I then recorded for the 10-min predator exposure phase the behaviour of the focal fish as follows.

As an immediate response to raising the gate of the predator compartment, focal fish typically either fled to the refuge compartment (N = 31) or associated with the stimulus shoal (N = 11), which I consider here a biological refuge from predation (cf. Godin 1986). I subsequently recorded the time taken for the fish to initially leave the physical refuge compartment and considered this latency time as the fish's initial refuge-emergence time during this second phase. Fish that did not initially emerge from the refuge, as defined above, for the entire 10-min phase were assigned a maximal emergence latency time of 10 min. Activity score, time spent in the open zone, time spent

shoaling, and time spent inside the predator compartment were only recorded for focal fish that had initially emerged from the refuge during the pre-predator exposure phase. This was done to avoid any skewing of the data set owing to those fish that remained in the refuge compartment throughout the initial 10-min pre-predator exposure phase. These latter fish (N = 19) were subsequently censored from the analysis. Finally, to compare the behaviour of the fish between treatments, I expressed separately general activity (the number of grid lines crossed), refuging time, shoaling time and open-zone time as difference scores (S_D), calculated as the value of the behavioural measure obtained for the pre-predator exposure phase (S_{pre}) minus its value obtained for the predator exposure phase (S_{exp}) such that $S_D = S_{pre}-S_{exp}$.

At the end of the behavioural trial, the focal fish was removed from the experimental arena, euthanized via cerebral percussion, weighed for wet body mass (to the nearest 0.5 g), measured for total length, and its external parasites enumerated. The fish's liver was also excised and weighed for the determination of the hepatosomatic index (HSI), following Busacker et al. (1990). The fish in the stimulus shoal were removed from the arena, placed into a separate holding tank and allowed to recover overnight. In between each successive behavourial trial, the water in the experimental arena was completely drained and refilled with fresh lake water to minimize any residual cues associated with focal fish excretions and/or the olfactory cues added to the experimental arena during the preceding trial.

4.3.4. Data analyses

All statistical analyses were conducted in R Studio (Version 1.1.456; R Studio Team 2015). Statistical significance was Bonferroni corrected to $\alpha = 0.007$ (i.e. $\alpha =$

0.05/7) to account for the multiple behavioural measures being recorded and analyzed for individual fish (Johnson and Sih 2007). Refuge emergence times from both the pre-predator and predator exposure phases were analyzed using a Cox proportional-hazards model (package "survival"; Therneau, 2015). Instances where the fish did not leave the refuge were included in the statistical model (with a maximum latency time of 10 minutes) but were considered as censored values in the Cox analysis. For the pre-exposure phase, the model included the main effects of implant treatment and the fish's body mass, ectoparasite load, HSI and trial time of day as covariates. For the predator exposure phase, the model additionally included the predation risk treatment (i.e. pike model present vs. absent) as a fixed effect, the interaction between the two treatments (implant treatment x predation risk treatment), and refuge location as a covariate to account for fish who initially fled to the refuge compartment or were already in either refuge, when the predator compartment gate was raised to start the predator-exposure phase. Fish that initially fled to the predator compartment, fled to the shoal or that remained in the open zone when the gate was raised were not included in emergence time analysis of the latter phase.

Difference scores for activity, total refuging time, shoaling time and open-zone time were analyzed using separate GLMs. All models included the main effects of implant treatment and predation risk treatment and their interactive term, as well as fish body mass, ectoparasite load, HSI, trial time of day as covariates. All difference score data were fitted to a Gaussian distribution. GLM's were subjected to AIC_C model simplification (Hurvich and Tsai 1989; Burnham and Anderson 2002). Time spent in the predator compartment was converted to a proportion (i.e. out of 10 min total) and was

analyzed using a beta regression model (package: ‘betareg’, V3.1-0; Cribari-Neto and Zeileis, 2009, 2010).

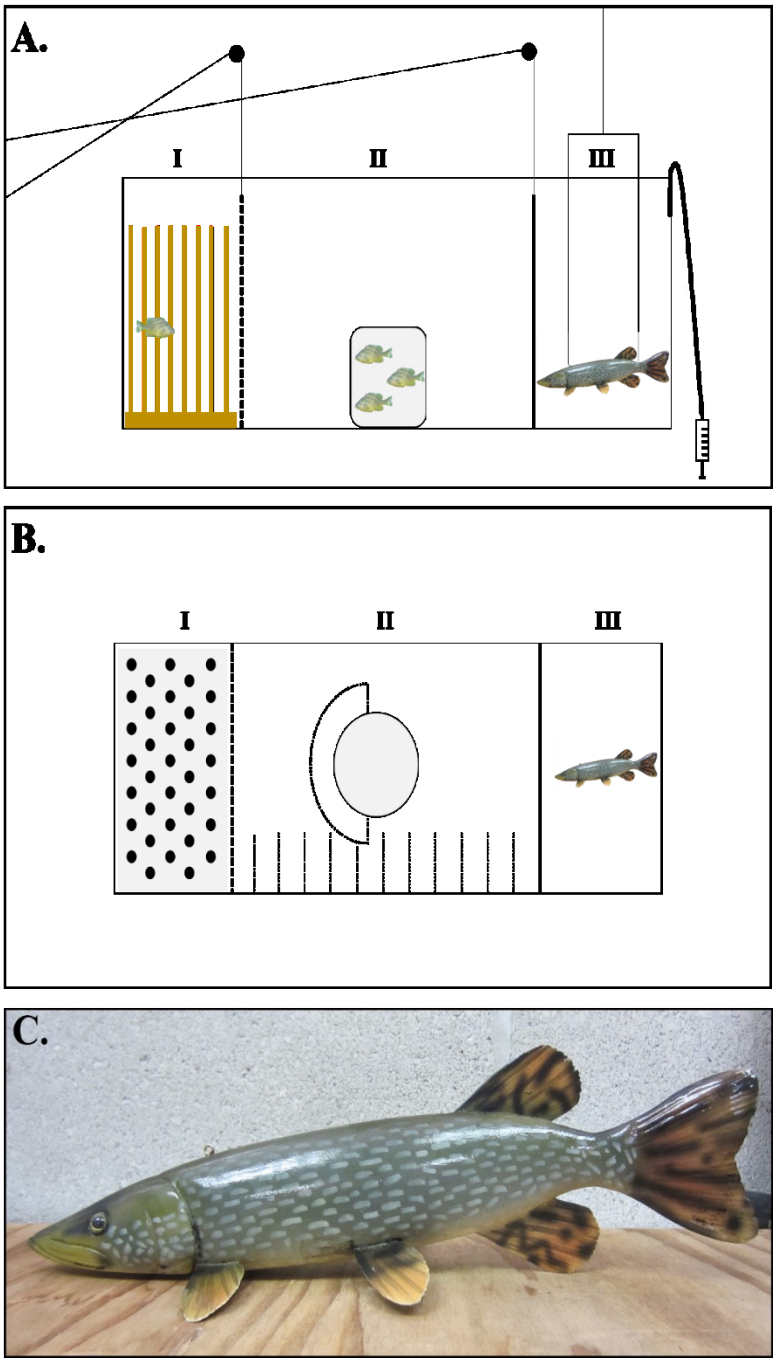


Figure 4.1: Schematic representation of the experimental arena used in the current study from a side view (**A**) and a top-down view (**B**). The experimental arena consisted of a physical refuge compartment (I) consisting of wooden dowels simulating emergent vegetation, an open zone (II) containing a stimulus shoal of three sunfish constrained in a

clear glass jar, and a predator compartment (III) containing a realistic model of a pike (C). A clear, perforated and removable Plexiglas gate (dotted line) separated compartments I and II, and an opaque removable Plexiglas gate (solid line) separated compartments II from III. Small dotted lines in compartment II represent small stones placed on the substratum used to record the activity of focal fish in the open zone, and the dotted semi-circle around one half of the jar denotes the shoal association zone.

4.5. Results

4.5.1. Refuge emergence

During the pre-predator exposure phase of the experiment, latency time to initially emerge from refuge was unaffected by cortisol ($z = -0.442$; $P = 0.659$) and predation risk treatments ($z = -0.180$; $P = 0.857$; Table 1, Figure 4.2A), nor was there an interaction between these two main effects ($z = 0.434$; $P=0.664$). There were no statistical effects of any of the covariates on refuge emergence times (all $Ps > 0.007$; Table 4.1).

Similarly, during the predator exposure phase, latency time to emerge from refuge was not affected by cortisol treatment ($z = 0.659$; $P = 0.510$) or predation risk treatment ($z = 1.224$; $P = 0.221$; Table 4.1, Figure 4.2B), nor was there a significant interaction between these two main effects ($z = -0.771$; $P = 0.441$; Table 4.1). No other covariate (all $Ps > 0.007$; Table 4.1) influenced refuge emergence times during the predator-exposure phase.

4.5.2. Activity and spatial use patterns

Difference scores for fish activity were generally positive across all of my treatment groups, indicating that fish exhibited higher activity levels during the pre-

predator exposure phase compared with the predator exposure phase (Figure 4.3). However, neither cortisol treatment (t = -0.787; P = 0.434) nor predation risk treatment (t = -1.354; P = 0.180; Table 4.1, Figure 4.3) affected the difference scores for fish general activity. Furthermore, there was no interaction between these two main effects (t = 1.317; P = 0.193; Table 4.1) and none of the covariates were significant predictors of activity patterns (all Ps > 0.007; Table 4.1).

Total time spent in refuge appeared to be comparable between the pre-predator exposure and predator exposure phases, as median difference scores approximated 0 (Figure 4.4A). Neither cortisol treatment (t = 1.199; P = 0.234) nor predation risk treatment (t = -0.423; P = 0.674; Table 4.1, Figure 4.4A) affected refuging time difference scores. These two main effects did not interact statistically (t = -0.173; P = 0.863, Table 4.1). While most of the covariates were not statistically significant in my model (all Ps > 0.007; Table 4.1), fish body mass did influence refuge use with larger bodied fish having more positive difference scores (t = 2.819; P = 0.006, Table 4.1).

Shoal use difference scores also generally approximated 0, suggesting that the fish exhibited comparable shoaling time during the pre-predator exposure and predator exposure phases (Figure 4.4B). Shoaling was not affected by cortisol treatment (t = 0.914; P = 0.364) or predation risk treatment (t = 1.506; P = 0.137; Table 4.1, Figure 4.4B), nor was there an interaction between these two main effects (t = -1.582; P = 0.118, Table 4.1). No covariates were significant predictors of shoaling behaviour (all Ps > 0.007; Table 4.1).

The difference scores for time spent in the open-zone were positive for all treatment combinations, indicating that the fish generally spent somewhat less time in the

supposedly risky open zone during the predator exposure phase than during the pre-predator exposure zone (Figure 4.4C). However, neither cortisol treatment ($t = 0.497$; $P = 0.621$) nor predation risk treatment ($t = -0.331$; $P = 0.742$; Table 4.1, Figure 4.4C) influenced the time that the fish spent in the open zone of the experimental arena. The interaction of these two main effects was also non-significant ($t = -0.991$; $P = 0.325$, Table 4.1). No covariates significantly affected the time spent in the open environment (all $Ps > 0.007$: Table 4.1).

Across all treatment groups, pumpkinseeds were generally risk-averse, spending minimal amounts of time within the predator compartment (medians ≤ 2 min; Figure 4.4D). I note that the range for this behavioural measure was relatively high during the predator exposure phase. Total time spent within the predator compartment was not affected by the cortisol treatment ($t = 1.736$; $P = 0.083$) or the predation risk treatment ($t = -1.403$; $P = 0.161$; Table 4.1, Figure 4.4D). All covariates, as well as the interaction of the main effects, were non-significant (Table 4.1).

Table 4.1: Summary statistics for all behavioural metrics measured. Mains effects include cortisol treatment (cortisol vs. sham control) and predation risk (pike model present vs. absent) and the covariates included in the models (body mass, ectoparasite count, trial time of day, hepatosomatic index [HSI], refuge status). Bolded values indicate statistically significant results ($\alpha = 0.007$). Test parameters are specific to the statistical model used, with the constant representing the Y-intercept of the model.

Behavioural measures		Test statistic	P value
Pre-predator Exposure Phase			
Refuge Emergence Time		*z value*	
	Cortisol treatment	-0.442	0.659
	Predation risk treatment	-0.180	0.857
	Interaction	0.434	0.664
	Body mass	-2.642	0.008
	Parasite count	0.487	0.626
	Time of day	0.749	0.454
	HSI	-1.105	0.269
Predator Exposure Phase			
Refuge Emergence Time		*z value*	
	Cortisol treatment	0.659	0.510
	Predation risk treatment	1.224	0.221
	Interaction	-0.771	0.441
	Body mass	-0.882	0.378
	Parasite count	0.185	0.854
	Time of day	0.017	0.986
	HSI	0.560	0.575
	Refuge status	1.640	0.101
Activity		*t value*	
	Constant	0.076	0.939
	Cortisol treatment	-0.787	0.434
	Predation risk treatment	-1.354	0.180
	Interaction	1.317	0.193

	Body mass	0.717	0.476
	Parasite count	2.065	0.043
	Time of day	-2.403	0.019
	HSI	0.006	0.996
Refuging time		*t value*	
	Constant	-1.371	0.173
	Cortisol treatment	1.199	0.234
	Predation risk treatment	-0.423	0.674
	Interaction	-0.173	0.863
	Body mass	2.819	**0.006**
	Parasite count	1.680	0.097
	Time of day	-0.505	0.615
	HSI	0.321	0.749
Shoaling time		*t value*	
	Constant	0.592	0.556
	Cortisol treatment	0.914	0.364
	Predation risk treatment	1.506	0.137
	Interaction	-1.582	0.118
	Body mass	-1.982	0.052
	Parasite count	-1.040	0.302
	Time of day	1.330	0.188
	HSI	-0.347	0.730
Open-zone time		*t value*	
	Constant	1.473	0.146
	Cortisol treatment	0.497	0.621
	Predation risk treatment	-0.331	0.742
	Interaction	-0.991	0.325
	Body mass	-1.050	0.298
	Parasite count	-1.489	0.141
	Time of day	1.357	0.179
	HSI	-1.447	0.153
Time in predator compartment		*z value*	
	Constant	-1.119	0.263
	Cortisol treatment	1.736	0.083
	Predation risk treatment	-1.403	0.161
	Interaction	-1.344	0.179
	Body mass	0.250	0.802
	Parasite count	0.563	0.573

Time of day	1.067	0.286
HSI	-0.364	0.716

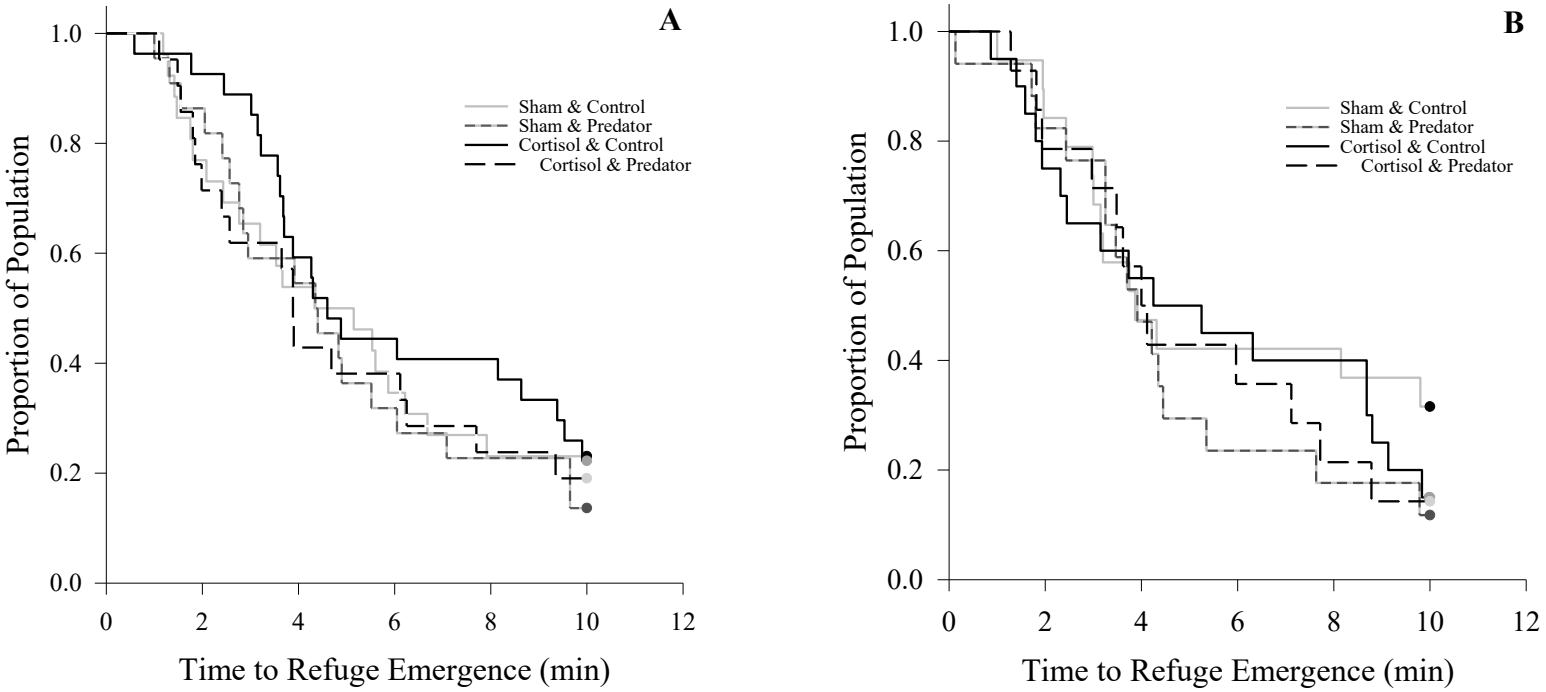


Figure 4.2: Survival curves of the latency time to emerge from refuge emergence times for focal fish during the pre-predator exposure phase (**A**) and the predator exposure phase (**B**) of the experiment for sham-control (grey lines; 5 ml kg^{-1} body weight, N = 36-48) and cortisol-treated (black lines; 25 mg kg^{-1} body weight, N = 34-48) pumpkinseed that were either exposed to a pike model paired with pike-derived olfactory cues (dashed lines, N = 31-43) or an empty predator compartment, paired with lake water olfactory cues, as the control (solid lines, left side, N = 39-53). Statistical significance was accepted at $\alpha = 0.007$.

