NUTRITIONAL CONDITION AND STRESS RESPONSE OF FISHES ALONG A GRADIENT OF HABITAT QUALITY IN THE ST. LAWRENCE RIVER: PHYSIOLOGICAL CONSEQUENCES OF ANTHROPOGENIC HABITAT DEGRADATION

BY

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THESIS

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ABSTRACT

Anthropogenic alterations to terrestrial habitat (e.g., urbanization, deforestation, agriculture) can have a variety of negative effects on riverine systems that flow through disturbed landscapes. These in-stream changes can alter aquatic population size and community structure; however, the underlying causal mechanisms remain poorly understood. Although patterns in population size presumably reflect underlying variation in organismal health and condition, such individual-level metrics are rarely evaluated in the context of ecosystem disturbance. Using physiological sampling, we can examine the effects of land use on the health of resident fishes, and improve our understanding of the mechanisms causing population declines. Therefore, the goal of my thesis is to define the relationship between human land use, habitat quality, and the physiological condition of stream fish. To accomplish this goal, I performed two distinct studies that examine these relationships in different ways. Chapter 1 quantified blood parameters relating to nutrition, oxidative stress, and the glucocorticoid (GC) stress response across streams differing in land-use practices at the watershed scale. Results from this study demonstrate natural lands (i.e., forests and wetlands) have stronger influences physiological condition than human land uses (e.g., agricultural and urban areas). Streams with the highest proportions of natural lands resulted in greater free energy, resistance to oxidative stress, and glucocorticoid function. In my second chapter, I used tissue cortisol extraction to examine the relationship between stream quality and the glucocorticoid function of a stream fish community. Results revealed that largemouth bass *Micropterus salmoides*, brown bullhead *Ameiurus nebulosus*, and logperch *Percina caprodes* had altered GC function as a result of anthropogenic stream degradation, whereas white sucker *Catostomus commersonii* and pumpkinseed *Lepomis gibbosus* did not. Together, my results show that different species residing in identical habitats can

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demonstrate a variety of responses to environmental stress, highlighting the variation in physiological ability to cope under poor environmental conditions, as well as the difficulty of predicting the GC dynamics in wild animals. These studies have the potential to provide managers, conservationists, and restoration practitioners with mechanistic information on the effects of land use practices on stream fishes, improving our ability to predict and mitigate the consequences of anthropogenic habitat degradation.

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CHAPTER 1: GENERAL INTRODUCTION

Human activities have degraded most ecosystems on earth, with over 83 % of terrestrial landscapes being under direct human impact (Meybeck 2004). This is particularly important for riverine systems because they are heavily influenced by surrounding environments (Allan & Flecker, 1993; Allan, 2004; Hynes, 1975). As a result, these ecosystems are becoming increasingly degraded from human activities, which is concerning for resident fauna considering their limited ability to disperse and escape the affected area (Allan 2004, Olden et al. 2010, Wang et al. 2011). Rivers and streams are home to some of the most diverse and unique ecosystems on the planet. However, habitat loss and degradation has led to a significant decrease in aquatic biodiversity worldwide (Allan and Flecker 1993). Freshwater fishes had the highest extinction rate among vertebrates in the 20th century (Burkhead 2012), while freshwater fauna are projected to have a future extinction rate five times that of their terrestrial counterparts (Ricciardi and Rasmussen 1999). Despite vast literature on human disturbances in lotic ecosystems (see Paul and Meyer 2001; Allan 2004), we lack information on the chain of cause and effect that links landscape changes to community responses, causing major problems for conservation and management.

Streams are heavily influenced by the landscapes through which they flow (Hynes, 1975). As a result, a large portion of anthropogenic influence on streams and their fauna arise from terrestrial activities rather than direct alterations within the stream (Meybeck 2003, Allan 2004). Natural terrestrial features, such as forests and wetlands, are beneficial to stream ecosystems. For example, they stabilize banks, reduce sedimentation, provide in-stream habitat, and regulate hydrologic and thermal regimes (Lenat and Crawford 1994, Richardson 1994, Allan 2004). Human actives often remove these natural features, resulting in drastic changes to stream

characteristics (Meybeck 2003). Urbanization and agriculture are two of the most influential anthropogenic disturbances on lotic habitat quality (Laub and Palmer 2009, Wang et al. 2011). Urbanization directly reduces the amount of riparian vegetation, increases the variety and amount of pollutants, and creates a large amount of impervious surfaces (Paul and Meyer 2001, Allan 2004). Impervious surfaces increase runoff, causing larger and more frequent periods of high flow and leading to habitat destruction, bank destabilization, erosion of streambeds, and sediment displacement (Lenat and Crawford 1994, Poff et al. 1997). Agricultural development has a similar suite of consequences, while also causing an increase in nutrient input and sedimentation of the adjacent waterways (Laub and Palmer 2009, Smith 2009). These effects of land use on streams have direct consequences for resident fauna. Transitions from natural to disturbed landscapes reduce abundance and diversity in macroinvertebrate and fish communities (Lenat and Crawford 1994, Paul and Meyer 2001, Allan 2004, Miserendino and Masi 2010, Wang et al. 2011). Due to the inseparable nature of streams and their watersheds, land use alterations have profound impacts on lotic systems and are a main cause for changes in stream communities (Allan 2004).

As a result of the prevalence of anthropogenic aquatic habitat degradation, much effort has recently focused on conserving aquatic ecosystems via management initiatives and restoration activities. Ecological restoration can be defined as the process of repairing damage caused by humans to the diversity and dynamics of indigenous ecosystems (Jackson et al. 1995). One of the largest efforts in restoration projects is riparian zone management (Bernhardt et al. 2007, Kondolf et al. 2007). The knowledge that riparian corridors have a large influence on streams (Gregory et al. 1991) has caused the majority of focus to be aimed away from understanding watershed-scale effects (Allan et al. 1997). This de-emphasis has led to a poor

understanding of watershed-scale effects, and has been recognized as major cause of failure in restoration efforts (Roni et al. 2008). To properly manage and conserve aquatic biodiversity, we must improve our understanding of how broad-scale human activities affect stream ecosystems.

Traditional methods to assess the relationship between human activities and stream ecosystems involve community monitoring. Using indices that quantify abundance and species richness, community assessments provide information on the end result of disturbance, but they provide no information on causal mechanisms, and can also be misleading. For example, Van Horne (1983) demonstrated that the link between habitat quality and population density is more complex than a simple positive correlation, meaning that a higher quality habitat does not necessarily have greater fish densities. This decoupling has been demonstrated in degraded environments that resulted in changes to species composition, but not abundance of organisms (Lenat and Crawford 1994, Davies and Jackson 2006). Conversely, species diversity and taxonomic distinctness indices can be poor reflections of anthropogenic disturbance (Heino et al. 2007), and studies that focus on the presence-absence of species have also been shown to provide an inaccurate representation of true species diversity (Mackenzie 2005). Clearly, the effects of human disturbance on fish communities is complex, and developing additional metrics to quantify this relationship could improve our ability to predict the impacts of watershed land use on stream ecosystems.

Physiological indices could help with quantifying the effects of terrestrial disturbance. Physiology represents the link between an organism and its environment, and has much to offer the field of ecological restoration (Ricklefs and Wikelski 2002, Cooke and Suski 2008). Physiological indices, particularly in terms of stress and nutrition, can reflect many aspects of habitat quality, including food availability (Congleton and Wagner 2006, van de Crommenacker

et al. 2011), land use practices (Blevins et al. 2013), pollution levels (Hontela et al. 1992), and restoration effectiveness (Adams and Ham 2011). Blood-based indices of health and condition can respond quickly to environmental conditions, as opposed to population or community metrics, which may take years to show a response. This is especially important in cases where populations residing in low quality habitats have decreased fitness and reproduction but are maintained through immigration from source populations (Pulliam and Danielson 1991, Dunning et al. 1992, Paul and Meyer 2001, Guisan and Thuiller 2005). In these cases, population-level metrics would not show a response, however, a physiological assessment would allow the detection of impaired organismal health and condition. Physiology also offers a series of metrics that are measured as continuous variables (e.g., concentration of a parameter in mg/L), providing a graded response to environmental conditions. Therefore, physiological indices can improve our understanding of how human land use practices shape aquatic communities by providing early warning signals, information on causal mechanisms, sensitive processes, and areas of ecological concern (Adams, 1990).

There are several blood-based nutritional metrics that can be used to quantify links between organisms and habitat quality, such as cholesterol, and total protein. These metrics can decrease in response to sustained fasting, and may not immediately increase after feeding (Farbridge and Leatherland 1992, Congleton and Wagner 2006). As food availability is known to have a large impact on perceived habitat quality (Mägi et al. 2009, Jenkins and Keeley 2010), and is often used as an indication of habitat quality (Dhondt 2010, van de Crommenacker et al. 2011), these indices represent excellent surrogates, or complements, for traditional habitat quality metrics. Similarly, oxidative stress is responsive to the quality of habitat in which an animal resides. Reactive oxygen species, generated through cellular metabolism, can damage

proteins, lipids, and nucleic acids and speed the aging and death of cells (Harman 1956, Halllwell and Gutteridge 1985, Beckman and Ames 1998). To combat oxidative damage, animals have evolved extensive antioxidant machinery, a substantial portion of which is exogenously-derived from food (van de Crommenacker et al. 2011). Because of this, low habitat quality can lead to increased oxidative stress (van de Crommenacker et al. 2011) and increased susceptibility to disease (Beckman and Ames 1998). A quantification of total antioxidant capacity and cellular oxidative damage can be used to assess food availability (van de Crommenacker et al. 2011), food quality (Costantini 2008), and pathogen burden (Costantini and Dell'Omo 2006).

Stress hormones and the stress response are also important indicators of organismal health, and can directly relate to habitat quality. Catecholamines and glucocorticoids are the two main hormonal groups that form the endocrine stress response. The catecholamines, epinephrine and norepinephrine, comprise the initial response, commonly called the fight-or-flight response because they immediately increase the readiness and activity of an animal (Romero and Butler 2007). Glucocorticoids, cortisol and corticosterone, are much slower at responding to a stress event, usually taking three to five minutes for increased concentrations in the blood to be detected (Romero and Butler 2007). Glucocorticoids induce short-term responses beneficial to an animal's survival (e.g., liberating energy reserves). Of potentially greater importance, however, are the consequences of repeated or long-term glucocorticoid expression, which alter behavior, inhibit growth and reproduction, and impair immune function (Romero 2004). Because of this, exposure to a chronic stressor may become detrimental to an organisms health, fitness, and inevitably their survival. For example, animals residing in disturbed or polluted environments often display elevated baseline cortisol levels (Martínez-Mota et al. 2007).

