

**Consequences of an experimental cortisol elevation on the behaviour, condition and survival of creek chub (*Semotilus atromaculatus*) across a gradient of habitat quality**

By

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## **Dedication**

To my parents, who have always pushed me to pursue higher education and have supported me throughout my entire academic career. Who, since childhood, have stressed the importance of education and ensured it was among my top priorities - despite my greatest efforts to resist. To Rebecca, for your unwavering support and encouragement, it has always helped to know that I have you in my corner.

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## **Abstract**

Aquatic ecosystems are becoming increasingly altered as a result of human land-use practices, exposing fish to a variety of environmental stressors. This thesis tested whether exposure of creek chub (*Semotilus atromaculatus*) to experimental chronic cortisol elevations affected their behaviour, health, condition, and survival across a gradient of habitat quality, outside of the reproductive period. Using biotelemetry methods, exogenous cortisol manipulations were found to have no effect on the fine- and large-scale movements of creek chub despite exposure to a significant, ecological relevant challenge. Similarly, elevated cortisol levels had no effect on the health, and condition of creek chub within streams in reference, agriculture, and urban land-use sites. Results suggest that compensatory mechanisms enable wild fish to cope with stressors that in laboratory environments typically result in profound effects. However, as creek chub are a rather tolerant organism, caution must be taken when applying results to other species.

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## Co-Authorships

**Chapter 2: Effects of an experimental short-term cortisol challenge on the behaviour of wild creek chub (*Semotilus atromaculatus*) in mesocosm and stream environments.** A.M. Nagrodski, K.J. Murchie, K.M. Stamplecoskie, C.D., Suski, and S.J. Cooke

Although this study is my own, the research was undertaken as part of a collaborative effort, and each co-author played a valuable role in its completion. The project was conceived by Nagrodski, Cooke and Suski. Fieldwork was conducted by Nagrodski, Stamplecoskie, and Murchie. All data analysis was conducted by Nagrodski. Data were interpreted by Nagrodski, Cooke, and Suski. All writing was conducted by Nagrodski. All co-authors provided comments and feedback on the manuscript. The manuscript has been submitted to the *Journal of Comparative Physiology A*.

**Chapter 3: Health, condition, and survival of creek chub across a gradient of stream habitat quality following an experimental cortisol challenge.** A.M. Nagrodski, C.D. Suski, S.J. Cooke

Although this study is my own, the research was undertaken as part of a collaborative effort, and each co-author played a valuable role in its completion. The project was conceived by Nagrodski, Cooke and Suski. All data analysis was conducted by Nagrodski with assistance of Cooke and Suski. Data were interpreted by Nagrodski, Cooke and Suski. All writing was conducted by Nagrodski. All co-authors provided comments and feedback on the manuscript. The manuscript is in preparation for submission to *Science of the Total Environment*.

## Table of Contents

<b>Dedication</b> .....	<b>ii</b>
<b>Abstract</b> .....	<b>iii</b>
<b>Acknowledgements</b> .....	<b>iv</b>
<b>Co-Authorships</b> .....	<b>vi</b>
<b>List of Tables</b> .....	<b>ix</b>
<b>List of Figures</b> .....	<b>x</b>
<b>Acronyms</b> .....	<b>xi</b>
<b>Chapter 1: General Introduction</b> .....	<b>1</b>
<i>Fish Stress Response</i> .....	1
<i>Stress Response across Different Land Use Patterns</i> .....	3
<i>Hormone Implants to Manipulate Cortisol Titers</i> .....	4
<i>Research Objectives &amp; Predictions</i> .....	5
<i>Model Species</i> .....	6
<b>Chapter 2: Effects of an experimental short-term cortisol challenge on the behaviour of wild creek chub (<i>Semotilus atromaculatus</i>) in mesocosm and stream environments</b> .....	<b>8</b>
Abstract.....	8
Introduction.....	9
Materials and Methods.....	14
Experimental animals and cortisol treatments .....	14
Laboratory Study .....	15
Mesocosm Study .....	17
Field Stream Telemetry Study .....	18
Statistical Analysis.....	21
Results.....	23
Cortisol Validation.....	23
Mesocosm Study .....	24
Field Stream Telemetry Study .....	24
Discussion.....	25
<i>Intra-peritoneal Cortisol Injections, Validations and Limitations</i> .....	26
<i>Fine-Scale Activity</i> .....	27

<i>Large-Scale Activity</i> .....	28
<i>Summary and Future Direction</i> .....	30
Tables.....	33
Figures .....	35
<b>Chapter 3: Health, condition, and survival of creek chub across a gradient of stream habitat quality following an experimental cortisol challenge.....</b>	<b>41</b>
Abstract:.....	41
Introduction.....	42
Material and Methods .....	47
<i>Experimental animals and cortisol treatments</i> .....	47
<i>Site Selections</i> .....	49
<i>In-field setup</i> .....	50
<i>In-field sampling</i> .....	51
<i>Statistical Analysis</i> .....	52
Results.....	53
Discussion.....	55
Tables.....	61
<b>Chapter 4: General Discussion .....</b>	<b>66</b>
<i>Findings and Implications:</i> .....	67
<i>Future Research Directions</i> .....	68
<b>References.....</b>	<b>70</b>



## List of Tables

**Table 2-1.** Average number of detections and standard error for control (n = 8), cortisol- (n = 7) and sham-treated (n = 7) creek chub within the fine-scale, mesocosm study. Fish that died prior to 30 June, 2011 were not included in these calculations.

**Table 2-2.** Results of ANOVAs testing the effects of treatment on cortisol concentration (as part of the validation study) and behaviour (*i.e.*, activity and movement) of creek chub.

**Table 3-1.** Summary of riparian land-cover and associated classifications for study sites.

**Table 3-2.** The classification, date sampled, electrofishing effort for initial fish collection, and date re-visited for fish recapture at each of the study streams.

**Table 3-3.** Total number of creek chub captured, released and recaptured ~25 days post treatment per land use type.

**Table 3-4.** Results of two-way mixed ANOVAs, with stream as a random effect, nested within land use, testing the impact of land use on stress response following a plasma cortisol challenge. “†” refers to variables whose data were square root transformed to meet assumptions of normality, while “††” refers to variables whose data were log-transformed to meet assumptions of normality.

**Table 3-5.** Means ( $\pm$  SE) of response variables, ~25 days after treatment, across reference, agriculture and urban streams. “†” refers to variables whose data was square root transformed, while “††” refers to variables whose data was log-transformed.

## List of Figures

**Figure 2-1.** Schematic diagram of outdoor tank used for mesocosm experiment. Three PIT tag antennas, positioned equidistant apart, monitored small-scale movements around the tank and centralized refuge platform constructed of a raised patio block.

**Figure 2-2.** Location of study site, Mosquito Creek (45°16' N, 75°40' W) in Ottawa, Ontario, Canada. Heavy black lines intersecting the creek indicate roadways where water is passed through culverts. The black star indicates where radio-telemetered fish were released.

**Figure 2-3.** Plasma cortisol concentrations (mean  $\pm$  SE) of 3 day trial fish (*i.e.*, post control, cortisol and sham treatments) compared to fish subjected to a standardized stress protocol (labeled as “exhausted”) and baseline fish. One-way ANOVAs, with square-root transformations were used to assess differences between treatment groups. Different letters between treatment groups indicate statistical difference ( $\alpha=0.05$ ).

**Figure 2-4** Surviving creek chub from all treatment groups during the mesocosm experiment between 22 June 2011 and 11 July 2011. There was no statistical effect of treatment on survival rate.

**Figure 2-5.** Diel activity patterns of (a) control, (b) cortisol-, and (c) sham-treated creek chub during mesocosm experiments between 23 June 2011 and 29 June 2011. Stacked bars indicate the individual contribution of each fish, at each time period. Light and dark shading represents day- and night-time respectively.

**Figure 2-6.** Total distances and total linear range for telemetered creek chub from both treatments groups three days post-injection (3 November – 5 November 2010), and for the accumulative study period (3 November – 29 November 2010). No significant differences were found between treatments.

## **Acronyms**

ANOVA: Analysis of variance

ELISA: Enzyme-linked immunosorbent assay

GSI: Gonado-somatic index

HAI: Health assessment index

HPI axis: Hypothalamic-pituitary-interrenal axis

HSI: Hepato-somatic index

K: Condition factor

QUBS: Queen's University Biological Field Station

SD: Standard deviation

SE: Standard error

SSI: Splenic-index

## **Chapter 1: General Introduction**

### *Fish Stress Response*

Selye (1973) broadly defines stress as the “nonspecific response of the body to any demand made upon it”. Regardless of origin, he reasoned that, while unavoidable, stressors influence organisms to adopt more adaptive functions in attempts to re-establish some level of normalcy (Selye, 1973). Building upon these ideas, Barton and Iwama (1991) further advanced this definition in the context of fish by proposing that, when faced with a stressor that exceeds normal resting condition, fish may experience changes in biological condition that can alter their homeostatic state. In particular, when exposed to a stressor, fish can exhibit primary, secondary and tertiary responses which are defined by the cascade of metabolic and physiologic changes that attempt to restore homeostasis (Mazeaud *et al.* 1977). More specifically, the primary response is marked by the recognition of an altered state, initiating an endocrine response that includes the release of corticosteroids and catecholamines (Barton and Iwama, 1991). Rapid elevation of these stress hormones evokes the secondary response, which involves physiological adjustments (*e.g.*, metabolic, hematological and cellular changes) in an attempt to mediate the effects of the stressor (Barton and Iwama, 1991; Martíne-Porchas *et al.* 2009). Although tertiary responses do not always occur, these responses can involve changes in whole animal performance or modifications in behavioural patterns that can arise due to energy reallocation to cope with the increased energetic costs of stress (Barton, 2002). In this context, although fish stress responses can be adaptive, enabling the animal to cope with a stressful event, they can also be maladaptive and impact the fish’s overall health and well-being (Barton, 2002). More recently, stress has been

considered in the context of allostasis whereby challenges that disrupt the homeostatic state of an organism impose what is called an energetic or allostatic load (Schreck, 2010). As a result, when an individual is able to maintain a balance between energy input and expenditure and thus actively maintain stability through change, the individual is said to have achieved allostasis (Windfield, 2005). Under this more recent paradigm, the primary mediators that support homeostasis are the hormones released by the hypothalamic-pituitary-interrenal (HPI) axis (*e.g.*, catecholamines and cytokines; McEwen and Wingfield, 2003). However, there are limits to the adaptive response that can be exhibited by an organism, and, in the event that an individual experiences an energy imbalance, they are said to experience an allostasis overload. Two distinctly separate types of allostasis overload have been proposed in the literature. More specifically, type 1 allostasis overload occurs when an organism's energy demand exceeds its available energy supply, initiating the emergency life-history stage (Windfield, 2005). Comparatively, while few type 2 allostasis overload examples exist in natural populations, they involve situations where permanent energy imbalance (or allostasis overload) exists, however, sufficient energy/resources available to maintain this load means that the emergency life-history stage is not triggered (Windfield, 2005). Nevertheless, the types of stress encountered by fish can be from a diversity of sources, and are broadly characterized as chemical (*e.g.*, contaminant and pollutant exposure), physical (*e.g.*, handling and capture) and perceived (*e.g.*, presence of a predator) stressors (Barton, 2002).

## *Stress Response across Different Land Use Patterns*

While there has been, and continues to be, a large amount of research on the physiological and endocrine responses of fish when encountering a stressful event, the majority of literature focuses on cultured fish or wild fish that have been acclimated to laboratory conditions, with comparatively little work on wild fish in natural settings (Pankhurst, 2010). While this pattern is often attributed to difficulties associated with accessibility and suitability of sampling sites and the difficulty of controlling for environmental history (*i.e.*, it is convenient to order fish from a hatchery that are all of a common age, size, feeding regime, and environmental/rearing history), the study of stress in natural environments is important as wild individuals tend to experience stress responses triggered by broader environmental challenges that are difficult to identify and simulate under laboratory conditions (Pankhurst, 2010). Furthermore, as human populations grow and continue to negatively impact natural ecosystems (Radeloff *et al.* 2005; Vitousek *et al.* 1997), wild fish will likely continue to experience an increased range of anthropogenic stressors (Dietz *et al.* 2007). Understanding the consequences that anthropogenic environmental degradation has on fish stress response is important as freshwater fish assemblages are becoming some of the most threatened taxa in the world (Burton, 1995; Saunders *et al.* 2002). Although species can acclimate to changing environments, physiological flexibility can become maladaptive and have consequences on overall species condition and persistence (Piersma and Drent, 2003). Moreover, while inhabiting an unfavourable environment, perceived chronic stressors in the form of poor conditions (*e.g.*, poor water quality) can impede blood cortisol response and attenuate subsequent fish stress responses (Hontela *et al.* 1997; Barton, 2002). In particular,

continual inter-renal activity can down-regulate the HPI axis, responsible for the secretion of cortisol, causing the attenuation of responses to additional stressors (Barton, 2002).

### *Hormone Implants to Manipulate Cortisol Titers*

Experimental evaluations of stressors in the natural environment are needed to predict how fish will respond to a range of environmental stressors (Wikelski and Cooke, 2006). While a suite of stress stimuli exist, many studies interested in the consequences of stress in an animal have used experimental exogenous manipulation of cortisol titers, accompanied with monitoring of physiological parameters, to assess fish stress response (reviewed in Gamperl *et al.* 1994). The manipulation of cortisol is physiologically relevant as it is a key endocrine response of a stressed fish. It is also ecologically relevant in that manipulations that raise cortisol titers for several days would be similar to what might be expected during a hypoxic event (*e.g.*, Herbert and Steffensen, 2005), a short-term starvation (*e.g.*, McConnachie, 2010), exposure to supercooling and frazil ice (*e.g.*, Brown *et al.* 1999), droughts and floods (*e.g.*, Flodmark *et al.* 2002). In particular, the use of a single intra-peritoneal or intra-muscular injection of cortisol suspended in coconut oil or butter has been found to act as an effective method to evaluate semi-chronic (*i.e.*, ~3 days) levels of plasma cortisol in fish (Gamperl *et al.* 1994). As cortisol/coconut oil/butter implants function by releasing hormone over an extended period, challenging a fish with ecologically-relevant doses of cortisol serves as a controlled means of examining how fish respond to stress in the environment and contribute to our overall understanding of the ecology of stress. Although there have been several hundred studies that have used experimental cortisol manipulations to study

fish responses (reviewed in Gamperl *et al.* 1994), there are very few that have occurred outside the confines of a laboratory (see Dey *et al.* 2010; O'Connor *et al.* 2010).

### *Research Objectives & Predictions*

The overall goal of my thesis is to examine the effects of short-term cortisol evaluation on ecologically relevant endpoints (*e.g.*, survival, behavior, parasite burden) in wild, free-swimming fish. More specifically, this thesis includes two studies (*i.e.*, chapters) that use experimental manipulations of cortisol. The objective of Chapter 2 is to assess the effects of cortisol on the activity, movement, and survival of creek chub in a mesocosm (fine-scale activity) and stream (large-scale movement). Specifically, I test the null hypothesis that there is no difference in the behaviour (*i.e.*, including activity and movement) of control, cortisol- and sham-treated fish. Although studies in the past have established relationships between an elevated physiological stress response (production of plasma cortisol) with increased blood pressure and energy use, as well as reductions in food intake and immune function (reviewed in Wendelaar-Bonga, 1997), none of these results can be directly applied to fish mobility. Consequently, I predict that administering a short-term stressor in the form of a cortisol injection will decrease activity levels in creek chub relative to shams and controls. The objective of Chapter 3 is to quantify the effects of cortisol on the health (*i.e.*, parasite burden and gill fraying), condition (*i.e.*, condition factor, blood glucose, hepato-somatic [HSI], splenic [SSI], and gonado-somatic [GSI] indices), and survival of creek chub across multiple streams, representing a gradient of stream habitat quality (*i.e.*, urban, agricultural, and reference). More specifically, I test the null hypothesis that there will be no difference in the health, condition, and survival of control, cortisol- and sham-treated fish across a gradient of



stream habitat quality. As past research suggests that the administration of cortisol in teleost fish has negative metabolic consequences (reviewed in van der Boon *et al.* 1991) and as research in other taxa such as birds (Chávez-Zichinelli *et al.* 2010) and lizards (Homan *et al.* 2003) has shown that disturbed environments can retard cortisol response, I predict that the experimental cortisol challenge will result in negligible changes in health, condition and survival in undisturbed sites but comparatively more extreme negative consequences in urban and agricultural sites.

### *Model Species*

In the past, creek chub have been successfully used as sentinel species to monitor pollutant trends (Stahl and Sobat, 2000), study the influences of land-use on the physiological response of stream fish (Blevins *et al.* unpublished manuscript), and to evaluate the effects of land use and their associated environmental stressors on fish communities (Fitzgerald *et al.* 1999). As such, sentinel species are commonly used in studies interested in evaluating the effects of environmental changes over time. What makes creek chub useful as a sentinel species is their relatively high abundance, easily measured life history characteristics, ability to inhabit environments across a range of different habitats, lack of any major fishing pressure, and rapid growth and maturation (Scott and Crossman, 1973; Powles *et al.* 1977; McMahon, 1982). Furthermore, past research indicates that creek chub tend to exhibit leptokurtic movements (Skalski and Gilliam, 2000) that are highly associated with patches of cover, which on occasion can play a role in their distributions within streams (Belica and Rahel, 2008). Moreover, studies on the European chub (*Leuciscus cephalus*), a cyprinid closely related to the North American creek chub, has discovered that these fish are relatively tolerant to high

levels of circulating cortisol as the affinity of their binding sites with cortisol display an eightfold increase when compared with rainbow trout (*Oncorhynchus mykiss*; Pottinger *et al.* 2000). As proposed by the authors, this lower affinity of cortisol receptors may offset the adverse effects of the naturally high circulating cortisol levels found in the European chub (Pottinger *et al.* 2000). Additionally, while purely speculative, perhaps this low cortisol receptor affinity may make chubs more tolerant to stress and allow these fish to inhabit sub-optimal environments. Results from Suski *et al.* (unpublished data) directly support these results, as they apply to creek chub, as they found creek chub to exhibit relatively high naturally circulating cortisol levels when compared with more traditionally studied fish species (*e.g.*, salmonids, centrarchids). Additionally, work by Blevins *et al.* (unpublished manuscript) found that creek chub have similar physiological parameters across land uses (*i.e.*, agricultural and forested), however, animals originating from different land use patterns exhibit differential performance to thermal and hypoxia challenges. Interestingly, prolonged holding of fish at high temperatures removed landscape-levels differences, suggesting that creek chub may possess the ability to adjust their physiological responses to improve physiological performance in sub-optimal environments (Blevins *et al.* unpublished manuscript). As such, and in light of all the above, creek chub will provide an ideal model species for these studies.

