

**Effects of watershed land use on the seasonal energy dynamics, condition,  
and parasite communities of stream fish.**

By

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## **Dedication**

To my parents for their patience and understanding while raising a child obsessed with the natural world. You never knew what creature you would find hidden somewhere in your house next, yet you never discouraged my curiosity. For that I am forever grateful. To the Wards and their ongoing support and encouragement. I am so fortunate to have joined such an amazing family. And finally, to my partner Taylor, to whom I simply say, thank you, thank you, thank you.

## **Abstract**

While it is generally considered that habitat degradation negatively impacts resident fish, the physiological mechanisms behind this trend are not well understood. Applying scientific techniques to increase our understanding of how habitat influences individual physiology and how that, in turn, influences fish population dynamics is becoming an important approach to environmental monitoring. In Chapter 2, I quantify how watershed land use influences the over winter energy reserves and health of a small stream fish and demonstrate that land use influences these metrics differently for females and males of the model species. In Chapter 3, I investigate the parasite communities of juvenile fish residing in a range of impacted streams to identify potential bioindicators of habitat – and consequently fish – health. These data are the first of their kind for both species examined, and are also the first time these techniques have been used to address these unique research questions.

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## **Co-Authorship**

**Chapter 2: Does land use influence the overwinter body composition and health of a stream fish?** Jacqueline M. Chapman, Greg D. King, Cory D. Suski, and Steven J. Cooke

While this study is my own, the research was undertaken as part of a collaborative effort and each co-author played a valuable role in its completion. The project was conceived by Chapman, Cooke and Suski. Fieldwork was completed by Chapman and King, with logistical support from Cooke and Suski. All data analysis was conducted by Chapman with valuable input from King. Data were interpreted by Chapman, King, Cooke and Suski. All writing was conducted by Chapman. All co-authors provided feedback on the manuscript.

**Chapter 3: Variation in parasite community and health index of juvenile *Lepomis gibbosus* across a gradient of land use: Can parasites be used as an indicator of habitat quality?** Jacqueline M. Chapman, David J. Marcogliese, Cory D. Suski and Steven J. Cooke

While this study is my own, the research was undertaken as part of a collaborative effort and each co-author played a valuable role in its completion. The project was conceived by Chapman, Marcogliese, Cooke and Suski. Field and laboratory work was completed by Chapman with logistical support and guidance from Marcogliese and Cooke. All data analysis was conducted by Chapman. Data were interpreted by Chapman. All writing was conducted by Chapman. Chapman, Cooke and Suski provided comments and feedback on the manuscript.

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## **List of Acronyms**

**AOC: Area of Concern**

**ANOSIM: Analysis of Similarity**

**ANOVA: Analysis of Variance**

**GSI: Gonadosomatic index**

**H': Shannon Diversity Index**

**HAI: Health Assessment Index**

**HSD: Honestly Significant Difference**

**HSI: Hepatosomatic Index**

**K: Fulton's Condition Factor K**

**MI: Mean Intensity**

**NMDS: Nonmetric Multidimensional Scaling**

**P: Prevalence**

**PC: Principal Component**

**PCA: Principal Component Analysis**

**RAP: Remedial Action Plan**

**SD: Standard Deviation**

**SE: Standard Error**

**SEM: Standard Error of Mean**

**SIMPER: Similarity Percentage analysis**

## **Chapter 1: General Introduction**

### **The Current State of Freshwater Ecosystems**

Stream habitat and biodiversity are directly influenced by the condition of the surrounding terrestrial landscapes through which they flow (Hynes 1975). Consequently, freshwater ecosystems are negatively impacted by habitat alterations and contamination arising from human activities (Dudgeon et al. 2006), with current extinction rates for freshwater animals in North America predicted to be five times greater than terrestrial animals and three times greater than marine animals (Ricciardi and Rasmussen 2001). Specifically, resident ichthyofauna are experiencing a rapid decline in both abundance and diversity with 39% of all described species in North America now listed as imperilled (Threatened, Endangered or Vulnerable) (Ricciardi and Rasmussen 2001; Jelks *et al.* 2008). The need to understand the mechanisms driving the effects associated with anthropogenic degradation has become increasingly urgent, as freshwater faunal populations continue to decline despite attempts to mitigate these trends.

Environmental management initiatives have endeavoured to address issues created by human induced disturbance through ecological restoration (Miller and Hobbs 2007). To ensure both environmental and economic stability, the overarching goal of these initiatives is to effectively manage, conserve and/or restore fish populations (Miller and Hobbs 2007). In lotic environments rehabilitation often focuses on the improvement of habitat structural complexity, riparian vegetative communities, flow regimes, connectivity, and water quality, particularly in areas where watersheds have undergone extensive land use changes (Cowx and Gerdeaux 2004; Palmer et al. 2010). Of these

initiatives, habitat restoration is often used in areas where extreme impact has been noted to negatively affect fish populations. The need to view the catchment as a whole, and how catchment characteristics interact to effect resident fish, is necessary to address landscape scale restoration efforts often found in heavily degraded areas (Ellis et al. 2012). Watershed-scale measurements can include point-source contaminants (e.g. wastewater treatment plants, mines) and nonpoint-source factors such as watershed land use (e.g. proportion of agriculture, urban development).

At the watercourse scale, identification of degraded areas and monitoring of rehabilitated areas is often achieved by aquatic faunal community surveys, where a community is defined as an assemblage of interacting species (Kerr and Ryder 1977). Communities can be quantified as basic diversity (number of species in a given area), or through various indices such as the Index of Biological Integrity for fishes (Karr 1981) or the Hilsenhoff index for invertebrates (Hilsenhoff 1988). Although informative, community-level metrics have limitations to the causal inferences that can be made from such data (Ellis et al. 2012), and may be slow respond to change as individual health must be compromised to a point where it impacts fitness and thus population recruitment (Adams and Brown 1993). It is therefore crucial to develop our understanding of individual response to various environmental conditions and how this relates to organismal health and success, as these processes underlie the patterns of species distribution and abundance which are of ultimate concern for resource managers (Wikelski and Cooke 2006; Adams and Ham 2011). When monitoring degraded and rehabilitated systems, evaluating fish health will enhance our mechanistic understanding of changes observed within a community. This will yield a more detailed understanding

of biotic factors that influence why certain restoration efforts do not achieve their desired objectives (Cowx 2004; Miller and Hobbs 2007; Hobbs and Cramer 2008).

### **Individual Condition and Health as a Measurement of Habitat Quality**

Individual-level parameters relevant to organismal condition and health have been successfully used to identify how habitat degradation influences biotic responses in fish (reviewed in Wikelski and Cooke 2006). Measurements of individual physiology may be an effective way to assess the impact habitat degradation has on resident fish. This can further aid in the identification of beneficial restoration practices and so inform and prioritize future applications (Wikelski and Cooke 2006; Cooke and Suski, 2008). Indicators of this type can further be used to establish baseline information on populations and quantify changes that occur as a result of future environmental perturbation or restoration (Cooke and Suski 2008). Individual health metrics both integrate and indicate the overall state of the ecosystem, creating potentially effective tools for use in monitoring the health of freshwater ecosystems.

Analysis of proximate body composition (proportion of lipid, protein, mineral and water) is a method that can be used to quantify energy stores in fishes (Weatherly and Gill 1987). This method has been used to study body composition of various fish species in response to differences in feeding levels (Niimi and Beamish 1974; Elliot 1976), temperature (Elliot 1976), hormone levels (Weatherly and Gill, 1983) and more recently, seasonal variation in response to habitat quality (Murchie et al. 2010). In wild animals, energy stores exhibit seasonal cycles depending on the life stage and life history of the species in question. Primary energy storage in fishes is in the form of lipids, while protein

and glycogen are considered secondary energy sources reserved for energy deficits (Weatherly and Gill 1987). Thus, a simple measurement of the proportion of lipids within a fish can be used to understand overall energy stores in an individual.

Energy acquisition and allocation within an individual is subject to multiple environmental and physiological factors, including temperature, quality of forage, and phenology, all of which may fluctuate seasonally (Weatherly and Gill 1987). For example, non-migratory fish residing in northern clines are likely to have their highest energy stores at the onset of winter (Shul'man 1974; Shuter et al. 2012). For these fishes, winter is a period of decreased temperature, day length and food availability. During this time, the majority of fish rely on energy stores created during summer to offset their energy output deficit (Wilkins 1967, Niimi 1972). This is particularly true for those fishes that spawn in the spring, as overwinter gonadogenesis requires additional utilization of energy stores (Weatherly and Gill 1987; Shuter et al. 2012). Consequently, any factor that influences energy stores may affect both overwinter success and reproductive capacity, specifically in non-migratory northern species.

Environmental perturbation may influence the presence or absence of disease in fishes residing in impacted areas (Meyer 1997), often as a result of immunosuppression associated with increased stress (Sniezko 1974). Environmental indices have been created that incorporate gross pathological indicators of fish health (e.g. Adams et al. 1993), which incorporate the intensity of parasite infections in their measurements of fish health. This is under the general premise that all parasites react positively to fish immunosuppression and are not themselves influenced by changes in the surrounding environment (Marcogliese 2005). While this may be true for certain species, a large body

of evidence exists which demonstrates the variable responses of different parasite taxa to varying types of pollutants (Lafferty 1997, Blonar et al. 2009). As a result, parasites can indicate both fish host immunosuppression and a suite of environmental characteristics, as a single host may contain groups that indicate unique portions of the surrounding environment (Mackenzie 1999). As an example, monogeneans, a monoxenous ectoparasite infesting the gills of fishes, have been demonstrated to be sensitive to changes in water quality, particularly wastewater effluent and heavy metal contamination (Blonar et al. 2009). The use of this group of organisms as a tool for monitoring can further provide indication of free-living diversity, as many parasites are functionally coupled with obligate intermediate and definitive hosts; these relationships can further indicate changes in food web dynamics associated with ecosystem changes (Marcogliese 2003, 2004). Taken together, these characteristics allow for the use of parasites as bioindicators to be uniquely versatile, particularly in aquatic ecosystems.

### **Great Lake Areas of Concern – Cornwall**

The Laurentian Great Lakes comprise the largest lake system in the world, accounting for 21% of the world's fresh water. Following environmental degradation from large-scale industrial activity, agriculture, and urbanization during the 20<sup>th</sup> century, the Great Lakes are now a major focal point for restoration efforts in both Canada and the United States (Hartig and Thomas 1988; Hartig and Vallentyne 1989). Impacts of anthropogenic land use change have led to wide-scale habitat modification, eutrophication, exploitation, contamination and the introduction of invasive species throughout the system (Kelso and Minns 1996).

Management practices aiming to rehabilitate and restore ecosystems in the Great Lakes were initiated not only to manage the system as a whole, but also to target severely degraded localized areas. In 1985 the International Joint Commission, a Canadian and US agency, identified 43 ‘Areas of Concern’ (AOC) within the Great Lakes region of Canada and the U.S. (Hartig and Thomas 1988) and began to work together to help guide environmental improvement programs. AOCs were selected based on extreme environmental degradation, typically as a result of heavy metal contamination, nutrient loading and species decline. There are currently nine areas of concern in Canada, 25 in the United States and five shared by both nations (St. Lawrence RAP 1992). Within each area, a specific ‘Remedial Action Plan’ (RAP) is in place, with targets for environmental benchmarks such as contaminant concentrations, nutrient discharge, and the presence and abundance of sensitive species (Kelso and Minns 1996).

The Cornwall AOC, located on the shores of Lake St. Frances on the St. Lawrence waterway, is an area where a history of heavy metal and bacterial contamination, eutrophication, and habitat destruction is known to directly impact resident fauna (AECOM Canada Ltd., 2009; Hughes *et al.* 2010). Extensive research has been conducted by various agencies (Ontario Ministry of the Environment, Department of Fisheries and Oceans, Raisin Region Conservation Authority) to identify the major anthropogenic impacts within each watershed. These efforts have generated several reports that appraise and classify the level of degradation exhibited within each watershed, including GIS maps of land use and habitat types and monthly variation in phosphorous output. In combination with a variety of restoration efforts (ex. upgrading manure storage facilities, riparian zone planting) these metrics enable one to rank streams

such that they yield a gradient of stream habitat quality across the drainage basins within the Cornwall AOC.