Alternatively, animals within degraded environments may become acclimatized to the chronic stress of a poor environment and demonstrate normal baseline cortisol levels, but show an elevated response to a novel stressor, a process referred to as facilitation (Dobrakovová et al. 1993, Bhatnagar and Vining 2003). If a chronic stressor is relatively severe, facilitation may not occur (Dallman and Bhatnagar 2001), resulting in an impaired stress response from animals occupying exceptionally poor habitats. Yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*), for example, demonstrated an impaired stress response in areas with high levels of contaminants (Hontela et al. 1992). While this relationship is complex, the glucocorticoid stress response can reflect the health of an animal, as well as its environment. Therefore, physiological metrics allow researchers to assess the ability of an organism to perform ecological function within its environment, which is the true indicator of whether or not it is occupying a suitable habitat (Cooke and O'Connor 2010). Despite this potential, the use of physiological metrics in quantifying the effects of human land use practices has not received a great deal of attention, leaving its efficacy unknown.

Based on this background, the goal of my research is to define the relationship between human land use, habitat quality, and the physiological condition of stream fish. My thesis consists of two chapters that apply this knowledge to evaluate fish health across a gradient of habitat quality and land use practices. Chapter 1 one is an extensive survey of largemouth bass across a gradient of land use characteristics to define how land use drives stress and nutrition, while chapter 2 was an intensive study that examined an entire fish community between a degraded and pristine environment to quantify if and how different species respond to human disturbance. This has the potential to provide managers, conservationists, and restoration practitioners with mechanistic information on the effects of land use practices on stream fishes,

improving our ability to predict and mitigate the consequences of anthropogenic habitat degradation.

This research was conducted in the area of Cornwall, ON, Canada, using several tributaries of the St. Lawrence Seaway (Figure 1.1). This area has experienced dramatic degradation of aquatic habitats due to eutrophication, habitat destruction, and exposure to a variety of contaminants, such as mercury, PCB, and bacteria, largely caused by urbanization and agriculture development (AECOM Canada Ltd., 2009). In an effort to monitor these problems the U.S. and Canadian governments established the International Joint Commission (IJC) (Hartig and Thomas 1988). Since 1973, the IJC has identified Areas of Concern (AOC) where water quality has been degraded to the extent that it has or may impair the area's ability to support life (Hartig and Thomas 1988). With little being done to improve these AOC, in 1985 the eight Great Lakes states and the Province of Ontario decided it was necessary to develop a remedial action plan (RAP) for each AOC to better guide and monitor their progress (Hartig and Thomas 1988). As such, since 1985 there have been extensive efforts by several agencies to determine the main generators of anthropogenic degradation in this area. These efforts have resulted in an abundance of data on water chemistry variables, land use, and habitat type. In addition, there is also a minimally impacted stream within the same area that serves as a reference (Hoople Creek). Thus, the Corwall AOC is a system that consists of several streams in close proximity to each other with known land use practices, providing a valuable opportunity to measure the impacts of watershed land use. For these reasons, the Cornwall AOC was the perfect area to conduct my research.

CHAPTER 2: WATERSHED-SCALE LAND USE ACTIVITIES INFLUENCE THE PHYSOLOGICAL CONDITION OF STREAM FISH

Abstract

Land use changes within watersheds can have large effects on stream ecosystems, but the mechanistic basis of those effects remains poorly understood. While changes to population size presumably reflect underlying variation in organismal health and condition, such individual-level metrics are rarely evaluated in the context of ecosystem disturbance. To address this deficiency, we combined physiological sampling with geographic information systems to quantify the effects of land use on the health largemouth bass. More specifically, we first quantified blood parameters relating to nutrition, oxidative stress, and the glucocorticoid (GC) stress response from largemouth bass residing in eight watersheds. We then used Akaike's Information Criterion to define relationships between these blood parameters and land cover including forest, agriculture, urban area and wetlands. The proportion of forest cover in a watershed was the best predictor of blood parameters representing free energy and resistance to oxidative stress, whereas wetland proportion was the best predictor of GC function. Patterns in energy reserves were not influenced by any land use practices. Interestingly, anthropogenic land use categories, such as urban and agriculture, were not the best predictor for any blood parameters. Together, our results indicate that fish health is largely driven by natural features of a landscape rather than anthropogenic land uses. Furthermore, these findings suggest that physiological methods could supplement traditional population and community assessments to develop a more comprehensive understanding of ecosystem interactions and improve stream management.

Introduction

Habitat selection has important physiological consequences for animals (Huey 1991). For example, forage quality and availability can affect antioxidant capacity in avian species (Costantini 2008), and land use bordering streams can dictate an animal's ability to mount a stress response (Blevins et al. 2013). The consequences of habitat choice are particularly important in challenging habitats, where costs to maintain homeostasis may increase. If suboptimal habitat conditions persist over extended periods, allostatic overload may occur. This can negatively affect physiological processes including immune function, energy availability, and stress response, potentially hindering survival and reproduction (Romero et al. 2009). Although individual-level physiological characteristics underpin population-level processes (Ricklefs and Wikelski 2002), such physiological parameters are rarely incorporated into field assessments of landscape-level environmental change (Cooke and Suski 2008).

In natural systems, forests and wetlands provide beneficial services for stream ecosystems (Richardson 1994, Allan 2004) and contribute to healthy aquatic systems and community structure (Miserendino and Masi 2010, Blevins et al. 2013). As human populations increase, the destruction of natural habitats for urban and agricultural development not only impedes ecosystem services but also increases runoff, raising pollutant, sediment and nutrient loads in impacted watersheds (Meybeck 2004). Combined, these factors result in the degradation of aquatic systems, which can alter species distributions, reduce biodiversity and cause significant population declines (Allan 2004).

Improving our understanding of the physiological consequences of watershed land use has the potential to improve conservation and restoration efforts. Physiological indices, particularly in terms of stress and nutrition, offer a mechanistic understanding of many aspects of

habitat quality, including food availability (Congleton and Wagner 2006), land use influences (Nagrodski et al. 2012, Blevins et al. 2013), and pollutants (Hontela et al. 1992). Furthermore, these indices can respond more rapidly to environmental conditions than population-level metrics, in which effects are only evident after significant changes in mortality, reproductive output, or distribution (Ellis et al. 2011). The continuous nature of physiological variables (e.g., concentration of glucose in mg/L) also provides a graded response to environmental conditions, improving explanatory power. An improved understanding of the physiological consequences of watershed land use could therefore guide restoration efforts to the most effective strategies prior to population declines or extirpation (Cooke and Suski 2008).

Within this context, the objective of this study was to quantify the relationship between watershed-scale land cover and the physiological properties of resident fishes. This will allow us to determine land covers that most affect stream fish and the organismal processes that are affected. To accomplish this goal, we collected *Micropterus salmoides* (largemouth bass) from streams that varied in their watershed land use practices, and obtained blood samples to examine health and condition. *Micropterus salmoides* was chosen as the model species for this study because their sedentary nature during this season ensured residence in the streams where they were collected (Winter 1977). This research will improve our understanding of the effects of land use practices on physiological processes, generating mechanistic insight into the effects of land cover on stream fish populations, and enabling better prediction of population responses prior to decline.

Methods

Site Selection

This study took place in a portion of the St. Lawrence Seaway near Cornwall, ON (45° 01' 17.5" N, 74° 43' 50.42" W). To quantify the effect of land use on the physiological properties of *M. salmoides*, watersheds with various proportions of land use practices were selected. This area was chosen because of the available land use data, the abundance of watersheds in a small study area, and the variability in land use across watersheds.

Land use data were gathered by the Ontario Ministry of Natural Resources and compiled into the Southern Ontario Land Resource Information System (SOLRIS, *Land Classification Data* [computer file]. Version 1.2. Peterborough, Ontario: The Ontario Ministry of Natural Resources, 2008.). Using Quantum GIS Lisboa (1.8.0), this database was integrated with a GIS layer developed by the Raisin Region Conservation Authority that delineated watershed boundaries within the study area. The SOLRIS database divides land use variables into many classes; for the purposes of this study, these classes were reduced into eight general land use categories (Table 2.1). Once these general land use categories were established, the proportion each category occupied per watershed was determined (Table 2.2). Using these data, eight watersheds representing a range of land use practices were chosen for field sampling.

Field Sampling

The field-sampling component of this study was performed between July 12 and 21, 2012, in the eight watersheds described above. Using pulsed direct current (PDC) boat electrofishing gear (Smith-Root, INC., 2.5GPP Electrofisher System [01868]) *M. salmoides* (n = 9 - 12, totaling 84) were collected from the lower portions of each stream. Similar to sample sizes used in other studies relating physiology to habitat quality (Homan et al. 2003, Martínez-Mota et al. 2007, Blevins et al. 2013). Sampling occurred from the confluence of each tributary with the St. Lawrence Seaway to the most upstream portion accessible by boat, typically 1 - 2

km, except for Pattingale Creek, where only the lower ~150 m was able to be sampled by boat. If 10 fish were not caught on the first pass of the stream, at least 48 hours was allowed before returning to sample remaining fish. This delay allowed any potential cortisol elevations in disturbed but uncaptured fish to return to baseline levels (Mommsen et al. 1999).

Once a *M. salmoides* was stunned by the electrofishing gear, it was immediately netted and placed in a moist, foam lined trough with the ventral side exposed and gills partially submerged in fresh stream water. To define baseline plasma cortisol levels, approximately 1.0 mL of whole blood was drawn via caudal puncture into a sodium-heparinized 1.0 mL syringe. All blood was drawn within 3 minutes of the fish being stunned by electrofishing gear to ensure baseline cortisol concentrations were not influenced by sampling (Mesa and Schreck 1989, Maule and Mesa 1994, Romero and Reed 2005)..

To quantify cortisol and glucose responsiveness, we used a standardized challenge previously shown to induce maximal elevations of circulating cortisol in other *Micropterus* species (O'Connor et al. 2011). Each fish was subjected to 3 minutes of air exposure in a moist, padded, and covered container immediately following the initial blood sample. *M. salmoides* were then placed in a 'fish bag' in the stream for 25 minutes to allow circulating cortisol concentrations to reach maximal elevations. The 'fish bag' was a cylindrical sack (1 m long \times 0.2 m diameter) constructed from hypalon rubber (chlorosulfonated polyethylene synthetic rubber) with mesh on both ends to allow for continuous water exchange. After the 25 minute confinement in the fish bag, *M. salmoides* were bled a second time in a manner identical to that of the first before being weighed, measured, fin clipped to prevent resampling, and released. Immediately following withdrawal, extracted blood samples were spun in a centrifuge for 3 minutes at 6,000 rpm to separate plasma from erythrocytes (Liss et al. 2013). Following

centrifugation, the plasma layer was removed and divided into two other microcentrifuge tubes. All three samples were placed in a dry shipper charged with liquid nitrogen for future laboratory analysis.