## **Chapter 2: Effects of an experimental short-term cortisol challenge on the behaviour of wild creek chub (*Semotilus atromaculatus*) in mesocosm and stream environments**

### **Abstract**

Aquatic ecosystems are becoming increasingly altered as a result of human activities, exposing fish to a variety of environmental stressors. However, little is known about how these stressors impact wild fish and in particular, if and how stress may influence ecologically-relevant endpoints such as behaviour. Use of experimental cortisol implants is a common laboratory approach to examine the consequences of stress on fish, but such manipulations have rarely been used on wild fish in natural environments. In this study, the consequences of stress on the behaviour of wild creek chub (*Semotilus atromaculatus*) outside of the reproductive period were studied using a single intra-peritoneal injection of cortisol, suspended in coconut butter, to experimentally raise plasma cortisol levels within fish. Behaviour between cortisol-treated, sham-treated (injected with coconut butter) and control fish was compared in a mesocosm system, using a passive integrated transponder (PIT) array and in a natural stream system, using surgically implanted radio transmitters. While laboratory time-course studies revealed that the cortisol injection provided an ecologically relevant challenge, causing prolonged (~3 day) elevations of in plasma cortisol concentrations, we observed no difference in the fine-scale movements between cortisol-, sham-treated and control fish nor differences in the large-scale movements of cortisol-treated and control fish. Moreover, we observed no differences in diel activity patterns among treatments.

However, we observed differential mortality between treatments 10 days into the experiment, as cortisol treated fish exhibited nearly twice as many mortalities as shams and controls. These results suggest that although the experimental manipulation of cortisol titers was sufficient to cause mortality in some individuals, there were compensatory mechanisms that maintained behaviours (*i.e.*, including activity and movement) prior to death. This study represents one of the first to use experimental cortisol implants outside of a laboratory environment and during the non-reproductive period and yields insight into how wild animals respond to additional challenges using ecologically-meaningful endpoints.

## **Introduction**

As the world's population expands, increased demands on natural resources will lead to the degradation of ecological functions and conditions of natural landscapes (Vitousek, 1997; Foley *et al.* 2005). In the case of aquatic ecosystems, changes in adjacent land-use, particularly in the riparian zone, can alter watershed hydrology and biology, exposing wild fish to a wide range of anthropogenic stressors that are known to influence fish distribution, abundance, and community structure (Allan, 2004). Underlying changes in fish distribution and abundance is the assumption that environmental conditions have changed beyond some threshold that exceeds environmental tolerances and physiological capacity for a given species (Fry, 1947). As such, research has assessed, and will continue to evaluate, the physiological and endocrine responses of fish when exposed to a variety of environmental stressors (reviewed in: Barton and Iwama, 1991; Wendelaar-Bonga, 1997).

In general, existing literature has broadly categorized fish stress responses into primary, secondary and tertiary responses. While primary responses mark the recognition of a stressor by the central nervous system (CNS) and the subsequent neuroendocrine responses, secondary responses occur as a consequence of neuroendocrine responses which mediate alterations in metabolic pathways (Mazeaud *et al.* 1977). Although tertiary responses do not always occur in response to a stressor, these responses involve changes to whole-body activity and performance (Schreck, 1990). Up to a point, fish stress responses are thought to be adaptive and enable the animal to cope with imposed challenges (Schreck *et al.* 1997). However, while much is known about primary and secondary stress responses in artificial laboratory conditions, less is known about tertiary responses or the “ecology of stress” in wild fish. In efforts to assess the whole-body effects of stress on fish, research has begun to use the monitoring of an individual’s physiological metrics (*e.g.*, energy transfer, nutritional condition and metabolism) as indicators of an organism’s performance within an environment in the context of environmental monitoring and assessment (*e.g.*, Doherty *et al.* 2005; Gray and Munkittrick, 2005; Peterson *et al.* 2011). Physiological metrics are useful tools in assessing environmental impacts on species as they control the degree to which an organism can acclimate to an environment and respond to various environmental challenges (Ricklefs and Wikelski, 2002; Wikelski and Cooke, 2006; Cooke and O’Connor, 2010). As natural systems become more degraded or threatened, monitoring physiological parameters presents an opportunity to assess the effects of inhabiting an environment as well as to assess current and future rehabilitation and restoration efforts (Cooke and Suski, 2008).

A commonly used physiological metric when evaluating fish stress response, is the measurement of circulating plasma cortisol concentrations (Wendelaar-Bonga, 1997). Attributed to the stimulation of the hypothalamic-pituitary-interrenal (HPI) axis, cortisol is a valuable indicator of stress in fish as its release is triggered within minutes of an individual being exposed to a stressor, as a primary response, and cortisol can also be associated with immune function impairments following chronic elevation (Barton, 2002). Many studies interested in the consequences of stress in fish have used experimental exogenous manipulation of cortisol titers, accompanied with monitoring of physiological parameters, to assess how fish respond to the challenge (*e.g.*, Vijayan *et al.* 1996; DiBattista *et al.* 2005). In particular, the use of a single intra-peritoneal or intramuscular injection of cortisol suspended in coconut butter has been found to act as an effective method to evaluate chronic levels of plasma cortisol in fish (Gamperl *et al.* 1994). As cortisol/coconut butter implants function by releasing hormone over an extended period (usually 3 to 5 days; Gamperl *et al.* 1994), challenging fish with an ecologically-relevant dosage of cortisol serves as a controlled means of examining how fish respond to stress in the environment. Although there have been many studies that have used experimental cortisol manipulations to study fish responses (reviewed in Gamperl *et al.* 1994), there are few that have occurred outside the confines of a laboratory.

Outside of laboratory experiments, exogenous manipulations of cortisol titers have been useful in assessing the effects of a short-term stressor on the growth and survival of wild fish (O'Connor *et al.* 2011), the role of stress during parental care (O'Connor *et al.* 2009; Dey *et al.* 2010) and the carryover effects of stress on wild fish

(O'Connor *et al.* 2010). Although cortisol manipulations have been limited in their field applications, the marriage between field manipulations of cortisol titers and effects-based assessment models provide a good opportunity to investigate the implications that stress may have on the ecology of wild fish. Used to gain insight on the impacts of land use on the physiological function of an organism (*e.g.*, Doherty *et al.* 2005; Gray and Munkittrick, 2005), effect-based assessments can also be useful in assessing spatial and temporal movements of fish. While variations in factors such as fish size, body condition, and growth rate have been shown to affect fish mobility (Belica and Rahel, 2008), research has yet to examine the effects of an elevated cortisol challenge on the movements of wild stream fish. In fact, behaviour (*e.g.*, movement rates, activity levels) has rarely been used as an endpoint in examining how wild fish respond to stressors in the wild, although in a laboratory context, there are a number of studies that have documented altered behaviour in fish challenged via exogenous cortisol manipulation (*e.g.*, Øverli *et al.* 2002; DiBattista *et al.* 2005; Schjolden *et al.* 2009) or exposure to acute or chronic stressors (*e.g.*, Mesa, 1994; reviewed in Schreck *et al.* 1990). The few studies that do exist where cortisol titers have been experimentally manipulated in the wild typically have occurred during the reproductive period, and these studies have failed to observe alterations in behaviour (*e.g.*, O'Connor *et al.* 2009; Dey *et al.* 2010; McConnachie, 2010). From a life-history theory perspective, one would predict that baseline behaviour would be maintained during reproduction in the face of stress to facilitate reproductive success (Sapolsky *et al.* 2000). There is only a single study to our knowledge that has evaluated the behaviour of fish in the wild after cortisol injection outside of the reproductive period (*i.e.*, O'Connor *et al.* 2010) and this study also failed to

demonstrate differences in behaviour, except in the event of a secondary stressor (*i.e.*, winter hypoxia) whereas cortisol-treated fish exhibited less activity than control and sham-treated fish immediately before death. Movement studies play an important role in fish ecology as they enable researchers to gain insight on the spatial ecology of their focal species which can influence resource utilization (Lucas and Baras, 2000). Studies of this nature are valuable as they can be applied to a variety of systems and can provide understanding around the functional interactions between organisms and landscapes, although fish movement and activity as an ecologically-relevant endpoint have rarely been used when studying fish stress.

This study sought to determine whether a short-term experimental stressor can influence the behaviour (*i.e.*, including activity and movement) of wild fish. A single exogenous cortisol implant was administered to experimentally raise circulating plasma cortisol values and mimic the physiological effects of the stress response observed in teleost fish. Similar to Fitzgerald et al. (1999) who used creek chub (*Semotilus atromaculatus*) as a sentinel species, we used them as a model because they satisfied our size requirements, occur in high numbers within the system, lack major fishing pressures, have easily measured life history characteristics, and exhibit rapid growth and maturation. Here, the consequence of elevated cortisol values on fish behaviour was evaluated using a complementary set of mesocosm and field experiments. We tested the null hypothesis that there would be no differences in the behaviour of control, cortisol- and sham-treated fish. A mesocosm approach was used to investigate fine-scale movements (*e.g.*, diel activity patterns) using a passive internal transponder (PIT) antenna array (*e.g.*, Binder and McDonald, 2008), while a field component with radio telemetry



was used to assess large-scale movements of wild creek chub. Because past studies have found elevated cortisol levels to increase parameters such as blood pressure, energy use, food intake, and immune function in the laboratory (*e.g.*, Gregory and Wood, 1999; Lankford *et al.* 2005; Also see Barton, 2002), and have identified that stress alters behaviour (reviewed in Schreck, 1990), although typically not during the reproductive period (O'Connor *et al.* 2009; Dey *et al.* 2010), we predicted that administering a short-term stressor outside of the reproductive period would cause alterations in creek chub behaviour relative to shams and controls. This experimental approach to challenging fish will help to elucidate the potential mechanisms by which stress can influence the ecology of stream fish, an important knowledge gap as we attempt to understand and predict the consequences of environmental change on wild animals.

## **Materials and Methods**

### **Experimental animals and cortisol treatments**

Sampling of fish was performed under an Ontario Ministry of Natural Resources Scientific Collection Permit (Licence Number: 1061994) granted to S.J.C. Fish were processed with adherence to the guidelines set out by the Canadian Council on Animal Care as issued by Carleton University (B10-9). All creek chub were captured using standard electrofishing techniques, with a battery-powered backpack electrofisher (Halltech Aquatic Research Inc., model Ht-2000). Pulsed-DC electrofishing is concerned to be a safe methodological tool for studying creek chub and results by Gatz and Linder (2008) suggest this practice is unlikely to have meaningful biological effects on creek chub condition, growth, and movements. As creek chub generally spawn in the spring, beginning at temperatures of 12.8°C (Scott and Crossman, 1973), sampling was carried

outside this range to avoid any confounding effects of the reproductive period. For all experiments, cortisol-treated fish were given a single intra-peritoneal injection of 10 mg mL<sup>-1</sup> of cortisol (hydrocortisone; Sigma H2882, Sigma-Aldrich) suspended in coconut butter (*Cocos nucifera*) at 0.005 mL g<sup>-1</sup> body weight (reviewed in Gamperl *et al.*, 1994). Sham-treated fish were given a single intra-peritoneal injection of coconut butter at 0.005 mL g<sup>-1</sup> body weight. Control fish received no injections. Fish were then separately placed in aerated recovery bins, according to their treatment and the experimental protocol (see below).

## **Laboratory Study**

### *Blood sampling and cortisol assay*

Blood (~0.2 - 0.4 ml) was collected via caudal puncture, using a combination of sodium heparinized 1 ml syringes with 25 gauge, 38 mm needles and sodium heparinized 0.5 ml insulin syringes with 28.5 gauge, 13 mm needles (Becton Dickinson & Co. Tuberculin Slip Tip Syringes and ½ cc LO-DOSE U-100 Insulin Syringe, Franklin Lakes, NJ). Blood samples were preserved in water-ice slurries until centrifuged at 10,000 × gravity (g) for 6 minutes (Compact II Centrifuge, Clay Adams, Parsippany, NJ). The resultant plasma samples were flash frozen in liquid nitrogen and stored at -80°C until further analysis. Concentrations of cortisol in plasma were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit in accordance with manufacturer specifications (Assay Designs, Kit #900-071, Ann Arbor, Michigan) and a microplate spectrophotometer (Molecular Devices, Spectra Max Plus 384, Model #05362, Union City, CA). This product has low cross-reactivity with other hormones produced by fishes that could interfere with binding (Assay Designs Kit #900-071). Sink

et al. (2008) confirmed the accuracy and precision of this kit compared to conventional radioimmunoassay techniques, and recommended this particular kit for cortisol detection relative to other commercially available brands.

#### *Cortisol dosage validation*

On 18 July 2010 and 21 July 2010, blood cortisol levels for “baseline” fish, at resting state, from local area streams were obtained by lethally blood sampling wild creek chub ( $n = 18$ ;  $133.7 \pm 5.4$  mm;  $27.2 \pm 3.3$  g; mean  $\pm$  SD) within 2-3 minutes of capture via electrofishing, using techniques described above. On 27 May 2011, plasma cortisol level for individual creek chub exposed to a common stressor was determined by exposing wild creek chub outside of the reproductive period ( $n = 8$ ;  $131.1 \pm 5.7$  mm;  $22.0 \pm 3.8$  g; mean  $\pm$  SD) to a combination of chasing to exhaustion (~5 minutes) and air exposure (~30 seconds). Once exhibiting a loss of equilibrium and color change (*i.e.*, fish became pale), fish were placed in an aerated cooler for 30 minutes, allowing circulating cortisol values to peak. Afterwards, fish were blood sampled as per above and euthanized via cerebral percussion.

Using the mean maximal cortisol values generated via exhaustive exercise and chasing as a benchmark, *in vivo* laboratory experiments were subsequently employed to ensure the cortisol manipulation elicited a response within the natural physiological range of stressed individuals for this species. The cortisol dosage was validated by subjecting 36 creek chub, previously acclimated to an outdoor holding tank, to one of the three above treatments (*i.e.*, control ( $n = 12$ ;  $137 \pm 32.1$  mm;  $32.1 \pm 21.7$  g; mean  $\pm$  SD), sham ( $n = 12$ ;  $146.5 \pm 40.9$ ;  $38.8 \pm 29.6$  g; mean  $\pm$  SD) and cortisol ( $n = 12$ ;  $132.2 \pm 38.3$  mm;  $29.5 \pm 22.5$  g; mean  $\pm$  SD)). Acclimated fish were collected as per above, from local area

streams and were held in a ~4290L tank for a minimum of 1 week prior to experimentation, at Queen's University Biological Field Station (QUBS) in eastern Ontario, Canada (44°31' N, 76°20' W). Fish were fed (Nutrafin Max Sinking Pellets, Rolf C. Hagen Inc., NY) daily and debris was removed from the tank using a siphon in effort to maintain water quality. These fish were individually placed in opaque experimental chambers (~10 L), receiving a constant flow through of fresh water from Lake Opinicon. After 3 days, all the fish in the experimental chambers were removed individually and blood sampled as per Cook (2011). As experiments were conducted outside, water temperatures fluctuated between 17-26°C.

### **Mesocosm Study**

On 22 June 2011, after the reproductive period of creek chub, 30 fish acclimated to an outdoor holding tank, were assigned to one of the three treatment groups: (1) control (n = 10; 155.9 ± 6 mm; 38.9 ± 4.1 g; mean ± SD), (2) sham (n = 10; 154.5 ± 6.1 mm; 38.9 ± 3.6 g; mean ± SD) and (3) cortisol (n = 10; 150 ± 6.7 mm; 36.6 ± 5 g; mean ± SD). After treatment, each fish was implanted with a uniquely coded passive integrated transponder (PIT) tag (Texas Instruments Radio Frequency Identification; 23 mm, 0.6 g). Tags were injected into the intra-peritoneal cavity through a small (~3 – 4 mm) incision made off the ventral midline of each fish (*sensu* Binder and McDonald, 2008). Movements were monitored for 20 days using three PIT antennas, equidistant apart, in a 3.65 m diameter outdoor tank (Figure 2-1). The tank had a centralized refuge (24 cm × 24 cm) constructed from a single patio block while the substrate of the tank was covered in crushed white gravel. Reaching a depth of 41 cm (~290 L), the water temperature of

the tank was monitored every hour using two iButton DS1921Z thermal loggers (Maxim Integrated Products, Inc., Sunnyvale, CA; factory-stated resolution  $\pm 0.1^{\circ}\text{C}$ , accuracy  $\pm 1^{\circ}\text{C}$ ) encased in an inert synthetic plastic coating (Plasti Dip International, Blaine, MN). Average water temperatures within the tank were  $21.3 \pm 1.4^{\circ}\text{C}$  (range  $17.9 - 26.4^{\circ}\text{C}$ ). Fresh water from Lake Opinicon was cycled into the tank daily for ~15 minutes, using a pump and garden hose. In attempts to minimize human disturbance near the tank during experimentation, fish were fed (Nutrafin Max Sinking Pellets, Rolf C. Hagen Inc., NY) daily at approximately the same time and location although variations did occur. As the tank was located in a field, far from regular human activity, minimal effort was necessary to control for outside human disturbances.

Fish movements were quantified by the number of times an individual passed between two different PIT tag antennas (*sensu* Binder and McDonald, 2008). Radio frequency identification (RFID) Half Duplex Reader with Antenna Multiplexer (Oregon RFID, Portland, OR) was used to record the date, time, antenna number (1-3) and PIT tag number for each fish passing an antenna. A Palm Pilot (model No. m505; Palm, Sunnyvale, CA) was used to transfer data to a computer. Prior to analysis, data collected during the first 12 hours of the study was removed in attempts to reduce the effects of the handling and tagging procedure.

### **Field Stream Telemetry Study**

#### *Study site*

Experimental procedures were conducted in Mosquito Creek ( $45^{\circ}16' \text{N}$ ,  $75^{\circ}40' \text{W}$ ), a tributary of the Rideau River, located in Ottawa, Ontario, Canada (Figure 2-2).