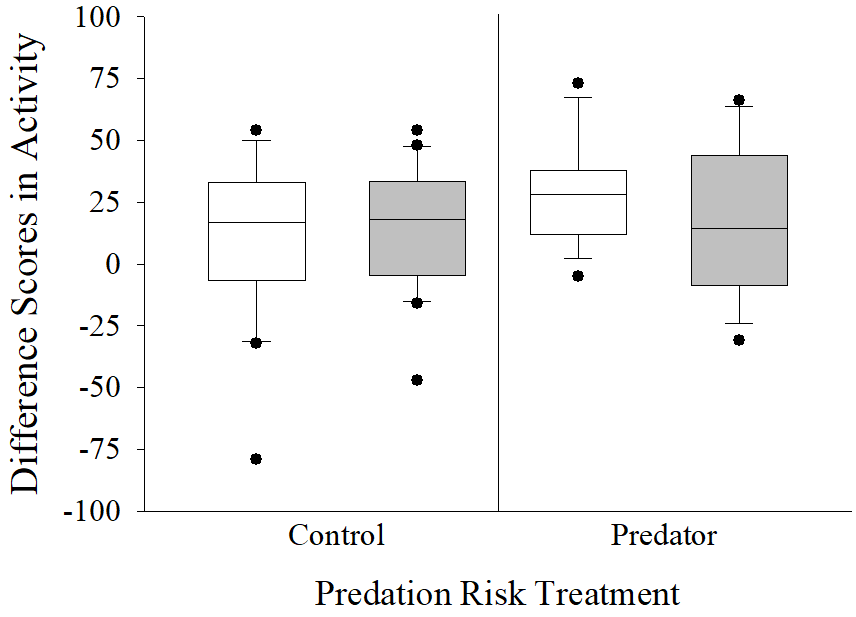


Figure 4.3: Box plot depicting difference scores for the total number of horizontal lines crossed (i.e. activity) for sham-control (white bars, 5 ml kg^{-1} body weight, N = 37) and cortisol-treated (grey bars, 25 mg kg^{-1} body weight, N = 37) pumpkinseed that were either exposed to a pike model paired with pike-derived olfactory cues (right side plots, 50 mL, N = 33) or an empty predator compartment, paired with lake water olfactory cues, as the control (left side, N = 41). Box plots depict the median difference score value, delineated by the interquartile range (1^{st} to 3^{rd} quantile) and an accompanying whisker that represents 1.5x beyond this range. Suspected statistical outliers are presented as black circles outside of the interquartile range. Statistical significance was accepted at $\alpha = 0.007$.

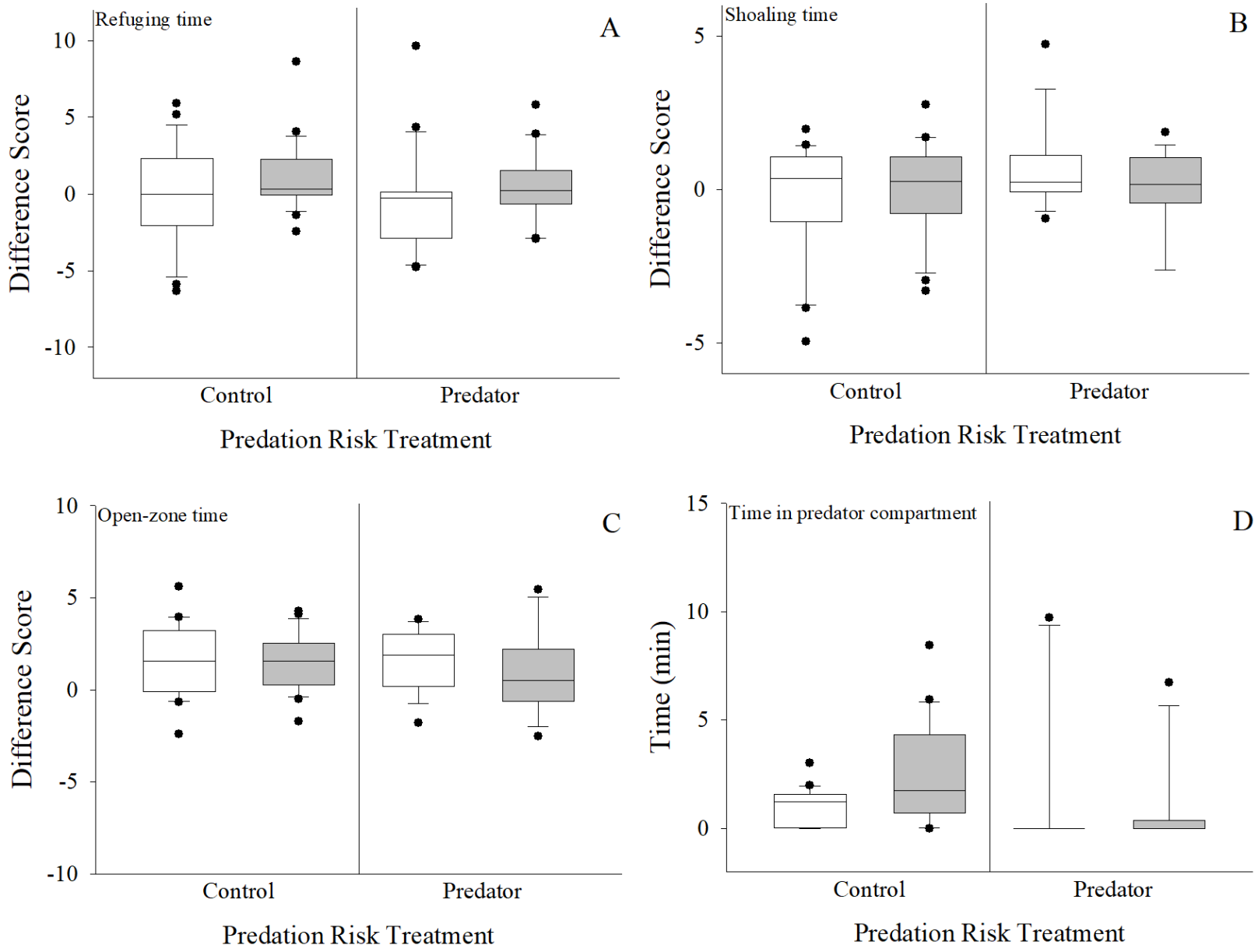


Figure 4.4: Box plots depicting the difference scores for total time spent in refuge (**A**), time associating with the shoal (**B**), time spent in the open zone (**C**), and the absolute time spent in the predator compartment (**D**) for sham-control (white bars, 5 ml kg^{-1} body weight, N = 37-46) and cortisol-treated (grey bars, 25 mg kg^{-1} body weight, N = 37-47) pumpkinseed that were either exposed to a pike model paired with pike-derived olfactory cues (right side plots, 50 mL, N = 33-40) or an empty predator compartment, paired with

lake water olfactory cues, as the control (left side, N = 41-53). Box plots are as described in the Figure 4.3 caption. Statistical significance was accepted at $\alpha = 0.007$.

4.6. Discussion

4.6.1. Cortisol's influence on risk-taking behaviours

Cortisol was expected to enhance risk-taking behaviours in my experimental fish given that cortisol can increase metabolic rate (Chan and Woo 1978; De Boeck et al. 2001; O'Connor et al. 2011), and that riskier behavioural phenotypes are often exhibited by fishes experiencing higher metabolic demands and/or energetic shortfalls (e.g. Giles 1983; Godin and Smith 1988; Godin and Sproul 1988; Gotceitas and Godin 1991; Krause et al. 1998; Killen et al. 2011). In contrast to my a priori predictions, risk-taking behaviours in pumpkinseed sunfish were unaffected by chronic cortisol elevation and a simulated apparent threat of predation. This finding is consistent with what has been observed in other wild-caught teleosts in this context (Lawrence et al. 2017; 2018a; Cull et al. 2015; Pleizier et al. 2015; Ch 3) and suggests that pumpkinseed were behaviourally resilient to the physiological effects of chronic cortisol elevation (cf. Piato et al. 2011; Schmidt et al. 2017a; Ch 3). Behavioural resilience to systemic physiological perturbations likely represents an adaptive response maintaining behavioural phenotypes that are optimally suited to current environmental conditions, thereby maximizing individual fitness (Schreck et al. 1997; Noakes and Jones 2016; Boonstra 2013b). In my current study, risk avoidance under an apparent threat of predation likely represented the most optimal behavioural phenotype given the potential costs of activity and exposure in

open habitat (i.e. predator induced mortality; Lima and Dill 1990; Godin 1997), especially given that there were no additional fitness-enhancing opportunities (i.e. foraging; see below) in the experimental arena. Consequently, being able to maintain consistent behaviours across differing physiological contexts ensures continued organismal success. Thus, I surmise that cortisol has no role in mediating predator-prey interactions in this particular context. My results indicate that pumpkinseed have a sufficient capacity to maintain behavioural phenotypes that share comparable risk burdens as sham-treated fishes.

While behavioural resiliency can permit an animal to cope with a physiological perturbation, the capacity to do so is limited. Therefore, it is important to highlight that, while I may have observed behavioural resiliency in my pumpkinseed, the capacity to do so is likely finite (Romero et al. 2009). Consequently, it could be that the pumpkinseed had sufficient capacity to behaviourally cope over the duration of my experiment (i.e. 48 h). Indeed, under the reactive scope model, the time course effects of the 'wear and tear' associated with the stress response, cortisol's actions reduce the animal's ability to cope over time (Romero et al. 2009). This effect is evident in chronically-stressed zebrafish (*Danio rerio*) whereby behavioural coping, with respect to shoal cohesion, was observed up to 7 days into the stress protocol and becoming behaviourally compromised thereafter (Piato et al. 2011). Similar effects have been observed in cortisol-treated teleosts as well. For example, behavioural resilience was observed in creek chub (*Semotilus atromaculatus*) wherein cortisol-treatment had no affect on activity and spatial use patterns, compared with respective controls (Nagrodoski et al. 2013). However, cortisol-treated chub did experience higher mortality rates than controls over a 10-day exposure

period suggesting a limited capacity to cope with physiological perturbations. Similarly, parental black bass treated with cortisol implants displayed comparable nest-tending behaviours to sham-controls (O'Connor et al. 2009; Dey et al. 2010; Zolderdo et al. 2016; Algera et al. 2017b). However, cortisol treatment often resulted in higher rates of nest abandonment, suggesting that fish had a limited capacity to cope with the effects of cortisol. Together, these results indicate that perhaps the potential effects of cortisol on pumpkinseed behaviour may become evident over more prolonged durations of elevated plasma cortisol levels. Thus, it would be of interest to determine if a time course for such an effect does indeed exist as well as explore some of the factors that may modulate coping capacity and thresholds in individual fish.

4.6.2. Predator fright responses in pumpkinseed

The presence of a pike model failed to elicit any alterations in pumpkinseed behaviour. This was unexpected as the threat of a predator generally corresponds with higher refuge use (Eklov and Persson 1995; Gotceitas et al. 1995; Krause et al. 2000), increased shoal cohesion and social association (Rehnberg and Smith 1988; Sogard and Olla 1997; Brown and Dreier 2002; Orpwood et al. 2008), and reduced activity patterns (Lawrence and Smith 1989; Wisenden et al. 2008; Dunlop-Hayden and Rehage 2011; Engstrom-Ost and Lehtiniemi 2004), all effective strategies in reducing predation risk (Lima and Dill 1990; Godin 1997). The lack of an effect of an apparent risk of predation on the behaviour of my pumpkinseed suggests that perhaps the pike model-olfactory cue pairing was not perceived as a significant threat of predation. This effect may be caused, in part, by a habituation of the focal fish to the olfactory cues used in this experiment as previous works have shown that prolonged exposure to various cues associated with

predator presence (e.g. olfactory, visual, and alarm cues) results in prey fish becoming habituated to the stimulus (Magurran and Girling 1986; Jarvi and Uglem 1993; Berejikian et al. 1999). This effect may also be rooted in the size disparity between the focal fish and the model itself. Size-dependent perception of risk is a well-established phenomenon occurring in various sunfish species (Werner et al. 1983; Werner and Hall 1988; Shoup et al. 2003) with increasing body size corresponding to lower vulnerability to predators (Werner et al. 1983; Werner and Hall 1988; Hill et al. 2004). Based on prior works, it appears as though my focal fish size class (~8 cm TL) was close to the threshold where sunfish exhibit a sharp decrease in predator vulnerability and a change in risk perception in the environment (Werner and Hall 1988; Hill et al. 2004). For example, large bluegill sunfish (~10-13 cm TL) did not alter their refuge use patterns in a mesocosm setting when a live predator, a largemouth bass, was present in the experimental arena. This was not the case for small bluegill (~6-8 cm TL) where refuging increased with the predator being present (Shoup et al. 2003). Thus, my pumpkinseed sunfish may not have perceived the pike model as a significant predation threat, which may help to explain why behavior was unaffected by predator treatment. However, there appeared to be an effect of an individual's body mass in mediating refuge use patterns, suggesting that there is likely a perception of risk in dictating pumpkinseed behaviour (cf. Dowling and Godin 2002; Brown and Braithwaite 2004; Polverino et al. 2016). I caution that these latter propositions remain speculative, as further work is needed to assess size-dependent perception of predation risk in juvenile pumpkinseed.

4.6.3. Conclusions

I tested the behavioural resiliency hypothesis which posits that an organism, in the face of significant physiological perturbations, is able to maintain a consistent behavioural phenotype in such a manner that optimizes overall fitness. To that end, I hypothesized that the metabolic effects of cortisol-treatment would result in greater risk-taking behaviour in pumpkinseed sunfish and would be too great for the animal to cope with. However, refuge and spatial use patterns as well as exploratory activity were unaffected by cortisol treatment. These data suggest that cortisol has no role in mediating predator-prey dynamics in my study species. I speculate that fish are behaviourally resilient to the physiological effects of cortisol treatment over the time-frame that these observations were made (48 h post-implant) providing support for the behavioural resiliency hypothesis. Indeed, in other works, the negative effects of chronic stress become evident over more extended durations than what was used in my study (>12 days; Piato et al. 2011; Pavlidis et al. 2015). As behavioural coping likely aids the individual in maximizing their fitness by maintaining behaviours that are appropriate to the given context (Romero et al. 2009; Boonstra 2013a,b), it would be of interest to ascertain if there exists a threshold of coping ability with pumpkinseed under cortisol treatment and a time course of such events. My hypothesis of higher risk-taking behaviours under cortisol elevations was rooted in the relationship between the fish's metabolism and its corresponding risk-taking behaviours which are often highly variable and contextual (Farewell and McLaughlin 2009; Biro et al. 2010; Killen et al. 2011, 2012; 2013; Polverino et al. 2016). Thus, it is possible that in this context, no such relationship between metabolism and behaviour exists in pumpkinseed sunfish under cortisol-treatment. However, I remain cautious in some of these interpretations as pumpkinseed

were not provided with foraging opportunities in my current study which has been shown to be an important feature in risk assessment studies (reviewed in Milinski 1993). Thus, further work is needed to fully appreciate the role of cortisol in mediating predator-prey interactions in sunfish. This would conceivably require experiments that address not only a time course of action but in also providing fitness enhancing opportunities (e.g. food) to tease apart some of the finer scale behavioural changes and decision-making processes under cortisol treatment. Until that point, I conclude that, alongside prior work on the topic (Ch 3), cortisol has no bearing on predator-prey interactions in wild-caught sunfish. Moreover, there is a need to conduct tests on a variety of vertebrate taxa to better understand the generality of the behavioural resiliency hypothesis.