Laboratory analyses

We quantified cholesterol (milligrams per deciliter) and triglycerides (mg dL⁻¹) with EnzyChrom Cholesterol Assay Kit [ECCH-100] and EnzyChrom Triglyceride Assay Kit [ETGA-200], respectively (BioAssay Systems, Haywood, CA, USA). Total antioxidant capacity (in mM) and lipid peroxide levels (in μ M malondialdehyde [MDA]) were quantified using Antioxidant Assay Kit [709001] and TBARS Assay Kit [10009055], respectively (Cayman Chemical, Ann Arbor, MI). Plasma cortisol (ng mL⁻¹) was measured using a Cortisol EIA Kit (Enzo Life Sciences ADI-901-071) previously validated for use on *M. salmoides* (Sink et al. 2008). Total protein (g dL⁻¹) was measured using a hand-held protein refractomenter (AST model 1250, Thomas Scientific, Swedesboro, New Jersey, USA), which has been certified for the use in the range of 0 – 12 g dL⁻¹ (Wells and Pankhurst 1999).

Statistical Analysis

To reduce the dimensionality of the physiological data and quantify relationships among parameters, a principal component analysis (PCA) on correlations was performed (Liss et al. 2013). Our ratio of samples to predictor variables met those recommended by (Grossman et al. 1991) and principal components (PCs) with eigenvalues >1 were used for analysis (Kaiser 1960). These PCs were rotated using varimax rotation on the maximum likelihood solution (Kaiser 1960, Liss et al. 2013). Variables with factor loadings > 0.4 were considered maximal

contributors to each PC (Kaiser 1960, Liss et al. 2013). Positive factor loadings indicate a positive correlation between the PC and raw data, while negative factor loadings indicate negative correlation. Rotated PCs were used as response variables in all subsequent analyses (Table 2.3). Once the rotated components were obtained normality and homoscedasticity were assessed using visual inspection of fitted residuals (Williams 1959); all data met necessary assumptions and no transformations were required.

Principal components were fitted to biologically relevant models chosen a priori that sought to define the relationship between land use practices and physiological response variables (Table 2.4). We did not include models with multiple land use types, as we were interested in determining the land use variables with the largest impact on *M. salmoides* physiological condition. While all fish were captured from the same stream, fish were kept as individual data points, as opposed to averaging stream values, to prevent artificially reducing the variance and retain information provided by each individual (Schank and Koehnle 2009). Watershed land use variables, considered treatments, were modeled against each principal component using linear and nonlinear regression to determine the best predictor variable. For this, land use variables were also modeled with their square term as biological responses to environmental conditions can often be nonlinear (Allan 2004, Mouillot et al. 2013). To account for the unequal size of our watersheds, we considered land use as a proportion of the total watershed area, rather than absolute area of each land use. Models were ranked using Akaike's Information Criterion adjusted for small sample size (AIC_c) to determine the best approximating model (Hegyi and Garamszegi 2010). Upon ranking models, those with a Δ_i AIC_c < 2 of the best-fit model were deemed to be a competitor for drawing inference (Mazerolle 2006).

Relative weight (W_r), a measure of fish condition that relates actual fish weight to the expected weight at a given length, was also compared between streams. This was done on all largemouth bass over the minimum recommended length of 150 mm with the W_r index using the RLP equation developed and presented by Murphy et al. (1991). Principal component analyses were performed using JMP 10.0, while regression models and AIC_c values were obtained using the maximum likelihood estimation in PROC MIXED with SAS 9.3 (SAS Institute, Cary, NC, USA). Rejection of the null hypothesis (α) for all tests was P \leq 0.05, and all values are reported as means \pm standard error (SE) where appropriate.

Results

Agriculture was the dominant land use practice in the watersheds examined, occupying 33 to 84 % of each watershed. Forests and wetlands were the next most abundant land use categories, and ranged from 8.7 to 18.1 %, and 2.2 to 27 %, respectively. Resource extraction and recreation were the two least abundant land uses, with both categories averaging ~ 2 % of total watershed area.

Physiological parameters were highly variable across streams (Table 2.3). Lipid peroxide level, a measure of cellular oxidative damage, was the most variable metric with a three-fold increase from the lowest to the highest-ranking watershed. The second most variable physiological metric was triglycerides, with watershed means ranging from 1.36 to 3.92 mg dL⁻¹. It is also worth noting that total antioxidant capacity was the third most variable metric, with the highest watershed mean being 2.5 times greater than the lowest. The two least variable parameters were total protein and cholesterol, with a respective 20 and 26 % increase from the lowest to the highest watershed means. One fish displayed a low cortisol and glucose response, with the baseline and responsiveness values interacting to generate a negative scope. We chose to not exclude this individual from analyses because both the baseline and post-stress values were within the range of baseline levels observed in this study and attributed the negative value to a muted stress response, recovery in the fish bag, or inherent error (+/- 8 %) in the cortisol assay (*Cortisol ELISA kit Product Manual* 2013). Both total length (Analysis of Variance [ANOVA], $F_{(7, 56)} = 1.7098$, P=0.2481) and weight (ANOVA, $F_{(7, 56)} = 1.7833$, P = 0.2351) of sampled *M. salmoides* did not differ across streams.

Principal component analysis produced 4 PCs with eigenvalues >1, which explained 55 % of the total variation in physiological parameters measured (Table 2.4). PC1 explained 18 % of the total variation and was characterized by positive factor loadings for baseline glucose and triglycerides, indicating readily available, or free, energy (German 2011) (Table 2.4). PC2 explained 14.1 % of total variation and was characterized by positive factor loadings for total protein and cholesterol, indicating energy reserves (Wagner and Congleton 2004, German 2011) (Table 2.4). PC3 explained 12.7 % of the total variation, and was characterized by a positive factor loading for total antioxidant capacity and a negative factor loading for lipid peroxide levels, therefore representative of oxidative stress resistance (Beckman and Ames 1998) (Table 2.5). Finally, PC4 explained 10.5 % of the total variation and was characterized by a negative factor loading for baseline glucose and positive factor loadings for cortisol and glucose responsiveness, indicating both current nutritional status and glucocorticoid responsiveness (Romero 2004).

Variation in PC1 (free energy) was best explained by the model containing the quadratic term for proportion of forest in a watershed (Table 2.5). No other models received a Δ_i AIC_c of < 2, and were therefore not considered competitive. The greatest PC1 scores occurred in watersheds with an intermediate proportion of forest (between 11 and 15 %), while *M. salmoides*

residing in watersheds with greater or less than this intermediate proportion displayed lower PC1 scores (Figure 2.1).

Variation in PC2 (energy reserves) did not receive substantial support from any model examined. Of the 12 models examined, nine were competitors for best-fit model receiving a Δ_i AIC_c of < 2. The top six were the linear models for each land use category, and the next three also contained the quadratic term for proportion of forest, urban, and agriculture (Table 2.5).

Variation in PC3 (resistance to oxidative stress) was explained by several competing models. The top ranked model contained the quadratic term for the proportion of forest in a watershed (Table 2.5). More specifically, the lowest PC3 scores occurred in watersheds with an intermediate proportion of forest area (between 12 and 16 %), and watersheds with proportions of forest greater or less than this intermediate amount showed higher PC3 scores for resident *M. salmoides* (Figure 2.2a). The first competing model contained the linear term for the proportion of forest in a watershed, demonstrating a positive relationship between the proportion of forest in the watershed and PC3 scores (Figure 2.2a). The next competing model contained the linear term for the proportion of recreational land, demonstrating a negative relationship between PC3 scores and the amount of recreational land in the watershed (Figure 2.2b). The final competing model contained the quadratic term for proportion of wetlands in a watershed. Similar to the relationship between PC3 and forests, the lowest PC3 scores occurred in watersheds with an intermediate proportion of wetland area, and watersheds greater or less than this intermediate proportion showed higher PC3 scores for resident *M. salmoides* (Figure 2.2c).

Variation in PC4 (baseline glucose and GC responsiveness) was explained by two competing models: proportion of wetland and proportion of agriculture in the watershed. The top model overall contained the proportion of wetland in the watershed (Table 2.5), which

demonstrated a positive relationship between PC4 scores and the proportion of wetland in the watershed (Figure 2.3a). The competing model for PC4 contained proportion of agriculture and other fields in a watershed. This model received a Δ_i AIC_c of 0.5 and PC4 scores demonstrated a negative relationship with the amount of agriculture and other fields in a watershed. (Figure 2.3b).

There were no differences in fish condition between the streams sampled in this study. Across all streams, 54 fish exceeded 150 mm (n = 3 – 11) with relative weights (W_r) ranging from 97.9 – 108.3 (Table 2.3). When compared across streams, no difference was found in resident largemouth bass W_r ($F_{(7, 46)}$ =0.77, P = 0.62).

Discussion

The proportion of forest and wetland were found to be the strongest watershed-scale drivers of physiological condition of resident *M. salmoides*. More specifically, for PC1, representing free energy, and for PC3, representing resistance to oxidative stress, the best-fit model was the proportion of forest in a watershed. For PC4, which contained baseline glucose and GC responsiveness, the best-fit model was the proportion of wetland in a watershed. The importance of these variables in our study is likely the result of forests and wetlands creating better habitat by generating more stable hydrologic regimes (Richardson 1994), reducing inputs (Allan 2004), and increasing forage availability and quality (Theodoropoulos and Iliopoulou-Georgudaki 2010, Miserendino et al. 2011). The relationship between the extent of these natural areas and *M. salmoides* physiology is particularly informative, as our analysis did not discern location of the land use practices within the watershed, meaning these natural areas have a large impact on streams even though they were not necessarily riparian. Together our results indicate

the proportion of forests and wetlands within a watershed are the strongest drivers of physiological condition in resident *M. salmoides* in relation to other land use types, such as agriculture and urban areas.

Interestingly, for some of the parameters examined, the relationship between land use (forested area in particular) and physiological metrics was parabolic rather than linear. More specifically, the best-fit model between land use type and PC1 (free energy) peaked in watersheds with intermediate proportions of forested area and decreased when proportions of forested area were higher or lower. PC1 contained the energy sources triglycerides and glucose, which have been demonstrated to decrease during periods of fasting and increase after feeding (Wagner and Congleton 2004, Congleton and Wagner 2006). As forests can increase water quality and aquatic community structure and diversity (including taxa M. salmoides use for forage), increasing triglyceride and glucose concentrations from low to intermediate proportions of forest is likely related to increasing water and forage quality/quantity (Miserendino and Masi 2010, Theodoropoulos and Iliopoulou-Georgudaki 2010). Decreased PC1 scores at high proportions of forests may indicate reduced feeding caused by changes in community and habitat structure, such as increased competition for food in higher quality habitats (Ward et al. 2006) or increased prey refugia if forests are creating more in-stream habitat (Savino and Stein 1989). While the mechanism is unclear, results clearly show that the proportion of forest in a watershed influences the free energy in *M. salmoides*.