To enhance adaptive management strategies within the RAP, information that can help identify processes relating individual and community response to habitat variables is fundamental. While several studies have addressed heavy metal contamination in sediments (Poissant *et al.* 2007; Delongchamp *et al.* 2008) and wildlife (Weis 2004; de Solla *et al.* 2007, 2008; Choy *et al.* 2008), little is currently known about the overall health of fishes within the area, and whether fish in more degraded areas are negatively impacted compared to fish in less degraded and partially restored areas. By helping to identify areas for further restoration that may produce maximal yields of both fish and other aquatic species, this information can enable cost effective decision making.

### **Research Objectives**

The general objective of this thesis was to study the effect of anthropogenic land use on the condition, energy dynamics and parasite burden of small stream fish in the heavily impacted area of Cornwall, Ontario. This work further endeavours to assess whether or not these measurements may serve as appropriate bioindicators of habitat health resultant from such land use. In Chapter 2, the overwinter energy stores of central mudminnow *Umbra limi* across a gradient of watershed land use were quantified. I also assessed differences between sexes with respect to timing of gonadogenesis and body composition. This was accomplished using proximate body composition analysis and health indices of fish collected from streams within the Cornwall AOC composed of varying proportions of agriculture and urban development. I expected fish in more heavily impacted areas to demonstrate decreased energy stores and decreased spring

condition, indicated by the proportion of lipids and various health indices. In Chapter 3, the parasite community of juvenile pumpkinseed *Lepomis gibbosus* was investigated to indicate both the health and condition of individual fish and provide insight into ecosystem-scale trends. I expected to recover parasites similar to those from a recent study conducted by Locke et al. (2012) on adult *L. gibbosus* collected from nearby shoreline of the St. Lawrence River, and that some of these parasites would increase in prevalence and intensity with increasing eutrophication (measured in total phosphorous). I anticipated areas with increased benthic diversity and lower watershed disturbance would have higher species diversity, a trend that has been demonstrated in previous research (e.g. Valtonen et al. 1997; Shah et al. 2013). In Chapter 4, the findings from Chapters 2 and 3 will be integrated and discussed, and potential avenues for future research and applications of results found here will be presented. Moreover, although research was focused on the Cornwall AOC, findings will be discussed in terms of their relevance to other systems and more broadly as they relate to habitat science, monitoring and restoration.

## **Chapter 2: Does land use influence the seasonal energy dynamics and health of a stream fish?**

### **Abstract**

Changes in land use within watersheds are known to influence fish populations in stream ecosystems, though the physiological mechanisms behind population-level changes remain elusive. Here we test the use of proximate body composition to assess the influence of land use on the health and reproductive status of stream fish. Geographic Information Systems (GIS) was used to characterize land use in six tributaries of the St. Lawrence River. Using central mudminnow *Umbra limi* as a model, spring and fall energy dynamics were quantified through proximate body composition analysis and health indices. These factors were then used in principal component analysis (PCA) to summarize fish condition and principal components compared to land use in each watershed. In general, mudminnow were composed of 76% water, 15% protein, 5% lipid, and 4% trace minerals, values consistent with other fish species studied to date. Significant differences were found in proximate body components and health indices between males and females, particularly in spring associated. These differences appear to be associated with life-history traits (e.g. spawning) and overwinter feeding behaviours. Central mudminnow energy stores, growth and condition were not significantly related to watershed land use, indicating this species would not be an appropriate bioindicator for fish health in degraded systems.

## **Introduction**

Freshwater ecosystems are currently experiencing unprecedented change as a result of anthropogenic activities and development (Dudgeon *et al.* 2006; Vörösmarty *et al.* 2010). For example, 39% of all described freshwater species are now listed as imperilled (Threatened, Endangered or Vulnerable; Jelks *et al.* 2008), extinction rates in North American freshwater systems are five times greater than terrestrial animals and three times greater than marine (Ricciardi and Rasmussen 1999), and between 56 and 83 freshwater fish species are expected to become extinct by 2050 (Burkhead 2012). Anthropogenic habitat alteration, including both degradation of water quality and physical habitat, is regarded a primary driver influencing the abundance, distribution and condition of fishes (Wang *et al.* 1997, Dudgeon *et al.* 2006). In particular, land use practices in a watershed such as urbanization and intensive agriculture in headwater source areas have been correlated with decreased habitat heterogeneity, water quality, and species diversity ( Schlosser 1991, Wang *et al.* 1997, Walser and Bart 1999).

Aquatic environments are physiologically demanding, as they are naturally variable due to seasonal hydrodynamics and the physicochemical properties of water (Fry 1971; Brett and Groves 1979). Resident ichthyofauna are particularly vulnerable to habitat degradation due to their intimate connection with their environment. Consequently, anthropogenically -derived environmental stress (e.g. increased water temperature, changes in hydrology, sedimentation) can interact and combine with the already arduous natural flux, potentially causing atypical shifts in individual energy partitioning between somatic growth, homeostasis, and reproduction, which can ultimately influence fecundity, i.e. overall fitness (Ross *et al.* 1985; Barton *et al.* 2002;

Adams and Hamm 2011). In order for fish populations to remain stable there must be adequate energy acquired and allocated towards reproduction (Wootton 1985) while meeting all other energetic requirements (Calow 1985; Jobling 1992). As such, understanding how anthropogenic alterations to habitat influence energy flow is essential for the effective management and evidence-based conservation of freshwater fish (Hansen et al. 1993; Lapointe et al. 2014).

In north-temperate stream habitats, fish populations are adapted to a unique suite of environmental challenges. Resident fishes experience extreme environmental changes on a fine temporal scale, with changes in hydrology, suspended sediment, temperature, and dissolved oxygen occurring in response to weather events (e.g. drought, floods). In addition, these fish must survive the annual challenge of winter – a period of decreased temperature, day length and food availability. During this time, the majority of fish rely on energy stores created during summer to offset their energy output deficit (Wilkins 1967, Niimi 1972). However, when an increased energetic demand occurs during winter due to external challenges, an increase in mortality has been demonstrated, mainly due to increased depletion of energy stores (Oliver et al. 1979, Driedger et al. 2008, Shuter et al. 2012). Challenges that are normally tolerable in summer months due to high food availability and warm water temperatures may become lethal if experienced during this physiologically demanding time of year (Shuter et al. 2012).

Analysis of proximate body composition (e.g. water, mineral and lipid) and various health indices is a method that has been employed to reveal the condition of fish in response to changes in diet and various environmental stressors (Wilkins 1967, Cunjak and Power 1986, Berg and Bremset 1995, Murchie et al. 2010). Each component

evaluates specific energy endpoints. For example, the relative proportion of lipids represents chemical energy stores, and change in protein content is considered a measure of realized growth (Busacker et al. 1990).

While recognized as a critical period in northern climates, stream fish winter biology is not well understood (Cunjak 1996, Shuter et al. 2012). The challenges placed upon fish throughout this season have been demonstrated to cause depletions in fish lipid and protein reserves (Newsome and Leduc 1975, Berg and Bremset 1995). Energy density and nutritional research to date has had a strong focus on the effects of diet, particularly in relation to aquaculture (Miglav and Jobling 1989, López et al. 2009), with little investigation into the relationship between habitat degradation and changes in proximate body composition related to overwinter stress in Northern stream fish.

Due to their ability to withstand extreme variation in environmental conditions, the central mudminnow (*Umbra limi*, Kirkland 1840) serve as an interesting model for relationships between land use derived degradation and energy dynamics in Northern stream fishes. A species that is found commonly across central and eastern North America, central mudminnow are both facultative air breathers capable of withstanding extreme temperature variations, allowing them to exploit habitats that other fish cannot withstand (Chilton et al. 1984, Martin-Bergmann and Gee 1985, Currie et al. 2009). In addition, mudminnow have been observed feeding throughout winter, although at a reduced rate and on less diverse prey items than during the summer (Chilton et al. 1984). For this reason they are often one of few species found in the headwaters of watersheds with high proportions of intensive agriculture, and their presence in these systems allows

the comparison of energy dynamics in highly impaired systems compared to those in a less impacted state.

Using central mudminnow as a model, the goal of this study was to quantify the effects of land use on overwinter energy dynamics and fish health/condition. To accomplish this goal, I coupled field sampling across replicate watersheds with laboratory analyses of body composition to define how watershed land use influences variation in body composition between sexes, as well as the energy dynamics pre- and post-winter. I hypothesized that fish collected from watersheds with a high proportion of agriculture and urban land use would have decreased energy stores and condition indices compared to fish collected from watersheds with lower proportions of these land use types, and that this relationship would be more evident in the spring as a result of the utilization of energy stores to maintain homeostasis throughout winter.

## **Methods**

### *Data Collection*

This study took place near Cornwall, ON, in tributaries of the St. Lawrence River (45° 01' 17.5" N, 74° 43' 50.42" W). Land use data were obtained from the Southern Ontario Land Resource Information System (SOLRIS) and were combined with a GIS layer defining watershed boundaries developed by the local Raisin Region Conservation Authority using Quantum GIS Lisboa (1.8.0). Because watersheds varied in size, the proportion of different land use categories was determined using total hectares allocated to each land use divided by the total watershed area (Table 2-1). Streams were selected to represent a variation in watershed land use, and to ensure collections of adequate

numbers of central mudminnow based on community surveys associated with a concurrent research project (unpublished data). Together, this yielded a total of six tributaries to the St. Lawrence that were included in this study.

### *Sample Collection*

Mudminnow were collected by backpack electrofishing (Haltech HT-2000, Guelph, ON, Canada) in November 2012 and April 2013. Fish were collected from replicate streams at road access points in the lower reaches of watersheds in an effort to ensure that fish were living in effluent associated with land use characteristics. Fall collection occurred prior to first ice over of the year (Nov 21-28, 2012), when water temperatures were  $\sim 4^{\circ}\text{C}$ , and spring collection occurred during spring freshet (April 25-30<sup>st</sup>, 2013) before water temperatures reached  $10^{\circ}\text{C}$ , when central mudminnow have not started spawning (Scott and Crossman, 1973). After capture, fish were euthanized in clove oil, bagged individually and placed immediately on ice for dissection at the end of the day. Length and weight were recorded prior to dissection, at which point gonads and liver were excised for weighing. Gonads and livers were then returned to the body cavity and fish were placed in individual bags for later processing. All research was carried out in accordance with the Canadian Council on Animal Care.

### *Laboratory Analysis*

To quantify proximate body composition of this small species, the entire fish was ground and homogenized using a small hand held blender.  $2 \text{ g} \pm 0.2 \text{ g}$  of homogenized whole body tissue was then placed into a crucible and dried overnight at  $80^{\circ}\text{C}$  to a constant weight to the nearest 0.0001 g. Samples were removed and allowed to cool in

desiccators, and then weighed again to the nearest 0.0001 g to quantify the proportion of water. This dried tissue was then ground into a fine powder using a quartz pestle and separated into two portions ranging between 0.12 – 0.24 g dependent on fish size. All samples were run in duplicate, and differences between replicates never exceeded 3%.

Whole body lipids were extracted using the chloroform-methanol technique developed by Bligh and Dyer (1959). The dried, fine powder samples were combined with chloroform, methanol and distilled water in a 1:2:0.8 ratio and placed in an ultrasonic bath for 15 minutes. An additional 1 mL of chloroform and 1 mL of distilled water were added, and samples were centrifuged at  $126 \times$  gravity for 10 minutes. The solvent layer containing the lipids was removed and filtered through sodium sulphate and quartz wool into preweighed aluminum boats. The extracted lipid solution was left overnight to allow chloroform to evaporate, then placed in a drying oven for 1 h at 60°C and weighed to the nearest 0.0001 g to calculate percentage of lipids by dry mass.