Chapter 5: Cortisol does not increase risk of mortality to predation in juvenile bluegill sunfish: a manipulative experimental field study

5.1. Abstract

The hypothalamic-pituitary-interrenal (HPI) or stress axis in teleost fishes produces their primary glucocorticoid, cortisol. Although generally an adaptive response, prolonged HPI axis stimulation can impair organismal performance. Previous work has shown that stressed teleosts have higher mortality to predation than unstressed conspecifics, suggesting a role for the HPI axis in modulating predator-prey interactions. My current study investigated whether elevated cortisol levels altered the predation rate of a wild-caught teleost fish, the bluegill sunfish (*Lepomis macrochirus*). Wild-caught, juvenile bluegill were given intraperitoneal implants of cocoa butter (i.e. sham), or cocoa

butter containing cortisol or cortisol and the glucocorticoid receptor antagonist RU486. After 24-h, fish were tethered along the bottom of the lake and their survival under natural predation was recorded following 24 h. A subset of fish was used to validate the efficacy of cortisol implants in this setting. No treatment effect on survival was observed, suggesting that elevated cortisol has minimal involvement in mediating predator-prey interactions in this context. However, experimental fish may have demonstrated resiliency to physiological perturbations owing to the relatively acute duration of my experimental series, and negative effects might be manifested over a more chronic period.

5.2. Introduction

When teleost fishes are subjected to a stressor, the hypothalamic-pituitary-interrenal (HPI) axis increases production of cortisol, the primary glucocorticoid in this taxon (Barton and Schreck 1987; Cook et al. 2012; reviewed in Gorissen and Flik 2016). Cortisol has multiple functions in teleosts, including enhancing energy substrate synthesis (i.e. gluconeogenesis & glycogenolysis), assisting in re-establishing hydromineral balance, and temporarily diverting energy away from non-essential processes (i.e. growth & reproduction; reviewed in Mommsen et al. 1999; Schreck and Tort 2016). These effects are believed to be achieved predominately through glucocorticoid receptor (GR) activation (reviewed by Mommsen et al. 1999). Together, the actions of cortisol ensure that individuals have access to the energy needed to offset the deleterious effects of the stressor (Mommsen et al. 1999; Romero et al. 2009; Schreck and Tort 2016). Thus, the HPI axis acts to co-ordinate a suite of adaptive mechanisms that help animals cope with challenges in the environment (Romero et al. 2009; Schreck and Tort 2016).

However, sustained elevations of plasma cortisol can be detrimental to organismal performance. In chronically stressed teleosts, for example, reductions in somatic growth (Sadoul and Vijayan 2016), energy reserves (e.g. hepatosomatic index [HSI]; Barton et al. 1987; Montero et al. 1999), reproductive capacity (Pankhurst 2016), and immune function (Yada and Tort 2016) have been observed. Interestingly, stress also appears to influence predator-prey interactions, with stressed teleosts suffering higher rates of predation compared with non-stressed conspecifics (reviewed in Mesa et al. 1994; Raby et al. 2014). Although the mechanism(s) underlying this effect remain unclear, stressors have been shown to cause reduced escape distances (Handelhand et al. 1996; Ryer 2004; McKenzie et al. 2008; Killen et al. 2015b) and altered shoal cohesion (Ryer 2004; Piato et al. 2011; Pavlidis et al. 2015), suggesting impairment of predator avoidance capacity (reviewed in Godin 1997).

The observation of higher predation rates among stressed teleosts suggests that the HPI axis has a role in modulating predator-prey interactions. However, at this time, studies addressing the specific role of cortisol in mediating predator-prey interactions are quite scant, representing an interesting avenue for future research, especially given cortisol's potential role in modifying life history attributes in fishes (Sopinka et al. 2016; Crossin et al. 2016). More specifically, in previous work chronic cortisol administration failed to alter mortality (Lawrence et al. 2017) and anti-predator behaviours (Lawrence et al. 2017; 2018a) in tethered schoolmaster snapper (*Lutjanus apodus*). Similarly, cortisol-treatment in checkered pufferfish (*Sphoeroides testudineus*) failed to impede defense capabilities (i.e. puff scores), which suggests that anti-predator responses may be unaffected (Cull et al. 2015; Pleizier et al. 2015). Thus, the purpose of the current

experiment was to test experimentally whether chronic cortisol elevation affects predator-induced mortality in a wild-caught, freshwater teleost fish, the bluegill sunfish (*Lepomis macrochirus*). I hypothesized that experimental elevation of cortisol levels would result in higher mortality in cortisol-treated fish compared with sham controls and RU486-treated fish. I tested this hypothesis with bluegill sunfish that were implanted with cocoa butter (i.e. a sham), or with cocoa butter containing cortisol, or cortisol and the GR antagonist, RU486. Twenty-four-hours post-implant, fish were tethered in the shallow nearshore waters of a lake and left undisturbed and exposed to natural predators for 24 h, following which the surviving fish were recovered and mortalities noted.

5.3. Methods

5.3.1. Fish collection, housing and implantation procedures

Juvenile bluegill sunfish (mean ± SE, wet mass = 5.4 ± 0.1 g) were seined from shallow nearshore habitats in Lake Opinicon (44.5590° N, 76.3280° W; Ontario, Canada) during the months of May and June of 2018 (under Ontario Ministry of Natural Resources and Forestry permit #1089028). Lake Opinicon is a shallow and weedy euphotic lake with a diverse predator community including teleost, avian and mammalian piscivores (Keast 1978; Keast et al. 1978). Seining was the preferred capture method to avoid possible selection of specific behavioural phenotypes (Wilson et al. 2011; Gutowsky et al. 2017), thus ensuring a random collection of fish in my samples. Following capture, fish were sorted by hand to select individuals of 5-8 cm total length, because previous work on sunfish predation used similarly-sized individuals with success (Gotceitas and Colgan 1989). Fish were promptly transported to the nearby Queen's University Biological Station (Elgin, ON, Canada) where they were held in large, outdoor

flow-through tanks (~435 L) supplied with fresh lake water (19.7 ± 0.3°C; >90% O_2 saturation) and exposed to natural sunlight and darkness. Animals were held under these conditions for 24 h prior to implantation. All experimental series were conducted in accordance with the guidelines set by the Canadian Council on Animal Care under administration of the Carleton University Animal Care Committee (AUP# 104262 and 106523).

Bluegill were implanted with cocoa butter containing either cortisol (hydrocortisone 21-hemisuccinate; 25 mg kg^{-1} body weight [BW]), or cortisol (25 mg kg^{-1} BW) with RU486 (Mifepristone; 50 mg kg^{-1} BW), or were implanted with cocoa butter alone (5 ml kg^{-1} BW) as a sham control. Implants were deposited into the peritoneal cavity through the ventral surface of the fish using a 1 ml syringe and an 18 G needle. The use of cortisol-treated cocoa butter implants has been validated for use in teleost fishes (Gamperl et al. 1994) and has been widely used in centrarchid fishes (e.g. O'Connor et al. 2011; McConnachie et al. 2012; Zolderdo et al. 2016; Algera et al. 2017a). The cortisol dosage used here was based on prior work (Brown et al. 1991; Algera et al. 2017a). Cocoa butter solutions containing cortisol were prepared following the methods of Hoogenboom et al. (2011). The RU486 dose (50 mg kg^{-1} BW) was based on prior work, where RU486 has been used extensively as an antagonist of glucocorticoid receptors (Vijayan et al. 1994; Bernier et al. 1999; Lawrence et al. 2017). I did not include a no-treatment (i.e. no implant) control group in my experimental design because I was primarily interested in the relative effects of exogenous cortisol rather than how stressors associated with fish handling may have affected predator-induced mortality. Furthermore, prior work indicated that plasma [cortisol] is comparable between sham-

and non-treated bluegill sunfish (McConnachie et al. 2012). Following implantation, fish were held in indoor, flow-through tanks (~211 L; T = 20.9 ± 0.4 C°; O_2 saturation >90%) for 24 h to ensure that the pharmaceutical agents reached biologically active concentrations in the blood prior to exposure to predation risk, as in Lawrence et al. (2017). Fish were not fed at any time while in captivity.

5.3.2. Assessment of predatory mortality in the wild

Tethering has been used effectively to assess the risk of predation on teleost fish in natural aquatic environments (see Rypel et al. 2007; Elvidge and Brown 2014; Lawrence et al. 2017). As in previous work (Rypel et al. 2007; Lawrence et al. 2017), tethers consisted of a single piece of monofilament fishing line (1.5 m in length; 2.72 kg-test monofilament) attached to the lower jaw of the fish. A small sewing needle was used to pierce the flesh in the jaw so that the tether could be knotted directly to the fish (Lawrence et al. 2017). Tethers were secured to the animal just prior to deployment in the field.

Tethering was conducted at one of four sites in Lake Opinicon, namely, Keast Beach (44°33'53.1"N 76°19'33.9"W), Cow Island (44°34'02.8"N 76°19'11.4"W), Birch Bay (44°33'56.0"N 76°19'40.1"W) and Eight Acre Island (44°33'34.3"N 76°19'20.4"W). These sites were selected because they have naturally occurring populations of *Lepomis spp.* and a diverse predator community. The sites were similar in depth (~1.5-1.8 m) and substratum composition, having a sandy/muddy bottom with sparse woody debris, small rocks and natural vegetation. At each site, a 60-m length of lead-core line with 5-m interval markers was anchored into the substrate. The interval markers ensured consistent spacing between individual tether lines. A random number generator was used to select

the particular tethering site. On a given day of experimentation, upwards of 12 fish were used, consisting of four individuals from each of the three treatment groups (i.e. N = 4/treatment/site). Sample sizes for each of the tethering treatments can be found in Table 5.1. Unbalanced sample sizes between my treatment groups for tethered fish are the result of uncontrollable occurrences in the field (e.g. overnight holding tank mortalities, fish establishing a nest overnight near a tethering site, missing tether stake, etc.). Animals were transported in coolers to their predetermined tether site and were fitted with tethers (see above). Tether lines were attached to a large metal stake (22.9 cm long) that was driven into the substrate at the predetermined location by hand by a snorkeller. The order in which animals were placed along the line, at 5-m separations, was such that no two adjacent fish were of the same treatment group (i.e. sham, cortisol, RU486+cortisol) and was changed daily using systematic randomization. Tethered fish were left for 24 h and survivors were collected the following day. Fish that were not present on their tether line at the time of collection were assumed to have been consumed by a predator. Fish that survived the overnight tethering period were euthanised as per my approved animal care protocol (AUP# 106523).

5.3.3. Validation of cortisol implants and physiological indices

A second group of bluegill (mass = 10.3 ± 2.1 g; N = 65; see Table 5.1 for specific sample sizes) were implanted as described above and held for 24 h or 48 h post-implant in individual, blacked-out compartments (as described by McConnachie et al. 2012) supplied with flowing water. A larger size class (size range 8-10 cm TL) was used here to ensure sufficient volumes of blood for analytical procedures. Blood (~100 µL) was collected via caudal venipuncture (23G needle on a 1 ml syringe; 10,000 USP units/

ml of sodium heparin; Sandoz, Boucherville, Canada) with the procedure taking less than 3 min to ensure samples were representative of baseline conditions (Lawrence et al. 2018b). Whole blood was immediately assessed for blood [glucose] using a portable glucose meter (Accu-Chek Compact Plus, Hoffman-La Roche Limited, Mississauga, ON, Canada) that has been validated for use in teleost fishes (Wells and Pankhurst, 1999; Serra-Llinares and Tveiten, 2012; reviewed in Stoot et al., 2014). The remaining blood was centrifuged (2 min @ 2,000 *g*; Mandel Scientific, Guelph, ON, Canada) and the plasma fraction was flash frozen and stored at -80°C for later analysis of plasma [cortisol]. Plasma [cortisol] was determined using a commercially available radioimmunoassay kit (ImmuChem Cortisol Coated Tube RIA Kit, MP Biomedicals, Solon, OH, USA) that has been validated for use in teleosts (Gamperl et al., 1994). Intra-assay variation was 5.8% and all samples were measured in a single assay. Following blood sample collection, fish were euthanized via cerebral percussion and weighed. The liver was dissected out and weighed to determine hepatosomatic index (HSI). HSI was calculated using the method of Busacker et al. (1990), where liver mass (m_L) is divided by total mass of the fish (m_F) and multiplied by 100%, $HSI=(m_L/m_F)*100\%$. HSI is a relevant index here because previous work has shown it to be under the regulatory control of cortisol (Mommsen et al. 1999) and it is commonly used as a stress index (Sopinka et al. 2016).

5.3.4. Data analyses

Statistical analyses of blood and tissue data from the cortisol implant validation portion of the study were conducted using SigmaPlot v11.0 (Systat Software Inc., San Jose, CA, USA). Each physiological metric was analyzed separately using an

independent two-way ANOVA model with the main effects of treatment (sham, cortisol, cortisol+RU486) and time (24 h & 48 h post-implant) as the fixed independent factors. An interaction term between treatment and time was also included in this model. Non-normal data were log transformed. Data are presented as means ± SE, unless otherwise noted, and statistical significance is reported at $\alpha = 0.05$.

I analyzed survival data using a generalized linear model (GLM) fitted to a binomial distribution in the R statistical environment (version 1.1.423; R Studio Team 2015). Here, treatment group (i.e. sham, cortisol, cortisol+RU486) was the main fixed factor in the model, with the tethering line site location in the lake (i.e. the four sites listed above) included as a covariate. Model fitting, using a likelihood ratio test (Zuur et al. 2009), was used to determine the overall effect of each model term (i.e. treatment group and site location) on fish survival. Using an effect size derived from a similar study on schoolmaster snapper (Lawrence et al. 2017) and G*Power (v 3.1.9.2; Heinrich Heine University of Dusseldorf , Düsseldorf, Northrhine-Westphalia, Germany; Faul et al. 2007; 2009), I carried out a post-hoc power analysis that revealed a power value of 0.865, which suggests that I had a high probability of observing an effect in this study if one indeed existed (Cohen 1992).

Table 5.1: Sample sizes for the physiological indices measured at 24 h and 48 h post-implant. The total number of fish that were tethered in the experiment is also included. Treatment groups consisted of fish implanted with cocoa butter as a sham control, cocoa butter containing cortisol (25 mg kg^{-1} body weight [BW]), or cocoa butter containing cortisol+RU486 (cortisol = 25 mg kg^{-1} BW & RU486 = 50 mg kg^{-1} BW).