The influence of land use on PC3 scores (resistance to oxidative stress) received the most competition of our physiological components. Five models had Δ_i AIC_c scores < 2; however, four of these five were the natural land uses, forests and wetlands. Similar to PC1, PC3 scores demonstrated a parabolic relationship with the proportion of forest and wetland in a watershed,

declining in watersheds with intermediate proportions and increasing at high and low proportions. PC3 was composed of a positive factor loading for total antioxidant capacity and a negative factor loading for lipid peroxide levels, therefore representing resistance to oxidative stress. Oxidation can damage proteins, lipids, and nucleic acids, increase susceptibility to disease, and speed the aging and death of cells, (Beckman and Ames 1998). Because a substantial portion of antioxidants are exogenously-derived from food, oxidative stress has been shown to relate to habitat quality, food availability, and food quality (Costantini 2008, van de Crommenacker et al. 2011). In the current study, decreased PC3 scores at intermediate proportions of forest and wetlands were likely related to poor quality forage, while increased PC3 scores at high proportions were likely related to higher quality forage (Miserendino and Masi 2010, Theodoropoulos and Iliopoulou-Georgudaki 2010). The elevated PC3 scores from watersheds with low levels of forest and wetlands in the watershed is not clear, but may have resulted from up-regulation of endogenously produced antioxidants in *M. salmoides* experiencing a mild but chronic shortage of exogenous antioxidants (Monaghan et al. 2009). This may not be the case, however, as the competing models of the proportion of forest and wetland demonstrate a positive linear relationship with resistance to oxidative stress, indicating that low levels of forests and wetlands result in decreased resistance to oxidative stress. The high ranking of the model containing recreational land suggests that these areas (e.g. golf courses, playing fields) may have a large impact on stream fish even at very low densities. Regardless of the mechanism, results from the current study clearly indicate that natural land cover and recreational land is an important driver of *M. salmoides* oxidative stress.

PC4, comprised of baseline glucose along with both cortisol and glucose responsiveness, was best predicted by the model containing proportion of wetland area in a watershed, with

which there was a positive linear relationship. Cortisol and glucose responsiveness represent the ability of *M. salmoides* to mount a GC stress response (Romero 2004). Glucose is released during a stress event and was negatively associated with cortisol and glucose responsiveness in this component. This suggests that *M. salmoides* inhabiting streams with a low proportion of wetlands in the watershed had elevated baseline stress levels and a reduced ability to mount a stress response relative to those from streams with high proportions of wetlands. Natural areas have previously been shown to increase a fish's ability to mount a glucocorticoid stress response (Blevins et al. 2013), which is critical to the short-term survival of an animal (Romero 2004). In our watersheds, higher proportions of wetland areas likely confer greater hydrological stability and reduced inputs from runoff, lowering the frequency of stress events caused by floods and chronic stress caused by pollutants (Hontela et al. 1992, Richardson 1994). The result of this PC clearly indicates that increased proportions of wetlands in a watershed increases the ability of *M. salmoides* to mount a proper GC stress response, likely resulting in increased survival and reproduction.

Baseline cortisol did not result in a maximally contributing factor loading for any PC, indicating that it did not account for a significant amount of the variance explained by any PC. This was unexpected as many studies have found baseline cortisol concentrations to be influenced by habitat quality(Homan et al. 2003, Martínez-Mota et al. 2007). However, Dickens and Romero (2013) examined existing literature on chronically stressed wild animals and found that there is not a predictable GC response to chronic stress and there can often be no change to baseline levels. Therefore, the small amount of variation explained by baseline cortisol concentrations does not necessarily mean it is an inconsequential variable or that watershed land use is not causing chronically stressed *M. salmoides*.

Interestingly, PC2, which was representative of energy reserves, did not appear to be influenced by any land use practices in this study. Examination of data revealed that nine models had Δ_i AIC_c values < 2, which indicates no strong support for any land use. This result is not too surprising as total protein and cholesterol, which comprise PC2, were the two least variable metrics, with a respective 20 and 26 % increase across all streams. Total protein and cholesterol respond to changes in nutritional status (e.g., food consumption, growth, and body condition) (Wagner and Congleton 2004). The lack of a land use effect on energy reserves could indicate that the land use practices within our study watersheds were not variable enough to result in any effects on body condition. Indeed, W_r, a measure of fish condition, agreed with this conclusion and was also consistent across streams. These results further demonstrate the importance of in-depth physiological assessments. Studies assessing condition factor would have concluded that there was no difference among streams within our study area; however, our results show that land cover within these watersheds is affecting the physiological condition of these fish. Although *M. salmoides* energy reserves show resilience to changes in watershed land use, the effects can still be seen in other parameters, which demonstrate the benefit of forests and wetlands.

Our results also indicate that the extent of anthropogenic land use had less impact on physiological properties than natural landscape features. Although land use characterized as "recreational" and "agriculture and other fields" did result in competitors for best-fit model in two PCs, no anthropogenic land use category resulted in a top model predicting physiological variables despite the large impact human land use has on stream ecosystems (see Meybeck 2004). This result is similar to other studies that found human land use to be a poor predictor of stream quality in areas of widespread anthropogenic influence (Allan 2004). Anthropogenic land

use is indeed widespread in our study watersheds, with disturbed land ranging from 54 - 87 %. As major degradation can occur at levels as low at 5 - 15 % (Paul and Meyer 2001), the lack human land use resulting in top models predicting *M. salmoides* physiology suggests that disturbances may have little influence once a certain level is reached.

The physiological properties of resident stream fishes are driven by the proportion of forest and wetland area in these watersheds. This finding demonstrates that physiological consequences of habitat use can be quantified (Huey 1991) and used to assess the health of a fish community to improve the understanding of the relationship between watershed-scale processes and stream quality. This improved mechanistic understanding can increase the success rate of restoration projects by enabling them to target the most influential land-use practices in their system (Cooke and Suski 2008). Determining natural land use at the watershed-scale created the best predictor models for the physiological condition of *M. salmoides* highlights their importance for resident stream fishes. Appreciating this relationship is critically important as a poor understanding of watershed-scale effects was found to be a major cause of restoration project failures worldwide (Roni et al. 2008). For these projects to be successful, practitioners must gain a mechanistic understanding by determining both the land use practices that affect the stream and the in-stream processes that are affected (Allan 2004). Our results show that, in these watersheds, it is the forests and wetlands that are affecting resident stream fish by influencing their feeding, resistance to oxidative stress and ability to mount a stress response. This result improves the understanding of watershed-scale land use effects and can be used by restoration practitioners to develop projects directed towards these natural areas and strategies that will most benefit the impaired processes.

CHAPTER 3: STRESS IN THE NEIGHBORHOOD: TISSUE GLUCOCORTICOID FUNCTION RELATIVE TO CHANGES IN STREAM QUALITY FOR FIVE SPECIES OF STREAM FISH

Abstract

Anthropogenic alterations to terrestrial habitat (e.g., urbanization, deforestation, agriculture) can have a variety of negative effects on watercourses that flow through disturbed landscapes. Currently, the relationship between stream habitat quality and fish condition remains poorly understood. The use of physiological metrics such as glucocorticoids (GCs) provides a powerful tool for quantifying these effects by relating the health of resident fishes to stream quality. To date, however, most studies that measure GCs tend to focus on a single, large-bodied species, rather than evaluating how GCs may be influenced differently between species in a fish community. In the current study, we used cortisol extracted from fish tissues to quantify effects of anthropogenic habitat degradation on the glucocorticoid function of five species of smallbodied and juvenile stream fish. Largemouth bass Micropterus salmoides, brown bullhead Ameiurus nebulosus, white sucker Catostomus commersonii, pumpkinseed Lepomis gibbosus, and logperch Percina caprodes were sampled from a reference and a degraded stream. Upon capture, fish were either euthanized immediately, to quantify baseline stress parameters, or following a standardized stressor, to quantify GC responsiveness. Results revealed that largemouth bass, brown bullhead, and logperch had altered GC function as a result of anthropogenic stream degradation, while white sucker and pumpkinseed did not. Together, our results show that different species residing in identical habitats can demonstrate a variety of responses to environmental stress, highlighting the variation in physiological ability to cope under poor environmental conditions, as well as the difficulty of predicting the GC dynamics in wild animals. Understanding the relationships between GC function, habitat quality, and

population-level processes will increase the ability of researchers and managers to predict how fish communities and aquatic ecosystems will be shaped by anthropogenic environmental change.

Introduction

Natural landscapes have been heavily modified on a global scale, mainly due to human expansion and the requisite resource acquisition, agriculture, and urban development (Vitousek et al. 1997, Foley et al. 2005). These activities have led to the degradation of most ecosystems as natural habitats are lost or fragmented (Fahrig 1997). Freshwater systems are especially susceptible to human induced habitat destruction (Allan 2004, Olden et al. 2010, Wang et al. 2011), and, as a result, freshwater fauna are among the most imperiled groups on the earth (Ricciardi and Rasmussen 1999). In their natural state, forests and wetlands contribute to healthy aquatic systems by stabilizing flow, decreasing erosion, and reducing sediment and nutrient inputs (Richardson 1994, Allan 2004, Miserendino and Masi 2010). Human activities, such as urbanization and agriculture, not only remove the benefits provided by these natural habitats, but further alter hydrology, geomorphology, and thermal regimes, while also increasing pollutant, sediment, and nutrient loads (Poff et al. 1997, Meybeck 2004, Allan 2004). Combined, these human-induced changes to natural landscapes have resulted in substantial habitat degradation of aquatic ecosystems (Allan 2004, Dudgeon et al. 2006).

Habitat choice has important physiological consequences for individuals (Huey 1991, Hendry et al. 2011), and reductions in the quality or quantity of suitable habitat can challenge the ability of individuals to persist within a system. This is an area of concern for conservation scientists and environmental managers because human disturbances often change important

habitat characteristics. For example, agriculture and urbanization are known to alter thermal regimes (Allan 2004), which can be problematic as fish are ectothermic and thermal refugia are an important resource for maintaining behavioral, physiological, and metabolic optima (Magnuson et al. 1979, Huey 1991). Land use alterations can also alter forage quality and availability (Allan 2004, Theodoropoulos and Iliopoulou-Georgudaki 2010, Miserendino et al. 2011), which can increase oxidative stress (van de Crommenacker et al. 2011), increasing disease and senescence (Beckman and Ames 1998). The changes to streams resulting from human land use activities place animals in stressful conditions that often push them to their physiological limits (Adams et al. 2002). Over time, these processes can result in changes to populations, communities, and eventually the biodiversity of the system (Adams 2002).