While initially flowing through agricultural fields, Mosquito Creek travels alongside a developing suburban area where all sampling and tracking took place. During the course of the project, ongoing large-scale road construction affected the watercourse in a few areas. Additionally, this reach includes several road crossings, culverts and stormwater inputs. For our purposes, land use within the study reach was classified as a “developing urban area” by using ArcView GIS (version 10, Environmental Systems Research Institute, Redlands, CA) and geospatial data mapping land use patterns of the National Capital Commission Region (City of Ottawa, 2005). Using a 50 m buffer and taking into account more recent developments not incorporated into data files, we roughly approximated land uses within our reach to be made up of: 57% urban/recreational, ~28% agriculture, ~9% forest, and ~6% other. Common riparian vegetation identified within the study area included sugar maple (*Acer saccharum*), basswood (*Tilia americana*), white ash (*Fraxinus americana*), American elm (*Ulmus Americana*), ironwood (*Carpinus caroliniana*), herbaceous vegetation and grasses. Along the entire reach, riparian vegetation alternated between areas dominated by deciduous trees, herbaceous vegetation and grasses. Common fish species encountered included creek chub, white suckers (*Catostomus commersoni*), common shiners (*Luxilus comutus*), pumpkinseed (*Lepomis gibbosus*), mottled sculpin (*Cottus bairdi*), northern redbelly dace (*Phoxinus eos*), rock bass (*Ambloplites rupestris*), brook stickleback (*Culaea inconstans*) and central mudminnow (*Umbra limi*).

#### *Implantation of radio transmitters*

On 1 November 2010 and 2 November 2010, 15 and 8 creek chub were captured respectively at Mosquito Creek at water temperatures of ~5°C. Fish were individually

anaesthetized in an induction bath of 70 ppm clove oil emulsified in ethanol (1 part clove oil to 10 parts ethanol; Anderson *et al.* 1997). Once unresponsive, fish were placed on a moist C-notched sponge surgery platform, in a supine position, where the gills were irrigated continuously with a recirculating maintenance bath of 20 ppm of clove oil in creek water. A small (1.5 – 2 cm) incision was made posterior of the pelvic girdle, slightly off the ventral midline, to implant a pulsed radio transmitter (Sigma Eight Inc., Newmarket, ON; model: Renata, 14 mm x 7 mm x 6 mm, 0.8 g, range of 1- 3% of body weight, antenna length 15 cm, total pulse duration 5ms, burst interval 3s, life expectancy 42 days, frequencies 148.32 – 149.8 Hz) into the coelomic cavity of each fish. Prior to surgeries, tags were programmed to transmit between 0600 and 1800 (*i.e.*, daylight tracking hours) to maximize battery life. From an anterior direction, a 20 gauge needle was externally inserted in the right side of the posterior end of the incision, to create a separate exit hole that the antenna wire was fed through (as described in Cooke *et al.* 2003). A blunt probe was used to ensure there was no damage to the viscera upon insertion of the needle. Incisions were closed with 2 simple interrupted sutures, using monofilament absorbable suture material (Ethicon 3-0 PDS II, Johnson and Johnson, New Jersey). All instruments, including radio-transmitters, were disinfected using iodine solution. All surgeries were completed within 5 minutes by the same surgeon. While under anesthesia, the total length and mass of all radio-tagged fish was taken to the nearest mm and g respectively. A roughly equal number of fish, from both sampling days, were assigned to one of the two treatment groups: (1) control (n = 12; 161.9 ± 5.4 mm; 40.6 ± 4 g; mean ± SD) and (2) cortisol (n = 11; 160.4 ± 5.6 mm; 40.6 ± 5.1 g; mean ± SD). Sham fish were excluded from this experiment however control fish also

experienced surgery which was a necessary component of the study but which itself represented a short-term stressor (Lower *et al.* 2005). All fish were released in a central location along the sampling reach (45°17' 0.3.14"N, 75°40' 0.8.55"W).

### *Tracking methods*

Portable receivers (SRX 600 and Biotracker, Lotek Wireless Inc., Newmarket, ON) were used to manually track radio tagged fish from 3 November to 30 November, 2010. Tracking was conducted every second day by walking along the bank of the creek while scanning for all frequencies. When found, signal strength and gain reduction methods were used for fine scale positioning of each fish. Once positioned, UTM coordinates (etrex, Garmin, Olathe, KS) and habitat were recorded for every fish. Fine scale positioning of fish occurred twice, on 22 November and 30 November, 2010. On both occasions, a subset of same radio tagged creek chub (n=13; 6-control, 7-cortisol) were positioned once between each of following time intervals: 0600-0900, 0900-1200, 1200-1500 and 1500-1800 hours.

### **Statistical Analysis**

Prior to field application, a one-way analysis of variance (ANOVA) was used to compare plasma cortisol concentrations of treatments within the cortisol validation study against exhausted, air exposed and “baseline”, resting state fish. Levene’s test of equality of error variance was used to ensure normality of sampling distribution (Zar, 1999). Circulating plasma cortisol concentrations were square-root transformed to meet the assumption of homogeneity of variance (Kolmogorov-Smirnov test). Type IV sum of squares methods were employed to account for the unequal sample sizes between



treatments ( $n_{\text{control}} = 8$ ,  $n_{\text{cortisol}} = 9$ ,  $n_{\text{sham}} = 9$ ,  $n_{\text{exhausted}} = 7$ ,  $n_{\text{baseline}} = 18$ ; *sensu* Shaw and Mitchell-Olds, 1993 but see Hanson *et al.* 2008). Differences among treatment groups were assessed using Tukey HSD post hoc tests of multiple comparisons to determine statistical significance between means.

During the field mesocosm study, fish movements were categorized as day- or night-time movements using a government database on regional sunrise/sunset (<http://www.nrc-cnrc.gc.ca/eng/services/hia/sunrise-sunset.html>). Daytime movements were characterized as occurring one hour post-sunrise to one hour pre-sunset whereas the remaining movements were classified as night-time movements. Two-way repeated measures ANOVAs (main effects: treatment, average detections per time period, treatment x average detections per time period) were performed for the first three days post-injection and over the entire study period (*i.e.*, 23 June 2011- 29 June 2011), taking into account variations in individual mortality rates (continuous covariate). Data from individuals dying within 7 days of the experiment were excluded from analysis. One-way ANOVAs followed by Tukey HSD post hoc tests were used to test for statistical significance between factors and interactions. Additionally, a Kaplan-Meier Survival Analysis log-rank test was performed to assess whether survivorship differed between treatment groups.

In the field telemetry study, distances travelled for each creek chub were calculated using a Network Analyst OD cost matrix in ArcMap (version 10, Environmental Systems Research Institute, Redlands, California). Movements were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests to ensure the assumptions of an ANOVA were met. The total distance

travelled and total linear range were compared between treatment groups using general linear one-way ANOVAs, taking into account variability in detection rates across individuals (continuous covariate). As total distance travelled and total linear range did not meet the assumptions of normality, values were log- and square-root transformed prior to analysis respectively. Because the above cortisol validation study found that the time course of plasma cortisol elevation peaked at approximately three days post-injection, nonparametric tests were performed to compare the total linear range during the first three days post-injection for each treatment. Additionally, nonparametric tests were used to evaluate the total distances travelled during the two 12 hour tracking periods, for both cortisol-treated and control fish.

Statistical analysis was carried out using IBM SPSS Statistics 19.0 (IBM Corporation, Armonk, NY) with exception to the Kaplan-Meier Survival Analysis which was performed using JMP, version 7.0.1 (SAS Institute, Cary, NC). Statistical significances for all analyses were set at  $\alpha = 0.05$ .

## **Results**

### **Cortisol Validation**

During the 3 day trial, cortisol injection had an effect on individual circulating plasma cortisol concentrations (Figure 2-3, Table 2-2; ANOVA,  $F = 9.3$ ,  $df = 4, 50$ ,  $p < 0.001$ ). More specifically, three days following cortisol injection, the plasma cortisol concentration of creek chub was similar to that of fish subjected to a standardized stress protocol (Tukey HSD,  $p = 0.916$ ), while cortisol-treated fish had significantly higher plasma cortisol concentrations than sham-treated (Tukey HSD,  $p = 0.009$ ), control

(Tukey HSD,  $p = 0.0016$ ) and baseline fish (Tukey HSD,  $p = 0.001$ ). Hence, we were successful in generating an ecologically-relevant physiological challenge using the cortisol injection.

### **Mesocosm Study**

Throughout the 20 day monitoring period, 16 of the 30 tagged creek chub died. More specifically, dead creek chub included 7 cortisol-treated, 5 sham-treated and 4 control fish. However, while mortality rates for the cortisol-treated fish were nearly twice that of the sham or control fish, there was no significant treatment effect on fish mortality rates (Figure 2-4; survival analysis: log-rank test,  $\chi^2 = 1.373$ ,  $df = 3$ ,  $P = 0.503$ ). During the entire study period (*i.e.*, 23 June 2011 to 29 June 2011), while all fish were found to exhibit significantly more activity during daytime hours rather than night (Figure 2-5, Table 2-1 and 2-2; repeated-measures ANOVA,  $F = 82.6$ ,  $df = 1, 19$ ,  $p < 0.005$ ), there was no effect of treatment on activity (Table 2-1; repeated-measures ANOVA,  $F = 1.77$ ,  $df = 2, 19$ ,  $p = 0.196$ ). Conversely, 3 days post-injections (*i.e.*, 23 June 2011 to 26 June 2011), fish activity was found to be consistent across diel periods (Table 2-2; repeated measures ANOVA,  $F = 0.9$ ,  $df = 1, 19$ ,  $p = 0.4$ ) and treatments (Table 2-2; repeated measures ANOVA,  $F = 1.0$ ,  $df = 2, 19$ ,  $p = 0.4$ ).

### **Field Stream Telemetry Study**

Cortisol treatment did not have any effects on the large-scale movements of adult creek chub. Over the course of the study (*i.e.*, 3 November 2010 – 29 November 2010), no significant difference was found between the total distances travelled (Figure 2-6, Table 2-2; ANOVA,  $F = 2.50$ ,  $df = 2, 18$ ,  $p = 0.11$ ) or the total linear range (Figure 2-6,

Table 2-2; ANOVA,  $F = 1.16$ ,  $df = 2, 20$ ,  $p = 0.33$ ) of control and cortisol-treated fish, when using the number of detections as a covariate. Furthermore, treatment had no effect on the total distances travelled (Table 2-2; ANOVA,  $F = 6.99$ ,  $df = 1, 17$ ,  $p = 0.62$ ) or the total linear range (Kolmogorov-Smirnov  $Z$ ,  $p = 0.319$ ) during the first two tracking sessions (*i.e.*, first three days post cortisol manipulation), when using the number of detections as a covariate. During the two 12 hour tracking sessions, no difference was found between the total distance travelled for cortisol-treated and the control fish on 22 November 2010 (Kolmogorov-Smirnov  $Z$ ,  $p = 1.0$ ) or on 30 November 2010 (Kolmogorov-Smirnov  $Z$ ,  $p = 0.804$ ). Of fish positioned 5 or more times, control fish exhibited upstream movement (31%), downstream movement (38%) and non-movement (31%) at similar frequencies to cortisol fish (upstream 34%, downstream 35%, and non-movement 30%).

## **Discussion**

This study used mesocosm and field telemetry methods to examine the effects of an experimental short-term cortisol challenge on the behaviour of wild creek chub outside of the reproductive period. These two approaches allowed us to examine both small-scale movements (*i.e.*, activity patterns) under controlled conditions and large-scale movements within an urban stream, to provide a more complete picture of the behaviour of fish when exposed to a stressor. Overall, we found that exogenous manipulations of plasma cortisol concentrations did not result in significant changes to fish activity, despite the fact that these manipulations were found to significantly raise circulating plasma cortisol concentrations during experiments that validated cortisol dosage. These results indicate that while our cortisol manipulation was effective at emulating a primary

stress response among subject fish, it was unable to produce a detectable tertiary stress response within our study parameters (*i.e.*, behaviour). Moreover, we did not observe any statistical evidence of treatment on mortality rate, which could further indicate there were little to no long-term secondary or tertiary affects of our cortisol treatment. However, mortality rates were generally high for cortisol-treated fish (~70% mortality after 3 weeks in the mesocosm experiment), and nearly twice that observed for sham and control treatments.

#### *Intra-peritoneal Cortisol Injections, Validations and Limitations*

The cortisol dose used to experimentally raise circulating plasma cortisol concentrations in creek chub was based on values commonly used to induce a stress response in teleost fish (Gamperl *et al.* 1994 but see low-dose cortisol treatment for largemouth bass in O'Connor *et al.* 2009). Analysis of pilot samples found this dosage to provide an ecologically-relevant cortisol challenge as experimentally stressed fish had similar mean cortisol values to those of fish chased to exhaustion. It is our belief that the cortisol injection emulated a semi-chronic (~3 to 5 day) stress response that would be similar in duration to what might be expected during ecologically-relevant events such as a hypoxic event (*e.g.*, Herbert and Steffensen, 2005), a short-term starvation (*e.g.*, McConnachie, 2010), exposure to supercooling and frazil ice (*e.g.*, Brown *et al.* 1999), droughts and floods (*e.g.*, Flodmark *et al.* 2002). While sham treatments were used to account for the stress of cocoa butter injections and the associated immune response with the addition of foreign matter in the body cavity, Hoogenboom *et al.* (2011) report that cocoa butter implants can act as a stressor, reducing the growth, egg and hatching size in

female brown trout (*Salmo trutta*). However, sham treatments were not included within our field telemetry component. Nevertheless, comparing movement and activity between solely control and cortisol-treated fish, while excluding sham treatments, is still important as control fish were also exposed to surgery (including anesthesia, laparotomy and wound closure) which is more invasive than the injection. As such, the only difference between controls and treatments in the telemetry study was the presence of the cocoa butter and cortisol rather than the injection procedure itself. We did observe mortality associated with all treatments in the mesocosm study. Although efforts were made to minimize handling stress, experiments were conducted on wild fish that were captured and transported to a research facility. Although not statistically different, the fact that cortisol-treated fish had mortality levels nearly twice that of control and sham fish indicates that the cortisol treatment did appear to increase mortality. Nevertheless, while these experimental limitations must be acknowledged, we believe that the results obtained from this study provide an accurate assessment of the long-term effects of an elevated cortisol challenge on the behaviour of wild stream fish.

#### *Fine-Scale Activity*

In general, there were no statistical differences in the behaviours (*i.e.*, activity and movement rate) of control, cortisol-, and sham-treated fish. However, during the first three days of the experiment, individuals from all treatment groups exhibited altered feeding patterns, as fish previously observed to readily feed during the pre-treatment, acclimation period were found to have reduced food intakes. However, as the experiment progressed, the number of fish observed feeding increased, although whether treatment affected the rate in which an individual returned to regular feeding behaviour was

untested. Similar short-term behaviours shared among treatment groups may suggest that these results more accurately represent the effects that tag insertion and its associated handling stress have on wild fish and consequently mask the short-term effects of each treatment. Furthermore, as anesthetic was not administered prior to treatment and tag insertion, our handling procedures would have activated the hypothalamic pituitary-interrenal (HPI) axis and caused the subsequent release of cortisol prior to the onset of treatment effect (Small, 2003). As reported by Hoskonen and Pirhonen (2006), repeated handling without anaesthetics, can cause reductions on individual food consumption when compared to unhandled control fish. Nonetheless, our cortisol injection would have elevated cortisol titers in cortisol treated fish which would have been consistently high and less transient than the stress associated with short term handling.

#### *Large-Scale Activity*

Exogenous cortisol manipulations, with paralleled control groups, were found to have no effect on the large-scale movements of wild creek chub, in an urban stream, as evidenced by treatment groups travelling similar distances throughout our study. Based on lack of differences between sham and control fish in an *a priori* study, sham treatments were not included within this portion of experimentation in efforts to reduce tag expenses. A limitation of excluding sham treatments is that we were unable to account for the effects of adding foreign matter into the body cavity (*i.e.*, cocoa butter). However, DiBattista et al. (2005) argues that sham treatments can be disadvantageous as they have a tendency to have unpredictable cortisol responses and as a consequence create complicated interpretations as the distinction of cortisol values between treatment groups become less definitive. Nevertheless, the lack of variation between the large-scale

movements of cortisol-treated and control fish was surprising as past research has found elevated blood cortisol values to significantly increase blood pressure and energy usage as well as cause reductions in food intake and immune function (reviewed in Wendelaar-Bonga, 1997). Although only loose connections have been suggested between blood pressure, energy usage, food intake and immune function on fish movements, we expected these effects would have a large enough impact to reduce the mobility of cortisol-treated fish.

As found in other studies (*e.g.*, Skalski and Gillam, 2000; Belica and Rahel, 2008), creek chub exhibited leptokurtic movement distributions, as study fish were found to have high turnover rates (*i.e.*, proportion of population that moves) with low displacement distances (*i.e.*, distance that each fish moves). As characteristic of leptokurtosis, a large proportion of fish, from both treatment groups, were found to remain within particular sections of the creek at relatively close proximity (~600 m) to the release site. Occasionally, individuals were found to move larger distances (~1-2 km) between consecutive tracking periods, however, few fish were found to be consistently mobile. Observationally, fish locations were closely associated with patches of habitat offering refugia in the form of backwater, plunge and lateral scour pools, containing structures such as woody debris, in-stream rock and macrophyte beds. However, the persistent leptokurtosis behaviour observed throughout the entire study period may suggest that population heterogeneity may have posed difficulties finding significant differences between treatment groups, which could have only been addressed with much larger sample sizes.