	Treatment Group					
	Sham		*Cortisol*		*Cortisol+RU486*	
Parameter	*24 h*	*48 h*	*24 h*	*48 h*	*24 h*	*48 h*
Plasma [Cortisol]	10	5	6	7	8	4
Blood [Glucose]	12	8	12	9	10	12
Hepatosomatic Index	12	9	12	9	11	12
Tethered Fish	57		54		47	

5.4. Results

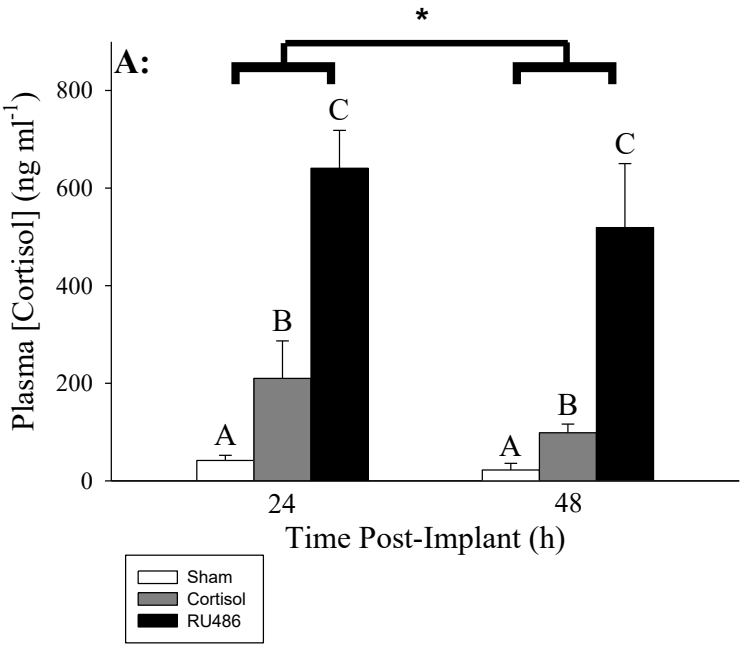
5.4.1. Implant validation and physiological responses

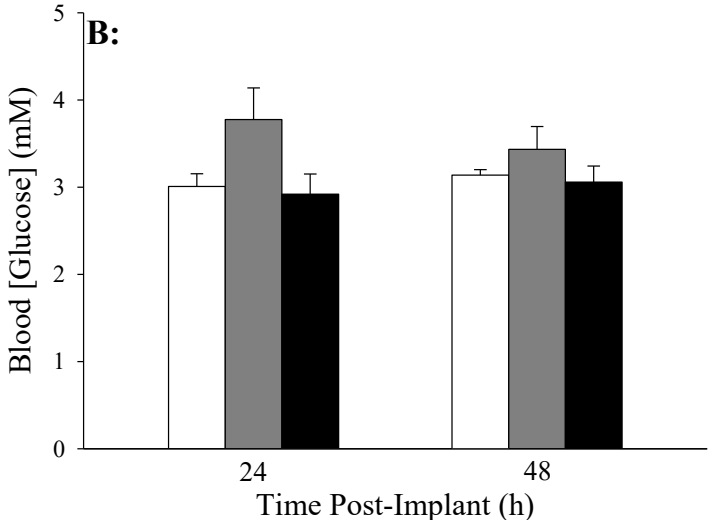
Treatment significantly affected plasma [cortisol] in bluegill ($F = 60.75$ df = 2,39; $P < 0.001$; Figure 5.1A). Plasma [cortisol] was 5- and 15-times higher in cortisol- and cortisol+RU486-treated fish, respectively, than in sham-treated fish, at 24 h post-implant. By 48 h, cortisol- and RU486+cortisol-treated fish exhibited plasma [cortisol] that was, respectively, 4.5- and 23-times higher than that of shams (Figure 5.1A). Although a significant effect of time on plasma [cortisol] was observed ($F = 5.69$; df = 1,39; $P = 0.023$), the interaction of treatment group and time was not significant ($F = 0.67$, df = 2,39; $P = 0.518$). Despite a trend for blood [glucose] to be elevated in cortisol-treated fish, treatment effects on blood [glucose] were not statistically significant ($F = 3.15$; df = 2,57; $P = 0.051$; Figure 5.1B). Time did not have significant effects on blood [glucose] ($F = 0.81$; df = 1,57; $P = 0.371$), nor was there an interaction between the main effects ($F = 0.39$; df = 2, 57; $P = 0.676$). Similarly, none of treatment ($F = 1.69$; df = 2,59; $P = 0.193$), time ($F = 1.94$; df = 1,59; $P = 0.169$), or the interaction of these factors ($F = 0.40$; df = 2,59; $P = 0.672$) had significant effects on HSI.

5.4.2. Tethering survival

Survival rates of sham-, cortisol- and cortisol+RU486-treated sunfish were comparable at 14.0%, 11.1% and 10.6%, respectively (residual deviance = 115.77; df = 2; $P = 0.844$). Furthermore, there was no effect of tethering site location on survival of bluegill (residual deviance = 112.81; df = 3; $P = 0.398$). Opportunistic use of video as

well as visual observations suggested that the primary predators included largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), northern pike (*Esox lucius*), rock bass (*Ambloplites rupestris*), brown bullhead (*Ameiurus nebulosus*) and common loons (*Gavia immer*), as these animals were observed feeding on tethered fish and/or were in the vicinity of tethering lines. Common snapping turtles (*Chelydra serpentine*) and western osprey (*Pandion haliaetus*) also inhabit the lake and may represent additional sources of predation. Survivors were often found within nearby vegetation upon collection following the 24-h predator exposure period.


*
A:
800
600
400
200
0
Plasma [Cortisol] (ng ml-1)
A
B
C
A
B
C
24
48
Time Post-Implant (h)
Sham
Cortisol
RU486


B:
5
4
3
2
1
0
Blood [Glucose] (mM)
24
48
Time Post-Implant (h)

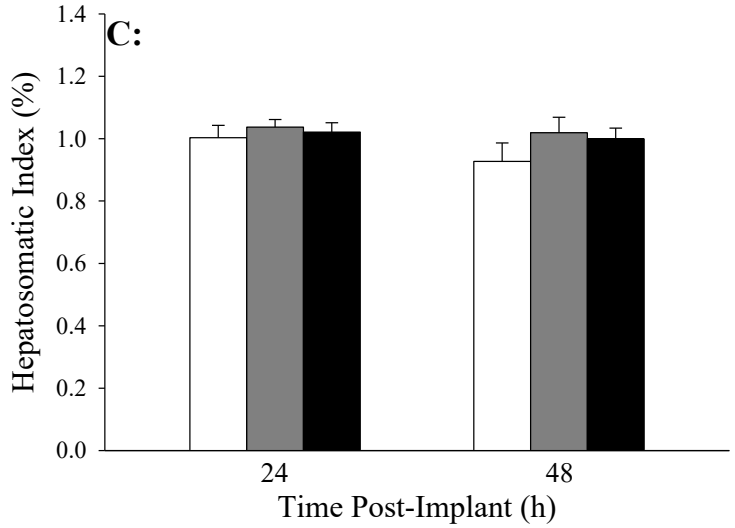

C:
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Hepatosomatic Index (%)
24
48
Time Post-Implant (h)

Figure 5.1.: Plasma [cortisol] (A), blood [glucose] (B) and hepatosomatic index (HSI; C) of sham-treated (white bars; 5 ml kg-1 body weight [BW] cocoa butter; N = 5-12), cortisol-treated (black bars; 25 mg kg-1 BW cortisol in cocoa butter; N = 6-12), and cortisol+RU486-treated (grey bars; 25 mg kg-1 BW cortisol & 50 mg kg-1 in cocoa butter; N = 4-12) bluegill sunfish sampled 24 h or 48 h post implant. Differences in the results between the two post-implant sampling times are denoted by an asterisk (*, $P < 0.05$), whereas treatment level effects within a sampling time are denoted by unique letters ($P < 0.05$). Statistical effects of time and treatment group on response variables were ascertained with a 2-way ANOVA model (see Results section).

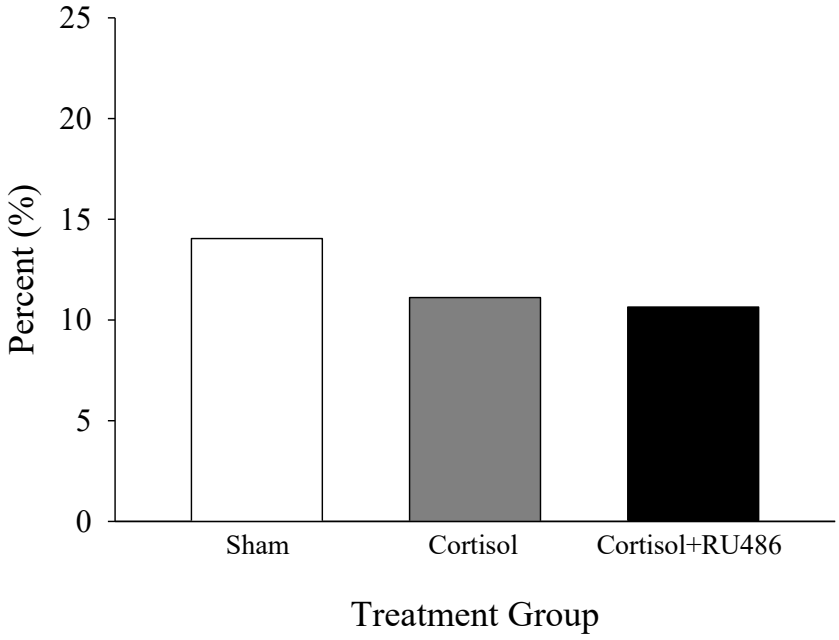


Figure 5.2.: Percent survival of sham-treated (white bars; 5 ml kg-1 body weight [BW] cocoa butter; N = 57), cortisol-treated (black bars; 25 mg kg-1 BW cortisol in cocoa butter; N = 54), and cortisol+RU486-treated (grey bars; 25 mg kg-1 BW cortisol & 50 mg kg-1 in cocoa butter; N = 47) bluegill sunfish after 24 h of being tethered in a nearshore lentic environment.

5.5. Discussion

5.5.1. Physiological effects of implants

Cortisol concentrations in cortisol-treated fish appeared to reach a peak of ~209 ng ml^{-1} at 24 h post-implant, declining thereafter, which is consistent with previous work on centrarchids (McConnachie et al. 2012). These plasma cortisol levels were well within the physiologically-relevant range, because peak values were similar to those measured in bluegill experiencing acute stressors (Wilson et al. 2011; Cook et al. 2012). Cortisol was elevated for substantially longer than would be the case for an acute stressor (Sopinka et al. 2015), but the physiological consequences of prolonged cortisol elevation would be expected to become more debilitating with time. Plasma cortisol titres in fish treated with cortisol+RU486 were substantially higher than those in either cortisol- or sham-treated animals. This difference could possibly stem from blockade of GR in the HPI axis itself by RU486, abolishing cortisol-induced negative feedback resulting in a rise in plasma cortisol levels (Bradford et al., 1992; Bernier et al., 1999; Reddy et al., 1995; Veillette et al., 1995; Lawrence et al. 2017). However, I am unsure of the exact reason driving this effect. Thus, effective blockade of GR was likely achieved in the bluegill studied here, and RU486-treated animals were probably not affected adversely by chronic cortisol elevation. Although, it must be highlighted that I did not create tether anchor points in the jaws of the fish used in physiological assessments. Conceivably, I would expect that adhering the tether to the fish would induce an acute stress response. As such, the fish used in the physiological assessment may not fully represent the physiological status of the tethered fish and would likely have lower plasma cortisol levels than the tethered animals thus limiting some of my interpretations.

Both blood [glucose] and HSI were unaffected by treatment. Cortisol treatment in teleosts (reviewed in Mommsen et al. 1999) and specifically in centrarchids (Dey et al. 2010; McConnachie et al. 2012; Zolderdo et al. 2016) typically results in elevated blood [glucose], because cortisol upregulates gluconeogenic enzymes (Chan and Woo 1978; Foster and Moon 1986; Vijayan et al. 1996; reviewed in Mommsen et al. 1999). However, this effect is not ubiquitous and cortisol treatment does not always lead to hyperglycemia (Davis et al. 1985; Andersen et al. 1991; Vijayan et al. 1994). Although marginally non-significant statistically ($P = 0.051$), the clear trend in my results for elevated blood [glucose] in cortisol-treated fish is consistent with an impact of cortisol treatment on gluconeogenesis. In agreement with literature demonstrating that RU486 treatment blocks the effect of cortisol on gluconeogenic activity (Vijayan and Leatherland 1992; Reddy et al. 1995), blood [glucose] in cortisol+RU486-treated bluegill was similar to that observed in sham-treated fish, adding to evidence that the desired blockade was effective. As observed previously in teleosts (Storer 1967; Leatherland 1987; Mommsen et al. 1992; Vijayan et al. 1994), HSI was unaffected by cortisol treatment in the present study, suggesting that hepatic glycogen reserves, for which HSI can serve as a proxy (Chellappa et al. 1995; Lambert and Dutil 1997), likely were not affected by cortisol treatment.

5.5.2. Cortisol and predation mortality

Contrary to prediction, cortisol treatment had no impact on the vulnerability of tethered bluegill sunfish to predation; all implant groups experienced similar survivorship. Because of cortisol's potential role in modulating anti-predator behaviour (e.g. Ryer 2004; Hawlena and Schmitz 2010; Raby et al. 2014; Pavlidis et al. 2015), a

greater risk of mortality to predation was predicted for cortisol-treated fish compared with fish in the other two treatment groups. Previous empirical studies are consistent with my negative findings. For example, cortisol treatment failed to alter survivorship and anti-predator behaviour in the schoolmaster snapper (Lawrence et al. 2017; 2018a). Similarly, cortisol treatment failed to alter anti-predator behaviour in checkered pufferfish (Cull et al. 2015; Pleizier et al. 2015) and pumpkinseed sunfish (*Lepomis gibbosus*; Chapter 3).

Collectively, these findings suggest that cortisol may not be an important mediator of predator-prey interactions in teleost fishes. However, I am cautious in this interpretation because cortisol implants in vertebrates often produce variable behavioural responses that are context dependent (Crossin et al. 2016; Sopinka et al. 2015). In these instances, physiological disturbances do not necessarily result in behavioural-level effects, suggesting a disconnect between these two levels of scale. For example, cortisol-treated creek chub (*Semotilus atromaculatus*) displayed no change in fine-scale spatial use patterns despite chronic elevation of plasma [cortisol] (Nagrodski et al. 2013). Similarly, negative findings were noted for some (O'Connor et al. 2009; Dey et al. 2010; Zolderdo et al. 2016; Algera et al. 2017b; Lawrence et al. 2018b), but not all (O'Connor et al. 2010; Algera et al. 2017a) studies of cortisol-treated centrarchids. Such conflicting effects highlight the inherent complexity and context-dependent nature of physiology-behaviour interactions (Killen et al. 2013; Crossin et al. 2016; Sopinka et al. 2015). Thus, in the particular context of the present study, it may be that changes in plasma cortisol alone are insufficient to alter bluegill behaviour, which may require additional input associated with the stress response or an additional environmental challenge to yield

cortisol/stress-related effects on predator-induced mortality (Killen et al. 2013; Spagnoli et al. 2016).

Alternatively, the time frame selected for this study may have been too short for the deleterious effects of cortisol to manifest. For example, in chronically-stressed zebrafish (*Danio rerio*), behavioural impairment became apparent at 14 days of chronic stress with the animal being able to cope over more acute durations (~7 days; Piato et al. 2011). Likewise, behavioural performance can be maintained under cortisol treatment, but the fish suffer consequences at a later time (i.e. mortality, loss of fecundity, etc.; O'Connor et al. 2009; Nagrodski et al. 2013; Zolderdo et al. 2016; Algera et al. 2017b). However, I am limited in the interpretation of our findings in the current study because behavioural traits were not measured. It would therefore be of interest to repeat my current experiment over a longer period and with a behavioural assessment to ascertain whether duration of elevated cortisol affects anti-predator capacity. It should also be noted that I cannot completely rule out that RU486 completely abolished cortisol's effects on behaviour (i.e. not all receptors bound/effectively blocked) nor that there are other behavioural effects associated with RU486's actions on the focal fish. Thus, caution must be exercised when interpreting these results.

5.5.3. Conclusions

I tested the hypothesis that cortisol treatment increases predator-induced mortality in juvenile bluegill sunfish. Despite elevated plasma cortisol levels, no effects of treatment were observed on survival of tethered sunfish. My results suggest that chronic cortisol elevation may not increase susceptibility to predation in this species. However, context-dependent effects, disconnects between physiological and behavioural processes,

and/or the relatively acute experimental duration may also explain the lack of effect. I acknowledge in addition that tethering, as an experimental methodology, can limit natural predator-avoidance tactics as well as making organisms more conspicuous to a predation, which may act to mask treatment-level effects (reviewed in Lawrence et al. 2017). Nevertheless, tethering serves as an important tool in estimating relative rates of predation in wild fish in ecologically-relevant settings (Rypel et al. 2007; Elvidge and Brown 2014; Lawrence et al. 2017). Further study is needed to address how cortisol influences fine-scale risk-taking and anti-predator behaviours.