To predict how human disturbance will influence populations, it is important to consider the capacity for adaptation, as well as individual tolerance limits and the potential for acclimatization in novel conditions. A number of techniques are available to answer these questions, including broad-scale examinations, common garden or reciprocal cross experiments, artificial selection, and modeling (Hoffmann and Sgrò 2011). For example, broad-scale examinations have found breeding date in birds to be a plastic response to climate change (Gienapp et al. 2008), and a common garden experiment found plasticity in physiological performance as it relates to habitat quality (Blevins et al. 2013). While these techniques are useful for characterizing the physiological ability of organisms to respond to anthropogenic environmental change, one problem is that many of these studies will only examine a single species that exists across wide geographic ranges, requiring the ability to tolerate variable conditions. Therefore, these studies tend to focus on generalist species and essentially overlook more sensitive species that may be at risk (Falconer 1990, Conover and Schultz 1995, Nagrodski

et al. 2012, Blevins et al. 2013). In reality, anthropogenic environmental change does not influence single species, nor do all species show the same response, especially when considering specialists are adapted to a more narrow range of environmental conditions (Minns et al. 1994, McComb et al. 2008, Mouillot et al. 2013). This is an important aspect as human land use alterations cause drastic changes in a short time-frame, and specialists may lack the physiological scope to adequately respond, causing population declines (Chevin et al. 2010). To improve our ability to predict the effects of environmental change on biodiversity, it is important that we move beyond a single-species approach and consider the community in studies of organism tolerance.

Using organism-level biological indicators to examine communities, rather than specieslevel, can help us gain a broader understanding of how habitat changes influence ecosystems and biodiversity. Biological indicators can be used to assess organism health (e.g., nutrition and energetics; Barton et al. 2002; Congleton & Wagner 2006) and physiological function (i.e., the stress response; Hontela et al. 1992; Barton et al. 2002), which directly impact growth, survival, and reproduction (Adams et al. 1989; Adams 2002; Cooke & Suski 2008). Examination of bioindicators can provide early warning signals of environmental stressors, and identify the mechanisms responsible for changes seen at the population and community level (Adams et al. 1989, 2002). Evaluating the physiological condition of multiple sympatric species can therefore identify areas of ecological concern and species most sensitive to, and robust against, anthropogenic environmental change.

Evaluating physiological stress is a valuable bioindicator for quantifying the effects of environmental perturbations in a community. The glucocorticoid (GC) stress response is an adaptive mechanism that increases energy available for activities necessary for short-term

survival by stimulating widespread catabolic activities such as proteolysis and gluconeogenesis (Mommsen et al. 1999, Romero 2004). However, chronic activation of the stress response can become maladaptive by using energetic resources necessary for normal organismal functions, resulting in reduced growth, reproduction, and immune function (Mommsen et al. 1999, Barton 2002), and can ultimately increase mortality (Selye 1973, Schreck 2000). Therefore, altered GC function of a population can be detrimental to its persistence (Mommsen et al. 1999, Romero and Butler 2007, Dickens and Romero 2013). As both baseline and post-stress cortisol levels can be affected by human disturbances (Hontela et al. 1992, Homan et al. 2003, Martínez-Mota et al. 2007, Blevins et al. 2013), an examination of GC function of multiple species in stream fish communities can improve our ability to predict the consequences of human-induced, rapid environmental change.

The objective of the current study was to quantify the effects of human induced habitat degradation on the glucocorticoid function of a stream fish community. We accomplished this goal by quantifying baseline and post-stress cortisol levels for five sympatric fish species residing in two watersheds that differed in land use characteristics (Table 3.1). These species varied in their taxonomic and ecological traits, allowing us to quantify fishes differing in trophic guild and evolutionary history are affected by habitat degradation (Table 3.2). We chose to examine glucocorticoid function using tissue extracts (Sink et al. 2007), as opposed to plasma. This provided a greater size range of fishes that could be sampled as blood collection from small fishes (< 90 mm) becomes challenging, and many of the fish used in this study were below this range (Table 3.3). Together, results from this study will improve our understanding of how communities are shaped by environmental disturbances.

Methods

Site selection

The field-sampling component of this study was performed in October 2012, in tributaries of the St. Lawrence River, near Cornwall, ON, Canada (45° 01' 16.59" N, 74° 43' 49.24" W). To quantify the impacts of stream quality use on glucocorticoid function, it was first necessary to define study sites that varied in environmental characteristics. For this land use and a variety of other stream characteristics were examined. Land use data (Table 3.1), were gathered by the Ontario Ministry of Natural Resources and compiled into the Southern Ontario Land Resource Information System (SOLRIS). Using Quantum GIS Lisboa (1.8.0), the SOLRIS database was integrated with a GIS layer developed by the Raisin Region Conservation Authority that delineated watershed boundaries within the study area. Analyses of land use data identified two streams, Hoople and Gunn Creek, which were ideally suited for this study as they differed greatly in land use characteristics and were in close geographic proximity. Hoople Creek's (45° 01' 19.09" N, 74° 59' 32.37" W) watershed consisted of about 41 % forests and wetlands, while Gunn Creek's (45° 11' 30.57" N, 74° 22' 32.38" W) watershed had <10 % of forests or wetlands (Table 3.1). A large portion of Gunn Creek's watershed consists of agriculture. As a result of land use practices, Gunn Creek had an average total phosphorus (TP) concentration exceeding 100 μ g/L during the summer months, while Hoople Creek averaged less than half of that (39 µg/L) (Ontario Ministry of the Environment 2009). Gunn creek also showed signs of fecal contamination and potentially high levels of disease causing organisms in the water, indicated by elevated levels of *E. coli* bacteria loads, reaching twice the level of Hoople Creek (8.3 CFU/mL and 3.9 CFU/mL, respectively) (Raisin Region Conservation Authority). Furthermore, the Ontario Ministry of the Environment regularly uses Hoople Creek as a reference stream when assessing stream quality because it is considered one of the most pristine

streams in this region (Ontario Ministry of the Environment 2009). For these reasons, Hoople Creek was considered to be of high habitat quality and served as a reference stream in this study, while Gunn Creek was considered to be low quality and served as the degraded stream.

Field sampling

Using a backpack electrofisher (Halltech Aquatic Research Inc., HT-2000 Battery Backpack Electrofisher), we collected largemouth bass (*Micropterus salmoides*) (n = 15 from Gunn Creek and 13 from Hoople Creek), white sucker (*Catostomus commersonii*) (n = 14 from Gunn Creek and 20 from Hoople Creek), brown bullhead (*Ameiurus nebulosus*) (n = 5 from Gunn Creek and 20 from Hoople Creek), pumpkinseed (*Lepomis gibbosus*) (n = 21 from Gunn Creek and 20 from Hoople Creek) and logperch (*Percina caprodes*) (n = 12 from Gunn Creek and 20 from Hoople Creek) and logperch (*Percina caprodes*) (n = 12 from Gunn Creek and 20 from Hoople Creek) from both study creeks. These species were chosen because they reside in both streams, are taxonomically diverse, and occupy a variety of ecological niches (Table 3.2). To reach sufficient sample sizes, both streams required multiple sampling trips. We waited at least 24 hours between sampling efforts to allow any potential cortisol elevations in disturbed but uncaptured fish to return to baseline levels (Mommsen et al. 1999).

Once fish of the target species were stunned by the electrofishing gear, they were immediately netted and assigned to either the baseline or stress treatment. The first fish captured from each species (per stream) was randomly assigned a treatment, and subsequent fish were alternately assigned each treatment. Fish in the baseline treatment were euthanized via cerebral percussion within 30 seconds of being stunned by the electrofisher, which should precede any cortisol elevation related to electroshocking, handling, or sampling (Mesa and Schreck 1989, Maule and Mesa 1994, Romero and Reed 2005). Fish assigned to the stress treatment were

subjected to a standardized challenge previously shown to induce maximal elevations of circulating cortisol in fish (O'Connor et al. 2011, Cook et al. 2012). For this, each individual was subjected to 3 minutes of air exposer while being held in a moist, padded, and covered container, and then transferred to a 20 L bucket filled with fresh stream water to allow circulating cortisol levels to rise. The time lag between the onset of a stressor and maximal plasma cortisol concentrations varies from species to species, and has not been determined for all target species in the current study. Based on previous literature, 25 minutes was chosen as the elevation period for largemouth bass (O'Connor et al. 2011), 40 minutes for pumpkinseed (Cook et al. 2012), and 30 minutes for logperch, white sucker, and brown bullhead (Limsuwan et al. 1983, Acerete et al. 2004). At the conclusion of the elevation period, fish were euthanized via cerebral percussion. Once euthanized, all fish were weighed, measured, wrapped in aluminum foil, and stored in a dry shipper charged with liquid nitrogen for laboratory processing. A small number of fish (n = 9 of 34 total white sucker, spread with 6 from Gunn and 3 from Hoople and n = 1 brown bullhead from Gunn of 25 total) were too large for a whole-body sample to be taken. Therefore, these fish were cut in half and the posterior body section was brought back to the lab for cortisol processing. This was not expected to influence cortisol values as cortisol is not stored in any tissue or organs after production, and circulates throughout the entire body acting on a number of tissues and organs (Mommsen et al. 1999, Romero and Butler 2007).

Laboratory analysis

Cortisol was extracted from the collected tissues using a modification of the ethyl ether/vegetable oil method outlined and validated by Sink et al. (2007). Briefly, fish tissue was homogenized in 3 mL phosphate buffered saline (PBS), plus an extra 1 mL for fish over 10 g.

After homogenization, $100 \,\mu$ L of vegetable oil per gram of fish tissue was added to the sample. The vegetable oil had been previously analyzed to ensure is did not contain cortisol and was added to increase the final extract volume, ensuring each sample could be assayed individually. For fish up to 6.5 g, 7 mL of ethyl ether was then added to the homogenate; for every gram over 6.5 g, samples received 1 mL extra, up to a maximum of 15 mL. The sample was then centrifuged for 10 minutes at 3000 rpm and placed in a -80° C freezer for 2 hours. The unfrozen ethyl ether portion was then decanted into a separate test tube and evaporated under a gentle stream of gaseous nitrogen for 2 hours, yielding a lipid extract containing cortisol. This extraction procedure was performed a second time on the thawed homogenate, and the second lipid extract was added to the first. This extract was stored at -20° C until a commercially available enzyme linked immunosorbant assay (ELISA) was performed to quantify cortisol concentrations (Enzo Life Sciences, Cortisol EIA Kit [901-071], BioAssay Systems) (Sink et al. 2007, 2008). This assay has a detection limit of 0.0567 ng mL⁻¹ and while the majority of fish had levels well above this limit, extract from several individuals in the baseline group had cortisol concentrations below the detection limit. Extract concentrations from these fish were assigned values equal to the detection limit prior to calculations of cortisol per gram of fish weight.