Trace minerals were analyzed using  $2 \text{ g} \pm 0.2 \text{ g}$  of homogenized wet tissue, or the remaining tissue if fish were small. Crucibles were again weighed to quantify proportion of water, then combusted at 600°C for 2 hrs in a muffle furnace. Samples were removed from the furnace and cooled in a desiccator prior to weighing. To determine protein content, the following equation was used:

$$C_P = 100 - (C_W + C_L + C_A)$$

with  $C_W$ ,  $C_L$  and  $C_A$  representing the proportions of water, lipid and mineral, respectively (Berg *et al.* 1998; Murchie *et al.* 2010).

Fish health indices were calculated using the following equations:

$$\text{Fulton's } K = \text{whole body weight (g)} / [\text{total length (mm)}]^3 \times 10^5$$

$$\text{HSI} = \text{liver weight (g)} / \text{total body weight (g)} \times 100$$

$$\text{GSI} = \text{gonad weight (g)} / \text{total body weight (g)} \times 100$$

### *Statistical Analysis*

All analysis was conducted using JMP 9® V 9.0.1 (SAS Institute, Cary, North Carolina). Normality and equivalence of variance was assessed using Shapiro-Wilks and Levene's tests, respectively. Results are presented as mean  $\pm$  standard deviation when appropriate. Two-way analysis of variance (ANOVA) followed by Tukey-Kramer HSD was used to determine the relationships between sex and season on proximate body composition. Due to significantly different variances, health indices were tested using student's t-tests when normally distributed, while non-parametric Wilcoxon tests were used where applicable. Whole body water content has previously been reported as an potential surrogate to assess lipid content in various fish species (e.g. Caulton and Bursell 1977; Trudel *et al.* 2005; Murchie *et al.* 2010). To determine whether this technique would be appropriate for use with proximate body assessments involving central mudminnow, regression analyses on arcsine-transformed data were conducted. Further investigation into the relationship of the proportion of lipids with minerals and proteins was investigated to understand any observed variation in this relationship.

In order to summarize the overall effect of land use on fish health, a multivariate principle component analysis (PCA) was conducted to determine the relationship between land use and central mudminnow health indices and body composition. We chose to compute separate principle components (PC) for males and females in fall and

spring due to the large differences in body composition found between sexes and seasons, and to address the question of how land use interacts differently across sexes and seasons. PC's with eigenvalues  $\geq 1.0$  were included in analysis, and variables with factor loadings  $> |0.4|$  were considered maximal contributors (Kaiser 1960). Land use variables were then modeled against the median PC values for each watershed to define the relationship between fish body composition, condition, and land use practices. Bonferonni adjustments for multiple comparisons were used for regressions where multiple comparisons were tested.

## **Results**

### *Watershed land use*

Agriculture was the most predominant land use across all streams in this study. Gray's Creek had the highest proportion of urban development, ~5 times higher than the next highest watershed, likely a result of close proximity to the city of Cornwall. The largest watershed in this study was Hoople Creek, which has the lowest proportion of agriculture and highest proportion of forest (Table 2-1).

### *Proximate body composition and health indices*

A total of 278 central mudminnow ( $73.9 \pm 13.2$  mm standard length) were captured, with 264 being included in analysis. Those that were not included were either too small or under-developed to process ( $n=4$ ), were contaminated or lost during laboratory processing ( $n=2$ ) or identified as statistical outliers using the maximum normed residual test when data was reasonably normal ( $n=6$ ). In general, this species is composed of 76% water, 5% lipid, 15% protein and 4% trace minerals. For both sexes,

all examined constituents varied significantly by sex and season (% water  $F_{(3, 257)} = 76.66$ ,  $P < 0.0001$ ; % mineral  $F_{(3,257)} = 16.11$ ,  $P < 0.0001$ ; % lipid  $F_{(3, 257)} = 19.16$ ,  $P < 0.0001$ ; % protein  $F_{(3, 257)} = 7.55$ ,  $P < 0.0001$ ). Two-way ANOVA revealed significant relationships between proportion of lipids and water and fish sex, proportion of water and mineral and season, and overall significant interactions between sex and season for all body constituents (Table 2-3). Tukey-Kramer HSD revealed significant differences in proximate body composition between fall and spring for each sex (Figure 2-1).

Condition factor (k) ranged from 1.30 – 2.61, with males scoring higher than females in the fall ( $t=5.38$ ,  $df = 149.0$ ,  $P < 0.0001$ ) and females higher than males in the spring ( $t=2.59$ ,  $df = 95.6$ ,  $P = 0.011$ ). Within the sexes, females had a higher condition score in the spring ( $t=3.103$ ,  $df = 95.86$ ,  $P = 0.0013$ ) and males a lower condition score in the spring ( $t=4.640$ ,  $df = 131.53$ ,  $P < 0.0001$ ). When comparing sexes, females had significantly higher HSI scores in both fall and spring (Wilcoxon  $\chi^2 = 97.1013$ ,  $P < 0.0001$ ; Wilcoxon  $\chi^2 = 62.3153$ ,  $P < 0.0001$ ). Males had higher GSI values than females in the fall (Wilcoxon  $\chi^2 = 9.8273$ ,  $P = 0.0017$ ) while females had much higher GSI values than males in the spring (Wilcoxon  $\chi^2 = 84.3391$ ,  $P < 0.0001$ ; Figure 3). Males did not increase in GSI values in spring compared to fall (Wilcoxon  $\chi^2 = 0.1145$ ;  $P = 0.74$ ) (Figure 2-2).

As multiple comparisons were made for each body composition component, bonferroni correction of statistical significance was set at  $\alpha = 0.008$ . Significant negative relationships between the proportion of whole body lipids and water were found in females at fall ( $\arcsine(\%lipid) = 0.59 - 0.61 * \arcsine(\%water)$ ;  $r^2=0.45$ ,  $F=52.73$ ,  $P < 0.0001$ ), weaker in spring ( $\arcsine(\%lipid) = 0.33 - 0.32 * \arcsine(\%water)$ ;  $r^2=0.15$ ),

F=10.01;  $P=0.003$ ), and the relationship between proportion of lipid and water in males was weak in fall ( $\arcsin(\%lipid) = 41.21 - 0.47 * \arcsin(\%water)$ ;  $r^2=0.11$ ,  $F=9.98$ ,  $P=0.002$ ) and non-significant in spring ( $F=0.056$ ,  $P = 0.81$ ) (Figure 2-3). In contrast, significant negative relationships between the proportion of protein and lipids were comparatively strong for males in the fall ( $\arcsin(\%protein) = 0.499 - 0.435 * \arcsin(\%lipid)$ ;  $r^2=0.40$ ,  $F=52.97$ ,  $P<0.0001$ ) and present in spring ( $\arcsin(\%protein) = 0.489 - 0.518 * \arcsin(\%lipid)$ ;  $r^2=0.17$ ,  $F=10.38$ ,  $P=0.002$ ). For females, the relationship between protein and lipid was significant, although weaker than that found in males in both fall ( $\arcsin(\%protein) = 0.45 - 0.25 * \arcsin(\%lipid)$ ;  $r^2=0.17$ ,  $F=13.62$ ,  $P=0.0005$ ) and non-significant in spring ( $\arcsin(\%protein) = 0.48 - 0.30 * \arcsin(\%lipid)$ ;  $r^2=0.07$ ,  $F=4.7$ ,  $P=0.034$ ). No significant relationships were found between mineral and lipid content for either sex in either season.

#### *Principal component analysis and land use regressions*

Principal component analysis produced two eigenvalues  $> 1.0$  for females in both fall and spring (Table 2-3). Fall female PCs were composed of groups indicating biological relationships between body constituents as (1) energy stores and (2) sustained growth, and explained a total of 60.0% of the variance in the model. The relationships between body constituents revealed through the spring female PCA were similar though explained different variances in the model such that (1) sustained growth explained the greatest amount of variation in the model, followed by (2) energy stores, with a total of 53.81% of model variation explained by the two PCs. GSI and condition factor did not contribute to female fall PCs, while spring PCs were composed of only % mineral, lipid and protein.

For male central mudminnow, PCA produced three PCs with eigenvalues  $>1.0$  for fall and two for spring. Fall PCs were composed of functional groups demonstrating different relationships between body constituents and health indexes compared to females. Fall PCs combined to explain 66.09% of the total variation in the model, and were grouped in biologically function groups (1) energy stores, (2) condition and gonad development and (3) sustainable growth (Table 2-3). Spring PCs followed slightly different patterns and explained 83.9% of the total variation in the model. The greatest amount of variation was described by (1) sustained growth and condition, followed by (2) energy stores. Fall PCs did not include hepatosomatic index, and hepatosomatic index and % water did not contribute to spring PC's.

All principal components were found to vary significantly among sites except spring energy stores for both males and females (Table 2-5). However, no significant relationship was found when median PC values for each watershed were regressed with each land use (agriculture, urban, forest and wetlands/open water)..

## **Discussion**

This research has been a first effort to describe the seasonal body composition of central mudminnow and outline the physiological effects of land use on this small stream fish residing in heavily degraded areas. Females and males were found to have different overwintering strategies to facilitate successful gonadogenesis in preparation for spring spawning; females increased in proportions of lipid and protein over winter compared to males, who decreased in lipids and proteins, corresponding to a decrease in overall

condition factor. These results highlight a physiological link between life history strategies and indicate that variation in energy allocation patterns may differ between males and females of the same species. Although no significant relationship between land use was discovered, energy stores and growth patterns appear to be influenced by the stream from which the fish were removed.

### *Watershed land use*

The study area is one of the most anthropogenically-disturbed areas in the Great Lakes drainage basin; all streams included in this study have some extent of channelization and tiling of headwaters associated with intensive agriculture, and all have a combined disturbed area (agriculture and urban development) >60% of the watershed. For this reason, it unfortunately was not possible to include a true ‘reference creek’ within the same physiogeographic region. Gray’s Creek watershed includes the eastern portion of the city of Cornwall, so while it includes the lowest proportion of agriculture it has the highest proportion of urbanization. This is the only watershed included in the study that includes a protected forest located in a conservation area; all other forested areas are privately owned woodlots or portions of municipal land (e.g. highway buffer zones).

As land use variables are proportions and thus inherently related to one another, land uses may equally predict fish condition and thus the identification of a single land use as the primary driver of any variable must be interpreted with great caution (Herlihy *et al.* 1998; Allan 2004). Further, anthropogenic development is inherently related to natural landscape features, as the suitability of the land for specific uses will dictate their spatial distribution (Allan 2004). As a result, multiple confounding factors may be

influencing the relationships observed between fish body composition, health indices and watershed land use. Indeed, results may represent interaction effects with other land use types and geomorphology. Due to the nature of sampling (i.e., lethal), distribution of model species, and extensive laboratory work associated with the research questions, a relatively small number of streams were used compared to traditional landscape ecology papers (e.g. Richards *et al.* 1996; Wang *et al.* 1997; Trautwein *et al.* 2011). This may account for the seeming lack of relationship between the observed variables and watershed land use. However, most previous work across landscapes has focused on community-level metrics rather than mechanistic aspects of organismal condition and health studied here.

#### *Proximate body composition and health indices*

In this study we found the strength of the relationship between the proportion of water and lipids in central mudminnow to vary by sex and season, with females in particular demonstrating more consistent relationships between lipid and water content compared to males. To our knowledge, this is the first reporting of season and sex-based variation in the strength of this relationship. Trudel *et al.* (2005) noted only seasonal variation, though it was not so great as to significantly reduce the predictive strength of their model. Murchie *et al.* (2010) reported differences in the strength of this relationship between tissues of bonefish *Albula vulpes* (liver, gonad and whole body), but did not find any differences between sexes. These variations indicate that while a strong relationship between body water content and lipid content may suggest water can be used as an effective and economical surrogate for lipid analysis, time of year, sex, and species must be taken into consideration.

Interestingly, male central mudminnow appear to have a stronger relationship between the proportion of protein and lipid components, particularly in the fall. Lipid stores are considered to be a more efficient form of energy storage when compared to protein, as lipids are more easily metabolized and can be depleted without reducing overall performance of the fish (Weatherly and Gill 1987; Jobing 1992). The lack of relationship with the proportion of water within male central mudminnow suggests that there may be sex-based differences in lipid metabolism in this species.