Chapter 6: General conclusions and future directions

My overarching hypothesis was that the metabolic alterations associated with chronic stress (i.e. a homeostatic overload state) would promote increased risk-taking behaviours and/or compromised anti-predator behaviours resulting in higher predator-induced mortality in centrarchid fishes. This hypothesis was not supported by my data chapters. In Chapter 2, I did observe a higher SMR that corresponded with cortisol-treatment in pumpkinseed. However, because of a concurrent rise in the fish's MMR, cortisol treatment did not elicit a change in aerobic scope. Aerobic scope was comparable between sham- and cortisol-treated fish suggesting similar aerobic power. Despite a higher SMR under cortisol-treatment, sunfish risk-taking and anti-predator behaviours were unaffected in both isolated behavioural trials (Ch 3) and the microcosm study (Ch 4) thus refuting my hypothesis. Consequently, I found no evidence for a change in predator mortality when the HPI axis was modified pharmacologically; all treatment groups had comparable survival (Ch 5). Thus, despite significant physiological alterations, my thesis

refutes my hypothesis and suggests that chronic elevations in cortisol have no role in modulating predator-prey interactions in this context. From fisheries management and basic biological perspectives, these data are interesting in that it appears that fish may have a certain capacity to cope with stressors in the environment and that perhaps chronic stress, in a wild context, is not as a maladaptive trait as previously thought. Below, I summarize my principle findings providing mechanistic explanations for the observed phenomena and a course for future work that should be addressed in the context of my thesis. This synthesis is based around some of the key themes that my research highlighted.

6.1. The implications of the stress response in mediating predator-prey dynamics in fish

In this dissertation, I found that cortisol had no role in mediating predator-prey dynamics in teleost fish and is not likely the causal factor behind the differential predation rate observed between stressed and unstressed teleosts (reviewed in Mesa et al. 1994 & Raby et al. 2014). Unlike the data presented in this dissertation, prior works have shown that risk-taking behaviours, in the context of predator-prey interactions, are positively correlated with increasing energetic distress often in the form of food deprivation, parasitism, and higher metabolic expenditures (e.g. Giles 1983; Godin and Smith 1988; Godin and Sproul 1988; Gotceitas and Godin 1991; Krause et al. 1998; Killen et al. 2011). Such activities make the animal more susceptible to a predation event thus enhancing overall predation risk (i.e. P(death)). Surprisingly, despite a significant alteration in my sunfish's SMR (Ch 1), there were no effects of cortisol-treatment on the animal's behavioural phenotype with respect to both risk-taking and anti-predator behaviours (Ch 3 & 4). Consistent with this effect, no change in survivorship was noted

between my treatment groups (Ch 5) indicating that cortisol, and likely the HPI axis at large, has no role in mediating predator-prey interactions in this particular context. Below, I will discuss probable reasons for the disconnect between physiological and behavioural scale processes, in the context of predator-prey interactions, as well as addressing future avenues of research to investigate this effect more thoroughly.

In controlling predator-prey interactions, cortisol on its own may be insufficient to cause significant changes in a fish's risk of predation. In many of the works assessing stress-predation relationships, the stressors that the animals are exposed to (e.g. handling, air exposure, salinity, etc.) are likely to elicit an upregulation of multiple physiological systems alongside the HPI axis. For example, teleosts exposed to a shift in water salinity often exhibit alterations in ionic status, heat shock protein expression, and growth-related factors (e.g. growth hormone & insulin like growth factor) alongside a rise in blood cortisol titres (reviewed in McCormick et al. 2001 & Gonzalez 2012). The corresponding effect of a salinity change can result in a higher incidence of predator mortality (Handelhand et al. 1996). This example highlights that under a stress response, the HPI axis rarely functions in isolation and works in tandem with several axes and systems that facilitate survival under the specific physiological/environmental challenge (reviewed in Schreck and Tort 2016). Consequently, this indicates that while cortisol is important in mediating a stress response, it alone may not be the sole factor in driving the higher incidence in predator mortality under stress that is observed in the literature (Mesa et al. 1994; Raby et al. 2014). This is supported by my dissertation research in that, despite significant changes to the metabolic physiology of the fish, cortisol-treatment alone was unable to alter risk-taking behaviour and predator mortality in the sunfish studied here. In

further support of this notion, similar observations were made in complementary work to my thesis in schoolmaster snapper with respect to anti-predator behaviours and mortality (Lawrence et al. 2017; 2018a). It is possible that cortisol in tandem with some other physiological input may be required to alter risk-taking behaviours and/or rates of predation. Indeed, cortisol-treatment in vertebrates appears to have a variable influence on the animal's behavioural dynamics and suggests that there is a disconnect between the HPI axis and higher orders of biological scale with cortisol-behavioural relationships being incredibly complex and highly context dependent (Sopinka et al. 2015; Crossin et al. 2016). I speculate that this effect could serve in a beneficial manner as it likely prevents maladaptive behavioural responses (i.e. higher risk-taking) from being expressed with a rise in blood cortisol titres alone. This would be of particular importance for fish that experience diel (Bry 1982; Cousineau et al. 2014) or seasonal (Carruth et al. 2002) alterations in baseline plasma cortisol levels which aid in facilitating important life history events that are not associated with the stress response. Thus, only in circumstances where the animal receives multiple physiological inputs associated with the stress response does a change in behaviour occur that makes the animal increasingly vulnerable to a predator in some manner. Together, the lack of an influence of cortisol on predator-prey interactions may well represent an adaptive mechanism ensuring optimal fitness.

An alternative explanation for my results is that stress-induced changes in predation are the result of a completely different physiological input that was not investigated in this thesis. Indeed, a brief review of the literature indicates that there are a diversity of stressor types involved in causing higher predation rates including toxicants

(e.g. mercury, DDT, fluorene; Hatfield and Anderson 1972; Kania and O'Hara 1974; Painter et al. 2009), physical stressors (e.g. handling, confinement, angling events etc.; Jolley and Irby 1979; Mesa 1994; Danylchuk et al. 2007) and changes in water chemistry (e.g. temperature, salinity; etc.; Coutant et al. 1974; Jarvi 1989; Handelhand et al. 1996). Consequently, it could be that the physiological mechanism(s) underpinning this effect could be equally diverse and may not necessarily involve the HPI axis. It could be that cortisol, while being an adaptive mechanism for resisting physiological dysregulation, could simply be a correlative factor that accompanies stressor exposure while having no role in behavioural modification in the context of predator-prey dynamics. While this remains a speculative notion, it would be interesting to test the relative contribution of other physiological inputs associated with the stress response to discern potential mechanisms driving the stress-predation observations in the literature. A starting point may be to conduct a metanalysis of the literature to address which physiological aspects of the stressor (e.g. hormones, ions, energy substrates) or stressor types (e.g. temperature, handling, air exposure, etc.) that have the strongest bearing on such predator-prey relationships (i.e. greatest effects sizes). This could substantially narrow the focus of future work and allow for more specific hypotheses and predictions to be generated in this context. Follow up work could then include an experimental series addressing how the selected parameters of interest (e.g. changing blood $[K^+]$, $[Na^+]$, or pH, water chemistry changes, etc.) influences behavioural metrics relevant in a predator-prey setting. This could also be done in conjunction with cortisol manipulations to ascertain potential interactive and synergistic effects of the HPI axis with other stress related factors as discussed above. Together, this series of experiments would attempt to address

the first prediction of cortisol requiring an additional physiological input (i.e. synergism) to alter risk-taking behaviours as well as establishing other potential stress related parameters of interest for further study. As this thesis only explored the specific actions of cortisol in affecting predator-prey interactions, incorporating further aspects of the stress response would go a long way to broadening the scope of this work allowing for a more thorough understanding of the processes and mechanisms that govern predator-prey interactions following stressor exposure.

6.2 The role of metabolism in dictating risk-taking and anti-predator behaviours

Consistency in risk taking behaviours between cortisol- and sham-treated fish represents the contextual nature of metabolism-behavioural interactions. Under the 'metabolic hypothesis' proposed by Brown and Braithwaite (2004), higher basal metabolic expenditures tend to facilitate the development of riskier behavioural phenotypes as the animal must seek out foraging opportunities to meet heightened metabolic demands (Brown and Braithwaite 2004; Biro and Stamps 2010; Killen et al. 2013). This hypothesis is supported by a number of works where higher metabolic expenditures correspond with greater degrees of risk-taking behaviour and, presumably, a higher level of perceived predation risk (Krause et al. 1998; Metcalfe et al. 1998; Dowling and Godin 2002; Brown and Braithwaite 2004; Petrie and Ryer 2006). Interestingly, despite showing higher SMR, cortisol-treated sunfish in this dissertation did not show an alteration in risk-taking behavioural metrics contrasting the metabolic hypothesis (Brown and Brathwaite 2004) and juxtaposes with the findings of the aforementioned works on the topic (Krause et al. 1998; Metcalfe et al. 1998; Dowling and Godin 2002; Brown and Braithwaite 2004; Petrie and Ryer 2006). However, it is

important to realize that many of the observations underpinning the metabolic hypothesis use body mass/size as a proxy for metabolic rate as it assumed that mass specific metabolic rate generally scales inversely with body mass (White et al. 2006; Killen et al. 2010). Thus, the metabolic rate of focal fish is implied rather than actually measured in the aforementioned works presenting a major assumption that body size related changes in risk are the product of metabolic consequences rather than a size-dependent perception of risk (Brown and Braithwaite 2004). This is also further complicated by the fact that body mass-metabolism scaling in teleosts is often quite variable and is highly species and context dependent (Glazier 2005; 2006; Killen et al. 2010). A recent study by Polverino et al. (2016) attempted to address metabolism-risk-taking interactions by measuring how risk-taking behaviours scale with body mass and routine metabolic rate in zebrafish. As in this dissertation, they found that while decreasing body mass did scale with higher risk-taking behaviours, there were no relationships associated with the animal's routine metabolic rate or its body condition (measured as Fulton's condition factor). Other works have found similar occurrences whereby there was either no correlation between the two (Farwell and McLaughlin 2009; Killen et al. 2011) or that it only occurred under specific contexts often in the form of an additional stressor (Killen et al. 2011; Killen et al. 2012). These results are consistent with what I observed in my dissertation work where body mass did appear to influence risk taking behaviours but the overall treatment level effects of cortisol (i.e. higher SMR) appeared to be non-existent. As well, like these works, I found that body mass did play a role in mediating some of the risk-taking behaviours such as exploratory activity (Table 3.1) and refuging time (Table 4.1) while there was generally no effect of condition factor (i.e. HSI) in this respect. Thus, under this scenario,

I would expect that cortisol alone would be insufficient to alter risk-taking behaviours and, consequently, have no influence on predator-prey interactions at this level of scale. Furthermore, my original assumptions that cortisol-induced behavioural changes are the product of metabolic effects may not have been entirely valid given that it seems that direct metabolism-behavioural relationships appear to be highly contextual occurring under very specific circumstances. Indeed, this effect appears to be quite prevalent in cortisol-behaviour experiments whereby direct linking between physiological and behavioural scale processes is highly variable in the literature and often does not scale in a direct fashion (reviewed in Sopinka et al. 2015; Crossin et al. 2016). As alluded to earlier in this chapter (see Section 6.1), perhaps an additional physiological disturbance or input is required to observe such a relationship between metabolism and behaviour in this setting (Killen et al. 2011; 2012).

While my thesis research attempted to investigate relationships between physiology and behaviour, in the context of predator-prey interactions, this work could be further improved upon. Unlike previous works on the topic (e.g. Killen et al. 2011; Polverino et al. 2016), I did not measure metabolic and behavioural phenotypes on the same individuals such that my results represent an average effect of cortisol-treatment rather than addressing individual responses. I feel it would be worth re-investigating my behavioural metrics in fish where the specific metabolic parameters are known. Not only would this contribute to validating the metabolic hypothesis, in the context of cortisol effects, but would also strengthen my arguments regarding metabolism-behavioural relationships in this dissertation. It is also worth considering that conducting such an experiment may help to tease apart some of the effects of individual responses to cortisol-

treatment that may be serving to 'mask' some of the finer scale changes in behaviour (Killen et al. 2013). Specifically, it has been established that sunfish do exhibit personality syndromes that occupy a bold-shy continuum (Wilson and Godin 2009a,b; Wilson et al. 2011; Binder et al. 2016) which may result in differential sensitivity of the HPI axis and to glucocorticoids in general (Johansen et al. 2011; Raoult et al. 2012). Additionally, stressors can result in personality switching in the environment (Frost et al. 2013) which may have implications for altering the personality and/or behavioural traits of individual fish in this experiment. As alluded to earlier, stressors can strengthen metabolism-behavioural relationships which do not normally occur under normal environmental conditions (Killen et al. 2011; 2012). Consequently, bold-shy personality traits and individual-scale effects are likely to have an important role in dictating cortisol responsiveness and the resulting behavioural modifications in this dissertation work. Thus, investigating concurrent metabolic and risk-taking behavioural metrics would greatly improve the resolution to this work by allowing me to observe how more fine scale population level differences in sunfish behaviour, at the level of the individual, can dictate the responsiveness to a stressor and the implications of such in mediating predator-prey interactions here. Perhaps, at this level, there may be indeed an effect of cortisol in mediating predator-prey interactions specific to certain sub-populations of fish that is being masked by the population at large. These notions remain entirely speculative but integrating individual scale effects should act to strengthen the conclusions provided by this thesis. It also represents a timely area of research given that investigating the role of the individual is becoming increasingly popular in contemporary fisheries biology (Killen et al. 2013; Metcalfe et al. 2016; Ward et al. 2016).

The role of the animal's aerobic scope in mediating predator-prey dynamics still remains uncertain at this time. Aerobic scope is believed to be the primary metric associated with an organism's performance and represents the amount of metabolic power available to fitness-enhancing functions. As this scope is considered to be finite (i.e. aerobic scope = MMR-SMR), life history traits are often partitioned within the confines of available aerobic scope energy. Consequently, increased metabolic loading (e.g. stressors, disease, etc.) or reductions in overall aerobic scope can potentially result in decreased organismal performance and life-history trade-offs particularly if the majority of the aerobic scope is utilized (Guderley and Portner 2010; Sokolova 2013). In this dissertation, I predicted that part of the observed rise in predator mortality under stress may result from a constrained aerobic scope under cortisol-treatment that would compromise the fish's ability to maintain anti-predator activities. However, this was not the case as aerobic scope was maintained with cortisol-treatment and there were no effects on any of the anti-predator metrics that were observed here. Thus, at this time, it is difficult to ascertain the role of aerobic scope in maintaining anti-predator behaviours as scope was unchanged. Currently, data regarding the role of aerobic scope in maintaining anti-predator behaviours in teleosts is rather scant. There is some evidence to suggest that partitioning of aerobic scope energy has implications for predator avoidance capacity (Lankford et al. 2005; Arnott et al. 2006; Killen et al. 2007; 2015a,b; reviewed in Guderley and Portner 2010). Furthermore, fish released following exhaustive exercise such as in the case of a fish-related stressor (Jolley and Irby 1979; Ryer et al. 2004; Danylchuk et al. 2007; reviewed in Raby et al. 2014), typically exhibit high rates of predation. Presumably, the animals would have a large proportion of their scope allocated

towards physiological recovery (i.e. excess post-exercise oxygen consumption [EPOC]; Scarabello et al. 1991; Wood 1991; Donaldson et al. 2010) suggesting a role of aerobic scope in dictating anti-predator activities (Killen et al. 2015b). Although, I can provide no clear conclusions regarding the specific role of aerobic scope in mediating anti-predator behaviours as there were no discernable differences between my treatment groups. However, my data does suggest that under a chronic elevation in cortisol, sunfish should be able to sustain comparable degrees of anti-predator behaviours to that of a resting fish as aerobic scope is conserved. It is also worth noting that even if aerobic scope was constrained by cortisol's metabolic effects, the animal may elect to enter a refuge environment to offset the metabolic costs associated with maintaining predator vigilance behaviours (Fischer 2000; Finstad et al. 2004; Millidine et al. 2006). Thus, the animal could behaviourally regulate its aerobic energy budget in a way that would likely maximize its fitness.