### *Summary and Future Direction*

As far as we are aware, this study represents the first attempt to test whether an experimental short-term stressor has an effect on the small- and large-scale movements of wild, adult stream fish. Overall, these results provide evidence indicating that exogenous cortisol manipulations do not significantly impact behaviours related to locomotor activity. This finding is particularly interesting given that there are few studies of how experimental cortisol elevation influences behaviour of fish outside of the reproductive period. All of the studies that we are aware of that involve cortisol manipulation prior to or during reproduction have failed to document behavioural differences relative to controls (*e.g.*, O'Connor *et al.* 2009; Dey *et al.* 2010). Even though some cortisol-treated fish in those studies abandoned parental care, prior to that they maintained care at the same level as control fish. Only a study by O'Connor *et al.* (2010) noted reductions in movement following cortisol injection, but that occurred in largemouth bass in the winter, well outside of the reproduction period, and immediately prior to total mortality associated with a winter-kill event. Moreover, in that study no behavioral differences were noted until nearly 4 months after initial cortisol manipulation. The absence of variation between control, cortisol- and sham-treated fish in the current study may suggest that compensatory mechanisms exist that allow stress-induced fish to behave similarly to their experimental counterparts, even outside of the reproductive period. That finding is somewhat notable as life-history theory would predict that fish that are reproductively active should maintain behaviour in the face of stressors (Sapolsky *et al.* 2000), but not so for fish outside of the reproductive period. However, while compensatory mechanisms can be adaptive in that they can help an individual, operating

under allostatic conditions to survive a stressful event, they can also be maladaptive as they tend to have negative implications on necessary life processes (Schreck *et al.* 2001). In particular, a potential trade-off between reproductive success, in terms of gamete or progeny quality, for potential survival has long been identified as a possible consequence of compensatory mechanism (*e.g.*, Pickering *et al.* 1987; Schreck *et al.* 2001; Ostrand *et al.* 2004). While our work did not assess the fecundity of treatment fish, it is important to note that although exogenous manipulations did not affect behaviour, other whole-body effects of cortisol elevations are possible. Clearly there is a need for additional research that extends across multiple seasons and years.

As indicated by their ability to inhabit streams throughout a range of different landscape patterns, creek chub are known to be a relatively robust fish, with a wide distribution (Scott and Crossman, 1973). Our results speak to the resilience of creek chub as we found that fish activity and movement remain unaffected when given an additional exogenous cortisol challenge. However, we did observe considerable mortality for cortisol-treated fish in the mesocosm study suggesting that there was a cost associated with the cortisol treatment. While the mean circulating plasma cortisol concentrations for our stress-induced and baseline fish were similar to values reported in the European chub (*Leuciscus cepalus*), our cortisol values were found within the range known to cause immunosuppression as well as inhibition of growth and reproduction in salmonids (Pottinger *et al.* 2000). Comparative analysis, performed by Pottinger *et al.* (2000), indicates that while the number of total binding sites in gill tissue are similar between trout and chub, the affinity of binding sites for cortisol are eightfold higher in trout. This low cortisol receptor affinity in chub may make these fish more tolerant to stress and

explain the lack of response found among our exogenous cortisol manipulations. Future experiments should focus on evaluating other sublethal metrics related to organismal condition and in understanding the mechanisms that led to mortality. There is a need for additional studies that use ecologically-relevant endpoints such as behaviour and habitat use in wild fish to study the effects of stressors on wild fish.

## Tables

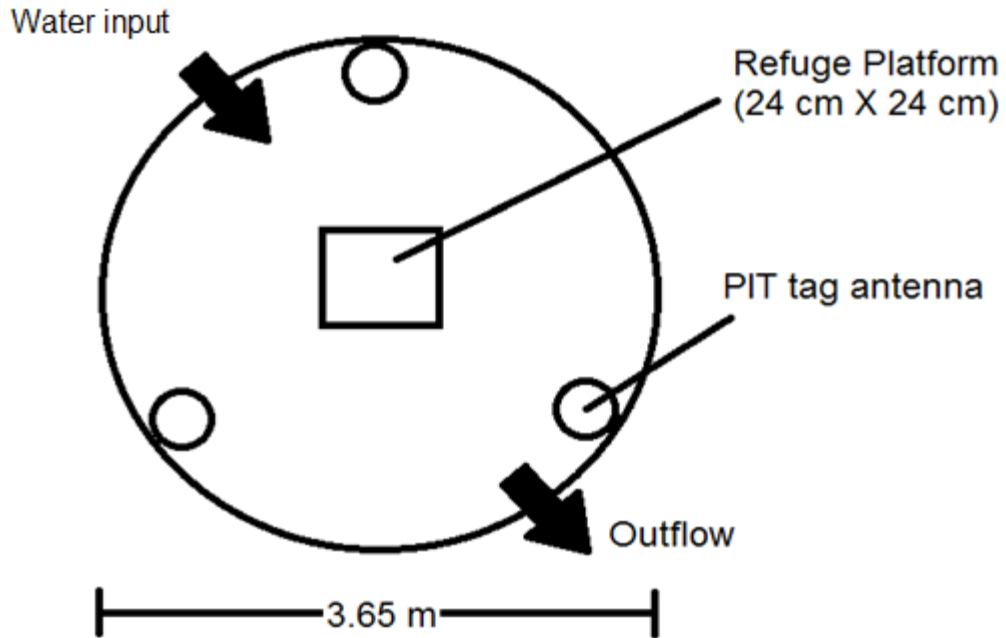
**Table 2-1.** Average number of detections and standard error for control (n = 8), cortisol- (n = 7) and sham-treated (n = 7) creek chub within the fine-scale, mesocosm study. Fish that died prior to 30 June, 2011 were not included in these calculations.

	Average number of detections $\pm$ SE					
	Control		Cortisol		Sham	
	Day	Night	Day	Night	Day	Night
6/23/2011	63.1 $\pm$ 19.6	87.1 $\pm$ 17.5	35.7 $\pm$ 11.5	45.4 $\pm$ 19.8	33.4 $\pm$ 10.3	41.3 $\pm$ 13.9
6/24/2011	96.5 $\pm$ 19.0	61.5 $\pm$ 12.0	48 $\pm$ 10.3	47.7 $\pm$ 8.7	44.7 $\pm$ 12.0	46.1 $\pm$ 12.0
6/25/2011	121.7 $\pm$ 1401	84.7 $\pm$ 12.2	97.9 $\pm$ 21.6	81.3 $\pm$ 17.7	61.3 $\pm$ 11.7	59.4 $\pm$ 3.7
6/26/2011	177.7 $\pm$ 19.2	49.1 $\pm$ 12.9	161.4 $\pm$ 39.7	44.3 $\pm$ 13.2	130.1 $\pm$ 19.2	34.3 $\pm$ 8.5
6/27/2011	166.9 $\pm$ 30.4	22.2 $\pm$ 3.5	141.4 $\pm$ 31.3	25.6 $\pm$ 5.3	151.7 $\pm$ 13.7	28.4 $\pm$ 6.1
6/28/2011	81.2 $\pm$ 19.0	16.4 $\pm$ 2.9	57 $\pm$ 10.4	17.3 $\pm$ 2.1	62.9 $\pm$ 4.5	19.1 $\pm$ 3.3
6/29/2011	55.7 $\pm$ 10.8	16.4 $\pm$ 4.2	47.3 $\pm$ 22.7	12.7 $\pm$ 5.8	66.7 $\pm$ 11.1	20.4 $\pm$ 5.2

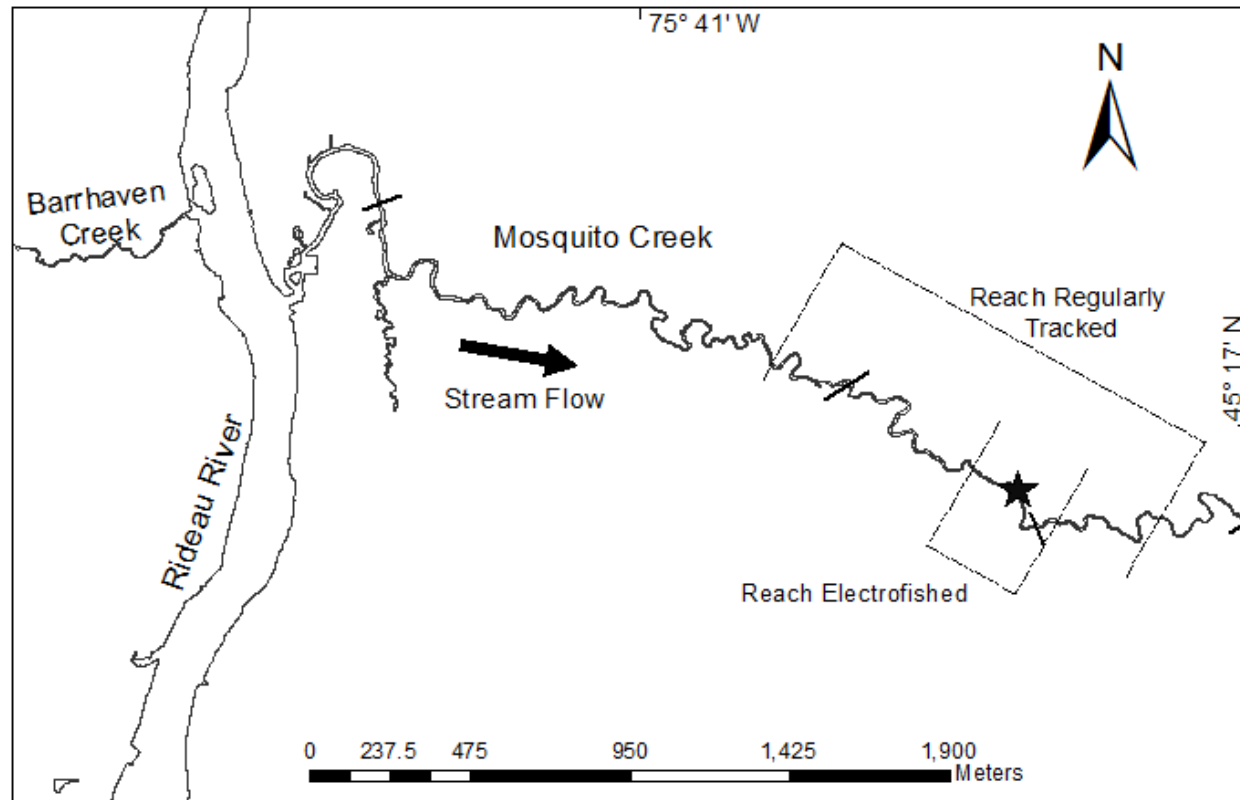
**Table 2-2.** Results of ANOVAs testing the effects of treatment on cortisol concentration (as part of the validation study) and behaviour (*i.e.*, activity and movement) of creek chub.

Experiment	Factors	Time period							
		3 days				Entire study period			
		d.f.	SS	F	P	d.f.	SS	F	P
Cortisol Validation	Whole model	4	2753.9	9.3	<b>&lt;0.001</b>				
	Treatment	4	2753.9	9.3					
	Error	50	3681.0						
Mesocosm Study	Average detections per time period	1	307.9	0.9	0.4	1	26691.4	82.6	<b>&lt;0.005</b>
	Average detections per time period × treatment	2	696.8	1.0	0.4	2	630.4	1.0	0.4
	Error	19	6678.4			19	6136.5		
Telemetry field study – <i>total distance travelled</i>	Whole model	2	3.6	7.0	0.006	2	3.5E6	2.5	0.1
	Number of detections	1	3.5	13.2	0.002	1	2.4E5	4.2	0.05
	Treatment	1	0.07	0.2	0.6	1	1.3E7	0.9	0.3
	Error	17	4.4			18			
Telemetry field study - <i>total linear range</i>	Whole model					2	496.6	1.2	0.3
	Number of detections					1	253.0	1.2	0.3
	Treatment					1	167.5	0.8	0.4
	Error					20	4275.4		

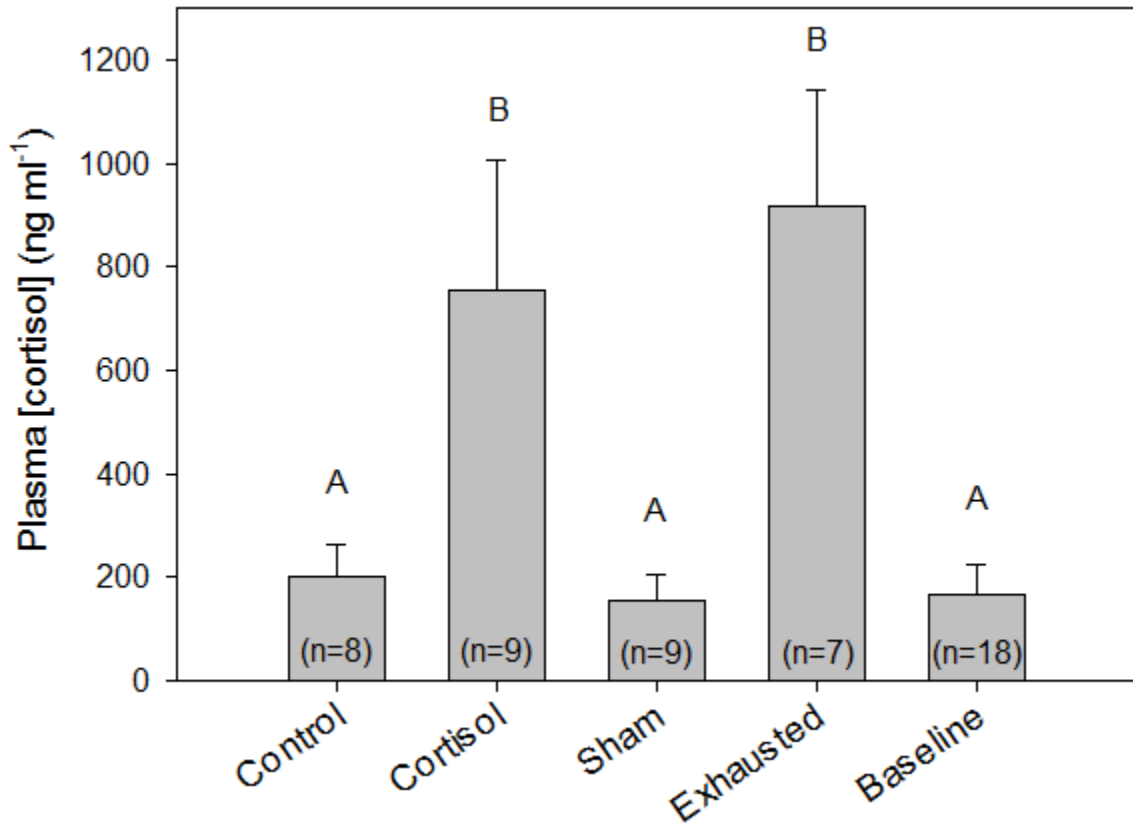
## Figures



**Figure 2-1.** Schematic diagram of outdoor tank used for mesocosm experiment. Three PIT tag antennas, positioned equidistant apart, monitored small-scale movements around the tank and centralized refuge platform constructed of a raised patio block.

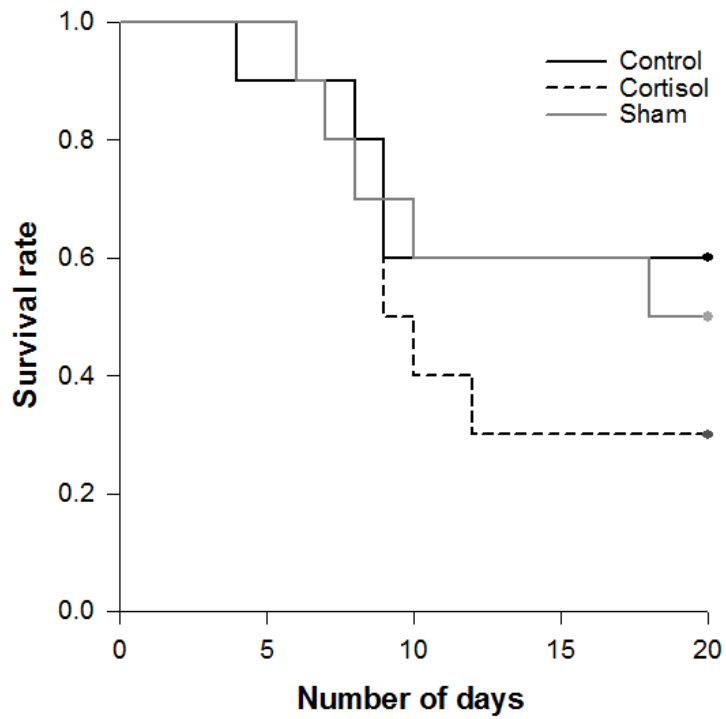


**Figure 2-2.** Location of study site, Mosquito Creek (45°16' N, 75°40' W) in Ottawa, Ontario, Canada. Heavy black lines intersecting the creek indicate roadways where water is passed through culverts. The black star indicates where radio-telemetered fish were released.

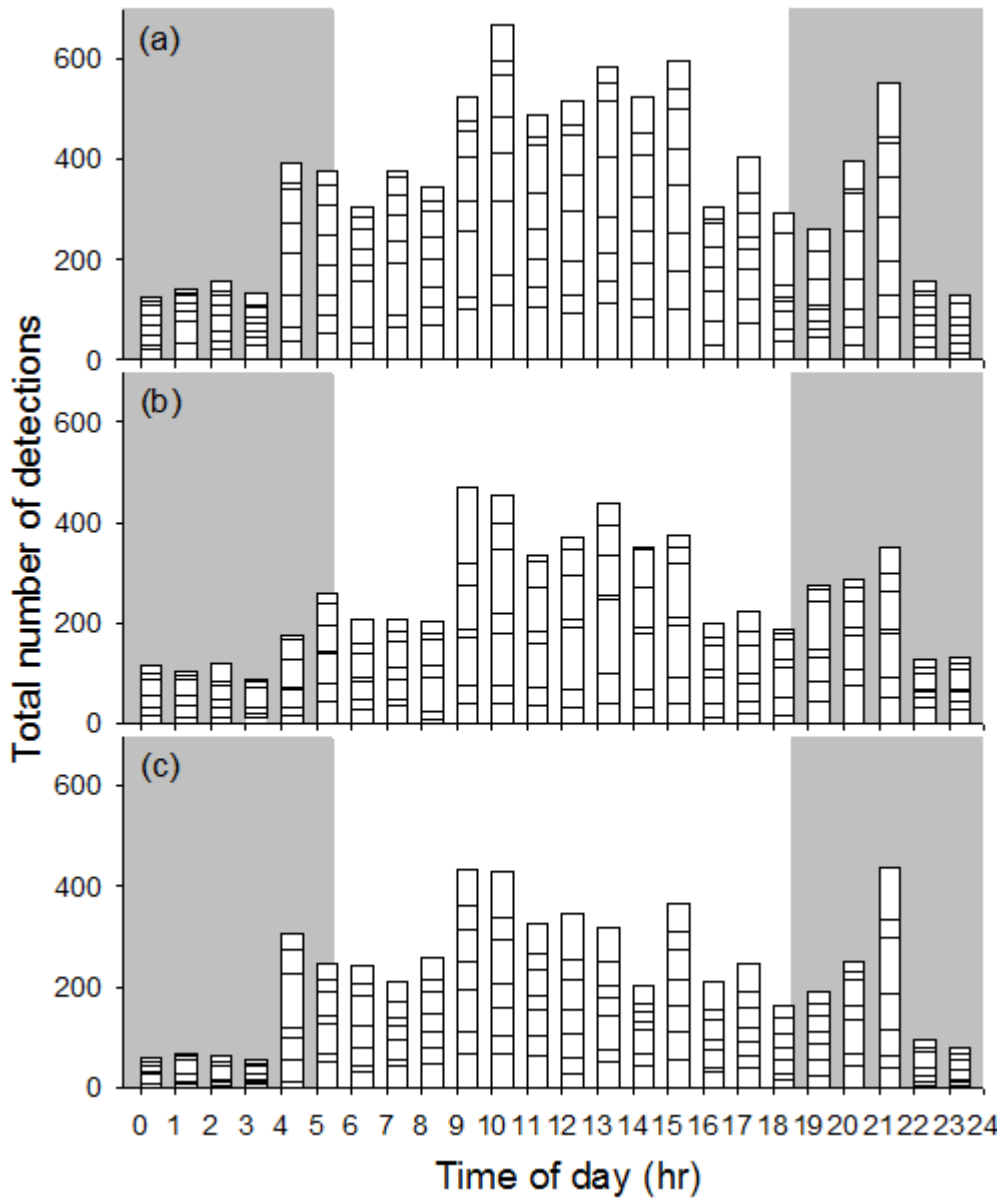


**Figure 2-3.** Plasma cortisol concentrations (mean  $\pm$  SE) of 3 day trial fish (*i.e.*, post control, cortisol and sham treatments) compared to fish subjected to a standardized stress protocol (labeled as “exhausted”) and baseline fish. One-way ANOVAs, with square-root transformations were used to assess differences between treatment groups. Different letters between treatment groups indicate statistical difference ( $\alpha=0.05$ ).