Seasonal differences in male and female health indices are consistent with previous studies addressing reproductive and feeding behaviour of this species (Martin 1982, Chilton *et al.* 1984). Females and males were found to have opposite effects of winter on condition factor values; females began winter with a lower condition than that which they ended, while male condition decreased in spring. That trend was also reflected in the seasonal changes in proportion of protein (Figure 2-2). This is likely contributable to the differences in overwinter reproductive and feeding strategies between the two sexes; male mudminnow begin gonadogenesis in the summer, entering winter at an intermediate to late stage of development, while females show little gonadal development over the summer and fall periods (Chilton *et al.* 1984; Martin-Bergmann and Gee, 1985). Both male and female mudminnow are active throughout winter, capable of maintaining swimming activity and rapid digestion rates at relatively low temperatures allowing for growth year round, albeit slower in winter (Chilton *et al.* 1984; Mertin-Bergmann and Gee 1985); Females however are destined to gain a large proportion of weight through egg production over winter, increasing the weight to length ratio used to assess condition.

In addition, females are more likely to be piscivorous than males, a portion of their diet that may be an integral factor for the overwinter development of gonads (Martin 1982).

Energy storage is an extremely vital part of seasonal adaptation (see Shuter et al. 2012), with the proportion of lipids indicating the amount of energy available for future use for growth, reproduction and general homeostasis (Kaufman *et al.* 2007). Energy reserves in the form of lipids are particularly important for females of species that spawn in the early spring such as central mudminnow. As the liver is a main storage site for energy in the form of glycogen, and the primary site of lipid biosynthesis (Henderson and Tocher 1987), the increased HSI index observed in female mudminnow may be an adaptive strategy for successful overwintering coupled with oogenesis (Henderson *et al.* 2000). In addition, males having a higher GSI index in the fall were previously noted by Martin (1982), who attributed an observed increase in female overwinter piscivory to the metabolic demands of egg production not experienced by males, who begin development in fall when benthic prey is more abundant.

#### *Principal component analysis and land use regressions*

The correlation of watershed land use with water quality data in previous research indicates that in this area, there is a strong relationship between anthropogenic disturbance and habitat quality. Ongoing community assessments in these areas have further demonstrated that many species have been largely extirpated from streams in areas with high proportions of disturbance (AECOM 2009; RRCA 2006), leaving more generalist species such as white sucker *Catostomas commersonii*, brown bullhead *Ameiurus nebulosus*, and central mudminnow (Scott and Crossman 1973). Faunal homogenization in disturbed areas has been demonstrated to occur as species adapted to

warm, sediment and nutrient rich conditions replace those that are adapted to cool, clear, nutrient-low conditions (Rahel 2002). Generalist species able to effectively reproduce in heavily impacted areas likely have adaptive strategies that allow for maintained homeostasis in such conditions.

Indeed, disturbance tolerant species such as central mudminnow have been demonstrated to benefit from increased levels of watershed agriculture, increasing in abundance compared to undisturbed streams (e.g. Wasler and Bart 1999, Wang *et al.* 2003, Trautwein *et al.* 2011). In addition, Blevins *et al.* (2013) demonstrated improved physiological performance in creek chub *Semotilus atromaculatus* – a generalist species - from agricultural creeks compared to forested creeks when faced with acute thermal challenges, suggesting fish living in these conditions are physiologically ‘primed’ to cope with stressors found in these systems. Mudminnows are physiologically well adapted to the extreme thermal variation and hypoxic conditions often found in streams impacted by agriculture (Martin-Bergmann and Gee, 1985; Currie *et al.* 2009) and were one of few species found in the headwaters of the most impacted streams in the Cornwall AOC (*unpublished data*), as such it is possible these species also have local adaptations associated with watershed land use. This localized adaptation may maximize performance depending on the surrounding environment, so differences in PC groupings that are observed in this research may account for more localized habitat traits unobservable at the watershed scale.

Overall variations in physiology occur as a response of organisms to their environment, with the primary purpose of maintaining growth and reproduction (Adolph, 1956). With respect to fish community, generalist species such as this have been

demonstrated to respond positively to intermediate proportions of degradation (Wang *et al.* 1997; Allan 2004). As central mudminnow are an extremely hardy species, it appears they may be physiologically unaffected by non-point source pollution associated with watershed land use with respect to their proximate body composition and overall condition. As such, the analysis of proximate body composition and related health indices to evaluate the influence of watershed land use may be better suited to those fish that have been demonstrated to have decreased populations in response to anthropogenic development.

### *Conclusions*

Through this study we have identified sex-based differences in proximate body composition and health indices and how those differences relate to central mudminnow life history. We have further demonstrated that central mudminnow proximate body composition does not appear to be influenced by watershed land use, likely a result of this species ability to maintain physiological functions in relatively extreme conditions. Further research investigating these metrics in more sensitive species may increase our understanding of how the individual-level physiological metrics studied here relate to population- and community-level processes, however sensitive species may not persist in adequate numbers in particularly degraded areas. As a result, research may focus on relocation of sensitive species to these areas in contained environments (E.g. wire cages) to observe differences in body composition and health indices associated with habitat quality.

## Tables

**Table 2-1:** Proportion of land use within each watershed extracted from the Southern Ontario Land Resource Information System (SOLRIS) data system. Boundaries were defined using a watershed GIS layer provided by the Raisin Region Conservation Authority. All values except total watershed area represent proportions of the watershed dedicated to each land use.

<b>Watershed</b>	<b>Total Watershed Area (ha)</b>	<b>Agriculture and Other Fields</b>	<b>Urban</b>	<b>Forest</b>	<b>Wetland and Open Water</b>
Hoople	9534.8	53.5	5	13.9	26.7
Grays	4450.8	33.0	35.3	8.7	16.9
Finney	3182.0	77.9	4.7	13	4.2
Ferguson	1479.3	81.8	3.53	11.6	2.6
Gunn	1037.5	87.3	4.5	5	2.9
Wood	3013.6	83.9	2.8	10.8	2.2

**Table 2-2.** Whole proximate body composition (% water, lipids, mineral and protein) of central mudminnow *Umbra limi* sampled in fall and spring in creeks within the Cornwall Area of Concern in Eastern Ontario, Canada (mean  $\pm$  standard deviation).

Season	Site	Sex	N	Water	Lipids	Mineral	Protein	
Fall	Hoople Creek	M	14	76.6 $\pm$ 1.3	5.7 $\pm$ 1.6	3.2 $\pm$ 0.9	14.5 $\pm$ 1.4	
		F	15	77.6 $\pm$ 0.8	4.7 $\pm$ 1.2	3.4 $\pm$ 1.2	14.3 $\pm$ 1.4	
	Gray's Creek	M	13	76.4 $\pm$ 0.6	3.9 $\pm$ 0.7	3.3 $\pm$ 1.0	16.4 $\pm$ 1.2	
		F	8	77.2 $\pm$ 0.7	4.2 $\pm$ 1.0	2.8 $\pm$ 1.1	15.8 $\pm$ 1.3	
	Finney Creek	M	16	76.9 $\pm$ 0.7	4.5 $\pm$ 1.0	3.4 $\pm$ 1.0	15.2 $\pm$ 1.2	
		F	12	76.9 $\pm$ 0.8	5.1 $\pm$ 1.2	4.1 $\pm$ 1.3	14.0 $\pm$ 1.4	
	Wood Creek	M	19	76.1 $\pm$ 1.2	6.3 $\pm$ 1.0	3.1 $\pm$ 0.6	14.5 $\pm$ 1.5	
		F	14	76.2 $\pm$ 0.9	6.5 $\pm$ 0.9	3.5 $\pm$ 1.2	13.9 $\pm$ 1.1	
	Gunn Creek	M	9	76.4 $\pm$ 0.9	4.2 $\pm$ 1.0	3.2 $\pm$ 1.4	16.1 $\pm$ 0.9	
		F	9	77.0 $\pm$ 1.2	4.2 $\pm$ 1.2	3.2 $\pm$ 0.9	15.6 $\pm$ 0.9	
	Ferguson Creek	M	13	76.6 $\pm$ 0.7	5.3 $\pm$ 0.7	2.6 $\pm$ 1.2	15.6 $\pm$ 0.9	
		F	9	76.2 $\pm$ 1.1	6.9 $\pm$ 0.8	2.4 $\pm$ 0.7	14.5 $\pm$ 1.1	
	Spring	Hoople Creek	M	9	77.6 $\pm$ 0.7	4.5 $\pm$ 0.6	4.7 $\pm$ 1.6	13.1 $\pm$ 1.7
			F	9	74.2 $\pm$ 0.7	7.0 $\pm$ 1.1	3.1 $\pm$ 0.9	15.6 $\pm$ 1.9
Gray's Creek		M	10	76.5 $\pm$ 1.1	3.4 $\pm$ 0.5	5.0 $\pm$ 2.0	15.1 $\pm$ 2.4	
		F	14	75.0 $\pm$ 1.1	5.4 $\pm$ 1.0	4.8 $\pm$ 2.3	14.8 $\pm$ 2.8	
Finney Creek		M	9	77.4 $\pm$ 0.9	3.9 $\pm$ 0.5	6.0 $\pm$ 2.1	12.7 $\pm$ 1.8	
		F	11	75.1 $\pm$ 0.7	5.9 $\pm$ 1.1	3.0 $\pm$ 0.5	16.0 $\pm$ 1.2	
Wood Creek		M	7	77.2 $\pm$ 0.6	4.4 $\pm$ 1.5	3.7 $\pm$ 0.3	14.8 $\pm$ 1.3	
		F	11	74.3 $\pm$ 1.6	6.0 $\pm$ 1.5	3.4 $\pm$ 0.8	16.4 $\pm$ 1.5	
Gunn Creek		M	13	76.8 $\pm$ 0.7	4.2 $\pm$ 1.2	4.0 $\pm$ 1.2	15.1 $\pm$ 1.6	
		F	8	74.2 $\pm$ 0.8	5.9 $\pm$ 1.6	3.7 $\pm$ 0.8	15.8 $\pm$ 1.4	
Ferguson Creek		M	5	77.6 $\pm$ 0.6	3.5 $\pm$ 0.5	5.2 $\pm$ 2.1	13.7 $\pm$ 2.8	
		F	8	74.5 $\pm$ 0.6	5.3 $\pm$ 1.5	5.5 $\pm$ 2.5	14.7 $\pm$ 3.0	

**Table 2-3.** Results of a two-way ANOVA comparing proximate body composition of central mudminnow *Umbra limi* proximate body composition with sex, season, and sex × season interactions as effects. Significant values are presented in bold.

Variable	Sex			Season			Sex × Season		
	F	df	P	F	df	P	F	df	P
% Lipid	37.57	1	< <b>0.0001</b>	37.57	1	0.097	44.30	1	< <b>0.0001</b>
% Water	72.80	1	< <b>0.0001</b>	42.25	1	< <b>0.0001</b>	133.20	1	< <b>0.0001</b>
% Mineral	3.13	1	0.078	39.69	1	< <b>0.0001</b>	7.18	1	<b>0.008</b>
% Protein	2.21	1	0.14	0.022	1	0.882	21.92	1	< <b>0.0001</b>

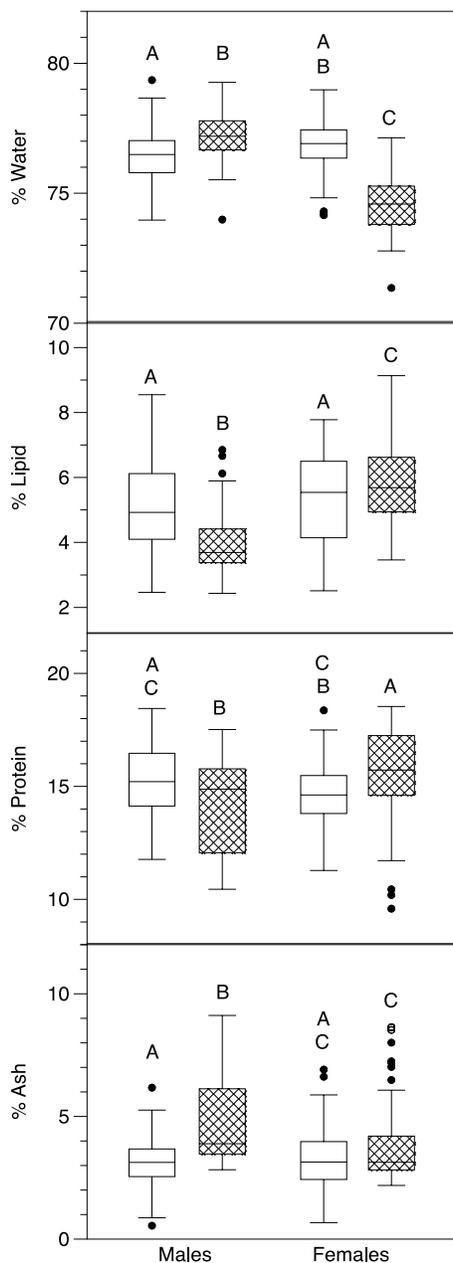
**Table 2-4.** Factor loadings for principle components summarizing seasonal proximate body composition (% water, protein, lipid and mineral) and health indices (hepatosomatic index [HSI], gonadosomatic index [GSI] and condition factor K) for male and female central mudminnow *U. limi* sampled from streams within the Cornwall AOC in Eastern Ontario. Variables were loaded into 4 principal components and factor loadings of  $> |0.4|$  were considered maximal contributors to each PC.