Further experimentation would be required to assess the role of aerobic scope in sustaining anti-predator behaviours. While there was no clear indication of an influence of aerobic scope in moderating anti-predator avoidance here, prior work has shown there to be a possible link between these two metrics (Lankford et al. 2005; Arnott et al. 2006; Killen et al. 2007; 2015a,b). This avenue of research could prove to be incredibly interesting and relevant given that human activity may be producing environmental conditions where aerobic scope may be compromised (Lankford et al. 2005; Fitzgibbon et al. 2007; Eliason et al. 2011; reviewed in Clark et al. 2013). Indeed, this experimental series could be carried out in a concurrent fashion with the aforementioned proposed research regarding individual responses on predation risk behaviours. Briefly, this would

involve characterizing the aerobic scope and other relevant metabolic parameters (e.g. SMR, MMR, recovery effort, etc.) of an individual fish and correlating that with the animal's capacity to engage in anti-predator activities. Ideally, anti-predator behavioural tests would be restricted to assessments of the animal's vigilance behaviours including flight initiation distance tests similar to that of Killen et al. (2015b). To further strengthen the role of metabolic scope in mediating anti-predator activities, assessments of vigilance behaviours could be conducted under circumstances where scope is likely constrained such as during a post-exercise event (Wood 1991) or post-prandial (e.g. specific dynamic action [SDA]; Auer et al. 2015) to address if higher aerobic scope confers a greater capacity to engage in vigilance behaviours or if there are energetic trade-offs between metabolic recovery and anti-predator behaviours. This work would be highly complementary to my dissertation allowing me to further investigate the role of metabolism, from an energy allocation perspective, in driving stress-predation relationships.

6.3. Behavioural resiliency under stressors

In this thesis dissertation, I formalized the concept of the behavioural resiliency hypothesis and attempted to test its validity in the current context. This hypothesis was developed under consideration from a number of works that found that teleosts were able to retain a relatively normal degree of behaviour despite experiencing physiological perturbations resulting from stressors (Paito et al. 2011; Pavlidis et al. 2015; Schmidt et al. 2017a,b). For example, exposure to high environmental CO_2 results in a number of physiological changes in Atlantic cod (*Gadus Morhua*; de Souza et al. 2014; Schmidt et al. 2017b). Despite this, behavioural metrics associated with predator avoidance, general

activity patterns, and laterality scores are unaffected by CO_2 treatment suggesting that these animals are able to behaviourally cope under this environmental context (Jutfelt and Hedgarde 2013; 2015; Schmidt et al. 2017a). Furthermore, adverse effects to shoaling (Piato et al. 2011) and feeding behaviours (Pavlidis et al. 2015) were not observed until a much later time point if at all in zebrafish subjected to chronic stress protocols to which the authors have attributed the notion of behavioural resilience in this context (Piato et al. 2011). Together, these works suggest that teleosts have a certain capacity to behaviourally cope with stressors, buffering the effects of stress-induced physiological perturbations. To that end, I developed the behaviour resiliency hypothesis which states that behavioural phenotypes should be maintained under stressor exposure as means of retaining behaviours that are optimally suited for the current environmental context. Furthermore, behavioural coping in this context should act in an adaptive fashion ensuring a high degree of organismal fitness. This differs from phenotypic plasticity as plasticity generally confers a difference in behavioural phenotypes in response to an alteration in environmental conditions (Komers 1997). As behavioural coping strategies are likely rooted within the animal's broader ability to cope with stressors, the reactive scope model would predict that this coping ability is finite and is highly contextual such that modifying factors such as prior stress history and individual scale differences could conceivably modify the limits of coping (Romero et al. 2009).

In this dissertation, support was found for the behavioural resiliency hypothesis. Here, I observed a marked difference in the physiological status of cortisol-treated pumpkinseed which included higher SMR, ammonia excretion rates and energy substrate formation (Ch 2). Thus, this suggested that cortisol-treated sunfish should experience a

higher degree of risk-taking behaviours resulting from increased energetic expenditures (e.g. Giles 1983; Godin and Sproul 1988; Gotceitas and Godin 1991; Krause et al. 1998). However, in line with the behavioural resiliency hypothesis, all behavioural metrics were unaffected by cortisol-treatment (Ch 3 & 4) suggesting that sunfish here were able to behaviourally cope with the significant physiological perturbation. Given the current context, behavioural resiliency here likely represents an optimal adaptive strategy such that higher risk-taking behaviours would impart a higher perceived predation risk. As this may result in a possibly higher incidence of mortality, behaviourally coping by cortisol-treated sunfish represents a means of ensuring a high degree of fitness. My results are consistent with prior work on stress-predation interactions whereby shoaling cohesion (Piato et al. 2011) and feeding behaviours (i.e. a proxy for risk-taking; Pavlidis et al. 2015) were not negatively affected by chronic stress in a timeframe comparable to this thesis. Furthermore, checkered pufferfish suffered no ill effects to any behavioural metrics associated with anti-predator behaviours and performance indices under cortisol-treatment (Cull et al. 2015; Pleizier et al. 2015). Indeed, my effects are also in line with a large proportion of the centrarchid literature whereby cortisol-treatment has little bearing on modifying behavioural metrics in these fish (O'Connor et al. 2009; Dey et al. 2010; Zolderdo et al. 2016; Algera et al. 2017b). Despite there being a large body of support for the behavioural resiliency hypothesis, it is important to note that there is likely an associated trade-off and/or finite ability to maintain behaviour in the face of physiological dysregulation (Romero et al. 2009). For example, by 14 days of the chronic stress protocol, zebrafish shoaling cohesion became compromised (Paito et al. 2011). Similarly, while nesting behaviours were comparable between cortisol- and sham-treated black

bass, nest-abandonment rates were much higher in cortisol-treated fish to which animals would incur a significant fitness cost (O'Connor et al. 2009; Dey et al. 2010; Zolderdo et al. 2016; Algera et al. 2017b). Nagrodski et al. (2013) also found that while spatial movement patterns were preserved in cortisol-treated creek-chub, these same fish exhibited higher mortality rates. Together, this indicates that the animal's ability to cope is likely finite and that maintaining a consistent suite of behaviours with physiological perturbations comes at a cost of some sort.

In this thesis, I observed no apparent costs or trade-offs associated with the fish's behavioural resiliency but that is not to say that none may exist outside the scope of this work. I would expect that perhaps my sunfish would experience reductions in somatic growth (Gregory and Wood 1999; O'Connor et al. 2011) or possibly a higher incidence of inherent mortality (i.e. not predation related; Pickering and Pottinger 1989; Gregory and Wood 1999; Nagrodski et al. 2013) if these animals were observed for a longer period than my dissertation work (i.e. beyond 96 h post-implant). Given that these sorts of changes can have tangible impacts onto the animal's wellbeing and fitness (Sneddon 2016), it may be worth investigating further into the associated costs of maintaining behavioural resiliency. By extension, as I proposed that behavioural coping is likely to be finite, it would be of interest to observe how this resiliency holds up over a more chronic duration of exposure. This would address the questions of is this capacity finite and at what point does this effect become evident? As chronic exposures of sunfish to stressors in the environment may last longer than my experimental duration (i.e. 48 h post-implant), this research direction could be highly relevant in generating data for anticipating when the deleterious effects of stress may occur; a point of interest for

fisheries managers and aquatic conservation in general. Ideally, this experiment would look to assess risk-taking behaviours with cortisol- and sham-treated fish at discrete time intervals post-implant (i.e. 24 h, 48 h, 96 h, 168 h, etc.) to observe the threshold when behaviour coping deteriorates in sunfish. This experiment would also need to incorporate an individual scale approach to account for intraspecific differences in coping ability, physiological temperament and prior stress history.

6.4. Cortisol's role in mediating foraging dynamics

Foraging dynamics are an important factor dictating predator-prey interactions in fish. As mentioned earlier, teleosts experiencing increasing degrees of hunger tend to exhibit riskier behavioural phenotypes such as earlier refuge emergence and longer durations spent at foraging patches (Gotceitas and Godin 1991; Godin and Crossman 1994; Petrie and Ryer 2006; Killen et al. 2011). Thus, during this dissertation, the original goal was to include foraging opportunities in my behavioural trials to help tease apart the metabolic effects of chronic cortisol elevations on risk taking behaviours. In such a circumstance, I would anticipate that cortisol-treated fish would modify their behaviour to optimize nutritional acquisition to meet greater basal expenditure at the sacrifice of taking on a greater degree of risk in line with the aforementioned studies. However, I could not implement this aspect into my experimental design as wild-caught sunfish did not feed reliably in a captive setting. Thus, some of the homogeneity in my behavioural data may be explained by a lack of foraging opportunities within the experimental setup. In my current work, there were no net benefits, from a fitness perspective, to engage in riskier behaviours which may help to explain comparable behavioural phenotypes between sham- and cortisol-treated fish (Godin 1997). In future

experiments, I believe that including foraging opportunities are required to fully address the role of cortisol in mediating risk-taking behaviours as the decision to engage in riskier behaviours in teleosts is often weighed against the benefits of foraging prospects (reviewed in Milinski 1993). For example, a number of works have shown that teleosts are more likely to feed under a higher perceived predation risk provided that the foraging opportunities are better than a comparatively lower predation risk feeding patch (reviewed in Milinski 1993) in support of the μ/g rule (Gilliam 1982; Werner and Gilliam 1984; Gilliam and Fraser 1987; Lima and Dill 1990). While previous work was able to get sunfish to feed in captivity over long acclimation durations (Wilson and Godin 2007a), a better approach that is more ecologically relevant may be to conduct foraging risk-taking assessments, in the context of cortisol's effects, in the natural environment. This experiment would likely make use of bluegill sunfish as previous work has shown that their home and core ranges are relatively small (~0.11-0.72 ha) such that animals would conceivably stay within a small bay area of the lake (reviewed in Warren 2009). The experiment would involve using the same implantation approach used in this thesis and visually marking the fish in such a manner to identify the treatment group (e.g. Visible Implant Elastomer [VIE] tags or spine clips). After a 24-h incubation period, fish would be released into the experimental bay whereby there would be a number of minnow traps in a methodological approach similar to that of Brown and Godin (1999). Here, the perceived risk associated within each of the minnow traps could be modified using olfactory cues and would consist of sponges soaked with a conspecific alarm cue (i.e. high perceived predation risk), a sham (i.e. distilled water, low risk) or a novel olfactory cue (e.g. lemon extract, novel risk; Elvidge et al. 2016). Concurrently, using a

balanced experimental design, these minnow traps would be baited with differing degrees of food representing no food, a low-density foraging patch or a high-density foraging patch. After a set duration (probably 48 h after bluegill release), the traps would be retrieved and the number of cortisol and/or sham treated fish in the trap could be counted up to determine which type of risk & foraging environments that each of these treatment groups selected for under a natural and ecologically relevant setting. Based on prior works, we may expect a higher proportion of cortisol-treated fish to be found in traps containing food rewards regardless of the perceived predation risk level; sham-treated fish should avoid high risk traps except when feeding gains are at their highest. Complimentary work could also look at metabolic parameters of fish recovered in the minnow traps to ascertain metabolic correlates with risk-taking behaviours (e.g. Metcalfe et al. 2016). Thus, this would be an excellent follow up experiment that could allow me to better tease apart the effects of cortisol-treatment and risk-taking behaviours in centrarchid fishes while addressing a significant knowledge gap in this thesis.

6.5. *Cortisol's role in modulating metabolic parameters*

In the literature, cortisol implants are widely used as a means of mimicking the conditions of chronic stress whereby plasma cortisol titres remain elevated for a prolonged period (Gamperl et al. 1994; Sopinka et al. 2015). In teleosts, this methodological approach has been used in a number of contexts and settings to understand the relationship between alterations in the animal's physiology and behaviour. For example, cortisol-behaviour interactions have been thoroughly investigated in nesting black basses as a means of understanding human impact on spawning success (e.g. O'Connor et al. 2009; Dey et al. 2010; Zolderdo et al. 2016). Despite this fact, we

understand very little with respect to how whole animal metabolism (i.e. oxygen consumption) is afflicted by chronic elevations in plasma cortisol levels as well as how cortisol can alter the specific metabolic parameters associated with organismal performance (i.e. SMR, MMR, aerobic scope, etc.). From the limited studies to date, we have some evidence to suggest that basal expenditures (e.g. De Boeck et al. 2001; O'Connor et al. 2011) and that general MO_2 (Chan and Woo 1978; Morgan and Iwama 1996; Liew et al. 2013) are higher in cortisol-treated teleosts. No data in the current literature appears to investigate the effects of cortisol on any other metabolic rate parameter such as MMR, aerobic scope, and EPOC.

Given the lack of data in the literature, one of the central goals of this dissertation was to address how prolonged elevations in plasma [cortisol] affects metabolic rate parameters associated with organismal performance and behaviours (e.g. Biro and Stamps 2010; Guderley and Portner 2010; Killen et al. 2013). In Chapter 1, I had hypothesized that cortisol-treatment would result in a higher SMR with a corresponding reduction in aerobic scope provided no change in MMR occurred. Here, the first predictive effect was shown to be correct in that SMR was higher under cortisol-treated pumpkinseed against respective controls and is in general agreement with previous works on the topic (De Boeck et al. 2001; O'Connor et al. 2011). I surmised that this effect is a likely a product of cortisol's regulatory actions on the metabolic functioning of the fish all of which are necessary for physiological adjustments for stressor mitigation. This may include an upregulation of such energetically taxing processes including general protein transcription/translation, ionoregulatory processes, and gluconeogenic activity, amongst a suite of other physiological adjustments during the stress response (reviewed in

Mommsen et al. 1999 & Schreck and Tort 2016). In support of this, I did observe the development of a hyperglycemia, net deposition of glycogen in the liver, and higher ammonia excretion under cortisol treatment suggesting that basic maintenance functions were higher in cortisol-treated fish. Interestingly though, in contrast to previous works on the topic (reviewed in Mommsen et al. 1999), I found that cortisol-treatment resulted in reduced activities of enzymes mediating gluconeogenic activities (see Appendix A). This result is quite puzzling and is at odds with what I observed in the rest of my physiological data suggesting that further work may be required to address this phenomenon. This could potentially include the use of molecular techniques to address transcriptional or translational activities of these enzymes and/or investigate other aspect of the enzymes' kinetic profiles (e.g. k_m). Regardless, this dissertation provides some of the first work characterizing cortisol's effects on SMR in a wild-caught teleost fish.

To date, no prior works have addressed the role of cortisol in modulating either MMR or aerobic scope in teleost fishes. Here, I found that aerobic scope was conserved under cortisol-treatment despite higher SMR (Ch 1). This contrasts what has been found under periods of chronic stress in fish whereby a chronic stress regime results in a higher SMR with a concurrent reduction in aerobic scope (Lankford et al. 2005). Unlike the aforementioned work, I did see a higher MMR under cortisol-treatment (Ch 1) which likely explained the preserved aerobic scope here. As discussed earlier, the maintaince of aerobic scope under cortisol-treatment would conceivably allow for the animal to maintain a high degree of performance in its environment which may help to explain, in part, why anti-predator behaviours were consistent across my treatment groups. With respect to cortisol's effects on MMR, I'm not entirely sure of the mechanism driving this

effect. Cortisol itself doesn't appear to have a direct influence on cardiovascular operation (reviewed in Mommsen et al. 1999) which is often the physiological system dictating MMR in teleosts (Norin and Clark 2016). However, cortisol may be influencing MMR in a more indirect manner by either enhancing tissue catecholamine sensitivity (Reid et al. 1992; Perry and Reid 1993; Reid et al. 1996; reviewed in Perry and Capaldo 2011) or by increasing mitochondrial aerobic capacity (Foster and Moon 1986; Tripathi and Verma 2003). I did attempt to follow up with the latter by investigating protein expression levels of cytochrome C oxidase in the livers of the pumpkinseed used in Chapter 1. Unfortunately, difficulties with anti-body specificity prevented this work from going forward and I was unable to quantify these changes. Thus, given the importance of MMR in dictating organismal performance and physiological limits (Norin and Clark 2016), and its potential role in mediating predator-prey interactions (Lankford et al. 2005; Killen et al. 2007), future works should look to address the specific mechanisms by which cortisol alters MMR. This should include molecular work investigating changes in the expression of relevant metabolic enzymes as well as changes in catecholamine receptor expression levels on cardiovascular tissue. Further, this could also include using isolated cardiac tissues with a cortisol rich medium to investigate cortisol's direct influence on cardiovascular tissues as the current literature seems scant in such studies.