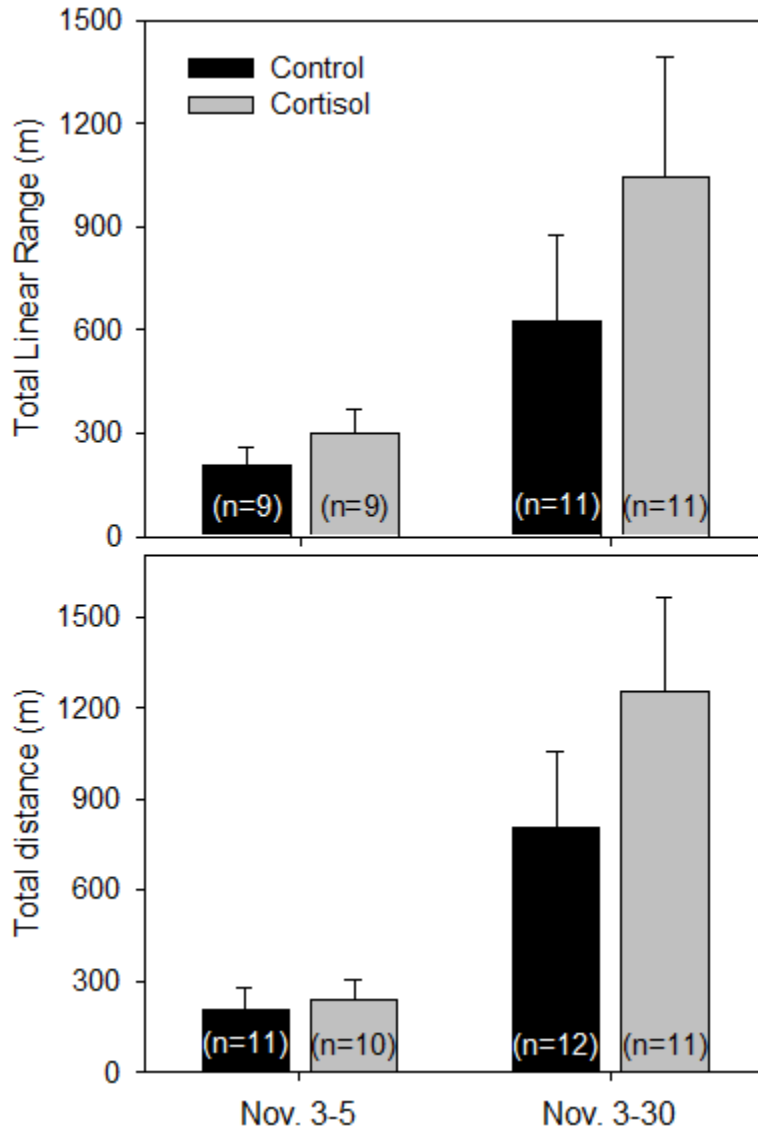




**Figure 2-4.** Surviving creek chub from all treatment groups during the mesocosm experiment between 22 June 2011 and 11 July 2011. There was no statistical effect of treatment on survival rate.



**Figure 2-5.** Diel activity patterns of (a) control, (b) cortisol-, and (c) sham-treated creek chub during mesocosm experiments between 23 June 2011 and 29 June 2011. Stacked bars indicate the individual contribution of each fish, at each time period. Light and dark shading represents day- and night-time respectively.



**Figure 2-6.** Total distances and total linear range for telemetered creek chub from both treatments groups three days post-injection (3 November – 5 November 2010), and for the accumulative study period (3 November – 29 November 2010). No significant differences were found between treatments.

### **Chapter 3: Health, condition, and survival of creek chub across a gradient of stream habitat quality following an experimental cortisol challenge**

#### **Abstract:**

Human land-use activities related to urbanization and agricultural practices can lead to alteration in stream habitat quality which has the potential to negatively influence fish populations. To date, there have been few attempts to understand if variation in habitat quality influences the health and condition of fish or their ability to respond to multiple stressors. The purpose of this study was to examine how fish residing in streams adjacent to different types of land-use practices respond to an experimental but ecologically relevant stressor. Creek chub (*Semotilus atromaculatus*) from replicate streams that were associated with agricultural and urban development, as well as reference sites were subjected to a single intra-peritoneal injection of cortisol, suspended in coconut butter, to experimentally raise circulating plasma cortisol concentrations for ~3 days. We compared the survival, health, and condition of cortisol-treated, sham-treated (injected with coconut butter), and control fish across the different land use types. While our overall survival analysis failed to detect differences, closer evaluation of survival within agricultural sites revealed that, while not statistically significant (*i.e.*,  $p = 0.06$ ), cortisol-treated fish had ~50% more mortality than control and sham-treated fish. We did not observe any significant differences in health (*i.e.*, parasite burden), or condition (*i.e.*, condition factor, hepato-somatic [HSI], splenic, and gonado-somatic indices, blood glucose) of fish relative to land-use or experimental treatment with one exception; in agricultural watersheds, we observed that sham-treated fish had a higher

HSI relative to control fish. Despite experimentally elevating cortisol titers in fish for an extended period, and using fish from streams across a gradient of habitat quality, none of the ecologically-relevant sublethal endpoints we measured were affected, although there was some evidence of higher mortality in the cortisol treated fish in our most disturbed stream type (*i.e.*, agricultural). Overall, these results suggest that in the wild, there are compensatory mechanisms that enable fish to persist despite being exposed to a significant challenge. Creek chub are regarded as a rather tolerant organism so it is unknown if other species would respond differently, or if greater levels of experimental stress (*i.e.*, higher levels of cortisol or longer duration of elevation) or habitat degradation would result in negative alterations in fish health, condition, and survival. This study is one of the first to use experimental cortisol implants to study how fish respond to experimental stress challenges in the wild and provides insight as to how multiple stressors may affect fish in streams relative to different types of adjacent land use and stream habitat quality.

## **Introduction**

The habitat and biological diversity of stream ecosystems are strongly influenced by the surrounding terrestrial landscapes in which they flow (reviewed in Allen, 2004). In particular, the riparian zone, defined as the interface between terrestrial and aquatic ecosystems (Gregory *et al.* 1991), plays an important role in controlling the effects that catchment activities have on neighbouring aquatic ecosystems (Quinn *et al.* 2001). Consequently, as human development promotes the transition from undisturbed to more human-dominated landscapes, research has and continues to focus on the effects that human land use practices have on stream conditions (*e.g.*, Lenat and Crawford, 1994;

Niyogi *et al.* 2003; Roy *et al.* 2003). For example, anthropogenic alterations to terrestrial landscapes have been found to influence sediment loads (Henley *et al.* 2000), alter watershed hydrology (Allen *et al.* 1997), increase water temperature (LeBlanc, 1997), create imbalances in nutrient inputs (Mainstone and Parr, 2002), and increase pollution (Clements *et al.* 2000) within adjacent aquatic ecosystems, with the extent that anthropogenic activities influence natural landscapes varying according to the type and severity of disturbance (*e.g.*, agriculture, forestry, and urbanization). In general, anthropogenic alterations and associated changes in water quality and stream habitat force organisms that reside in these systems to be exposed to more extreme environmental conditions.

For a species to persist in a particular environment, essential habitat requirements need to be satisfied. Although species can be found to inhabit a range of different environments, life-history traits and associated physiological tolerances and capacities make particular environments more favourable to inhabit than others. At some point, phenotypic flexibility and adaptive life-history responses to unsuitable environments become maladaptive and represent a significant allostatic load (Wingfield, 2005), causing the organism to be at a competitive disadvantage (Piersma and Drent, 2003). When faced with a stressor, species inhabiting sub-optimal environments may be less capable of adequately responding to the challenge (Wingfield, 2005) and may experience greater negative consequences (*e.g.*, reduced food intake and immune function) as a result of allostatic overload (Schreck, 2010) than when exposed to a stressor in an optimal environment. For example, research evaluating the stress response of birds across different land use patterns (*i.e.*, urban, suburban and industrial) revealed that while all

birds had a large capacity to respond to stressors, individuals from industrial areas could become compromised if frequently exposed to high intensity stressors (Chávez-Zichinelli *et al.* 2010). In a conceptually similar study evaluating the stress response of salamanders within areas of varying habitat quality, Homan *et al.* (2003) was able to detect differences in corticosterone concentrations among individuals inhabiting sites with varying degrees of deforestation. Researchers found that individuals inhabiting more disturbed areas had lower baseline and stress-induced corticosterone concentrations than individuals from less disturbed sites. As proposed by the authors, a lower stress response among individuals within disturbed sites may indicate a significant reduction in the ability of an individual to cope with additional stressors in the future (Homan *et al.* 2003). However, from an aquatic standpoint, similar research as it pertains to fish is uncommon (but see Adams and Ham 2011), though could prove valuable as more stream habitats become impacted by anthropogenic disturbances (Walsh *et al.* 2005). Furthermore, as freshwater fish are among the most threatened vertebrates on Earth (Burton 1995; Saunders *et al.* 2002), research coupling fish stress response to a range of land use patterns could help inform management decisions on how to mitigate the impacts of human activities on aquatic landscapes.

As aquatic ecosystems continue to be affected by human land-use practices, there is an increasing need to assess the degree in which anthropogenic activities impact natural riverscapes and their resident fish populations. The application of *in situ*, effects-based assessments provides an opportunity to broadly monitor the biological responses of resident fish, when exposed to site-specific conditions characterizing different land use patterns (*e.g.*, Doherty *et al.* 2005; Gray and Munkittrick, 2005). Effects-driven

assessments are valuable when examining the consequences of inhabiting particular environments as the effects of all stressors, within the system, are pooled together, generating an “accumulated environmental state” rather than evaluating the impacts of stressors individually. Within effects-based assessments, monitoring physiological indicators (*e.g.*, energy transfer, nutritional condition and metabolism; see Adams and Ham 2011) has been identified as an appropriate method to assess the performance of an organism within its environment as physiology is often regarded as the driving force behind organismal responses to environmental change (Wikelski and Cooke 2006; Cooke and O’Connor, 2010). In particular, studies concerned with the consequences of stress on fish (*e.g.*, Pickering *et al.* 1987; Fevolden *et al.* 1993) commonly use circulating plasma cortisol concentrations as a means to quantitatively evaluate individual stress responses (Wendelaar-Bonga, 1997; Barton, 2002), albeit typically in the laboratory and/or in response to environmental toxicants and pollutants (*e.g.*, Adams and Greely, 2000; Almeida *et al.* 2005; Camargo and Matinez, 2006). On occasion, the stress response can extend to tertiary responses, which represent the changes to whole-body activity and performance (Schreck, 1990). An alternative approach to studying stress involves the experimental manipulation of cortisol titers to raise circulating cortisol to levels observed in stressed fish (Gamperl *et al.* 1994). While many studies have found exogenous manipulation of cortisol titers, accompanied with monitoring of physiological parameters, useful in assessing the consequences of stress on fish, few experiments have applied this methodology outside the confines of a laboratory (*e.g.* Vijayan *et al.* 1996; DiBattista *et al.* 2005). Of the existing field research using exogenous cortisol manipulations, none have assessed whether the consequences of experimental challenges vary across systems



of differing habitat quality. Studies of this nature can contribute to the understanding of the ecological consequences of stress on fish and provide insight as to whether alterations in habitat quality can influence the ability of fish to respond to multiple stressors.

In this study we compared the health, condition, and survival of control, cortisol- and sham-treated fish inhabiting streams within reference, agricultural, and urban landscapes. Cortisol-treated fish received a single exogenous, intra-peritoneal injection of cortisol suspended in coconut butter, which has been found to be an effective method in experimentally studying how chronic levels of plasma cortisol in teleost fish influence various biological endpoints (reviewed in Gamperl *et al.* 1994). Moreover, as cortisol/coconut butter implants function by slowly releasing hormone over an extended period (~3 to 5 days), challenging fish with an ecologically- and physiologically-relevant cortisol dose provides a controlled means of examining how fish respond to stress, across different land use patterns. Creek chub (*Semotilus atromaculatus*) were used as our sentinel species as they occur in high numbers, inhabit streams of varying habitat quality, lack major fishing pressures and exhibit rapid growth and maturation (Scott and Crossman, 1973; Fitzgerald *et al.* 1999). An experimental approach was used to evaluate fish response to an experimental stressor as past research by Blevins *et al.* (unpublished manuscript) has established that while baseline physiological parameters of creek chub are consistent across different land use patterns (*i.e.*, agricultural and forested), fish originating from different land use patterns exhibit differential performance to thermal and hypoxia challenges. However, results of this study also suggested that creek chub exhibit the ability to improve physiological performance within suboptimal environments as prolonged holding at high temperature removed landscape-level differences (Blevins *et*

*al.* unpublished manuscript). Nevertheless, here we tested the null hypothesis that there is no difference in the health, condition, and survival of control, cortisol- and sham-treated fish across undistributed, agricultural and urban landscapes. However, as past studies have found creek chub behaviour and growth to be influenced by pollution (Katz and Howard, 1955) and turbidity levels (Gradall and Swenson, 1982), we predict that the health, condition and survival will vary among habitat types, with reference sites faring better than fish in agricultural or urban streams. Moreover, we anticipate that the response to the experimental cortisol challenge will result in negligible changes in health, condition and survival in reference sites but comparatively more extreme negative consequences in agricultural and urban sites.

## **Material and Methods**

### *Experimental animals and cortisol treatments*

All fish were sampled under an Ontario Ministry of Natural Resources Scientific Collection Permit (Licence Number: 1061994; granted to S.J.C) and were processed in adherence to the guidelines set out by the Canadian Council on Animal Care, as issued by Carleton University (B10-9). As creek chub generally spawn in the spring beginning at temperatures of 12.8°C (Scott and Crossman 1973), all sampling occurred outside this range to avoid confounding effects of the reproductive period. Captured fish were individually anaesthetized in an induction bath of 70 ppm clove oil emulsified in ethanol (1 part clove oil to 10 parts ethanol; Anderson *et al.* 1997). Once unresponsive, fish were randomly distributed across the following three groups: cortisol-treated, sham-treated and control. Cortisol-treated fish received a single intraperitoneal injection of 10 mg mL<sup>-1</sup> of cortisol (hydrocortisone; Sigma H2882, Sigma-Aldrich) suspended in coconut butter

(*Cocos nucifera*; Sigma C1758, Sigma-Aldrich, St. Louis, MO) at 0.005 mL g<sup>-1</sup> body weight (reviewed in Gamperl *et al.* 1994; but see O'Connor *et al.* 2010). Sham-treated fish received a single intra-peritoneal injection of pure coconut butter at 0.005 mL g<sup>-1</sup> body weight, while control fish were handled in a manner identical to treatment fish, but received no injections. This method of experimentally raising circulating plasma cortisol concentrations to physiologically- and ecologically-relevant level is an established technique used to study the effects of stressors on fish (Gamperl *et al.* 1994). The dosage administered was confirmed by Nagrodski *et al.* (unpublished data) to experimentally raise cortisol levels in creek chub to 753 ± 256 ng/ml, for ~ 3 days relative to control values of 203 ± 60 ng/ml. The cortisol dosage was intended to emulate the stress that might be encountered during ecologically-relevant events such a hypoxic event (*e.g.*, Herbert and Steffensen, 2005), a short-term starvation (*e.g.*, McConnachie, 2010), exposure to supercooling and frazil ice (*e.g.*, Brown *et al.* 1999), droughts and floods (*e.g.*, Flodmark *et al.* 2002). After treatment, fish were placed in separate, aerated recovery bins (~ 52L). All fish were also identified individually using fluorescent orange VI Alpha Tags (Northwest Marine Technology, Inc., Turnwater, WA) and collectively, according to treatment, by small partial caudal fin clips (Rounsefell and Kask, 1945). While cortisol- and sham-treated fish received upper and lower fin clips respectively, control fish received both upper and lower fin clips. Prior to field application, a laboratory pilot study found that VI Alpha Tag had an ~88% retention rate after 14 days when implanted in the cheek epidermis of creek chub in an outdoor holding tank (~ 4290 L) at Queen's University Biological Field Station (QUBS; 44°31' N, 76°20' W).