	Fall Females		Spring Females		Fall Males			Spring Males	
	PC1	PC2	PC1	PC2	PC1	PC2	PC3	PC1	PC2
<b>Biological grouping</b>	<b>energy stores</b>	<b>sustained growth</b>	<b>sustained growth</b>	<b>energy stores</b>	<b>energy stores</b>	<b>reproductive investment, condition</b>	<b>sustained growth</b>	<b>condition</b>	<b>energy stores</b>
<b>Eigenvalue</b>	2.51	1.62	2.15	1.61	1.97	1.39	1.26	2.45	1.37
<b>Cumulative % variance</b>	35.91	24.09	30.76	23.05	28.17	19.93	17.99	34.93	19.62
<i>Loadings</i>									
<b>Hepatosomatic Index</b>	<b>0.515</b>	0.149	-0.071	0.387	0.367	0.284	-0.255	<b>0.463</b>	0.349
<b>Gonadosomatic Index</b>	0.214	0.036	0.346	-0.026	-0.175	<b>0.528</b>	0.003	-0.087	<b>0.441</b>
<b>% Water</b>	<b>-0.469</b>	-0.020	-0.399	-0.341	-0.223	0.278	<b>0.584</b>	0.256	-0.162
<b>% Mineral</b>	-0.067	<b>-0.701</b>	<b>-0.548</b>	0.173	0.295	<b>-0.500</b>	<b>0.458</b>	0.363	-0.284
<b>% Lipid</b>	<b>0.554</b>	-0.074	0.159	<b>0.645</b>	<b>0.580</b>	0.333	-0.216	0.249	<b>0.714</b>
<b>% Protein</b>	-0.174	<b>0.690</b>	<b>0.555</b>	-0.386	<b>-0.544</b>	-0.142	<b>-0.476</b>	<b>-0.596</b>	-0.019
<b>Condition Factor K</b>	0.357	0.059	0.287	0.371	-0.178	<b>0.427</b>	0.333	0.404	-0.259

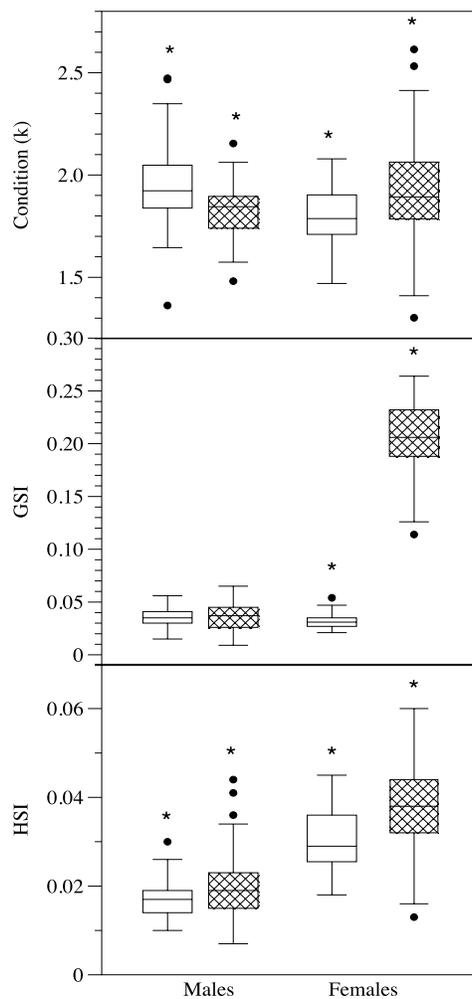
**Table 2-5.** One-way ANOVA comparing principal component scores across sites. Principal components representing biological groupings associated with maximally contributing loadings including proximate body composition and health index variables of central mudminnow *Umbra limi* collected in Cornwall, Ontario. Bonferonni correction for multiple comparisons was applied at  $\alpha = 0.01$ .

Sex	Season	PC	Biological Grouping	F	Site	
					df	P
Females	Fall	PC1	energy stores	11.00	5	<0.0001
		PC2	sustained growth	3.85	5	0.0043
	Spring	PC1	sustained growth	4.12	5	0.0031
		PC2	energy stores	1.10	5	0.37
Males	Fall	PC1	energy stores	8.35	5	<0.0001
		PC2	reproductive investment	4.21	5	0.002
		PC3	sustained growth	8.35	5	<0.0001
	Spring	PC1	condition	5.45	5	0.0005
		PC2	energy stores	2.57	5	0.039

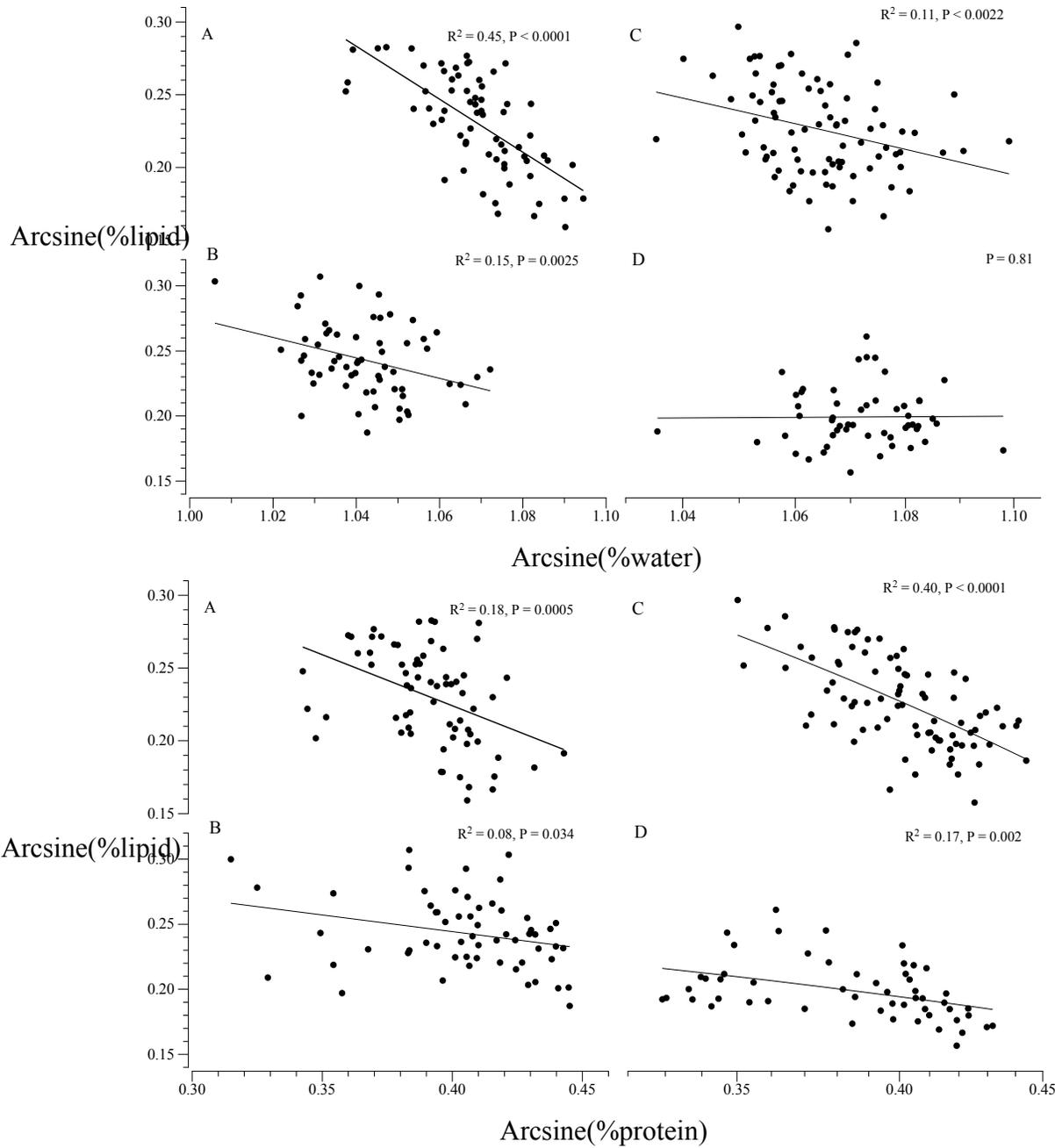
## Figures



**Figure 2-1.** Seasonal variation in proximate body composition in central mudminnow *U. limi* collected from streams within the Cornwall AOC in Eastern Ontario. Spring values are indicated by the hatched boxplots while fall are unmarked. Significantly different values are not connected by the same letters.



**Figure 2-2:** Seasonal variation in hepatosomatic (HSI) and gonadosomatic (GSI) indices of central mudminnow *U. limi* sampled from streams in the Cornwall AOC in Eastern Ontario. Spring values are indicated by hatched boxplots. Significant tests compared values within sexes, and asterisks indicate significantly different values. See text for supporting statistics.



**Figure 2-3.** Estimated relationships between arcsine transformed % lipid and % water and % protein by sex and season in central mudminnow *U. limi* collected from streams within the Cornwall AOC in eastern Ontario. See text for equations. All samples were run in duplicate. A) fall female B) spring females; C) fall males D) spring males

### **Chapter 3: Variation in parasite community and health index of juvenile *Lepomis gibbosus* across a gradient of watershed land use: Can parasites be used as an indicator of habitat quality?**

#### **Abstract**

Parasites of fishes have been shown to display variation in community structure and species abundance in response to pollutants. As a result, the use of parasites as bioindicators of habitat degradation has been suggested and successfully applied over recent years. Here, helminth parasites of juvenile pumpkinseed *Lepomis gibbosus* from three streams representing a gradient of habitat degradation were examined to determine variations in parasite communities. Health assessment indices were also conducted on fish to quantify the influence of habitat on the observed health of individuals. A total of 22 taxa were recovered from examined fish, comprising 13 digeneans, 3 cestodes, 2 acanthocephalans, 5 monogeneans and 1 crustacean. Parasite diversity was lower in the most disturbed stream, following a similar pattern to invertebrate diversity, while individual abundance was highest in the most disturbed stream. There was no significant difference in health indices among streams. Analysis of similarities (ANOSIM) and Bray-Curtis dissimilarity in species composition (SIMPER) identified *Posthodiplostomum* *ssp.* and *Actinocleidus* *sp.* as the species driving parasite community dissimilarity between the three streams. These taxa are relatively easy to identify to genus level and thus may be appropriate for use as indicators of environmental health, where increased abundance would suggest negative changes in habitat quality. However, larger scale study including more streams would be necessary to establish baseline community data before such implementation would be plausible.

## **Introduction**

Stream ecosystems impacted by high levels of anthropogenic development in their watershed are of great concern for environmental managers as they are currently among the most threatened environments in the world (Richter et al. 1997; Malmqvist and Rundle, 2002). Due to the increasing pressures of urban and environmental development, restoration efforts are often limited or ineffective (Palmer et al. 2010), while intensification of agriculture and urban development associated with increased crop production and population growth is an unavoidable reality linked to growing populations and regional economies (Lambin and Meyfroidt 2011). Environmental managers use a variety of aquatic monitoring techniques to quantify the health of these disturbed aquatic ecosystems.