6.6. Implications for fisheries management

In today's world, centrarchid fishes are under increasing pressures from human activities in their environment. In many freshwater ecosystems in North America, such challenges often include shifts in water quality parameters (Mills et al. 2003; Palmer et al. 2011, Sondergaard and Jeppesen 2007), increased interactions with invasive species

(Mills et al. 2003; Kerr et al. 2005), and higher recreational fishing pressures (Cooke and Cowx 2004; Cooke and Murchie 2015) amongst a multitude of others. As we know that stress, in the broad sense, can influence predator-prey dynamics in fishes (Mesa et al. 1994; Raby et al. 2014), understanding the basic mechanisms driving this effect is of importance in developing a stronger predictive framework for assessing the impacts of human activities on local fish populations. Indeed, stock assessment models can often include predator mortality as a means of improving demographic models (Livingston and Methot 1998; Tjelmeland and Lindstrom 2005; Moustahfid et al. 2009). Similarly, incorporating some of the relationships between stress and predation garnered in this dissertation may serve in a beneficial capacity to fisheries managers looking to address the relative effects of a particular stressor on the populations of centrarchid fishes. Furthermore, metabolic characterizations determined in this thesis may also be useful in addressing population scale effects including in improving bioenergetic modeling under chronic stressor exposures. In this instance, it may improve the predictive power of these models to assess how stressors in the environment may have implications for the animal's various life history traits (e.g. somatic growth, reproductive investment, daily nutritional requirements, etc.) and the resulting consequences of this at the population level in a similar manner to that in O'Connor et al. (2011). On that note, as this work largely looked at a model centrarchid fish in its juvenile life stage, the pumpkinseed sunfish, the results of my thesis could likely be broadly applicable to other juvenile centrarchid species that are of high economic value in the recreational angling sector such as the black basses and crappie. Together, the results of this thesis could be of high interest to the fisheries management sector in improving model predictably both in terms of

population scale effects as well as in assessing the relative impacts of chronic stress in centrarchid fishes.

6.7. General summary

The research presented in my dissertation found little support for the involvement of the HPI axis and, more broadly, chronic stress in influencing predator-prey dynamics in centrarchid fishes thus refuting my overarching hypothesis. While cortisol-treatment caused a higher SMR, there were no corresponding effects on risk-taking behaviours or predator-induced mortality. The variable nature of the stressors involved in affecting predator mortality (e.g. toxicants, temperature, physical stressors; Mesa et al. 1994; Raby et al. 2014) may suggest that other physiological inputs/systems associated with the stress response may be mediating this effect. As well, the linkage between metabolic and behavioural scale responses, with respect to risk-taking behaviours, appears to be highly contextual in the literature (Killen et al. 2011; 2012) and may not apply in this particular stetting. As well, aerobic scope was preserved under cortisol-treatment suggesting that these animals should have a similar capacity to perform in the environment relative to controls. Clearly, more work is needed in this regard and should be coupled with investigating individual scale effects as these may be acting to mask treatment-level effects here (Killen et al. 2013). Behavioural resiliency, the maintenance of behaviour despite physiological perturbation, appeared to be occurring thereby limiting the effects of cortisol on risk-taking behaviours. However, as this effect should be finite (Romero et al. 2009), I would expect to see a higher degree of risk-taking behaviours and/or compromised anti-predator behaviours at exposure times greater than what was ascertained here. The consistency in risk-taking behaviours under cortisol-treatment may

also be explained by the lack of foraging opportunities provided in my behavioural trials. Together, my results indicate that chronic stress, at the level of changes in plasma cortisol, has no role in mediating predator-prey interactions in wild-caught centrarchid fishes. Despite this, these results are still likely to prove useful to fisheries managers in ascertaining the relative impacts and consequences of chronic stress at the population level. Thus, this dissertation provides a foundational work for assessing the interaction of physiological, behavioural and ecological scale processes which is becoming an increasing relevant topic in the contemporary Anthropocene (Madliger et al. 2017). As human activity continues to encroach on aquatic environments, understanding the link between stress and higher biological processes (i.e. behaviour, population dynamics, community function, etc.) is becoming increasingly important in today's world and in fully understanding the 'ecology of stress' (Boonstra 2013a,b).

Appendix A. Activity profiles of gluconeogenic enzyme under chronic cortisol treatment in wild-caught pumpkinseed

Cortisol functions as the primary corticosteroid in teleost fishes through regulatory control via the hypothalamic-pituitary-internal (HPI) axis (reviewed in Mommsen et al. 1999). Cortisol functions primarily in the reallocation of energy reserves away from non-essential functions (e.g. growth and reproduction; Sadoul and Vijayan, 2016; Pankhurst, 2016) as well as promoting the *de novo* synthesis of glucose via gluconeogenesis (Mommsen et al. 1999). In the case of the latter, a suite of enzymes are often found to be upregulated including glutamate dehydrogenase (GDH), aminotransferases and phosphoenolpyruvate carboxykinase (PEPCK; reviewed in

Mommsen et al. 1999). This work sought to characterize how sustained elevations in cortisol affected gluconeogenic activity in the liver of a wild-caught teleost fish, the pumpkinseed (*Lepomis gibbosus*; Linnaeus 1758). Information regarding hepatic gluconeogenic enzyme profiles is quite lacking in the literature with the current research largely focusing on captive/domestic species. We expected that cortisol-treatment would elicit higher activities of gluconeogenic enzymes. To test this, fish were implanted with cocoa butter containing cortisol (25 mg kg^{-1} body weight; BW) or alone as a sham. Over a 96-h period, fish were sampled for liver tissues in accordance with the procedures outlined in the Materials and Methods section of this work. Liver tissue was used for the assessment of maximal activities of enzymes mediating protein deamination and gluconeogenesis. Liver tissue was sonicated in a chilled, glycerol buffering solution (50% glycerol, 10 mM Hepes, 0.5 mM EDTA, 20 mM K_2HPO_4, 1 mM DTT; pH 7.5) and then centrifuged (4°C; 11,500 *g*) for 5 minutes. Activities of glutamate dehydrogenase (EC 1.4.1.2), alanine aminotransferase (EC 2.6.1.2), and aspartate aminotransferase (EC 2.6.1.1) were determined using the methods of Mommsen et al. (1980). The alanine aminotransferase assay was modified to have 0.0745 units ml^{-1} of lactate dehydrogenase in the reaction buffer. Phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.32) was determined as in Borowiec et al. (2015). Samples were sonicated in a chilled, buffering solution (1 mM sodium EDTA, 20 mM HEPES, 0.1% Triton X-100; pH 7.0) and centrifuged (10,000 *g* for 5 min). The supernatant was assayed using the conditions indicated in Borowiec et al. (2015). All tissue assays were carried out at room temperature (~22°C) using a microplate reader.

Cortisol-treatment resulted in lower maximal activities for all enzymes assayed here (Figure A.1). This is in stark contrast to the current body of literature where cortisol-treatment often elicits higher activities of these enzymes in teleost fishes and are key enzymes mediating cortisol-induced gluconeogenesis (reviewed in Mommsen et al. 1999). We are unsure of why this is the case and is quite puzzling. However, it does represent one of the first characterizations of cortisol's influence on these enzymes in a wild-caught teleost fish and highlights the need for assessments of such parameters on wild derived teleosts.

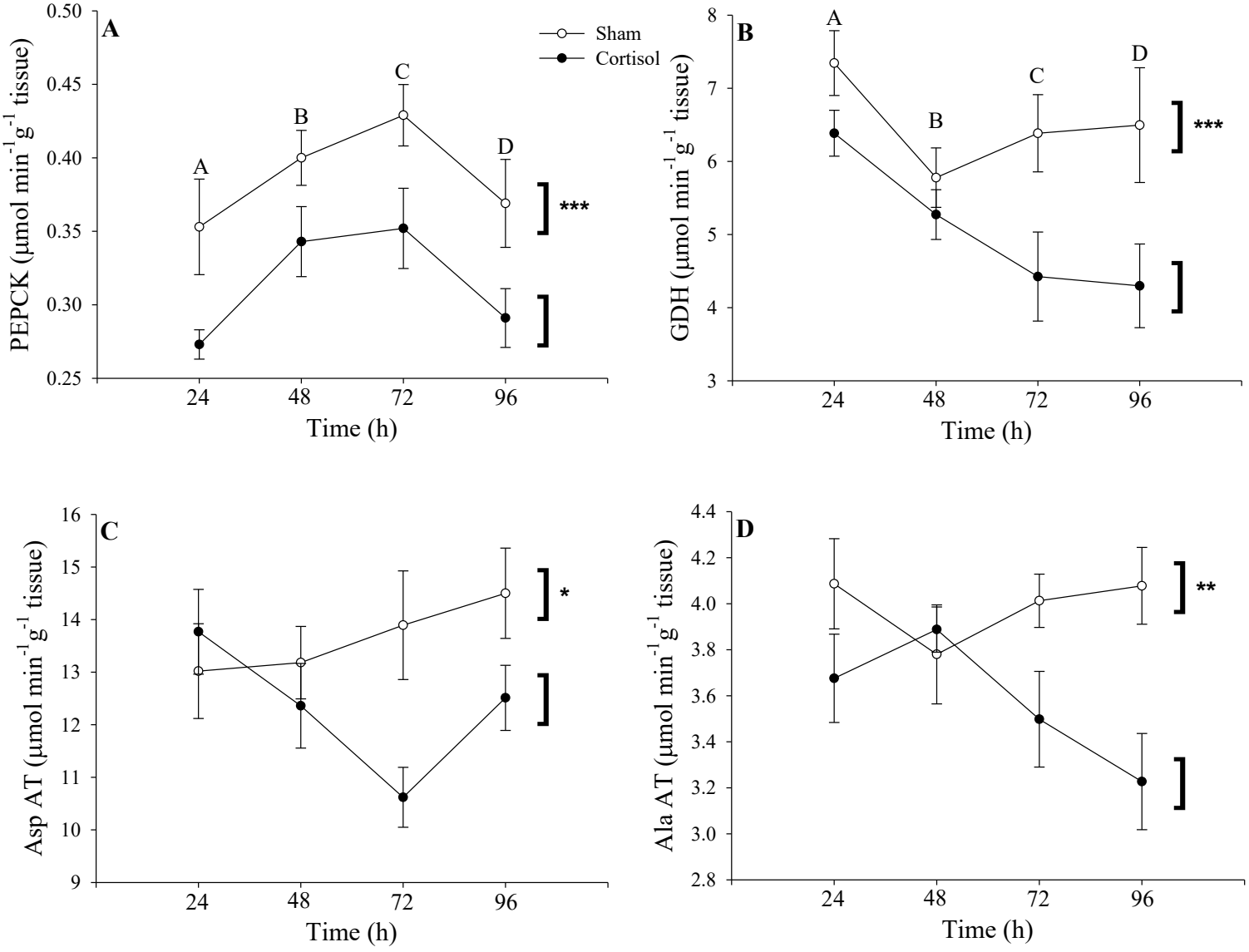


Figure A.1: Activities of hepatic (**A**) phosphoenolpyruvate carboxykinase (PEPCK; N≤9), (**B**) glutamate dehydrogenase (GDH; N≤9), (**C**) alanine aminotransferase (Ala AT; N≤9), and (**D**) aspartate aminotransferase (Asp AT; N≤9) for sham- (5 ml kg-1 BW cocoa butter) and cortisol-treated (25 mg kg-1 BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) over a 96-h sampling period. Values are shown as means±1 SEM. Statistical significance was accepted at $\alpha=0.05$ with differences between treatment groups represented by an asterisk (* $P<0.05$, *** $P\leq0.001$). Capital letters denote a statistically significant effect of time ($P<0.05$).

Appendix B. Abstracts of non-thesis publications during doctoral studies

1. **Lawrence, M. J.**, Stemberger, H. L., Zolderdo, A. J., Struthers, D. P., & Cooke, S. J. (2015). The effects of modern war and military activities on biodiversity and the environment. *Environmental Reviews*, *23*(4), 443-460.

War is an ever-present force that has the potential to alter the biosphere. Here we review the potential consequences of modern war and military activities on ecosystem structure and function. We focus on the effects of direct conflict, nuclear weapons, military training, and military produced contaminants. Overall, the aforementioned activities were found to have overwhelmingly negative effects on ecosystem structure and function. Dramatic habitat alteration, environmental pollution, and disturbance contributed to population declines and biodiversity losses arising from both acute and chronic effects in both terrestrial and aquatic systems. In some instances, even in the face of massive alterations to ecosystem structure, recovery was possible. Interestingly, military activity was beneficial under specific conditions, such as when an exclusion zone was generated that generally resulted in population increases and (or) population recovery; an observation noted in both terrestrial and aquatic systems. Additionally, military technological advances (e.g., GPS technology, drone technology, biotelemetry) have provided conservation scientists with novel tools for research. Because of the challenges associated with conducting research in areas with military activities (e.g., restricted access, hazardous conditions), information pertaining to military impacts on the

environment are relatively scarce and are often studied years after military activities have ceased and with no knowledge of baseline conditions. Additional research would help to elucidate the environmental consequence (positive and negative) and thus reveal opportunities for mitigating negative effects while informing the development of optimal strategies for rehabilitation and recovery.

2. Chapman, J. M., Algera, D., Dick, M., Hawkins, E. E., **Lawrence, M. J.**, Lennox, R. J., Rous, A.M., Souliere, C.M., Stemberger, H.L.J., Struthers, Vu, M., Ward, T.D., Zolderdo, A.J., Vu, M., Cooke, S.J. (2015). Being relevant: practical guidance for early career researchers interested in solving conservation problems. *Global Ecology and Conservation*, *4*, 334-348.

In a human-altered world where biodiversity is in decline and conservation problems abound, there is a dire need to ensure that the next generation of conservation scientists have the knowledge, skills, and training to address these problems. So called ‘‘early career researchers’’ (ECRs) in conservation science have many challenges before them and it is clear that the status quo must change to bridge the knowledge–action divide. Here we identify thirteen practical strategies that ECRs can employ to become more relevant. In this context, ‘‘relevance’’ refers to the ability to contribute to solving conservation problems through engagement with practitioners, policy makers, and stakeholders. Conservation and career strategies outlined in this article include the following: thinking ‘big picture’ during conservation projects; embracing various forms of knowledge; maintaining positive relationships with locals familiar with the

conservation issue; accepting failure as a viable (and potentially valuable) outcome; daring to be creative; embracing citizen science; incorporating interdisciplinarity; promoting and practicing pro-environmental behaviours; understanding financial aspects of conservation; forming collaboration from the onset of a project; accepting the limits of technology; ongoing and effective networking; and finally, maintaining a positive outlook by focusing on and sharing conservation success stories. These strategies move beyond the generic and highlight the importance of continuing to have an open mind throughout the entire conservation process, from establishing one's self as an asset to embracing collaboration and interdisciplinary work, and striving to push for professional and personal connections that strengthen personal career objectives.

3. Zolderdo, A. J., Algera, D. A., **Lawrence, M. J.**, Gilmour, K. M., Fast, M. D., Thuswaldner, J., Willmore, W.G., & Cooke, S. J. (2016). Stress, nutrition and parental care in a teleost fish: exploring mechanisms with supplemental feeding and cortisol manipulation. *Journal of Experimental Biology*, *219*(8), 1237-1248.

Parental care is an essential life-history component of reproduction for many animal species, and it entails a suite of behavioural and physiological investments to enhance offspring survival. These investments can incur costs to the parent, reducing their energetic and physiological condition, future reproductive capabilities and survival. In fishes, relatively few studies have focused on how these physiological costs are mediated. Male smallmouth bass provide parental care for developing offspring until the brood reaches independence. During this energetically demanding life stage, males cease active

foraging as they vigorously defend their offspring. Experimental manipulation of cortisol levels (via implantation) and food (via supplemental feeding) in parental males was used to investigate the fitness consequences of parental care. Improving the nutritional condition of nest-guarding males increased their reproductive success by reducing premature nest abandonment. However, supplemental feeding and cortisol treatment had no effect on parental care behaviours. Cortisol treatment reduced plasma lymphocyte numbers, but increased neutrophil and monocyte concentrations, indicating a shift in immune function. Supplemental feeding improved the physiological condition of parental fish by reducing the accumulation of oxidative injury. Specifically, supplemental feeding reduced the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) on DNA nucleotides. Increasing the nutritional condition of parental fish can reduce the physiological cost associated with intensive parental activity and improve overall reproductive success, illustrating the importance of nutritional condition as a key modulator of parental fitness.

4. Gallagher, A. J., **Lawrence, M. J.**, Jain-Schlaepfer, S. M., Wilson, A. D., & Cooke, S. J. (2016). Avian predators transmit fear along the air–water interface influencing prey and their parental care. *Canadian Journal of Zoology*, *94*(12), 863-870.