### *Site Selections*

To quantify how reference (what was considered “pristine” sites), agricultural and urban land use patterns affect the health, condition and survival of resident fish when exposed to an experimental cortisol challenge, 3 replicate streams for each land use pattern were selected. Considering a 100 meter buffer around each catchment, ArcView GIS (version 10, Environmental Systems Research Institute, Redlands, CA) and geospatial data mapping land use patterns of the Rideau Valley and South Nation Regions were used to select appropriate replicate study streams based on the following predetermined parameters: reference streams had to be surrounded by: < 5% developed area, < 30% cropland, and < 25 kilometers of road running through the catchment, agricultural streams had to be surrounded by: > 40% cropland, < 20% developed area, and < 25 kilometers of road running through the catchment, and urban streams had to be surrounded by > 30 developed area, and > 30 kilometers of road running through the catchment. Satisfying these parameters, Stevens Creek (45° 05’ N, 75° 47’ W), Keelers Creek (44° 48’ N, 75° 34’ W), and Hobbs Drainage (45° 10’ N, 75° 56’ W) comprised the reference sites, Middle Castor (45° 12’ N, 75° 34’ W), East Castor (45° 09’N, 75° 19’ W), and a tributary of the Jock River (45° 12’ N, 75° 52’ W) made up the agricultural sites, while Sawmill Creek (45° 20’ N, 75° 37’ W), Shields Creek (45° 15’ N, 75° 33’ W) and a stream off the Castor River (45° 14’ N, 75° 28’ W) made up the urban sites (Table 3-1, adapted from database provided by the Ministry of the Environment).

### *In-field setup*

Standard battery-powered backpack electrofishing (Halltech Aquatic Research Inc., Guelph, ON; model Ht-2000) was used to collect creek chub at each of the above study sites. Pulsed-DC electrofishing is considered a safe tool for studying stream fish, and results by Gatz and Linder (2008) suggest this practice is unlikely to have meaningful biological effects on creek chub condition, growth, or movements. Streams were sampled individually at approximately the same time everyday (~09:00), however, variations occurred due to differences in travelling distances (Table 3-2). In an effort to reduce sampling bias across habitat types, creeks within different land use patterns were sampled alternately (*i.e.*, urban – agricultural – reference; Table 3-2), however, on one occasion the sampling order of a reference stream and an agricultural stream (*i.e.*, Hobbs Drainage and a Tributary of the Jock River) were reversed. At each site, prior to electrofishing, a sampling reach of approximately 100-150 meters was chosen, however, on every occasion additional sampling was performed to increase sample size (reach lengths reported in Table 3-1; electrofishing efforts reported in Table 3-2). While electrofishing, creek chub were collected and held in multiple bait pails that were periodically left submerged in the stream, with efforts made to minimize crowding. Once electrofishing was completed, all fish were gathered at a centralized location along the reach and held in aerated bins. Once treated and allowed to recover (as per above), all fish were released in a centralized location that offered some protection/cover for fish.

### *In-field sampling*

Electrofishing techniques were used to recapture study fish from each site ~ 25 days after injections, however variations did occur (Table 3-2). Additionally, many streams were sampled twice in an effort to increase recapture rates. At this time, electrofishing efforts were extended further than previously sampled. When recaptured, ~0.2 - 0.4 ml of blood was collected via caudal puncture, using a combination of sodium heparinized 1 ml syringes with 25 gauge, 38 mm needles and sodium heparinized 0.5 ml insulin syringes with 28.5 gauge, 13 mm needles (Becton Dickinson & Co. Tuberculin Slip Tip Syringes and ½ cc LO-DOSE U-100 Insulin Syringe, Franklin Lakes, NJ). Blood glucose levels were assessed using ~ 10 µl of whole blood with a hand-held glucose meter (ACCU-CHEK glucose meter; Roche Diagnostics, Basel, Switzerland) as previously validated by Cooke et al. (2008). Directly after blood sampling, fish were euthanized using cerebral percussion. Fish were placed individually into labeled Ziploc bags and transferred to a cooler of ice before being transported to an indoor facility for necropsy-based condition assessments. Euthanized fish were assessed for hepato-somatic index (HSI), condition factor (K), splenic somatic index (SSI), gonadosomatic index (GSI) and a modified health assessment index (HAI) ~ 6-8 hours post-capture (as described in Adams *et al.* 1999 and Barton *et al.* 2002 but see McConnachie, 2010). More specifically, based on liver measurements, a major glycogen reserve in fish, the hepato-somatic index ( $HSI = (\text{liver mass}/\text{body mass}) \times 100$ ) was used as an indicator of individual energy and nutritional status (Chellappa *et al.* 1995). Similarly, condition factor ( $K = \text{mass} \times \text{length}^{-3}$ ) was used as an alternative, all-encompassing means of assessing nutrimental state (*i.e.*, energy storage and feeding activity) as variations in

length-weight measurements were monitored between treatment groups (Bolger and Connolly, 1989; Barton *et al.* 2002). Commonly employed to assess immune function and disease resistance (*e.g.* Hadidi *et al.* 2008), the splenic index (SSI = (spleen mass/body mass)  $\times$  100) was included to determine whether cortisol treatment impaired fish resilience compared to sham-treated and control groups. The gonadosomatic index (GSI = (testis or ovarian mass/body mass)  $\times$  100) was used to assess alterations in reproductive endocrine function to indicate a potential consequence of reproductive impairment of a treatment group (Thomas, 1988). Lastly, a modified health assessment index was employed to quantitatively assess the effects that elevated cortisol levels have on fish health, across different landscape patterns. To this end, index variables suspected to differ among treatment groups were assessed, based on necropsy observations, and were assigned numerical values according to the condition of the organs or tissues (*sensu* Adams *et al.* 1993). The variables chosen included: total parasite load (each organ [*i.e.*, gills, liver, kidney and spleen] affected was given a score of 0=none, 5=low, 10=moderate, 15=high), gill condition (fraying was given a score of 0=none, 5=low, 10=moderate, 15=high), and dermal fungus (cover was scored based on 0=none, 5=low, 10=moderate, 15=high) as previously outlined in McConnachie (2010).

### *Statistical Analysis*

Recapture rates of cortisol-, sham-treated and control fish were compared across land use types by using a  $3 \times 3$  chi-square test. A two-way mixed analysis of variance (ANOVA), with land type (*i.e.*, undisturbed, agricultural or urban) entered as a fixed effect, and stream (nested within land type) entered as a random effect, was used to test for differences among response variables across land use types (*i.e.*, blood glucose

concentrations, HSI, K, SSI, GSI and HAI; Zar, 1999). Levene's and Shapiro-Wilk tests were used to ensure the assumptions of normality of the sampling distribution and homogeneity of variance were met (Zar, 1999). In the event that one of these assumptions was violated, square-root and log-transformations were employed. When a significant main effect or interaction was present, a Tukey-Kramer HSD post hoc test was employed to separate means. All statistical analyses were performed using JMP version 4 (SAS Institute, Cary, NC, USA), with exception to the chi-square test which was performed using IBM SPSS Statistics 19.0 (IBM Corporation, Armonk, NY). The level of significance for all tests was  $\alpha = 0.05$ .

## **Results**

Over the course of this study, contingency table analysis showed that there was no difference in recapture rates among land use types (Table 3-3; Chi-square,  $df = 2$ ,  $p = 0.51$ ) or treatment (Chi-square,  $df = 2$ ,  $p = 0.95$ ). Nevertheless, it is noteworthy that within agricultural streams, cortisol-treated fish were recaptured at a rate of almost half that of the control and sham-treated fish, however, differences were not statistically significant (Chi-square,  $df = 2$ ,  $p = 0.060$ ).

Land type (Table 3-4; ANOVA,  $F = 0.6$ ,  $df = 2, 6$ ,  $p = 0.56$ ), treatment (Table 3-4 and 3-5; ANOVA,  $F = 0.5$ ,  $df = 2, 241$ ,  $p = 0.61$ ) or their interaction (Table 3-4; ANOVA,  $F = 1.5$ ,  $df = 4, 241$ ,  $p = 0.12$ ) had no influence on fish glucose concentrations. Similarly, condition factor was also not affected by land type (Table 3-4; ANOVA,  $F = 1.7$ ,  $df = 2, 6$ ,  $p = 0.26$ ), treatment (Table 3-4 and 3-5; ANOVA,  $F = 0.61$ ,  $df = 2, 255$ ,



$p = 0.54$ ) or the interaction between treatment and land type (Table 3-4; ANOVA,  $F = 0.09$ ,  $df = 4$ , 255,  $p = 0.98$ ).

While HSI did not significantly differ across land types (Table 3-4; ANOVA,  $F = 0.7$ ,  $df = 2$ , 6,  $p = 0.53$ ), treatment (Table 3-4 and 3-5; ANOVA,  $F = 8.8$ ,  $df = 2$ , 254,  $p < 0.01$ ) and the interaction between treatment and land use (Table 3-4; ANOVA,  $F = 3.8$ ,  $df = 4$ , 254,  $p < 0.01$ ) had an effect on HSI. More specifically, within the agricultural watersheds, the HSI of fish receiving a sham treatment was  $\sim 20\%$  greater than fish in the control treatment (Table 3-4; Tukey HSD,  $p < 0.05$ ). Conversely, SSI was not found to be significantly different across land types (Table 3-4; ANOVA,  $F = 5.1$ ,  $df = 2$ , 6,  $p = 0.051$ ), treatments (Table 3-4 and 3-5; ANOVA,  $F = 1.1$ ,  $df = 2$ , 254,  $p = 0.33$ ), or between their interactions (Table 3-4; ANOVA,  $F = 0.6$ ,  $df = 4$ , 254,  $p = 0.66$ ). Likewise, no land type (Table 3-4; ANOVA,  $F = 0.3$ ,  $df = 2$ , 6,  $p = 0.75$ ), treatment (Table 3-4 and 3-5; ANOVA,  $F = 0.48$ ,  $df = 2$ , 253,  $p = 0.62$ ) or interaction effect (Table 3-4; ANOVA,  $F = 1.1$ ,  $df = 4$ , 253,  $p = 0.35$ ) was found to effect GSI values.

No difference in total parasite load was found across land use (Table 3-4; ANOVA,  $F = 0.2$ ,  $df = 2$ , 6,  $p = 0.81$ ), treatment (Table 3-4 and 3-5; ANOVA,  $F = 2.8$ ,  $df = 2$ , 205,  $p = 0.61$ ) or their interactions (Table 3-4; ANOVA,  $F = 0.6$ ,  $df = 4$ , 205,  $p = 0.65$ ). Gill fraying was also unaffected by land use (Table 3-4; ANOVA,  $F = 0.3$ ,  $df = 2$ , 6,  $p = 0.73$ ), treatment (Table 3-4 and 3-5; ANOVA,  $F = 1.9$ ,  $df = 2$ , 254,  $p = 0.15$ ) and their interaction (Table 3-4; ANOVA,  $F = 0.16$ ,  $df = 4$ , 254,  $p = 0.96$ ).

## Discussion

Our approach involved identifying streams that were broadly characterized as either reference, urban, or agricultural, and then experimentally manipulating the cortisol concentration of creek chub in replicates of each stream type. Generally speaking, agricultural streams were relatively shallow, contained little foliage within the riparian zone, and possessed few forms of structure within the watercourse, which can provide cover and protection to resident fish. Agricultural activity requires clearing of foliage, which can lead to increases in water temperature, sediment load, and nutrient inputs (Muscutt *et al.* 1993; Matson *et al.* 1997). Of our chosen study sites, agricultural streams were found to contain the lowest percentage of forest habitat, which has been found to decrease the overall abundance of fish as the length of non-forested riparian patches increases (Jones *et al.* 1999). Indeed, anecdotally we observed the lowest abundance of creek chub in agricultural streams (Nagrodski, unpublished data). Comparatively, urban streams were found to have small buffered areas (ranging between 0 – 30m), consisting of mostly trees and foliage, separating the surrounding residential areas from the watercourse. While sampling our study sites, urban streams were also found to have the highest number of culverts, stormwater inputs, length of road through the catchment (> 30km), and in-stream litter (mainly in the form of residential dumping and litter). Consequences associated with high levels of urbanization surrounding streams include flashier hydrographs, elevated concentrations of nutrients and contaminants, and altered channel morphology which can translate to effects on population dynamics (reviewed in Walsh *et al.* 2005). Although our reference sites contained the most forested land cover, some levels of development made it impossible to consider them as pristine

environments. Nonetheless, land-use activities and their associated impacts on habitat quality were certainly reduced relative to the other two land-use types. Hence, we are confident that fish in the three stream types experienced differential challenges and environmental conditions prior to exposing them to our experimental stressor.

Despite chronically elevating circulating plasma cortisol concentration for a ~3 day period, we did not observe a significant effect of land-use type or treatment on survival when all factors were incorporated into a contingency table analysis. Nonetheless, if one looks only at the agricultural site, the cortisol treated fish were recaptured ~50% less frequently than the controls and the shams. While this result was not statistically significant (*i.e.*,  $p = 0.060$ ), it is ecologically relevant as this finding is consistent with our prediction in that agricultural streams were the most degraded and survival was reduced in cortisol-treated fish relative to control and sham-treated fish. Although there is not much literature to use for interpretation, it is worth noting that this finding is consistent with work on birds and amphibians where stress responses are mediated by habitat quality (Homan *et al.* 2003; Chávez-Zichinelli *et al.* 2010).

Overall, we failed to recapture ~75% of fish that we tagged in the experiment. We used recapture as a proxy for survival and there were several limitations with that approach. First, it assumes that our marks will be retained. However, as VI Alpha tags exhibited poor retention rates in the field, we relied largely on fin clips to differentiate between treatments groups. Our initial goal was to also measure growth of individuals but significant loss of VI Alpha tags meant that we could not track individuals through time and thus could only rely on condition factor to assess energy status. In particular, the loss of VI Alpha tags prohibited us from detecting whether growth depression

occurred across treatment groups, which has been documented as a consequence of stress in fish (*e.g.*, Ali *et al.* 2003). Another issue with mark recapture approaches is that, to evaluate survival, one must assume that animals are restricted to the study reach. That was not the case in the current study, and other work by our group (Nagrodski, Chapter 2) has revealed using radio telemetry that creek chub are somewhat mobile and tend to exhibit leptokurtic movement distributions, as fish had high turnover rates (*i.e.*, proportion of population that moves) with low displacement distances (*i.e.*, distance that each fish moves). A third assumption of mark-recapture studies is that gross behaviour is similar among treatments (*i.e.*, cortisol, sham and control) such that probability of recapture is equal among groups. In Chapter 2 we revealed that behaviour was similar between control and cortisol-treated fish, suggesting that this third assumption was not violated. As such, we are confident in the relative differences among groups and systems is a proxy for survival, but suggest that future research quantify survival of individuals, possibly with passive integrated transponders or radio telemetry, such that fish survival can be evaluated over greater temporal and spatial scales.

We did not observe any significant differences in health (*i.e.*, parasite burden) or condition (*i.e.*, condition factor, hepato-somatic, splenic, and gonado-somatic indices, blood glucose) of creek chub relative to land-use or experimental treatment. However, one exception occurred within the agricultural watersheds, where we observed that sham-treated fish had HSI values that were approximately 20 % higher than control fish. Although an increase in HSI values can suggest exposure to contaminants (*e.g.*, Fletcher *et al.* 1982), while a decrease is an indicator of chronic environmental stress (*e.g.*, Lee *et al.* 1983), we believe this difference is more likely attributed to the relatively low number

of recaptured fish within the agricultural watersheds. In light of the fact that all other response variables do not vary, and that both sham and control treatments have functionally very similar effects on fish, it seems unlikely that this result is ecologically significant. Nevertheless, the lack of variation across treatments groups is surprising as previous work has shown chronic elevation of cortisol levels can lead to negative consequences for fish, including reduce body weight and condition factor (Davis *et al.* 1985), hepatic glycogen content (Peters *et al.* 1980), and immune function (Maule *et al.* 1989). Moreover, as studies in the past have also been able to make connections between deleterious environmental conditions and reduced growth (Pickering, 1993), immune function (Bly and Clem, 1992), and gill morphology (Laurent and Perry, 1991) our results indicating that fish respond similarly to cortisol manipulation across land type is equally surprising. It is possible that given our inability to recapture ~75% of fish that were experimentally manipulated that our physiological and condition-based sampling focused on the healthiest fish that were still alive. However, given that recapture rates were rather consistent across most treatments, this would likely only be a reasonable scenario for the cortisol-treated fish in the agricultural treatment where we did observe evidence of treatment-induced mortality.

Results from the current study suggest that, in the wild, there may be compensatory mechanisms that enable creek chub to persist despite exposure to significant challenge(s). Similarly to mechanisms proposed by Wingfield and Sapolsky (2003), we reason that the compensatory mechanisms employed by creek chub may include: (i) down-regulation of central nervous system or glucocorticoid receptors, (ii) blockage of the hypothalamic-pituitary-adrenal axis thus reducing further secretion of

glucocorticosteroids and accentuating the consequences of the stress response, or (iii) stimulation of an alternate axis capable of counteracting the consequences associated with elevated glucocorticoids. However, as creek chub are regarded as a rather tolerant organism it is unknown if other species would respond differently, or if greater levels of experimental stress or habitat degradation would result in negative alterations in fish health, condition, and survival. Pottinger et al. (2000) has identified the European chub (*Leuciscus cephalus*), a close relative of the creek chub, as having high blood cortisol levels with low cortisol receptor affinity, which may offset some adverse effects associated with high circulating plasma cortisol concentrations. Although these results do not directly apply to creek chub, our results suggest that this species may possess a trait similar to the European chub, making them more tolerant as opposed to other traditional species studied (e.g., salmonids, centrarchids). As noted by Pottinger et al. (2000), similar strategies for glucocorticoid resistance has been documented in some species of rodents and New World primates which may also make them more resistant to stressful events (e.g., Taymans et al. 1997; Reynolds et al. 1997; Hastings et al. 1999).

When encountering environmental stress associated with anthropogenic alterations to natural landscapes, animals can exhibit physiological and behavioural responses that can have population level effects on resident fish species (Ricklefs and Wikelski, 2002). Given that we may have observed reduced survival in cortisol-treated fish in the most degraded (i.e., agricultural streams), there was still remarkable resilience of creek chub to what we regard as a significant stressor in that health, condition, and physiology were not affected (i.e., only HSI significant but not for cortisol). While it is difficult for our results to be extrapolated to other fish species, any compensatory

mechanisms that allowed creek chub to overcome our cortisol challenge potentially exist in other species. As such, further experimentation to determine whether fish native to reference streams have similar reactions to challenges, when transferred to urban or agricultural streams, may be of benefit as reference fish with reduced familiarity to anthropogenic stressors may have a lower capacity to cope with multiple imposed challenges.