Statistical analysis

This study employed both laboratory and field techniques to assess the effects of stream quality on resident fishes using a paired catchments While the limitations of our study design are recognized, we felt that studying additional species (in lieu of additional streams) was essential to quantify a community response to habitat degradation.

For analyses, we were interested in three different metrics related to intraspecific stress: baseline cortisol concentration, post-stress cortisol concentration, and glucocorticoid responsiveness (post-stress – baseline); we considered these three metrics to be discrete statistical hypotheses. To determine if baseline and post-stress cortisol levels differed between streams, a Welch's t-test was performed separately for each species (Ruxton 2006). Homogeneity of variances is not an assumption of this test; however, the assumption of normality was assessed using a visual inspection of the fitted residuals. If this assumption was not met, data were rank transformed prior to performing a Welsh's t test (Zimmerman and Zumbo 1993). To approximate GC responsiveness values within a species, we subtracted mean baseline cortisol concentrations for a stream from the post-stress levels of individual fish sampled from the same stream. Responsiveness values were then compared using a Welch's ttest on ranked or raw data, dependent on normality of the data. All statistical analyses were performed using JMP 10.0 (SAS Institute, Cary, NC, USA). Rejection of the null hypothesis (α) for all tests was $P \le 0.05$, and all values are reported as means \pm standard error (SE) where appropriate.

Results

Baseline cortisol levels were unaffected by habitat quality for all species examined except largemouth bass (Figure 3.1a), where a higher baseline cortisol concentration was present in the degraded stream (Gunn Creek) relative to the reference stream (Hoople Creek) ($t_{(12.5)} = -2.24$, P = 0.022). There were no differences in baseline cortisol concentrations for brown bullhead ($t_{(1.5)} = 0.43$, P = 0.72), logperch ($t_{(12.04)} = 0.79$, P = 0.44), pumpkinseed ($t_{(16.89)} = -0.52$, P = 0.61), or white sucker ($t_{(15.1)} = -0.30$, P = 0.78) across the two streams examined (Figure 3.1 b-e).

Following the stressor treatment, both brown bullhead and logperch demonstrated a difference in post-stress cortisol between streams (Figure 3.1b, c). More specifically, post-stress cortisol concentrations in brown bullhead were 3-times greater in the reference stream than in the degraded stream ($t_{(7.7)} = 3.86$, P = 0.0026). In contrast, logperch had higher post-stress cortisol concentrations in the degraded stream than in the reference stream, with the mean value from the degraded stream being nearly double that of logperch from the reference stream ($t_{(7.2)} = -1.97$, P = 0.044). There was no difference in post-stress cortisol concentrations for largemouth bass ($t_{(10.6)} = 1.03$, P = 0.33), pumpkinseed ($t_{(11.7)} = -0.54$, P = 0.6) or white sucker ($t_{(6.6)} = 0.058$, P = 0.96) between streams (Figure 3.1a, d, e).

Responsiveness values, defined as post-stress cortisol concentration minus baseline, were then examined. Both brown bullhead and logperch demonstrated a difference in post-stress cortisol between streams (Figure 3.1b, c). More specifically, responsiveness values in brown bullhead were greater in the reference stream than in the degraded stream ($t_{(7.7)} = 3.41$, P = 0.0048). In contrast, logperch had higher responsiveness values in the degraded stream than in the reference stream ($t_{(7.2)} = -2.33$, P = 0.0258). There was no difference in responsiveness values for largemouth bass ($t_{(10.6)} = 1.5$, P = 0.15), pumpkinseed ($t_{(11.7)} = -0.45$, P = 0.66) or white sucker ($t_{(6.6)} = 0.056$, P = 0.59) between streams (Figure 3.1a, d, e).

Discussion

Human-induced habitat alterations significantly influenced the glucocorticoid stress axis for three of the five fish species examined. Relative to the reference stream, largemouth bass demonstrated elevated baseline levels in the degraded stream, brown bullhead showed an impaired ability to mount a stress response, and logperch exhibited a greater stress response. Cortisol is released under stressful conditions including acute stressors such as failed predation events (Romero 2004) and fisheries interactions (i.e., capture and release; Suski et al. 2003) or chronic stressors such as habitat fragmentation (Martínez-Mota et al. 2007) and water quality degradation (Hontela et al. 1992). This is an adaptive response that alters a suite of organismal processes (e.g., behavior, energy allocation) and increases the likelihood of short-term survival (Barton 2002, Romero 2004). Elevated baseline cortisol levels, observed in largemouth bass, likely resulted from chronic activation of the stress axis. This has been shown to occur when animals inhabit sub-optimal habitats, such as those with extreme temperatures, pollution, or insufficient food resources (Dallman and Bhatnagar 2001, Romero 2004). If an animal is unable to acclimatize to these sub-optimal conditions, chronic cortisol release can lead to impaired immune function and the exhaustion of energy stores, which are necessary for growth and reproduction (Mommsen et al. 1999, Romero 2004). In contrast, brown bullhead residing in the degraded stream demonstrated a reduced maximal stress response relative to individuals from the reference stream. A reduction in the stress response can be caused by several factors, including attenuation, where elevated GC concentrations interact with receptors to inhibit continued release (Barton 2002), or environmental pollutants that inhibit cortisol production or secretion (Hontela et al. 1992, Leblond et al. 2001, Davies and Jackson 2006). Regardless of the mechanism, an acute stress response liberates energy and prioritizes homeostasis to increase survival during short-term stressors, so the lack of a stress response may lead to decreased survival in these populations (Barton 2002, Romero 2004). Finally, logperch demonstrated an exaggerated (or facilitated) response, which is sometimes seen when animals acclimate to a repeated stressor and then experience a novel stressor (Barton, Weiner, & Schreck 1985; Schreck 2000). In these animals, negative feedback signals are likely not strong enough to inhibit the combined stimulus of chronic and novel stressors (Dallman and Bhatnagar 2001). As logperch are the most

sensitive and specialist species examined in this study, this result is interesting as it suggests that these logperch do not have the ability to properly control cortisol release via negative feedback mechanisms. Together, our results show that different species residing in identical habitats can demonstrate a variety of responses to environmental stress, highlighting the variation in physiological ability to cope under poor environmental conditions, as well as the difficulty of predicting the glucocorticoid dynamics in wild animals (Sih et al. 2011, Dickens and Romero 2013).

Interestingly, two of the five species examined did not demonstrate altered GC function despite residing in different quality habitats. More specifically, both pumpkinseed and white sucker showed no difference in either baseline or post-stress cortisol concentrations when compared across reference and degraded streams. Previous studies have demonstrated variation in the physiological properties of resident organisms in response to reductions in habitat quality. For example, removal of terrestrial habitat was found to affect baseline and post-stress corticosterone concentrations in spotted salamanders *Ambystoma maculatum* (Homan et al. 2003). Similarly, Blevins et al. (2013) found that creek chub *Semotilus atromaculatus* collected from degraded watersheds (those dominated by agricultural land use) maintained physiological performance with a reduced stress response relative to creek chub collected from streams within forested watersheds (more pristine environments).

There are two hypotheses that could explain the lack of differences in the stress response for pumpkinseed and white sucker despite variation in habitat quality across the two sites examined. First, both species may have broad tolerances and the conditions in these streams do not approach the threshold for them to illicit a stress response. For example, both species appear to be habitat generalists (Table 3.2) and previous work has shown that generalist species are less

impacted by changes to environmental conditions than are specialists, likely because generalists have wider tolerance ranges, even though they may be less adapted to any single environment (Minns et al. 1994, Chevin et al. 2010). Alternatively, pumpkinseed and white sucker may possess a large capacity to display phenotypic plasticity, relative to other species in this study, which would allow individuals residing in degraded environments to minimize the costly activation of the acute stress response. Not only can the capacity for plastic physiological changes vary across species (Hendry et al. 2008, Chevin et al. 2010), but also variable environments are more likely to result in plastic changes to organisms relative to more stable environments (van Tienderen 1997, Baythavong 2011). Phenotypic plasticity can also occur based on the relative cost of inducing plastic changes to phenotype, competitive interactions, and evolutionary history (van Tienderen 1997; Hoffmann & Sgrò 2011; Sih, Ferrari, & Harris 2011; Seebacher & Franklin 2012). It is therefore possible that habitat characteristics from the degraded environment (i.e., increased water temperature, more variable flow regime, more variable temperatures – see Blevins et al. 2013) resulted in plastic changes in the magnitude of the stress response in pumpkinseed and white sucker, thereby negating any inter-site differences in the stress response. Such plastic changes would be beneficial because they could eliminate any negative effects of chronic cortisol elevation, while still maintaining the ability to produce an acute stress response. Regardless of the mechanism, results from the current study clearly demonstrate that reduced stream quality did not influence the GC stress axis in some of the species examined.

Defining interspecific variation in the ability to respond to environmental challenges is critical for predicting 'winners' and 'losers' in the face of anthropogenic change (Somero 2010, Angelier and Wingfield 2013). Current research suggests that phenotypic plasticity, the capacity

to experience changes to phenotypes, is critically important for the persistence of species through human induced environmental change (Hendry et al. 2008, 2011, Hoffmann and Sgrò 2011). Quantifying the capacity for animals to display plasticity in traits can be done with a number of different tests and experiments; however, studies of this nature often only examine a single species across an environmental or longitudinal gradient (Hoffmann and Sgrò 2011). Often, for a species to survive across a range of environments, they must inherently be plastic with regards to one or more phenotype, or a broad generalist that can tolerate a wide range of conditions. This may then generate results that are not truly indicative of all species residing in an area or community. In the current study, we examined the response of multiple species when exposed to differing levels of anthropogenic disturbance and documented a variety of physiological responses. While these changes may have a genetic basis, plasticity has been shown to play a major role in an organism's ability to cope with changing environments, especially with regards to rapid anthropogenic disturbance. As such, this result was likely due to species specific levels of plasticity that dictate their ability to cope behaviorally and physiologically with an altered environment. Our results suggest that pumpkinseed and white sucker possess a greater ability to cope with human disturbances and may fair better than other species in our study as they continue to spread. These types of studies can determine which species are most able to survive in changing environments and greatly increase our ability to predict the outcome of human disturbance (Cooke et al. 2013, Angelier and Wingfield 2013).