The non-consumptive consequences of predators on prey behavior, survival, and demography have recently garnered significant attention by ecologists. However, the impacts of top predators on free-ranging prey are challenging to evaluate because the most common fright response for prey is to leave the area of risk. Additionally, the top-

down impacts of avian predators on aquatic environments are surprisingly overlooked. Here we investigated the non-consumptive effects of avian predators on parental care in pumpkinseed *(Lepomis gibbosus* (L., 1758)) through use of a realistic model of a predatory bird, the Osprey (*Pandion haliaetus* (L., 1758)). Our predator model exacted dramatic metabolic fright responses and inducible defenses in experimental fish resulting in significant behavioral changes with respect to their parental care. Key parental behaviors including in-nest rotations and egg and nest maintenance were noticeably altered by predator treatments demonstrating as much as an order of magnitude difference in parental performance, suggesting that even transient predation risk might decrease reproductive fitness. Our data provide important new insights on how the landscape of fear operates along the air–water interface and suggests that avian predators may have greater controlling effects on fish populations than previously thought.

5. **Lawrence, M. J.**, Eliason, E. J., Brownscombe, J. W., Gilmour, K. M., Mandelman, J. W., & Cooke, S. J. (2017). An experimental evaluation of the role of the stress axis in mediating predator-prey interactions in wild marine fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *207*, 21-29.

The stress axis in teleost fish attempts to maintain internal homeostasis in the face of allostatic loading. However, stress axis induction has been associated with a higher predation rate in fish. To date, the physiological and behavioral factors associated with this outcome are poorly understood. The purpose of the present study was to investigate

the impact of experimental cortisol elevation on anti-predator behavior and physiological responses to predator presence. We hypothesized that semi-chronic cortisol elevation would increase susceptibility to predation by increasing stress-induced risk-taking behaviors. To test this hypothesis, schoolmaster snapper were given cocoa butter implants without cortisol (sham) or with cortisol (50 mg/kg body weight) and tethered to cover. Fish were exposed to either a lemon shark or control conditions for 15-min. Space use and activity were recorded throughout and fish were terminally sampled for blood. Cortisol implantation, relative to shams, resulted in higher blood glucose and plasma cortisol concentrations with a lower plasma lactate concentration. Shark exposure, relative to controls, elicited higher blood glucose and lactate concentrations but had no effect on plasma cortisol concentration. No interactions were detected between shark exposure and cortisol treatment for any physiological trait. Behavioral metrics, including shelter use and activity, were unaffected by either cortisol implantation or shark exposure. Physiological responses to cortisol implantation likely resulted from enhanced gluconeogenic activity, whereas alterations under predator exposure may have been the product of catecholamine mobilization. Further work should address context-specific influences of stress in mediating behavioral responses to predation.

6. **Lawrence, M. J.**, Zolderdo, A. J., & Cooke, S. J. (2017). The consequences of war on the natural environment. In W.H. Wiist & S.K. White (Eds), *Preventing War and Promoting Peace: A Guide for Health Professionals* (pp. 48-60). Cambridge, UK: Cambridge University press.

Military activities can have significant impacts on environmental health and ecosystem structure. Chapter Four looked to address the negative influences of conflict and military activities on the afflicted ecosystems over acute and chronic time scales while secondarily evaluating its positive impacts. Here, war represents an overwhelmingly negative influence on the environment. Over acute timescales, military activities can result in elevated rates of flora/fauna mortality as well as contributing to environmental degradation (e.g. soil compaction, altered physical chemistry & hydrology, etc.). Chronic impacts remain over a number of generations/years and can result in fitness level consequences (e.g. fecundity, sub-lethal toxicity, etc.), long term shifts in ecosystem structuring and the persistence of harmful chemicals in the environment (e.g. lead, dioxins, radioisotopes, etc.). Positive influences are often highly context dependent and are generally restricted to the protection of species/biodiversity through military exclusion of human activities. Together, military activities represent a considerable threat to ecological stability and functioning and, going forward, efforts should be made to address the potential impacts of war and conflicts on environmental stability and ecosystem functioning.

7. Algera, D. A., Brownscombe, J. W., Gilmour, K. M., **Lawrence, M. J.**, Zolderdo, A. J., & Cooke, S. J. (2017). Cortisol treatment affects locomotor activity and swimming behaviour of male smallmouth bass engaged in paternal care: A field study using acceleration biologgers. *Physiology & Behavior*, *181*, 59-68.

Paternal care, where the male provides sole care for the developing brood, is a common form of reproductive investment among teleost fish and ubiquitous in the Centrarchidae family. Throughout the parental care period, nesting males expend energy in a variety of swimming behaviours, including routine and burst swimming, vigilantly monitoring the nest area and protecting the brood from predators. Parental care is an energetically demanding period, which is presumably made even more difficult if fish are exposed to additional challenges such as those arising from human disturbance, resulting in activation of the hypothalamic-pituitary-interrenal axis (i.e., elevation of cortisol). To study this situation, we examined the effects of experimental manipulation of the stress hormone cortisol on locomotor activity and behaviour of nest guarding male smallmouth bass (*Micropterus dolomieu*). We exogenously elevated circulating cortisol levels (via intracoelomic implants) and attached tri-axial accelerometers to wild smallmouth bass for three days. During the recovery period (i.e., ≤4 h post-release), cortisol-treated fish exhibited significantly reduced locomotor activity and performed significantly less burst and routine swimming relative to control fish, indicating cortisol uptake was rapid, as were the associated behavioural responses. Post-recovery (i.e., > 4 h post-release), fish with high cortisol exhibited lower locomotor activity and reduced routine swimming relative to controls. Fish were less active and reduced routine and burst swimming at night compared to daylight hours, an effect independent of cortisol treatment. Collectively, our results suggest that cortisol treatment (as a proxy for anthropogenic disturbance and stress) contributed to altered behaviour, and consequently cortisol-treated males decreased parental investment in their

brood, which could have potential fitness implications.

8. Prystay, T. S., Eliason, E. J., **Lawrence, M. J.**, Dick, M., Brownscombe, J. W., Patterson, D. A., Crossin, G.T., Hinch, S.G., & Cooke, S. J. (2017). The influence of water temperature on sockeye salmon heart rate recovery following simulated fisheries interactions. *Conservation Physiology*, *5*(1).

Selective harvest policies have been implemented in North America to enhance the conservation of Pacific salmon (*Oncorhynchus spp*.) stocks, which has led to an increase in the capture and release of fish by all fishing sectors. Despite the immediate survival benefits, catch-and-release results in capture stress, particularly at high water temperatures, and this can result in delayed post-release mortality minutes to days later. The objective of this study was to evaluate how different water temperatures influenced heart rate disturbance and recovery of wild sockeye salmon (Oncorhynchus nerka) following fisheries interactions (i.e. exhaustive exercise). Heart rate loggers were implanted into Fraser River sockeye salmon prior to simulated catch-and-release events conducted at three water temperatures (16°C, 19°C and 21°C). The fisheries simulation involved chasing logger-implanted fish in tanks for 3 min, followed by a 1 min air exposure. Neither resting nor routine heart rate differed among temperature treatments. In response to the fisheries simulation, peak heart rate increased with temperature (16°C = 91.3 ± 1.3 beats min^{-1}; 19°C = 104.9 ± 2.0 beats min^{-1} and 21°C = 117 ± 1.3 beats min^{-1}). Factorial heart rate and scope for heart rate were highest at 21°C and lowest at 16°C, but did not differ between 19°Cand 21°C. Temperature affected the initial rate of

cardiac recovery but not the overall duration (~10 h) such that the rate of energy expenditure during recovery increased with temperature. These findings support the notion that in the face of climate change, efforts to reduce stress at warmer temperatures will be necessary if catch-and-release practices are to be an effective conservation strategy.

9. **Lawrence, M.J.**, Jain-Schlaepfer, S., Zolderdo, A.J., Algera, D.A., Gilmour, K.M., Gallagher, A.J., & Cooke, S. J. (2018). Are 3-minutes good enough for obtaining baseline physiological samples from teleost fish. *Canadian Journal of Zoology*, *96*, 774–786.

A prerequisite to studying the physiological status of wild animals is the ability to obtain blood samples that reflect the condition prior to capture or handling. Based on research in avian taxa, it is recommended that such samples be obtained within 3 min of capture; however, this guideline has not been validated in wild teleosts. The present study addresses the time course of physiological changes in a number of blood metrics across six species of freshwater fish. Fishes were caught using a standardized angling protocol and held in a water-filled trough prior to the collection of a blood sample, via caudal phlebotomy, between 0.5 and 11 min after capture. Changes in whole-blood glucose and lactate concentrations, hematocrit, and plasma cortisol concentrations were assessed. Change-point analyses indicated that blood lactate concentrations and hematocrit did not deviate from baseline values until ~2–5 min of handling for all species, whereas blood glucose concentrations generally did not deviate significantly from baseline over the 11

min test period. In all species, plasma cortisol concentrations began to increase above baseline between ~4 and 8 min after capture. Thus, to ensure that blood samples are representative of baseline conditions across multiple metrics, we recommend that sampling be limited to less than 2 min in teleost fishes.

10. **Lawrence, M. J.**, Eliason, E. J., Brownscombe, J. W., Gilmour, K. M., Mandelman, J. W., Gutowsky, L. F., & Cooke, S. J. (2018). Influence of supraphysiological cortisol manipulation on predator avoidance behaviors and physiological responses to a predation threat in a wild marine teleost fish. *Integrative Zoology*, *13*(2), 206-218.

The hypothalamic-pituitary-interrenal (HPI) axis, through corticosteroid secretion, is an integral mechanism regulating internal homeostasis when vertebrates are faced with a stressor. However, continued HPI-axis stimulation can produce homeostatic overload, where corticosteroids are detrimental to organismal function. This overload condition may play an important role in mediating predator-prey interactions, because chronically/previously stressed animals may have higher rates of predator-induced mortality. However, the mechanism(s) underlying this observation are unknown. Using fish as models, we hypothesized that chronic stress would increase predation susceptibility owing to a poor physiological state (e.g. homeostatic overload) with corresponding suboptimal changes in predator-avoidance behaviour. As cortisol is also required in low quantities to help regulate basic metabolic functions in fish, we expected that a glucocorticoid receptor antagonist (GR; e.g. homeostatic failure) may produce

similar effects. Schoolmaster snapper (*Lutjanus apodus*) were given intraperitoneal implants of cocoa butter impregnated with nothing (sham; 5 ml/kg body weight (BW)), cortisol (50 mg/kg BW) or the GR antagonist RU486 (100 mg/kg BW). At 24-h post-implantation, fish were tethered to the seafloor and observe for behavioural metrics associated with predation. Blood samples were collected from a subset of fish to assess the physiological consequences of the implants. Cortisol- and RU486-implanted fish both had significantly higher plasma cortisol concentrations than sham fish, with blood glucose and plasma urea being elevated only in the former. Further, anti-predator behaviours and predation mortality did not differ significantly among treatments. Despite changes in physiological state, predation susceptibility was unaffected, a finding that may reflect the complex relationships linking the physiology and behaviour of an organism as well as potential tethering artefacts.

11. Twardek, W. M., Lennox, R. J., **Lawrence, M. J.**, Logan, J. M., Szekeres, P., Cooke, S. J., Trembly,K., Morgan, G.E., & Danylchuk, A. J. (2018). The Postrelease Survival of Walleyes Following Ice-Angling on Lake Nipissing, Ontario. *North American Journal of Fisheries Management*, *38*(1), 159-169.

Natural resource agencies have developed catch-and-release regulations for Walleyes *Sander vitreus* of prohibited size and number to reduce mortality in many recreational fisheries. The efficacy of such regulations is contingent upon the released fish surviving, but survival data on Walleyes captured by ice-angling are lacking. We estimated the survival of Lake Nipissing (Ontario, Canada) Walleyes that were captured by both active

and passive ice-angling methods using a variety of hook types and lures baited with Emerald Shiners *Notropis atherinoides*. We also assessed the role of de-hooking methods on the survival of deeply hooked Walleyes. After the angling event, Walleyes (n = 260) were held for 24 h in a submerged holding pen to estimate postrelease survival. Average mortality after the 24-h holding period was 6.9%. Fewer Walleyes captured by active angling were deeply hooked (9.3%) than passively caught fish (50.4%), and deeply hooked Walleyes were observed to have more frequent postrelease mortality (14.8%) than shallow-hooked Walleyes (3.0%). There was no significant difference in mortality rates of Walleyes caught by passive angling (9.8%) or active angling (2.8%); mortality rates of fish caught on circle hooks (6.1%), J-hooks (8.2%), and treble hooks (5.6%) also did not differ. Neither air temperature nor the presence of barotrauma had a significant effect on mortality of captured Walleyes. Survival did not significantly differ between deeply hooked fish that had the line cut (11.1%) and those that had the hook removed (22.6%). Results from this study suggest a relatively high incidence of Walleye survival after catch-and-release angling through the ice.

12. Abrams, A. E., Rous, A. M., Brooks, J. L., **Lawrence, M. J.**, Midwood, J. D., Doka, S. E., & Cooke, S. J. (2018). Comparing Immobilization, Recovery, and Stress Indicators Associated with Electric Fish Handling Gloves and a Portable Electrosedation System. *Transactions of the American Fisheries Society*, *147*(2), 390-399.

Fish sedation facilitates safer handling of fish during scientific research or fisheries

assessment practices, thus limiting risk of injury to fish and reducing stress responses. In recent years, there has been growing interest in using electricity to sedate fish; two methods include (1) lower-voltage, non-pulsed-DC fish handling gloves (FHGs) that tend to only sedate fish while the gloves are touching the animal; and (2) a comparatively high-voltage, pulsed- DC Portable Electrosedation System (PES) that leads to galvanonarcosis. This study compared the physiological consequences of exposure to FHGs and PES in teleost fish. Bluegills *Lepomis macrochirus* and Largemouth Bass *Micropterus salmoides* were exposed to FHGs, PES, or a handling control for a 3-min simulated surgery. Blood was then sampled at 0.5 and 4.5 h postexposure and was analyzed for blood glucose, blood lactate, and plasma cortisol concentrations. Opercular rates were monitored during surgery, at 2 min postsurgery, and 0.5 h postsurgery. At 24 h postsurgery, time to exhaustion (via a standardized swimming chase protocol) was assessed. Fish exposed to FHGs tended to exhibit lower opercular rates than fish that were sedated with the PES during simulated surgery. Cortisol levels of Largemouth Bass treated with FHGs were higher than those of fish sedated with the PES. Glucose levels recorded for Bluegills at 4.5 h postsurgery were higher with FHGs than with the PES. In both species, lactate was lower for fish treated with FHGs than for those treated with the PES. At 24 h posttreatment, Bluegills sedated with FHGs exhibited a longer time to exhaustion than those subjected to the PES, whereas Largemouth Bass sedated with the PES exhibited a longer time to exhaustion than those sedated with FHGs. Physiological responses to treatments were inconsistent between species. Further investigation to determine the optimal electrosedation method is required.

13. Roche, D., Amcoff, M., Morgan, R., Sundin, J., Andreassen, A. H., Finnøen, M. H., **Lawrence, M.J.**, Henderson, E., Norin, T., Speers-Roesch, B., Brown, C., Clark, T.D., Bshary, R., Leung, B., Jutfelt, F., & Binning, S. A. (2019; *Pre-print*). Replication alert: behavioural lateralisation in a detour test is not repeatable in fishes. https://doi.org/10.32942/osf.io/6kcwa.

Behavioural lateralisation, defined as the asymmetric expression of cognitive functions, is reported to enhance key fitness-relevant traits such as predator escape performance, multitasking abilities, and group coordination. Therefore, studies reportingnegative effects on lateralisation in fish due toenvironmental stressors such as ocean acidification, hypoxia,and pollutants are worrisome. However, such studies have focussed on population-level measures, without validating whether lateralisation is consistent within individualsacross time. We conducted a multi-species, international assessment of the repeatability (R) of lateralisation in four previously studied fishspeciesusing thecommon detour test, and re-analysed a published dataset (on guppies) using new statistical methods. We expected the threeshoaling species to exhibit greater within-individual consistency in lateralisationthan their non-shoaling counterpartsgiven previous reports of stronger lateralisation in group-living fishes. However, both absolute andrelative lateralisation scores were highly non-repeatable inall five species ($0.01<R<0.08$). Thus, the commonly used detour test does not appear to be appropriate for quantifying behavioural lateralisation in fishes, calling into question inferences drawn by many published studies, including our own. As a consequence, potential anthropogenic

effectson lateralisation as a proxy for adaptive brain functioning need to be assessed with alternative paradigm

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