This study is one of the first to use experimental cortisol implants to study how fish respond to experimental stress challenges in the wild, and provides insight as to how multiple stressors may affect fish in streams relative to different types of adjacent land use and stream habitat quality. While the capacity of creek chub to respond to stressors is encouraging, in the face of increased human development, experiments determining how these results apply to other species would provide further insight when making management decisions. With increased interest in studying the consequences of habitat quality on organismal condition and survival, as well as a growing toolbox for doing so (Homyak, 2010), we would anticipate more of such studies in the near future.

## Tables

**Table 3-1.** Summary of riparian land-cover and associated classifications for study sites.

Stream	Classification	Reach length (m)	Road Length (km)	%Riparian Land Cover		
				Crop	Forest	Urban
Sawmill Creek	Urban	336.6	30.1	3.0	55.9	32
Shields Creek	Urban	501.5	35.9	10.0	13.1	54.8
Castor Stream	Urban	510.5	32.8	19.0	34.5	38.3
Middle Castor	Agricultural	534.7	4.6	40.1	30.5	16.5
East Castor	Agricultural	610.0	14.4	74.1	25.1	0
Tributary of Jock	Agricultural	739.3	0.4	86.9	13.1	0
Stevens Creek	Reference	437.6	2.0	22.0	62.0	0
Keelers Creek	Reference	260.0	8.1	26.6	67.9	0
Hobbs Drainage	Reference	348.5	21.1	29.1	60	3.3

\* Values adapted from Eastern Ontario Reference Condition & Biocriteria Project database provide by the Ministry of the Environment



**Table 3-2.** The classification, date sampled, electrofishing effort for initial fish collection, and date re-visited for fish recapture at each of the study streams.

Stream	Classification	Initial Electrofishing Effort (sec)	Dates (in 2011)		Days In-between Sampling
			Injections	Recap	
Sawmill Creek	Urban	4945	June 07	July 02 & 04	25 & 27
Middle Castor	Agricultural	4195	June 09	July 05 & 06	26 & 27
Stevens Creek	Reference	3926	June 13	July 07 & 08	24 & 25
Shields Creek	Urban	2320	June 15	July 10	25
East Castor	Agricultural	3872	June 20	July 14 & 15	24 & 25
Keelers Creek	Reference	1883	June 21	July 16	25
Castor Stream	Urban	3700	June 24	July 19 & 20	25 & 26
Hobbs Drainage	Reference	3607	June 29	July 21	22
Tributary of Jock	Agricultural	3607	June 30	July 25 & 26	25

**Table 3-3.** Total number of creek chub captured, released and recaptured ~25 days post treatment per land use type.

	Captured Fish								
	Reference Streams			Agricultural Streams			Urban Streams		
	Control	Cortisol	Sham	Control	Cortisol	Sham	Control	Cortisol	Sham
Total Fish Captured	135	124	124	99	101	102	109	108	107
Fish Recaptured	35	36	37	26	16	30	27	32	21
Percent Recaptured (%)	25.9	29.0	29.8	26.3	15.8	29.4	24.8	29.6	19.6

**Table 3-4.** Results of two-way mixed ANOVAs, with stream as a random effect, nested within land use, testing the impact of land use on stress response following a plasma cortisol challenge. “†” refers to variables whose data were square root transformed to meet assumptions of normality, while “††” refers to variables whose data were log-transformed to meet assumptions of normality.

Variable	Source	df	F	P
Glucose (mmol L <sup>-1</sup> ) <sup>†</sup>	Land Use	2	0.6	0.56
	Treatment	2	0.5	0.61
	Land × Treatment	4	1.5	0.20
K	Land Use	2	1.7	0.26
	Treatment	2	0.6	0.54
	Land × Treatment	4	0.09	0.98
HSI <sup>†</sup>	Land Use	2	0.7	0.53
	Treatment	2	8.8	<b>0.0002</b>
	Land × Treatment	4	3.8	<b>0.0047</b>
SSI <sup>††</sup>	Land Use	2	5.08	0.051
	Treatment	2	1.1	0.33
	Land × Treatment	4	0.6	0.66
GSI	Land Use	2	0.3	0.75
	Treatment	2	0.5	0.62
	Land × Treatment	4	1.1	0.35
Total Parasite Load (out of 60) <sup>††</sup>	Land Use	2	0.2	0.81
	Treatment	2	2.8	0.061
	Land × Treatment	4	0.6	0.65
Gill fray (out of 15)	Land Use	2	0.3	0.73
	Treatment	2	1.9	0.15
	Land × Treatment	4	0.2	0.96

**Table 3-5.** Means ( $\pm$  SE) of response variables, ~25 days after treatment, across reference, agriculture and urban streams. “†” refers to variables whose data was square root transformed, while “††” refers to variables whose data was log-transformed.

Variable	Averages $\pm$ SE								
	Reference Streams			Agricultural Streams			Urban Streams		
	Control	Cortisol	Sham	Control	Cortisol	Sham	Control	Cortisol	Sham
Glucose (mmol L <sup>-1</sup> ) <sup>†</sup>	2.3 $\pm$ 0.07	2.3 $\pm$ 0.1	2.3 $\pm$ 0.1	2.7 $\pm$ 0.07	2.5 $\pm$ 0.2	2.6 $\pm$ 0.1	2.7 $\pm$ 0.1	2.8 $\pm$ 0.1	2.4 $\pm$ 0.1
K (10 <sup>-5</sup> $\pm$ 10 <sup>-7</sup> )	1.02 $\pm$ 1.3	1.03 $\pm$ 1.4	1.03 $\pm$ 1.6	1.01 $\pm$ 1.6	1.02 $\pm$ 1.9	1.03 $\pm$ 1.7	1.05 $\pm$ 1.9	1.07 $\pm$ 1.7	1.07 $\pm$ 2.2
HSI <sup>†</sup>	2.2 $\pm$ 0.1	2.8 $\pm$ 0.2	2.6 $\pm$ 0.1	1.9 $\pm$ 0.1	2.4 $\pm$ 0.1	2.4 $\pm$ 0.1	2.6 $\pm$ 0.1	2.5 $\pm$ 0.1	3.0 $\pm$ 0.1
SSI <sup>††</sup>	0.3 $\pm$ 0.01	0.3 $\pm$ 0.02	0.3 $\pm$ 0.02	0.3 $\pm$ 0.05	0.4 $\pm$ 0.05	0.4 $\pm$ 0.03	0.4 $\pm$ 0.02	0.4 $\pm$ 0.02	0.4 $\pm$ 0.02
GSI	0.8 $\pm$ 0.08	0.8 $\pm$ 0.09	0.6 $\pm$ 0.09	0.7 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.2	0.8 $\pm$ 0.1
Total Parasite Load (out of 60) <sup>††</sup>	16.4 $\pm$ 2.1	18.4 $\pm$ 2.2	15.6 $\pm$ 2.1	6.7 $\pm$ 1.0	12.6 $\pm$ 2.5	10.2 $\pm$ 2.0	15.2 $\pm$ 2.2	16.2 $\pm$ 1.9	15.7 $\pm$ 2.1
Gill Fraying (out of 15)	5 $\pm$ 0.8	6.4 $\pm$ 0.8	5.6 $\pm$ 0.8	5.3 $\pm$ 0.9	6.2 $\pm$ 1.5	6.2 $\pm$ 0.9	4.2 $\pm$ 0.8	6.4 $\pm$ 1.0	4.8 $\pm$ 0.9

## Chapter 4: General Discussion

Little is known about the “ecology of stress” in wild fish, as much of our understanding is based upon the physiological and endocrine responses of stress in captive or cultured teleost fish, under laboratory settings (Pankhurst, 2010). However, as an increased range of anthropogenic stressors are imposed upon wild fish, it is necessary to assess the level to which human land-use practices affect overall fish performance. As such, experimental evaluations of stressors within natural landscapes can provide us with an indication as to how wild fish will respond to future environmental stressors. In particular, monitoring an individual’s physiological metrics across different land use regimes (*e.g.*, urbanization and agriculture) allows inferences to be made about an organism’s performance within particular environments (Wikelski and Cooke, 2006; Cooke and Suski, 2008). This thesis is a compilation of two complementary studies that together provide greater insight into how stress can influence the physiology, health, behaviour, (including activity and movement) and survivorship of wild stream fish. More specifically, Chapter 2 evaluates how the experimental elevation of plasma cortisol concentrations affects the activity, movement, and survival of creek chub in a mesocosm (fine-scale activity) and stream (large-scale movement). Chapter 3 examines the effects of a chronic cortisol elevation on the health, condition, and survival of creek chub across replicate streams, representing a gradient of stream habitat quality (*i.e.*, urban, agricultural, and reference). Taken together, the results of this thesis contribute to our understanding of the ecology of stress on wild fish which could have management implications for future restoration and rehabilitation activities.

### *Findings and Implications:*

In Chapter 2, it was found that despite being subjected to an ecologically relevant cortisol challenge, causing prolonged (~3 day) elevations of in plasma cortisol concentrations, fine-scale movements between cortisol-, sham-treated and control fish as well as large-scale movements between cortisol-treated and control fish were consistent across treatments. However, differences in mortality were observed starting 10 days after treatment whereby cortisol-treated fish exhibited nearly twice as many mortalities as their sham and control counterparts. Results of this nature suggest that while the cortisol manipulations were sufficient to cause mortalities in some individuals, compensatory mechanisms may have helped them maintain behaviours (*i.e.*, activity and movement) prior to death. Individual variations among treatment groups were not unexpected or surprising as past studies with similar findings have attributed individual variation to individual's capacity to respond and survive a challenge (*e.g.*, van Raaji *et al.* 1996)

In Chapter 3, our overall survival analysis failed to detect differences, however, closer evaluation of survival within agricultural sites revealed that, while not quite statistically significant (*i.e.*,  $p = 0.06$ ), cortisol-treated fish had ~50% more mortality than control and sham-treated fish. Additionally, the health (*i.e.*, parasite burden), and condition (*i.e.*, condition factor, hepato-somatic, splenic, and gonado-somatic indices, blood glucose) of cortisol-, sham-treated and control creek chub was found to be similar across all studied land-use patterns, with one exception being found within the agricultural watersheds; as the hepato-somatic index (HSI) calculated for the sham-treated fish was approximately ~20% larger than that found in control fish. Nevertheless, despite subjecting fish to experimental elevations in cortisol titers for an extended period

(~3 days), and using fish from streams across a gradient of habitat quality, none of the ecologically-relevant sublethal endpoints we measured were affected. Results here may suggest that in the wild, compensatory mechanisms may enable fish to persist during even multiple stressors.

Together, these studies are among some of the first attempts to using experimental cortisol implants outside of a laboratory environment to study how wild stream fish respond to experimental challenges, during a non-reproductive period. Collectively, these results suggest that when subjected to multiple challenges, creek chub possess compensatory mechanisms that allow them to experience few ill effects (at least using the ecologically-relevant endpoints used here) while coping with multiple stressors, despite inhabiting area with drastically different types of adjacent land use. However, as creek chub are regarded as a rather tolerant organism it is unknown whether other species would respond differently, or if greater levels of experimental stress or habitat degradation would result in negative alterations in fish health, condition, and survival.

#### *Future Research Directions*

While this body of work has contributed to our understanding of the ecology of stress in wild fish, findings have also revealed several opportunities for further research. For example, while both Chapter 2 and 3 found chronic elevations of plasma cortisol concentrations to have negligible effect on the behaviour, and condition of creek chub, it is unclear whether these results can be applied to other species. Potentially, species that exhibit different movement patterns or inhabit different systems may be more or less susceptible to experimental cortisol manipulations. In addition, different fish species

have varying environmental tolerances and physiological capacities that would be expected to influence the sensitivity of fish to stressors. Even cortisol receptor sensitivity, stress responsiveness, and baseline levels of circulating cortisol would be expected to vary among species. Furthermore, as all experiments were carried out during the non-reproductive period of creek chub, we are unable to evaluate the effects of a chronic cortisol elevation on the reproductive success of creek chub within different land-use patterns. While this is largely speculative, possibly the lower abundances observed throughout our studied agricultural sites were a function of reduced reproductive success due to an inability to cope with multiple stressors during the spawning period. Future experiments should evaluate how a range of species with different levels of sensitivity to environmental change respond to experimental cortisol manipulations. In addition, future studies should monitor fish over longer time periods and evaluate the role of repeated stressors (*e.g.*, multiple injections) on fish.



## References

- Adams, S.M., A.M. Brown, and R.W. Goede. 1993. A quantitative health assessment index for rapid evaluation of fish condition in the field. *Transactions of the American Fisheries Society* **122**: 63-73.
- Adams, S.M., and M.S. Greely. 2000. Ecotoxicological indicators of water quality: using multi-response indicators to assess the health of aquatic ecosystems. *Water, Air & Soil Pollution* **123**: 103-115.
- Adams, S.M., and K.D. Ham. 2011. Application of biochemical and physiological indicators for assessing recovery of fish populations in a disturbed stream. *Environmental Management* **47**: 1047-1063.
- Ali, M., A. Nicieza, and R.J. Wootton. 2003. Compensatory growth in fishes: a response to growth depression. *Fish and Fisheries* **4**: 147-190.
- Allan, J.D., D.L. Erickson, and J. Fay. 1997. The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology* **37**: 149-161.
- Allan, J.D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Reviews of Ecology, Evolution, and Systematics* **35**: 257-84.

- Almeida, J.S., P.C. Meletti, and C.B.R. Martinez. 2005. Acute effects of sediments taken from an urban stream on physiological and biochemical parameters of the neotropical fish *Prochilodus lineatus*. *Comparative Biochemistry and Physiology Part C* **140**: 357-363.
- Anderson, W.G., R.S. McKinley, and M. Colavecchia. 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. *North American Journal of Fisheries Management* **17**: 301-307.
- Barton, B.A. 2002. Stress in Fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* **42**: 517-525.
- Barton, B.A., J.D. Morgan, and M.M. Vijayan. 2002. Physiological and condition-related indicators of environmental stress in fish. Pp. 111-148 in Adams, S.M (ed). *Biological indicators of aquatic ecosystem stress*. American Fisheries Society, Bethesda Maryland.
- Barton, B.A., and G.K. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases* **1**: 3-26.

- Belica, L.A.T., and F.J. Rahel. 2008. Movement of creek chubs, *Semotilus atromaculatus*, among habitat patches in a plains stream. *Ecology of Freshwater Fish* **17**: 258-272.
- Binder, T.R., and D.G. McDonald. 2008. The role of temperature in controlling diel activity in upstream migrant sea lampreys (*Petromyzon Marinus*). *Canadian Journal of Fisheries and Aquatic Science* **65**: 1113-1121.
- Blevins, Z.W., E.L. Effert, D.H. Wahl and C.D. Suski. 2010. The effects of land use disturbance on the physiological properties of a resident stream fish. *Unpublished manuscript*.
- Bly, J.E., and L.W. Clem. 1992. Temperature and teleost immune functions. *Fish & Shellfish Immunology* **2**: 159-171.
- Bolger, T., and P.L. Connolly. 1989. The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology* **34**: 171-182.
- Brown, R.S., J.C. Brodeur, G. Power, S.F. Daly, K.D. White, and R.S. McKinley. 1999. Blood chemistry and swimming activity of rainbow trout exposed to supercooling and frazil ice. Pp. 97-110 in: Doering, J.C. (ed). *Proceedings of the 10<sup>th</sup> Workshop of River Ice*.

- Burton, M.N. 1995. Have fishes had their chips? The dilemma of threatened fishes. *Environmental Biology of Fishes* **43**: 1-27.
- Camargo, M.M.P., and C.B.R. Martinez. 2006. Biochemical and physiological biomarkers in *Prochilodus lineatus* submitted to in situ tests in an urban stream in southern Brazil. *Environmental Toxicology and Pharmacology* **21**: 61-69.
- Chávez-Zichinelli, C.A., I. MacGregor-Forsa, P.T. Rohanab, R. Valdéz, M.C. Romanob, and J.E. Schondube. 2010. Stress response of the House Sparrow (*Passer domesticus*) to different urban land uses. *Landscape and Urban Planning* **98**: 183-189.
- Chellappa, S., F.A. Huntingford, R.H.C Strang, and R.Y. Thomson. 1995. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *Journal of Fish Biology* **47**: 775-787.
- Clements, W.H., D.M. Carlisle, J.M. Lazorchak, and P.C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. *Ecological Applications* **10**: 626-628.
- Cook, K.V. 2011. Individual variability and fitness consequences of stress-induced cortisol in wild fish. Master's Thesis. Carleton University, Ottawa, ON.

Cooke, S.J., B.D.S. Graeb, C.D. Suski, and K.G. Ostrand. 2003. Effects of suture material on incision healing, growth and survival of juvenile largemouth bass implanted with miniature radio transmitters: case study of a novice and experienced fish surgeon. *Journal of Fish Biology* **62**: 1366-1380.

Cooke, S.J., and C.M. O'Connor. 2010. Making conservation physiology relevant to policy makers and conservation practitioners. *Conservation Letters* **3**: 159-166.

Cooke, S.J., and C.D. Suski. 2008. Ecological Restoration and Physiology: as overdue integration. *BioScience* **58**: 957-968.

Cooke, S.J., C.D. Suski, S.E. Danylchuk, A.J. Danylchuk, M.R. Donaldson, C. Pullen, G. Bulté, A. O'Toole, K.J. Murchie, J.B. Koppelman, A.D. Shultz, E. Brooks, and T.L. Goldberg. 2008. Effects of different capture techniques on the physiological conditions of bonefish *Albula vulpes* evaluated using field diagnostic tools. *Journal of Fish Biology* **73**: 1351-1375.

Davis, K.B., P. Torrance, N.C. Parker, and M.A. Suttle. 1985. Growth, body composition and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, *Ictalurus punctatus* Rafinesque. *Journal of Fish Biology* **27**: 177-184.

Dey, C.J., C.M. O'Connor, K.M. Gilmour, G. Van Der Kraak, and S.J. Cooke. 2010.

Behavioral and physiological responses of a wild teleost fish to cortisol and androgen manipulation during parental care. *Hormones and Behavior* **58**: 599-605.

DiBattista, J.D., H. Anisman, M. Whitehead, and K. Gilmour. 2005. The effects of cortisol administration on social status and brain monoaminergic activity in rainbow trout *Oncorhynchus mykiss*. *The Journal of Experimental Biology* **208**: 2707-2718.

Dietz, T., E.A. Rosa, and R. York. 2007. Driving the human ecological footprint. *Frontiers in Ecology and the Environment* **5**: 13-18.