The use of tissue cortisol extraction proved to be a valuable tool that increased the range of fish sizes available for a study such as this, and enabled us to examine GC function in a taxonomically diverse group of species, including three orders, four families, and five genera. The application of tissue cortisol extraction allows scientists to address multiple novel research

questions regarding habitat characteristics and its effect on small species or early life stages. For example, researchers could compare GC function in both juveniles and adults to determine if life-history stages are affected differentially by environmental disturbances. This could be informative for robust, generalist species, as brief elevations in stress hormones in early life stages can substantially reduce life expectancy in avian species (Monaghan et al. 2011). Therefore, using tissue cortisol extraction to examine stress during this period may enable us to determine otherwise unseen causes of reduced survival and fecundity in cases where chronic stress is not readily apparent in adults. Utilizing this technique would greatly increase the habitats, life stages, and species able to be studied with physiological tools, thereby increasing their contribution to conservation ecology as a whole. While quantifying cortisol from tissue extracts has been used in laboratory studies (Sink et al. 2007), to our knowledge, the current study is the first to employ this methodology in the field.

By examining tissue cortisol in a stream community, our study revealed species-specific responses to the effects of stream quality on glucocorticoid function. We observed nearly the entire spectrum of responses, including no response, elevated baseline cortisol, a facilitated stress response, and an impaired stress response. While researchers have documented a variety of causes, we still cannot confidently predict the endocrine responses of chronically stressed wild animals (Dickens and Romero 2013). Without understanding the conditions that result in the various changes to GC function, it is hard to determine how animals are affected by changes to stream quality and to relate the findings of physiological studies to managers (Cooke and O'Connor 2010). From an ecological standpoint, any change in GC function – regardless of the direction – is potentially important (Dickens and Romero 2013). Studies quantifying baseline and post-stress levels can, therefore, be used to determine if populations are chronically stressed,

and by examining many populations within a community, we can determine species most able to cope with changing environments. Studies of GC function in communities have the ability to improve the basic understanding of the endocrine response to chronic stressors and the relationship between organism-, population-, and community-level processes. Understanding the synergistic relationships between GC function and habitat quality will increase the ability of researchers and managers to predict how ecosystems will be shaped by anthropogenic environmental change.

CHAPTER 4: GENERAL CONCLUSION

Terrestrial systems have a profound influence on stream ecosystems (Paul and Meyer 2001; Allan 2004). The extent of stream degradation from human activities has prompted widespread management and restoration initiatives to protect the natural diversity of aquatic fish communities (Bernhardt et al. 2007; Laub and Palmer 2009). However, we still lack the necessary scientific understanding of the relationships between landscape-level processes and organismal functions that dictate outcomes under human-induced environmental change (Cooke and Suski 2008; Roni et al. 2008). Physiological research can improve this understanding and enable more effective prediction and mitigation of the consequences (Wikelski and Cooke 2006; Cooke and O'Connor 2010). My thesis research uses physiological metrics to examine the effects of anthropogenic disturbance at several levels. My first chapter defines the relationship between land use at large spatial scales and the health and condition of stream fish, while my second chapter reveals interspecific differences in the physiological response to human-induced habitat degradation.

Results from my first chapter demonstrate that natural lands in a watershed have important influences on the physiological condition of stream fish. Watershed forest cover resulted in the best fist model for PCs representing largemouth bass nutrition (i.e., free energy and resistance to oxidative stress), and wetland cover resulted in the top land use practice predicting glucocorticoid function. Furthermore, results showed natural landscape features to have stronger relationships with physiological condition than human land uses, such as agricultural or urban areas. Energy reserves, however, were not related to any type of watershed land use, nor did body condition differ between streams. These findings have important management implications, as they demonstrate not only the importance of natural lands at broad

spatial scales, but also that physiological processes may be impaired even if fish are in good condition. Although riparian rehabilitation is a main focus of stream managers, my results show natural land cover has important benefits even if it is not immediately adjacent to the stream. I would predict fish communities, and stream ecosystems as a whole, to continue declining if humans continue to remove natural land but only protect and restore riparian areas. When considered with other results, the absence of changes to energy reserves demonstrates that nutrition and health of an animal may be impaired even if the effects are not readily apparent. I would predict the long-term fitness of largemouth bass to be lowered as a result of oxidative stress and altered glucocorticoid function, possibly resulting in population declines. As all fish retained sufficient energy stores, the cause of these population declines would remain unknown if physiological metrics were not analyzed. Without this mechanistic information, we will continue to lack the requisite knowledge for proper management and conservation of our fisheries.

The second chapter of my thesis demonstrated that the effects of anthropogenic habitat degradation on the glucocorticoid function of stream fish vary between species in a community. From the five species examined, we witnessed four different glucocorticoid profiles. Whether these differences are due to ecological or physiological traits, they demonstrate the importance of exploring species-specific effects of human disturbance. In this manner, we can determine the species (or functional guilds) that are most sensitive to human disturbance and direct conservation efforts prior to population declines. Furthermore, a determination of the underlying mechanisms that dictate why and how a species is affected can improve our ability to predict the outcomes of environmental change. Together, my thesis studies examine the interacting processes that shape stream communities. My first chapter demonstrates how watershed land use

affects the physiological condition of largemouth bass. While my second chapter demonstrates how species-specific traits and tolerances interact to determine glucocorticoid function and likely health and fitness. My research improves our understanding of the relationships between land use, stream fauna, and species-specific tolerances. This is important because North American freshwater fishes have one of the highest extinction rates in the world (Burkhead 2012). Examining the physiological effects of anthropogenic disturbance can improve the restoration and management of lotic systems, as well as our ability to predict the changes in populations and communities faced with anthropogenic environmental change.

TABLES AND FIGURES

Table 2.1: Groupings of the Southern Ontario Land Resource Information System (SOLRIS) land use categories used in this study. The SOLRIS database provides specific information for many land use types. For the purposes of this study, these have been grouped into the more broadly defined categories listed below.

Presented land use category	SOLRIS land use types included
Agriculture and other fields	All agricultural land, urban brown fields,
	forest clearings, etc.
Urban	Residential, Industrial, Commercial and
	Civic Areas, Highways, Roads
Forest	Coniferous Forest, Deciduous Forest,
	Forest, Mixed Forest, Hedge Row,
	Plantations-Tree Cultivated
Resource extraction	Pits, Quarries
Wetland	Bogs, Marshes, Swamps
Recreation	Golf courses, playing fields, etc.

Table 2.2: Total watershed area and abundance of each land use category (shown as percent of total watershed area) for each watershed used in this study. Watershed land use data were determined using Quantum GIS to integrate land use data from the Southern Ontario Land Resource Information System (SOLRIS) and a layer delineating watershed boundaries produced by the Raisin Region Conservation Authority.

	Total	Agriculture					
	Watershed	and Other			Resource		
Watershed	Area (ha)	Fields (%)	Urban (%)	Forest (%)	Extraction (%)	Wetland (%)	Recreation (%)
Finney	3182.0	77.9	4.7	13.0	0.0	3.9	0.2
Fraser	4485.7	47.7	6.7	19.9	0.0	24.5	1.1
Grays	4450.8	33.0	35.3	8.7	0.0	16.5	6.1
Hoople	9534.8	53.5	5.0	13.9	0.3	26.3	0.6
Pattingale	938.8	69.8	5.9	15.2	0.0	4.9	4.0
Raisin	57847.1	49.8	4.4	18.1	0.3	26.6	0.4
Sutherland	7913.6	76.2	3.2	11.7	0.4	8.2	0.2
Wood	3013.6	83.9	2.8	10.8	0.0	2.1	0.3

Table 2.3: Blood-based nutrition and stress parameters measured in largemouth bass *M. salmoides* (n = 9 - 12) caught in streams in the vicinity of Cornwall, ON, CA, July, 2012, with the standard error of the mean and sample size given in brackets below the mean. Maximal cortisol and glucose elevations were determined by subjecting *M. salmoides* to 3-min air exposure.

Metric	Finny	Fraser	Gray's	Hoople	Pattengale	Raisin	Sutherland	Wood	Grand Mean
Triglycerides (mg dL ⁻¹)	3 [0.4, 11]	1.7 [0.4, 10]	1.4 [0.3, 10]	3.3 [0.6, 10]	3.9 [0.6, 10]	2.7 [0.5, 10]	2.7 [0.4, 12]	2.8 [0.8, 11]	2.7 [0.2, 84]
Total protein (g dL ⁻¹)	4.4	4.6	4.5	4.4	4.7	5.2	4.7	4.9	4.7
	[0.2, 11]	[0.1, 10]	[0.1, 10]	[0.1, 10]	[0.2, 10]	[0.1, 9]	[0.1, 12]	[0.1, 11]	[0.1, 83]
Cholesterol (mg dL^{-1})	337.8	403.8	367.7	320.1	351.4	323.5	321.1	390.7	351.6
	[23.2, 11]	[30.1, 10]	[17.3, 10]	[30.1, 10]	[19.9, 10]	[27.7, 10]	[32.7, 12]	[13.8, 11]	[9.2, 84]
Total antioxidant capacity (mM)	1.5	1.4	1.1	0.8	0.7	1.6	1.1	1.3	1.2
	[0.1, 11]	[0.1, 10]	[0.1, 10]	[0.1, 10]	[0.1, 10]	[0.1, 10]	[0.1, 12]	[0, 11]	[0.1, 84]
Lipid peroxide levels (µM MDA)	30.6	14.5	21.9	37	22.6	12.2	26.1	23.2	23.3
	[4.6, 8]	[1.7, 9]	[1.9, 9]	[3.5, 8]	[2.4, 9]	[1.9, 9]	[2.1, 11]	[2, 10]	[1.2, 73]
Baseline cortisol (ng mL ⁻¹)	4.3	6	5.8	9.6	3.6	8.3	7.5	4.4	6.1
	[0.5, 11]	[1.5, 10]	[0.9, 10]	[2.6, 8]	[0.4, 9]	[1.2, 9]	[1.6, 12]	[0.7, 9]	[0.5, 78]
Maximal cortisol (ng mL ⁻¹)	85.6 [12.2, 11]	81.2 [13.6, 10]	130.9 [17.7, 10]	90.2 [20.2, 8]	94.4 [13.5, 9]	73.8 [14.8, 9]	76.4 [15.9, 12]	71.6 [15.8, 9]	88 [5.6, 78]
Baseline glucose (mg dL^{-1})	3.3 [0.4, 11]	2.4 [0.2, 9]	2.2 [0.3, 9]	3.4 [0.4, 7]	4.3 [0.8, 8]	2.9 [0.5, 8]	3.5 [0.4, 12]	3.1 [0.5, 9]	3.1 [0.2, 73]
Maximal glucose (mg dL ⁻¹)	9.4 [0.7, 11]	9.7 [1.2, 10]	7.1 [0.7, 10]	7.1 [1.1, 8]	7.7 [0.7, 8]	9.8 [1.3, 8]	8.7 [1.1, 12]	7.5 [0.7, 9]	8.4 [0.4, 77]
Cortisol responsiveness (ng mL ⁻¹)	81.4 [12.3, 11]	75.2 [14.3, 10]	125.1 [17.2, 10]	80.6 [20.4, 8]	90.8 [13.5, 9]	65.5 [15.1, 9]	68.9 [14.9, 12]	67.2 [15.5, 9]	81.8 [5.6, 78]
Glucose responsiveness (mg dL ⁻¹)	6.1	7	5	3.8	3.6	6.9	5.2	4.4	5.3
	[0.9, 11]	[1.4, 9]	[0.8, 9]	[1.7, 7]	[0.5, 8]	[1.3, 8]	[1, 12]	[0.7, 9]	[0.4, 73]
Relative weight (W _r)	102.0	102.1	102.7	104.7	101.8	101.9	102.2	102.7	102.5
	[1.4, 3]	[0.9, 8]	[1.0, 6]	[1.1, 5]	[1.0, 6]	[0.9, 7]	[0.7, 11]	[0.9, 8]	[0.3, 54]