Stream ecosystems are monitored through various sampling techniques, and community structure is often used as a surrogate for watercourse health. Indeed a variety of indices have been created to quantify fish and invertebrate communities, such as the fish index of biologic integrity (IBI; Karr 1981) or Hilsenhoff index for invertebrates (Hilsenhoff 1988). While these techniques are effective for comparative purposes, they are only able to identify changes once they have occurred at the population level, and are consequently slow to indicate environmental change (Adams et al. 1993). Moreover, the level of sampling needed to document changes in community structure can be extensive and various IBIs need to be locally calibrated (Frausch et al. 1984). As such, there has been interest in identifying other potential endpoints beyond fish and invertebrate community structure that have the potential to be indicative of aquatic ecosystem health.

The use of parasites in fish as bioindicators of ecosystem variation in the face of anthropogenic degradation has grown over recent years (see Lafferty 1997, Marcogliese 2005 and Blonar et al. 2009 for extensive reviews). It has been demonstrated that certain parasite taxa are more sensitive to environment perturbation than their host species, and thus a more sensitive indicator of various contaminants and trophic changes (Marcogliese 2005). Two groups identified as potential bioindicators are Monogeneans, monoxenous gill infesting ectoparasites, and Digeneans, heteroxenous trematodes that typically include at least one invertebrate in their life cycle and various free-living stages during their transmission between hosts. Monogeneans are considered to be good indicators of water quality as they are constantly exposed to the environment, primarily infesting gill tissue (Bagge and Valtonen, 1996; Zagar et al. 2012). In contrast, trematodes may indicate multiple characteristics, such as changes in food-web dynamics, invertebrate populations and densities, and water quality (Marcogliese and Cone 1997; Soucek and Noblet 1998; Marcogliese 2005). In addition to the complex relationships that may occur as a result of the parasites interactions with environmental and intermediate host dynamics, fish themselves that experience environmental stress can experience immunosuppression, increasing their susceptibility to parasite infection and disease (Lafferty and Kuris 1999; Marcogliese 2004). Assessing changes in the overall parasite species community in resident fish hosts can thus indicate potential changes in aquatic ecosystems on a variety of levels. For example, eutrophication associated with nutrient loading has been identified as a factor that influences overall parasite species composition in fish residing in affected waters (Valtonen et al. 1997; Zagar et al. 2012), and can either increase or decrease infection properties depending on the taxa's life history traits, i.e. the presence, absence and densities of intermediate or definitive hosts, and sensitivity of

hosts and parasites themselves to environmental change (Sures 2004). Overall parasite taxa diversity has also been used to measure restoration success. As an example, Huspeni and Lafferty (2004) monitored increasing trematode diversity over six years of post-restoration in a coastal salt marsh, where the increase in snail-borne trematodes indicated the return of various bird species to the area.

As fishes are hosts to a taxonomically diverse suite of parasites, with estimates running upwards of 30,000 parasitic helminth species alone (Williams and Jones, 1994), the importance of parasites in aquatic ecosystems is under-appreciated when monitoring is concerned (Marcogliese and Cone 1997). As a result of the high parasite diversity of intermediate trophic fishes – i.e., those that consume invertebrates and are prey for both predatory fishes and piscivorous birds – and the ability to estimate age and thus parasite recruitment time, juvenile fish are excellent models to investigate the potential efficiency of parasites as bioindicators for use in environmental monitoring; however, due to the time and knowledge required to sample and identify all parasites present within a host species, previous authors advocate monitoring only individual taxa after prior knowledge of their biology and distribution within a system (Blanar et al. 2009). The aim of the present study is to define relationships between habitat quality and parasite community structure, and to determine whether helminth parasites of fish could be used as a tool for environmental monitoring. In addition, we sought to quantify the relationship between parasite diversity, parasite abundance, and fish health.

## Methods

### *Fish collection*

Using previous reports of water quality from the Raisin Region Conservation Authority, we selected three streams in the Cornwall area of eastern Ontario, Canada that represented a range in habitat quality to test whether or not changes in habitat quality were reflected in parasite community (Table 3-1). Land use data were obtained from the Southern Ontario Land Resource Information System (SOLRIS) and were combined with a GIS layer defining watershed boundaries developed by the local Raisin Region Conservation Authority using Quantum GIS Lisboa (1.8.0). Total phosphorous levels and ranges were extracted from the Ontario Ministry of the Environment Report (2009), and benthic and bacteria scores from the Raisin Region Conservation Authority Watershed Report Cards (2006). To ensure fish were representative of stream conditions, fish were sampled from areas approximately 1.5 km upstream of the confluence, in the first wadeable portions of the streams. Fish were captured between June 4<sup>th</sup> and 13<sup>th</sup>, 2012, using a beach seine (22.6 × 1.15m; 5 mm mesh) and all *L. gibbosus* at each site were measured to construct length frequency histograms to determine the size class of age 0+ fish (recruitment from the previous summer). Fish collected were from the smallest age class and thus assumed to have had ~1 year for parasite recruitment. *L. gibbosus* has been demonstrated to have high site fidelity throughout their first year (McCairns and Fox 2004); therefore individuals captured were likely exposed to local water quality conditions reflective of watershed characteristics throughout their entire lives.

### *Health Assessment and Parasitological Examination*

Fish were collected and immediately euthanized by adding a mixture of clove oil and 10 % EtOH, put in individual bags and placed on ice for transport back to the lab. Within 2 hours of euthanasia, dissection based health assessment indices (HAI) were conducted (as per Adams et al. 1993, with modification as described below) to quantify the general health status of each fish population, and to quantify the influence that overall parasite infection levels have on this measurement. After external examination for abnormalities of the fins, eyes, scales, and gills, a single incision was made along the ventral portion of the fish to assess the colour, size and condition of major organs (alimentary tract, heart, liver, and spleen). Scores for each organ category ranged from 0-30 and were combined to produce a single HAI value for each fish. A score of zero indicates a fish that did not have any appreciable abnormalities, where a high HAI score reflects an unhealthy individual. Throughout this process care was taken not to disturb tissues to avoid damaging parasite fauna within the host. After the HAI was completed, fish were placed back in their original bag and stored at -20°C until examined.

Prior to dissection fish were weighed to the nearest 0.01g and fork length was recorded. Fish and bag contents were first rinsed and checked for external parasites using a dissecting microscope following routine parasitological techniques. Parasitological examination was conducted using protocol provided through Environment Canada's Freshwater Monitoring Protocols (Marcogliese 1998). All organs and tissues were examined for parasites, which were excysted, enumerated and fixed for later staining and identification when species were unknown. Cysts were either torn open manually using fine forceps or insect pins, or chemically dissolved in a dilute hypochlorite solution. This

is a useful technique that has previously been used for excysting multi-layered gelatinous cysts of digeneans such as *Apatemon spp.* Nematodes were also excysted and removed though not identified for this analysis. Parasites were identified using the keys in Gibson (1996), Beverly-Burton (1984) and Hoffman (1999).

### *Calculations and Statistical Analysis*

Quantitative descriptions of parasite infection properties used throughout are as in Bush et al. (1997) and can be defined as follows: Prevalence is the proportion of examined hosts infected with a given species, mean abundance is the mean number of individual parasites per host including all hosts examined, and mean intensity is the mean number of individuals of a given species found per infected host. Also included in this analysis are descriptors of parasite community, including component community richness – the assemblage of parasites found of the entire sample of hosts examined – and infracommunity – the assemblage of parasites found within an individual host. Differences in total abundance and HAI values among sites were tested using Analysis of Variance (ANOVA) and post-hoc Tukey HSD. Simple linear regression was used to test the relationship between total parasite abundance and HAI score. Differences in fork length, HAI and total parasite abundance relationships were tested using JMP® V 9.0.1. Assumptions of normality and equal variance were tested using Shapiro-Wilk and Levene's test, respectively.

Non-parametric species accumulation curves for sample-based data were constructed using EstimateS V 9.1.0 and were extrapolated by 25 samples to estimate required host sample size to account for all species (Colwell et al. 2012). Examinations of

hosts concluded when species accumulation curves plateaued, or increased at a gradient such that multiple hosts would need to be examined to add a single species to the model.

To compare parasite component communities of each stream, several methods to compare composition were employed. Firstly, Shannon-Weiner diversity index ( $H'$ ) was calculated for each stream to quantify species diversity and evenness. To further quantify observed differences, non-parametric statistics based on the Bray-Curtis dissimilarity index were used following procedures outlined in Oksanen et al. (2013). This analytical technique accounts for both abundance and species identity in a single measurement of dissimilarity. An analysis of similarities was then conducted (ANOSIM) to test if there was indeed a significant difference between streams. To quantify the contribution of each parasite species to the observed dissimilarity, Bray-Curtis dissimilarity in species composition (SIMPER) was calculated with paired-comparisons between the sites. This method indicates the species that drive the overall dissimilarity between streams. Finally, a non-metric multidimensional scaling ordination (NMDS) with 95% confidence intervals around the contributing sites was created to visually interpret the observed community dissimilarity. All analyses were conducted in R using the “vegan package”.

## **Results**

Watershed land use within the selected streams followed several patterns associated with habitat quality (Table 3-3). The proportion of forest and undisturbed lands was highest in Hoople Creek and lowest in Gunn Creek, while total phosphorous (TP), annual TP range, benthic and bacteria scores increased with increasing proportion of disturbed watershed. The proportion of agriculture and urban land use did not follow

similar patterns, as Gray's Creek was found to have the lowest proportion of agriculture and the highest proportion of urban land use. These combined however created an intermediate level of disturbed consistent with water quality and benthic score data.

Forty-nine fish (total length range of 42-56mm) were examined for total macroparasite community excluding nematodes. Both total length (Analysis of Variance [ANOVA],  $F_{(2,46)} = 1.58$ ,  $P = 0.22$ ) and weight (ANOVA,  $F_{(2,46)} = 1.47$ ,  $P = 0.24$ ) of sampled *L. gibbosus* did not differ significantly among streams. Total parasite abundance was significantly different among sites (ANOVA,  $F_{(2,46)} = 12.75$ ,  $P = <0.0001$ ), and post-hoc tests revealed total parasite abundance in Hoople Creek (mean = 14.69) to be significantly different from Gunn (mean = 37.06) and Gray's Creek (mean = 28.63) (Figure 3-2). While HAI scores decreased with decreasing habitat quality, differences were not statistically different (ANOVA,  $F_{(2,46)} = 2.405$ ,  $P = 0.10$ ). HAI was found to be a significant predictor of total parasite abundance for Gunn and Gray's Creek ( $r^2 = 0.32$ ,  $F_{(1,15)} = 6.56$ ,  $P = 0.023$ , total abundance =  $26.69 + 0.29 \cdot \text{HAI score}$ ;  $r^2 = 0.25$ ,  $F_{(1,15)} = 4.87$ ,  $P = 0.043$ , total abundance =  $19.71 + 0.31 \cdot \text{HAI score}$ ), while this relationship was non-significant for Hoople Creek ( $r^2 = 0.01$ ,  $F_{(1,14)} = 0.19$ ,  $P = 0.67$ ).