Doherty, C.A., B. Galloway, R.A. Curry, and K.R. Munkittrick. 2005. Performance of white sucker populations along the Saint John River Main Stem, New Brunswick, Canada: an example of effects-based cumulative effects assessment. *Water Quality Research Journal of Canada* **40**: 361-373.

Fevolden, S.E., R. Nordmo, T. Refstie, and K.H. Røed. 1993. Disease resistance in Atlantic salmon (*Salmo salar*) selected for high or low responses to stress. *Aquaculture* **109**: 215-224.

Fitzgerald, D.G., R.P. Lanno, and D.G. Dixon. 1999. A comparison of a sentinel species evaluation using creek chub (*Semotilus atromaculatus Mitchill*) to a fish community evaluation for the initial identification of environmental stressors in small streams. *Ecotoxicology* **8**: 33-48.

Fletcher, G.L., M.J. King, J.W. Kiceniuk, and R.F. Addison. 1982. Liver hypertrophy in winter flounder following exposure to experimental oiled sediments. *Comparative Biochemistry and Physiology, Part C* **73**: 457-462.

Flodmark, L.E.W., H.A. Urke, J.H. Hallraker, J.V. Arnekleiv, L.A. Vøllestad, and A.B.S. Poléo. 2002. Cortisol and glucose responses in juvenile brown trout subjected to a fluctuating flow regime in an artificial stream. *Journal of Fish Biology* **60**: 238-248.

Foley, J.A., R. DeFries, G.P. Asner, C. Barford, G. Bonan, S.R. Carpenter, F.S. Chapin, M.T. Coe, G.C. Daily, H.K. Gibbs, J.H. Helkowski, T. Holloway, N. Ramankutty, and P.K. Snyder. 2005. Global consequences of land use. *Science* **309**: 570-574.

Fry, F.E.J. 1947. *Effects of the Environment on Animal Activity*. Toronto, ON: University of Toronto Press.

Gamperl, A.K., M.M. Vijayan, and R.G. Boutilier. 1994. Experimental control of stress hormone levels in fish: techniques and application. *Reviews in Fish Biology and Fisheries* **4**: 215-255.

Gatz, A.J., and R.S. Linder. 2008. Effects of repeated electroshocking on condition,

growth, and movement of selected warmwater stream fishes. *North American Journal of Fisheries Management* **28**: 792-798.

Gradall, K.S., and W.A. Swenson. 1982. Responses of brook trout and creek chubs to turbidity. *Transaction of the American Fisheries Society* **111**: 392-395.

Gray, M.A., and K.R. Munkittrick. 2005. An effect-based assessment of slimy sculpin (*Cottus cognatus*) population in agricultural regions of northwestern New Brunswick. *Water Quality Research Journal of Canada* **40**: 16-27.

Gregory, S.V., F.J. Swanson, W.A. McKee, and K.W. Cummins. 1991. An ecosystem perspective of riparian zones. *BioScience* **41**: 540-551.

Gregory, T.R., and C.M. Wood. 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* **72**: 286-295.

Hadidi, S., G.W. Glenney, T.J. Welch, J.T. Silverstein, and G.D. Weins. 2008. Spleen size predicts resistance of rainbow trout to flavobacterium psychrophilum challenge. *Journal of Immunology* **180**: 4156-4165.

Hanson, K.C., C.T. Hasler, S.J. Cooke, C.D. Suski, and D.P. Philipp. 2008. Intersexual



variation in the seasonal behaviour and depth distribution of freshwater temperate fish, the largemouth bass. *Canadian Journal of Zoology* **86**: 801-811.

Hastings, N.B., M. Orchinik, M.V. Aubourg, and B.S. McEwen. 1999. Pharmacological characterization of central and peripheral type I and type II adrenal steroid receptors in the prairie vole, a glucocorticoid-resistant rodent. *Endocrinology* **140**: 4459-4469.

Henley, W.F., M.A. Patterson, R.J. Neves, and A.D. Lemly. 2000. Effects of sedimentation and turbidity on lotic food webs: a concise review for natural resource managers. *Reviews in Fisheries Science* **8**: 125-139.

Herbert, N.A., and J.F. Steffensen. 2005. The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. *Marine Biology* **147**: 1403-1412.

Homan, R.N., J.V. Regosin, D.M. Rodrigues, J.M. Reed, B.S. Windmiller, and L.M. Romero. 2003. Impacts of varying habitat quality on the physiological stress of spotted salamanders (*Ambystoma maculatum*). *Animal Conservation* **6**: 11-18.

Homyack, J.A. 2010. Evaluating habitat quality of vertebrates using conservation physiology tools. *Wildlife Research* **37**: 332-342.

- Hontela, A., C. Daniel, and J.B. Rasmussen. 1997. Structural and functional impairment of the hypothalamo-pituitary-interrenal axis in fish exposed to bleached kraft mill effluent in the St Maurice River, Québec. *Ecotoxicology* **6**: 1-12.
- Hoogenboom, M.O., J.D. Armstrong, M.S. Miles, T. Burton, T.G. Groothuis, and N.B. Metcalfe. 2011. Implantation of cocoa butter reduces egg and hatchling size in *Salmo trutta*. *Journal of Fish Biology* **79**: 587-596.
- Hoskonen, P., and J. Pirhonen. 2006 Effects of repeated handling, with or without anaesthesia, on feed intake and growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* **37**: 409-415.
- Jones, E.B.D., G.S. Helfman, J.O. Harper, and P.V. Bolstad. 1999. Effects of riparian forest removal on fish assemblages on fish assemblages in southern Appalachian streams. *Conservation Biology* **13**: 1454-1465.
- Katz, M., and W.C. Howard. 1955. The length and growth of 0-year class creek chub in relation to domestic pollution. *Transactions of the American Fisheries Society* **84**: 228-238.
- Lankford, S.E., T.E. Adams, R.A. Miller, and J.J. Cech. 2005. The cost of chronic stress: Impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. *Physiological and Biochemical Zoology* **78**: 599-609.

- Laurent, P., and S.F. Perry. 1991. Environmental effects of fish gill morphology. *Physiological Zoology* **64**: 4-25.
- LeBlanc, R. 1997. Modelling the effects of land use change on the water temperature in unregulated urban streams. *Environmental Sciences* **49**: 445-469.
- Lee, R.M., S.B. Gerking, and B. Jezierska. 1983. Electrolyte balance and energy mobilization in acid-stressed rainbow trout, *Salmo gairdneri*, and their relation to reproductive success. *Environmental Biology of Fishes* **8**: 115-123.
- Lenat, D.R., and J.K. Crawford. 1994. Effects of land use on water quality and aquatic biota of three North Carolina Piedmont streams. *Hydrobiologia* **294**: 185-199.
- Lower, N., A. Moore, A.P. Scott, T. Ellis, J.D. James, and I.C. Russell. 2005. A non-invasive method to assess the impact of electronic tag insertion and stress levels in fish. *Journal of Fish Biology* **67**: 1202-1212.
- Lucas, M.C., and E. Baras. 2000. Methods for studying the spatial behaviour of freshwater fishes in their natural environment. *Fish and Fisheries* **1**: 283-316.
- Mainstone, C.P., and W. Parr. 2002. Phosphorous in rivers – ecology and management. *The Science of the Total Environment* **282-283**: 25-47.

- Martínez-Porchas, M., L.R. Martínez-Córdova, and R. Ramos-Enriquez. 2009. Cortisol and glucose: Reliable indicator of fish stress? *Pan-American Journal of Aquatic Sciences* **4**: 158-178.
- Maule, A.G., R.A. Tripp, S.L. Kaattari, and C.B. Schreck. 1989. Stress alters immune function and disease resistance in Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Endocrinology* **120**: 135-142.
- Matson, P.A., W.J. Parton, A.G. Power, and M.J. Swift. 1997. Agricultural intensification and ecosystem properties. *Science* **277**: 504-509.
- McConnachie, S.H. 2010. Understanding the ecological consequences of stress in wild fish using exogenous cortisol implants. Master's Thesis. Carleton University, Ottawa, ON.
- McEwen, B.S. and, J.C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. *Hormones and Behavior* **43**: 2-15.
- McMahon, T.E. 1982. Habitat Suitability Index Models: Creek chub. U.S.D.I. Fish and Wildlife Service. F.W.S./OBS-82/10.4 pp. 23.
- Mesa, M.G. 1994. Effects of multiple acute stressors on the predator avoidance ability

and physiology of juvenile Chinook salmon. *Transactions of the American Fisheries Society* **123**: 786-793.

Mommsen, T.P., M.M. Vijayan, and T.W. Moon. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews on Fish Biology and Fisheries* **9**: 211-268.

Munkittrick, K.R., M.E. McMaster, C. Portt, W.N. Gibbons, A. Farwell, L. Ruemper, M.R. Servos, J. Nickle, and G.J. Van Der Kraak. 2000. The development of cumulative effects assessment tools using fish populations. Chapter 8, pp. 149-174. In: *Integrated Assessment of Ecosystem Health* (Eds: Scow, K.M., Fogg, G.E., Hinton, D.E., Johnson, M.L.), Lewis Publishers, Boca Raton, FL.

Muscutt, A.D., G. L. Harris, S.W. Bailey, and D.B. Davies. 1993. Buffer zones to improve water quality: a review of their potential use in UK agriculture. *Agriculture, Ecosystems & Environment* **45**: 59-77.

Niyogi, D.K., K.S. Simon, and C.R. Townsend. 2003. Breakdown of tussock grass in streams along a gradient of agricultural development in New Zealand. *Freshwater Biology* **48**: 1698-1708

O'Connor, C.M., K.M. Gilmour, R. Arlinghaus, C.T. Hasler, D.P. Philipp, and S.J.

- Cooke. 2010. Seasonal carryover effects following the administration of cortisol to a wild teleost fish. *Physiological and Biochemical Zoology* **83**: 950-957.
- O'Connor, C.M., K.M. Gilmour, R. Arlinghaus, S. Matsumura, C.D. Suski, D.P. Philipp, and S.J. Cooke. 2011. The consequences of short-term cortisol elevation on individual physiology and growth rate in wild largemouth bass (*Micropterus salmoides*). *Canadian Journal of Fisheries and Aquatic Science* **68**: 693-705.
- O'Connor, C.M., K.M. Gilmour, R. Arlinghaus, G. Van Der Kraak, and S.J. Cooke. 2009. Stress and parental care in a wild teleost fish: insights from exogenous supraphysiological cortisol implants. *Physiological and Biochemical Zoology* **82**: 709-719.
- Ostrand, K.S., S.J. Cooke, and D.H. Wahl. 2004. Effects of stress on largemouth bass reproduction. *North American Journal of Fisheries Management* **24**: 1038-1045.
- Øverli, Ø., S. Kotzian, and S. Winberg. 2002. Effects of cortisol on aggression and locomotor activity in rainbow trout. *Hormones and Behaviour* **42**: 53-61.
- Pankhurst, N.W. 2010. The endocrinology of stress in fish: an environmental perspective. *General and Comparative Endocrinology* **170**: 265-275.

- Peters, G., H. Delventhal, and H. Klinger. 1980. Physiological and morphological effects of social stress in the eel (*Anguilla Anguilla* L.). *Archive fur Fischereiwissenschaft* **30**: 157-180.
- Peterson, M.J., R.A. Efroymsen, and S.M. Adams. 2011. Long-term biological monitoring of an impaired stream: synthesis and environmental management implications. *Environmental Management* **47**: 1125-1140.
- Pickering, A.D. 1993. Growth and stress in fish production. *Aquaculture* **111**: 51-63.
- Pickering, A.D., T.G. Pottinger, J. Carragher, and J.P. Sumpter. 1987. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. *General and Comparative Endocrinology* **68**: 249-259.
- Piersma, T., and J. Drent. 2003. Phenotypic flexibility and the evolution of organismal design. *TRENDS in Ecology and Evolution* **18**: 228-233.
- Pottinger T.G., T.R. Carrick, A. Appleby, and W.E. Yeomans. 2000. High blood cortisol levels and low cortisol receptor affinity: is the chub *Leuciscus cephalus* a cortisol-resistant teleost? *General and Comparative Endocrinology* **120**: 108-117.

- Powles, P.M., D. Parker, and R. Reid. 1977. Growth, maturation, and apparent and absolute fecundity of creek chub, *Semotilus atromaculatus* (Mitchill), in the Kawartha Lakes Region, Ontario. *Canadian Journal of Zoology* **55**: 843-846.
- Quinn, J.M., P.M. Brown, W. Boyce, S. Mackay, A. Taylor, and T. Fenton. 2001. Riparian zone classification for management of stream water quality and ecosystem health. *Journal of the American Water Resources Association* **37**: 1509-1515.
- Radeloff, V. C., R. B. Hammer, and S. I. Stewart. 2005. Rural and suburban sprawl in the U. S. Midwest from 1940 to 2000 and its relation to forest fragmentation. *Conservation Biology* **19**:793-805.
- Reynolds, P.D., S.J. Pittler, and J.G. Scammell. 1997. Cloning and expression of the glucocorticoid receptor from the squirrel monkey (*Samimiri boliviensis boliviensis*), a glucocorticoid-resistant primate. *The Journal of Clinical Endocrinology & Metabolism* **82**: 465-472.
- Ricklefs, R.E., and M. Wikelski. 2002. The physiology/life-history nexus. *TRENDS in Ecology & Evolution* **17**: 462-467.
- Roper, B.B., J. J. Dose, and J.E. Williams. 1997. Stream Restoration: Is fisheries biology



enough? *Fisheries* **22**: 6-11.

Rounsefell, G.A., and J.L. Kask. 1945. How to mark fish. *Transactions of the American Fisheries Society* **73**: 320-363.

Roy, A.H., A.D. Rosemond, M.J. Paul, D.S. Leigh, and J.B. Wallace. 2003. Stream macroinvertebrate response to catchment urbanisation (Georgia, USA). *Freshwater Biology* **48**: 329-346.

Sapolsky, R.M., L.M. Romero, and A.U. Munck. 2000. How do glucocorticoids influence stress response? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* **21**: 55-89.

Saunders, D.L., J.J. Meeuwig, and A.C.J. Vincent. 2002. Freshwater protected areas: strategies for conservation. *Conservation Biology* **16**: 30-41.

Schreck, C.B. 2010. Stress and fish reproduction: The roles of allostasis and hormesis. *General and Comparative Endocrinology* **165**: 549-556.

Schreck, C. B. 1990. Physiological, behavioural, and performance indicators of stress. *American Fisheries Society Symposium* **8**: 29-37.

- Schreck, C.B., W. Contreras-Sanches, and M.S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* **197**: 3-24.
- Schreck, C.B., B.L.Olla, and M.W. Davis. 1997. Behavioural responses to stress. In: Fish Stress and Health in Aquaculture. Ed. Iwama GK, Sumpter J, Pickering AD, Schreck CB) pp. 145-169. Cambridge University Press, Cambridge, UK.
- Schjolden, J., D. Basic, and S. Winberg. 2009. Aggression in rainbow trout is inhibited by both MR and GR antagonists. *Physiology and Behavior* **98**: 625-630.
- Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Buletin. No 184, pp. 507-510.
- Selye, H. 1973. The evolution of the stress concept: the originator of the concept traces its development from the discovery in 1936 of the alarm reaction to modern therapeutic applications of syntoxic and catatoxic hormones. *American Scientist* **61**: 692-699.
- Shaw, R.G., and T. Mitchell-Olds. 1993. Anova for unbalanced data: an overview. *Ecology* **74**: 1638-1645.

Sink, T.D., R.T Lochmann, and K.A. Fecteau. 2008. Validation, use and disadvantages of enzyme linked immunosorbent assay kits for detection of cortisol in channel catfish, largemouth bass, red pacu, and golden shiner. *Fish Physiology Biochemistry* **34**: 95-101.

Skalski, G.T., and J.F. Gilliam. 2000. Modeling diffusive spread in a heterogeneous population: a movement study with stream fish. *Ecology* **81**: 1685-1700.

Small, B.C. 2003. Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. *Aquaculture* **218**: 177-185.

Stahl, J.R., and S.L. Sobat. 2000. Creek chub as a sentinel indicator species for relative regional assessment of mercury contamination and monitoring pollutant trends in Indiana rivers and streams. Indiana Department of Environmental Management. Office of Water Management, Assessment Branch, Biological Studies Section, Indianapolis, IN. pp. 37.

Taymans, S.E., A.C. DeVries, M.B. DeVries, R.J. Nelson, T.C. Friedman, M. Castro, S. Detera-Wadleigh, C.S. Carter, and G.P. Chrousos. 1997. The hypothalamic-pituitary-adrenal axis of prairie voles (*Microtus ochrogaster*): Evidence for

target tissue glucocorticoid resistance. *General and Comparative Endocrinology* **106**: 48-61.

Thomas, P. 1988. Reproductive endocrine function in female Atlantic croaker exposed to pollutants. *Marine Environmental Research* **24**: 179-183.

Van der Boon, J., G.E.J.M. van den Thillart, and A.D.F. Addink. 1991. The effects of cortisol administration on intermediary metabolism in teleost fish. *Comparative Biochemistry and Physiology Part A: Physiology* **100**: 47-53.

Van Raaji, M.T.M., D.S.S. Pit, P.H.M. Balm, A.B. Steffens, and G.E.E.J.M. van den Thillart. 1996. Behavioural strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. *Hormones and Behaviour* **30**: 85-92.

Vijayan, M.M., T.P. Mommsen, H.C. Glémet, and T.W. Moon. 1996. Metabolic effects of cortisol treatment in a marine teleost, the sea raven. *The Journal of Experimental Biology* **199**: 1509-1514.

Vitousek, P.M. 1997. Human domination of Earth's ecosystems. *Science* **278**: 21-21.

- Walsh, C.J., A.H. Roy, J.W. Feminella, P.D. Cottingham, P.M. Groffman, and R. Morgan. 2005. The urban stream syndrome: current knowledge and the search for a cure. *Journal of the North American Benthological Society* **24**: 706-723.
- Wendelaar-Bonga, S.B. 1997. The stress response in fish. *Physiology Review* **77**:591–625.
- Wikelski, M., and S.J. Cooke. 2006. Conservation physiology. *TRENDS in Ecology and Evolution* **21**: 38-46.
- Wingfield, J.C. 2005. The concept of allostasis: coping with a capricious environment. *Journal of Mammalogy* **86**: 248-254.
- Wingfield, J.C., and R.M. Sapolsky. 2003. Reproduction and resistance to stress: when and how. *Journal of Neuroendocrinology* **15**: 711-724.
- Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, N.J.