Table 2.4: Factor loadings for varimax rotated principle components summarizing stress and nutritional characteristics for largemouth bass *Micropterus salmoides* (n = 9 – 12) sampled from eight watersheds in the vicinity of Cornwall, ON, CA, July 2012. Variables were loaded into four principal components and factor loadings of > |0.4| were considered maximal contributors to each PC.

Variable	PC 1	PC 2	PC 3	PC 4
Baseline cortisol (ng mL ⁻¹)	-0.11	0.31	-0.03	-0.08
Total protein (g dL ⁻¹)	0.36	0.85	0.34	-0.15
Triglycerides (mg dL^{-1})	0.99	-0.12	-0.01	-0.12
Total antioxidant capacity (mM)	0.00	0.07	0.74	0.16
Lipid peroxide levels (µM MDA)	0.37	-0.06	-0.51	-0.10
Cholesterol (mg dL^{-1})	-0.13	0.64	-0.04	0.35
Baseline Glucose (mg dL ⁻¹)	0.56	0.01	-0.24	-0.58
Cortisol responsiveness (ng mL ⁻¹)	-0.17	0.13	-0.38	0.40
Glucose responsiveness (mg dL ⁻¹)	-0.03	-0.06	0.12	0.49
Variance Explained	18	14.1	12.7	10.5

Table 2.5: Model selection results for examination of factors affecting principal component scores of largemouth bass *Micropterus salmoides* in streams near Cornwall, ON, CA, July 2012. Models were ranked with Akaike's Information Criterion adjusted for small sample sizes (AIC_c).

			-2 Log-			
PC	Model ^a	K ^b	likelihood	AIC _c	$\Delta_i AIC_c$	Model Likelihood
PC1	%Forest ²	4	173.2	181.9	0	0.53
	%Agriculture and other fields	3	178.7	185.1	3.2	0.11
	%Agriculture and other fields ²	4	176.5	185.2	3.3	0.10
	% Urban	3	178.9	185.3	3.4	0.10
	%Wetland ²	4	177.3	186	4.1	0.07
	% Urban ²	4	178.6	187.3	5.4	0.04
	%Wetland	3	181.5	187.9	6	0.03
	%Recreation	3	182.8	189.2	7.3	0.01
	%Recreation ²	4	181.2	189.9	8	0.01
	%Forest	3	183.9	190.3	8.4	0.01
	%Resource Extraction	4	181.7	190.4	8.5	0.01
	%Resource Extraction ²	4	182.9	191.5	9.6	0.00
PC2	%Resource Extraction	3	179.3	185.7	0	0.12
	%Recreation	3	179.3	185.7	0	0.12
	%Agriculture and other fields	3	179.3	185.7	0	0.12
	%Wetland	3	179.4	185.8	0.1	0.11
	%Urban	3	179.4	185.8	0.1	0.11
	%Forest	3	179.5	185.9	0.2	0.11
	%Forest ²	4	178.1	186.8	1.1	0.07
	%Urban ²	4	178.1	186.8	1.1	0.07
	%Agriculture and other fields ²	4	178.8	187.5	1.8	0.05
	%Resource Extraction ²	4	179.1	187.8	2.1	0.04
	%Recreation ²	4	179.3	188	2.3	0.04
	%Wetland ²	4	179.4	188.1	2.4	0.04
PC3	%Forest ²	4	152	160.7	0	0.26
	%Forest	3	155	161.4	0.7	0.18
	%Recreation	3	155.5	161.9	1.2	0.14
	%Wetland ²	4	153.4	162.1	1.4	0.13
	%Wetland	3	156.3	162.7	2	0.09
	%Recreation ²	4	154.5	163.2	2.5	0.07
	%Resource Extraction	3	158.3	164.7	4	0.03
	%Urban	3	158.4	164.8	4.1	0.03
	%Agriculture and other fields	3	158.8	165.2	4.5	0.03
	%Urban ²	4	157.8	166.5	5.8	0.01
	%Resource Extraction ²	4	158.1	166.8	6.1	0.01
	%Agriculture and other fields ²	4	158.8	167.5	6.8	0.01

Table 2.5 (cont.)

			-2 Log-			
PC	Model ^a	K ^b	likelihood	AIC _c	$\Delta_i AIC_c$	Model Likelihood
PC4	%Wetland	3	133.7	140.1	0	0.28
	%Agriculture and other fields	3	134.2	140.6	0.5	0.22
	%Wetland ²	4	133.6	142.3	2.2	0.09
	%Forest ²	4	133.9	142.6	2.5	0.08
	%Agriculture and other fields ²	4	134.2	142.9	2.8	0.07
	%Forest	3	137	143.4	3.3	0.05
	%Urban	3	137	143.4	3.3	0.05
	%Resource Extraction	3	137.6	144	3.9	0.04
	%Recreation	3	137.8	144.2	4.1	0.04
	%Resource Extraction ²	4	135.7	144.4	4.3	0.03
	% Urban ²	4	136.1	144.8	4.7	0.03
	%Recreation ²	4	137.6	146.3	6.2	0.01

^a Models indicated with a square term (²) analyzed effects of both linear and quadratic variation ^b Parameter count includes intercept and variance.

Table 3.1: Watershed land use characteristics (shown as percent of total watershed area) for the two streams used in this study. Watershed land use data were determined using Quantum GIS to integrate land use data from the Southern Ontario Land Resource Information System and a layer delineating watershed boundaries produced by the Raisin Region Conservation Authority.

	Stream			
	Gunn Creek	Hoople Creek		
Land use category	(Degraded)	(Reference)		
Forest	5.0 %	13.9 %		
Wetland	2.9 %	26.3 %		
Agriculture and other fields	87.3 %	53.5 %		
Urban	4.5 %	5.0 %		
Recreation	0.0 %	0.6 %		
Resource extraction	0.0 %	0.3 %		
Total Watershed Area (ha)	1038	9535		

Table 3.2: A brief description of the species used in this study and their basic characteristics. Habitat and life history characteristics were obtained from www.fishtraits.info (Frimpong and Angermeier 2009) and feeding preferences were obtained from www.fishbase.org (Froese 1990).

Species	Preferred in-stream habitat	Preferred food	Lifespan (years)	Age at maturity (years)
Largemouth bass	Vegetation, muck, silt, clay, sand, gravel, or cobble substrates	Fish, frogs, crayfish	16	2.5
Pumpkinseed	Can be found in almost all habitats, except large rocky substrates	Fish and other vertebrates, fish eggs	8	2
White sucker	Can be found in almost all habitats, except large woody debris	Insects, diatoms, crustaceans, protozoa, algae	8	3
Logperch	Riffle; prefer gravel and cobble substrate	Larval and adult insects, fish eggs	4	1.5
Brown bullhead	Vegetation, silt, clay, sand, or gravel substrates	Algae, plants, mollusks, crayfish, worms, plankton, insects, fish, fish eggs	11	2.5

Table 3.3: Size ranges for the individuals used in this study. All samples were collected from a degraded stream and a reference stream in the vicinity of Cornwall, ON, CA in October of 2012. Length is presented as the mean, \pm standard error.

Species	Length (mm)	Length Range (mm)
Largemouth bass	80 ± 3	57 – 127
Pumpkinseed	65 ± 2	44 - 111
White sucker	113 ± 8	66 - 240
Logperch	83 ± 3	51 – 111
Brown bullhead	97 ± 8	63 - 207

Figure 1.1



Figure 1.1: Map of the Cornwall Area of Concern (AOC) showing the watershed boundaries of streams to be used in this research. This map was created using a GIS layer that delineated watershed boundaries developed by the Raisin Region Conservation Authority.

Figure 2.1



Figure 2.1: Relationship between the proportion of forested area in a watershed and PC1 scores (free energy) for largemouth bass *Micropterus salmoides*. *Micropterus salmoides* (n = 9 - 12) were collected from a total of 8 watersheds in the vicinity of Cornwall, ON, Canada, and sampled for blood in the field immidiately after electroshocking. Information on PC1 is given in Table 4.





Figure 2.2: Relationship between the proportion of a) forested area, b) recreational area, and c) wetland area in a watershed and PC3 scores (resistance to oxidative stress) for largemouth bass *Micropterus salmoides*. *Micropterus salmoides* (n = 9 - 12) were collected from a total of eight watersheds in the vicinity of Cornwall, ON, Canada, and sampled for blood in the field immidiately after electroshocking. Information on PC3 is given in Table 4.





Figure 2.3: Relationship between the proportion of a) wetland area and b) agricultural and other fields area in a watershed and PC4 scores (glucocorticoid function) for largemouth bass *Micropterus salmoides*. *Micropterus salmoides* (n = 9 - 12) were collected from a total of eight watersheds in the vicinity of Cornwall, ON, Canada, and sampled for blood in the field immidiately after electroshocking and again after being subjected to a standardized stressor. Information on PC4 is given in Table 4.





Figure 3.1: Baseline, post-stress, and responsiveness (baseline subtracted from post-stress) tissue cortisol values for the 5 species examined in our study. All samples were collected from a degraded stream and a reference stream in the vicinity of Cornwall, ON, CA in October of 2012. Sample sizes are indicated at the base of the bars. Significant differences ($\alpha < 0.05$) between streams, within a category, are indicated by an asterisk (*) above the bars.

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