Twenty-two taxa were identified from examined fish, comprising 13 digeneans, 3 cestodes, 2 acanthocephalans, 5 monogeneans and 1 crustacean (Table 3-2). Groups such as *Posthodiplostomum ssp.* and *Diplostomum sp.* have recently been demonstrated to contain cryptic species through DNA analysis (Locke et al. 2013); as such, no attempt was made to identify individuals past genus to avoid improper identification. Species accumulation curves indicated that the majority of parasite species were accounted for by the analysis of ~13 hosts (Figure 3-1). As between 16-17 hosts were examined, the

majority of parasite species present within YOY pumpkinseed in the systems were likely accounted for. The majority of parasites found were larval stage trematodes (metacercaria) and monogeneans, while larval cestodes (pleurocercoids) and glochidia were comparatively rare (Table 3-2). All individual hosts examined contained at least two taxa, with the highest parasite diversity within a single host 10 species. The most common parasites found across sites were *Posthodiplostomum* *ssp.* metacercaria and the monogenean *Actinocleidus* *sp.* The Acanthocephalan *Neoechinorhynchus* *tenellus* was moderately prevalent in two of the three streams, and often occurred at the same time as *Posthodiplostomum* *ssp.* Of the 22 taxa found, eight were shared between sites. The average number of taxa found per fish was highest in Gray's, the intermediate stream, and lowest in Hoople Creek, the least degraded (Table 3-3). For Shannon-Weiner diversity Hoople Creek scored the highest  $H'$  and Gunn the lowest, indicating an increase in diversity and species evenness with increasing condition in these streams. Gray's Creek fell consistently intermediate in terms of total abundance,  $H'$  and HAI values. In addition, Hoople Creek had lower overall prevalence values for the most common species shared among the sites (Table 3-2).

Several parasite taxa were found to vary in abundance: three monogeneans, three trematodes, and one acanthocephala. Three of these significant species were not found at all three localities, while *Posthodiplostomum* *ssp.* and *Actinocleidus* *sp.* were the most abundant at species found in Gunn and Gray's Creek, and moderately abundant at Hoople Creek. All cestode species were present in the most degraded stream, and *Proteocephalus* *sp.* was the only cestode recovered from all three streams. The global ANOSIM comparison result indicates the overall parasite species compositions of the streams were significantly different (R statistic = 0.43,  $P < 0.001$ ). These differences are qualitatively

displayed in the NMDS plot, where relative similarities between localities can be observed by the two distinct clusters of site-specific data points (Figure 3-3).

Further investigation using SIMPER analysis revealed the main species driving the differences between good and poor habitat quality were the most abundant species collected: the trematode, *Posthodiplostomum ssp.*, and the gill infesting monogenean, *Actinocleidus sp.* (Table 3-4). *Posthodiplostomum ssp* contributed the greatest dissimilarity to comparisons involving the most degraded sites, while *A. incus* was the top contributor to dissimilarity between the less degraded sites. In total, these two species contributed the greatest amount to the observed dissimilarity between the sites, followed by the digenean *Apatemon sp.* and acanthacephala *Neoechinorhynchus tenellus*; all other species contributed <5% to the total dissimilarity. Total dissimilarity values presented through SIMPER analysis confirmed trends observed in the NMDS plot and H' indexes.

## **Discussion**

This is the first study to examine the parasite communities of fish across a gradient of stream conditions. In this study we are assuming that the overall habitat structure and *L. gibbosus* parasite populations would be the same if not for anthropogenic influences associated with land use, and thus trends observed are a result of landscape alteration. This assumption is based on local knowledge of historical fish populations, the proximity of sample sites (and thus historical similarities in land cover, climate and water chemistry), and knowledge of host species distribution within the area.

Species recovered were similar to those found in previous research on adult *L. gibbosus* in the St. Lawrence River (Locke et al. 2013). This is also the first research to compare modified health assessment indices of a YOY stream fish to parasite abundance and diversity in degraded areas. Fish condition was marginally non-significant, likely due to high variation in HAI scores at each site.

Although Hoople Creek has the highest proportion of forest and least proportion of disturbed land, and has been used as a reference system in previous studies (Raisin Region Conservation Authority, personal communication), all streams sampled in the present study notably impacted. It is possible this reference stream is already passed a threshold for some species that would otherwise be present, thus excluding the most sensitive parasite species; however, as the goal of this study was to identify potential tools for monitoring ecosystem recovery and resilience, the findings address the overall research objectives to identify relative changes in parasite community and species indicative of habitat quality. Watershed land use within Gray's Creek did not follow similar patterns to those seen in Hoople and Gunn in that there was a larger proportion of urban development. As parasite infection dynamics appear to follow a pattern consistent with the proportion of watershed size, disturbed habitat, TP, bacteria and benthic scores, the proportion of land use may not be as predictive as direct measurements of habitat quality.

The majority of parasites encountered were larval stages, most notably trematode metacercaria. This is a trend consistent with other studies on fish parasite communities in the St. Lawrence River (Krause et al. 2010; Marcogliese et al. 2006; Thilakaratne et al. 2007; Locke et al. 2013), and is likely a result of the intermediate trophic status of hosts

sampled in these studies (Marcogliese 2004). Fish that are positioned in the middle of local food webs such as juvenile *Lepomis sp.*, consume mainly invertebrates (Scott and Crossman 1978) and provide not only nutrients but also transmission pathways for developing helminth parasites; these intermediate fish hosts are common prey for the parasite's respective definitive host (Marcogliese 2004; Marcogliese and Cone 2007; Valtonen et al. 2010). Esch (1971) proposed that larval forms of parasites that mature in piscivorous birds and mammals would dominate eutrophic systems. The present data supports this idea, as many larval parasites found were generally those that reach sexual maturity in higher trophic level fish and piscivorous birds, such as *U. ambloplites* in Kingfishers *Megaceryle alcyon* and *Posthodiplostomum ssp.* in herons, gulls and terns, all of which are locally common during ice-off periods. Adult trematodes identified in this study were *Azygia angusticauda* and *Crepidostomum cooperi*, both species that mature in the alimentary tract of fish and are acquired through consumption of Amphipod and Ephemeropteran prey (Hoffman 1956; Amin 1982).

Variation in parasite communities found in fish from degraded and less degraded watersheds in this study were consistent with trends in previously reported. Here, total abundance of parasites significantly increased in more eutrophic streams, indicated by high TP values, while species diversity was significantly different between sites and creased decreased with increasing degradation. A positive relationship between nutrient loading and fish parasitism has previously been reported in several species (Esch 1971; Hartmann and Nümann 1977; Marcogliese 2001; Marcogliese and Cone 2010; Zargar et al. 2012), and Mackenzie (1999) summarized effects of eutrophication as being overall positive for monogeneans, digeneans, cestodes and acanthocephalans; however a detailed meta-analysis conducted by Blonar et al. (2011) reported an overall significant negative

effect of eutrophication on digenetic trematodes, and no effect on other groups. The disparity in summarized results compared to individual studies suggests that changes in water quality associated with eutrophication may only benefit a minority of species, in particular generalist hosts such as *Posthodiplostomum ssp.* In addition, the cause for presence or absence of a given species at a site may be a result of multiple factors, as fish immunity, parasite survival and intermediate host populations are not independent from one another, thus making cause and effect conclusions tenuous (Poulin 1992; Marcogliese 2005).

The species found in all streams that significantly increased in abundance and prevalence in eutrophic habitats were also identified as species driving dissimilarity between localities, and thus identified as potential bioindicators for these streams (Table 3-4). These parasites are extremely different in their transmission pathways and physiology; *Posthodiplostomum ssp.* are generalist heteroxenous trematodes found in many freshwater fish species (Hoffman 1956; Gibson 1996), while *Actinocleidus sp.* are monoxenous ectoparasites specific to centrarchids and commonly found on *L. gibbosus* (Beverly-Burton 1981;1984, Locke et al. 2013). As a result, these taxa interact with their hosts and the environment in different ways, and thus may indicate unique characteristics of the ecosystem and health of host fish.

The *Posthodiplostomum ssp.* lifecycle involves snails of the genuses *Physa sp.* and *Physella sp.* as the first intermediate host where sporocyst development and cercaria release occur (Hoffman, 1956), so increased abundance of this parasite may indicate either an increase in number or density of snails. The cercariae enter the intermediate fish host by a free-living stage that is directly exposed to surrounding water quality. Upon

contact, cercariae penetrate the mucus and tissue at scale margins, boring through the integument and musculature by inverting and everting their oral suckers (Miller, 1954). Once through, individuals move to the inner body cavity through a combination of active and passive migration via the circulatory system, finally encysting in the liver, spleen, heart or peritoneum (Miller, 1954; Hoffman, 1956; Lewis and Nickum 1964). In contrast, the lifecycle of *Actinocleidus sp.* is direct and involves transfer directly from fish to fish, requiring no intermediate host to facilitate development or transmission (Beverly-Burton, 1981). These ectoparasites use their posterior oposthaptor to remain attached to the gill tissue throughout their entire life cycle, feeding on components of fish tissue and mucus. These helminthes are hermaphroditic and able to proliferate rapidly under ideal conditions, and some species have been negatively correlated with decreases in fish condition (Rohlenová et al. 2011), suggesting their presence in high abundance may be detrimental to host health.

Several synergistic mechanisms may explain the increase in *Posthodiplostomum ssp.* and *Actinocleidus sp.* prevalence and intensity in watersheds with high proportion of disturbed landscapes. Individual fish health is influenced by a variety of biotic and abiotic factors, including temperature (Bly and Clem 1992), pollutants (Duffy et al. 2002) and nutrient loading (Johnson et al. 2010). These factors can cause immunosuppression in fishes thus increasing the likelihood of infection of certain parasite taxa (Secombes and Chappell 1996; Rohlenova et al. 2011). Consequently, fish living in sub-optimal habitat resultant from high proportions of watershed disturbance may demonstrate higher rates of infection of specific parasites when compared to those residing in optimal conditions. Whether it is that fish in poorer condition are more easily infected, or that fish with higher parasite loads become in poor condition cannot be determined from the results

presented here. As systems disturbed systems tend to have higher and more frequent swings in water temperatures (Poole and Berman 2001), increased efficiency of penetration and movement may result in higher prevalence and intensity. The process of penetration and migration in *Posthodiplostomum sp.* infections is reported to occur at a faster rate in water temperatures  $\sim 25^{\circ}\text{C}$  (Miller 1954), while the proliferation rate of monogeneans increases with increasing temperatures (Tinsley 2004). Further, fish in sub-optimal water temperatures and poor water quality associated with effluents have been demonstrated to experience immunosuppression, which may ease penetration and migration through host tissue (Jokinen et al. 1995; Khan 2004; Smith 2009; Inendino et al. 2005). Watersheds dominated by agriculture also have an increased suspended sediment loads that may be a detrimental factor in Gunn Creek, where the substrate has undergone infilling and homogenization to soft clay with little structure. Suspended sediments have been demonstrated to negatively affect the fish mucous layer, where increased abrasive suspended solids may cause the erosion of mucus layer covering the gills and body (reviewed in Kemp et al. 2011). The mucus layer may play an important role in fish defense against infections, and compromise to this barrier may facilitate increased parasite attachment (Shephard 1994).

Decreasing species diversity coinciding with an increase in overall abundance demonstrates that the majority of parasites in this system are not able to persist in degraded habitats. A greater species diversity of trematodes in the less degraded Hoople Creek suggests potential alteration of invertebrate transmission pathways (Marcogliese 2004). For example, Hechinger et al. (2007) found a consistent positive correlation between trematodes and large benthic invertebrate species in coastal wetlands of California. Hoople Creek and Gray's Creek, high in component community and

infracommunity values (Table 3-3), scored higher on benthic diversity indices. This indicates disturbed areas such as Gunn Creek, an area with homogenous clay substrate and intense eutrophication, may not support invertebrates necessary for the life cycle of certain parasite species. Indeed, Gunn Creek was absent of glochidium, suggesting that freshwater clams are infrequent or absent in this system; a similar trend was seen in pulp-effluent polluted lakes in Finland by Valtonen et al. (1997). Increased nutrient loading associated with high proportions of agriculture may however create increased densities of snails (Shah et al. 2013), which could potentially explain the increase in *Posthodiplostomum ssp.* prevalence and intensity with increasing habitat degradation compared to other species. Shah et al. (2013) noted increased densities of planorbid and lymnaeid snails in a eutrophic lake, and attributed increased densities and spatial distribution of snails to elevated *Diplostomum* levels in fish hosts from the area.

### ***Conclusions***

Within this study area, measurements of habitat quality followed patterns similar to total disturbed land use. This study has demonstrated that parasite species may be effective indicators of the proportion of disturbed land use and consequent habitat quality, and the potential for certain species to be used as indicators of habitat quality in eastern Ontario. While the mechanism driving the differences in parasite community dynamics is not well understood, the fact that species diversity indices were highest in the least degraded stream indicates potential sensitivity to habitat quality changes similar to those seen in previous studies (e.g., Huspeni and Lafferty 2004). HAI scores were variable and while trending a decrease in fish condition as habitat quality decreased, the

use of such an assessment technique may not be appropriate to use on small young fish. The changes in abundance of two large and rather easily identifiable parasite species suggests that they may be appropriate bioindicators to monitor changes in habitat quality for this area. With the intensive effort required to extract and identify all parasites present within a host, small-scale parasite surveys such as this would be useful to establish baseline community compositions and focal species for use in monitoring. After such a survey, the incorporation of parasites into sampling regimes such as Before-After-Control-Impact designs may increase the breadth of information acquired from sampling regimes designed to monitor post-restoration ecosystem changes. Indeed, individual-level metrics (such as fish condition and parasites) have much to offer the evaluation of habitat quality and restoration activities (Cooke and Suski 2008).

## Tables

**Table 3-1.** Watershed land use, water quality and benthic scores for the three streams used in this study in eastern Ontario, Canada. Watershed data was determined using Quantum GIS and land use data from the Southern Ontario Land Resource Information System. Total phosphorous levels and ranges were extracted from the Ontario Ministry of the Environment Report (2009), and benthic and bacteria scores from the Raisin Region Conservation Authority Watershed Report Cards (2006). Benthic scores are presented as a Hilsenhoff index, where lower values equate to greater benthic diversity.

	<b>Hoople Creek</b>	<b>Gray's Creek</b>	<b>Gunn Creek</b>
Watershed area (ha)	9534.8	4450.8	1037.5
% Forest	13.9	8.7	5
% Water & Wetland	26.7	16.9	2.9
% Agriculture	53.5	33.0	87.3
% Urban	5.0	35.3	4.5
% Disturbed	58.5	68.3	91.8
Mean annual TP ( $\mu\text{g/L}$ )	54	66	82
Annual TP range ( $\mu\text{g/L}$ )	30	80	140
Benthic Score	5.4	6.73	7.08
Bacteria Score	39	56	83

**Table 3-2:** Prevalence (P) and mean intensity (MI) of macroparasites collected from overwintered YOY pumpkinseed in three streams of different habitat quality in Cornwall, Ontario.

Class	Species	Gunn		Gray's		Hoople	
		P	MI	P	MI	P	MI
<b>Monogenea</b>	<i>Cleidodiscus robustus</i>	0.118	1.5 ± 0.50	0.188	2.00 ± 0.58	0.312	1.4 ± 0.24
	<i>Cleidodiscus venardi</i>	0.294	1.6 ± 0.18	-	-	-	-
	<i>Onchocleidus ferox</i>	0.235	2.25 ±	0.500	2.38 ± 0.60	0.312	2.6 ± 0.69
	<i>Actinocleidus incus</i>	1	13.5 ± 2.30	1.000	14.00 ± 2.31	0.938	6.73 ± 1.17
	<i>Lyrodiscus sp.</i>	-	-	0.375	1.17 ± 0.17	0.312	1.2 ± 0.20
<b>Trematoda</b>	<i>Phyllodistomum sp. m</i>	0.118	2 ± 1.00	-	-	0.375	1.5 ± 0.20
	<i>Plagioporus sp. m</i>	-	-	-	-	0.125	4 ± 1.00
	<i>Posthodiplostomum sp. m</i>	1	15.29 ± 2.23	1.000	7.62 ± 3.9	0.25	1.25 ± 0.25
	<i>Azygia angusticauda</i>	0.176	1	-	-	0.125	1
	<i>Apatemon ssp. m</i>	0.294	1.2 ± 0.20	0.625	3 ± 1.17	0.5	4.25 ± 1.15
	<i>Uvulifer ambloplitis m</i>	0.294	2.2 ± 0.97	0.375	1.17 ± 0.34	0.25	1.25 ± 0.25
	<i>Clinostomum marginatum m</i>	-	-	0.250	1.25 ± 0.25	-	-
	<i>Crepidostomum cooperi</i>	-	-	-	-	0.125	1
	<i>Diplostomum ssp. m</i>	0.353	2.83 ± 1.01	0.188	3.00 ± 1.15	0.375	3.38 ± 0.83
	<i>Apophallus venustus m</i>	-	-	0.188	1.33 ± 0.33	0.188	2.67 ± 0.67
	<i>Crassiphiala bulboglossa</i>	-	-	0.125	1	-	-
	<b>Cestoda</b>	<i>Proteometra macrostoma pl</i>	0.059	2	-	-	-
<i>Haplobothrium globuliforme pl</i>		0.176	1	-	-	-	-
<i>Proteocephalus sp. pl</i>		0.059	11	0.125	2 ± 1.41	0.188	1
<b>Acanthocephala</b>	<i>Neoechinorhynchus tenellus c</i>	0.882	4.27 ± 0.67	0.500	1.5 ± 1.07	-	-
	<i>Leptorhynchoides thecatus c</i>	-	-	0.062	1	0.125	1
<b>Mollusca</b>	Unoinidae Glochidium	-	-	0.188	3 ± 0.33	0.188	2.33 ± 0.33

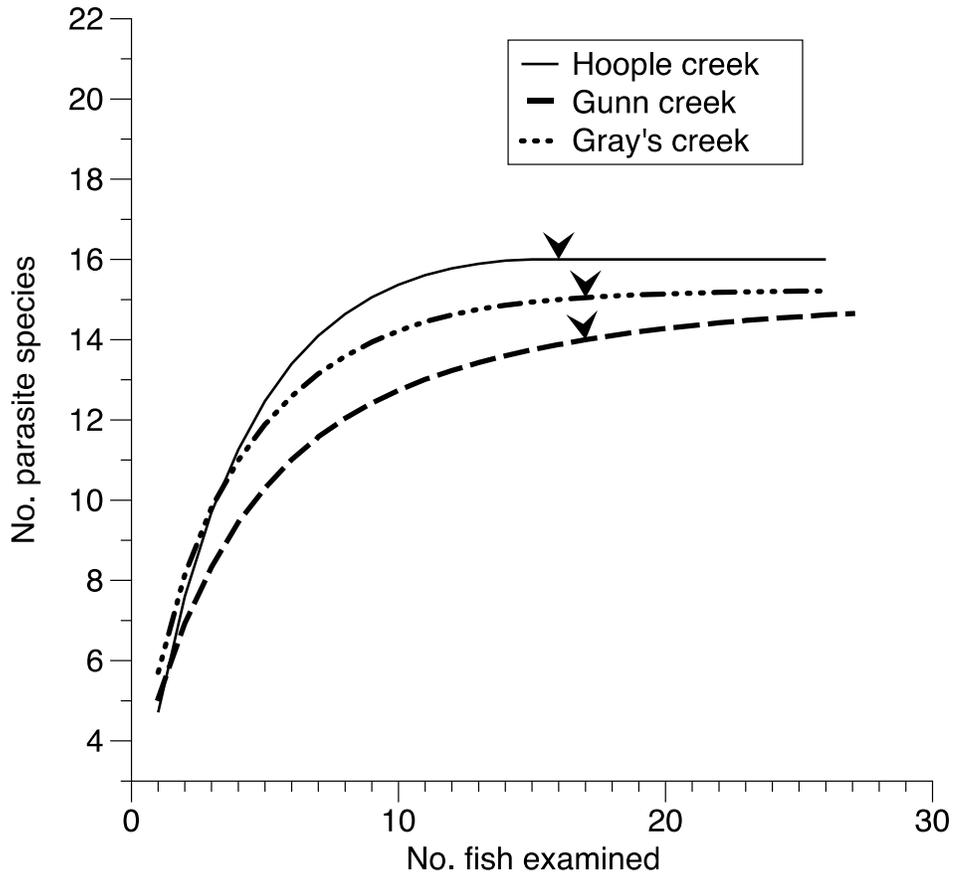
**Table 3-3.** Abundance and diversity data for both infracommunity and component community of parasites of *Lepomis gibbosus* collected from Eastern Ontario in early summer.

	<b>Gunn</b>	<b>Gray's</b>	<b>Hoople</b>
<b>Number of Fish Hosts Examined</b>	17	16	16
<b>No. of Parasite Taxa</b>	14	15	16
<b>Mean Taxa/fish</b>	5.06	5.69	4.69
<b>Common Taxa (prevalence &gt;50)</b>	3	3	1
<b>Intermediate Taxa (prev &lt; 10-50%)</b>	9	11	15
<b>Rare Taxa (prev &lt; 10%)</b>	2	1	0

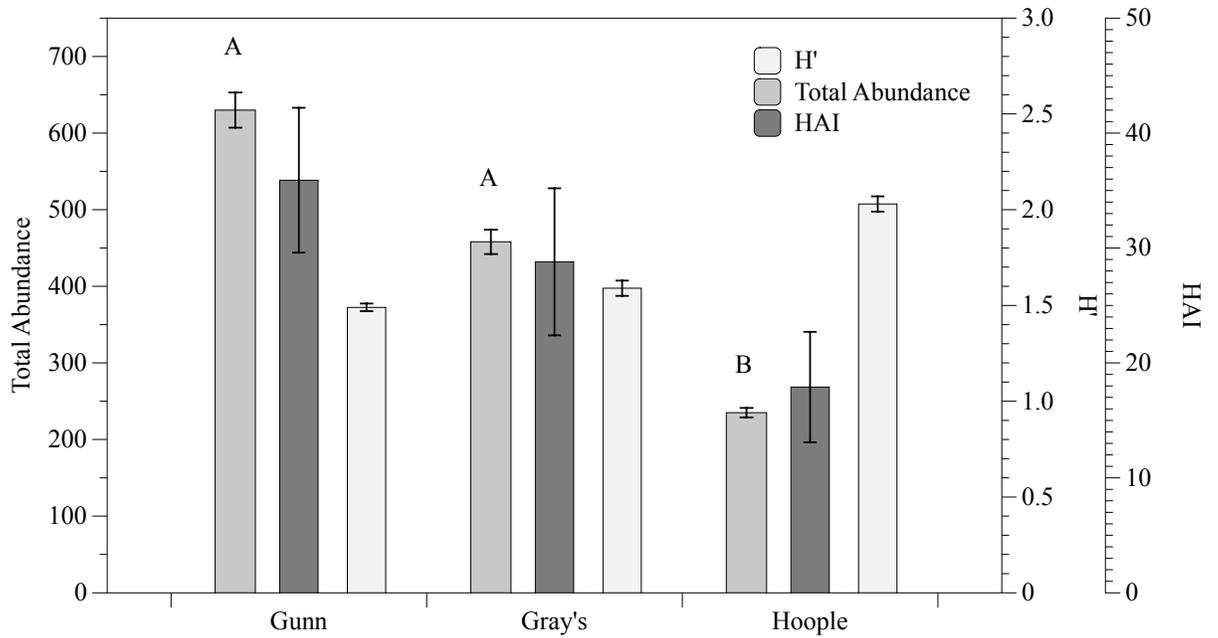
**Table 3-4:** Results of SIMPER analysis of parasite species extracted from overwintered YOY pumpkinseed *L. gibbosus* in three streams in Eastern Ontario. Only those species contributing >3% of dissimilarity are listed.

Site Comparisons	Total Dissimilarity	Species	Avg. A	Avg. B	Contribution
Gunn (A)	117.0	<i>Posthodiplostomum ssp.</i>	15.29	0.3125	<b>28.5</b>
Hoople (B)		<i>Actinocleidus incus</i>	13.47	6.31	<b>17.2</b>
		<i>Neoechinorhynchus tenellus</i>	3.76	0	<b>7.6</b>
		<i>Apatemon sp.</i>	0.35	0	<b>4.6</b>
		<i>Diplostomum ssp.</i>	1	1.44	<b>3.7</b>
Gunn (A)	66.2	<i>Posthodiplostomum ssp.</i>	15.29	7.63	<b>14.2</b>
Gray's (B)		<i>Actinocleidus incus</i>	13.47	14	<b>14</b>
		<i>Neoechinorhynchus tenellus</i>	3.76	0.75	<b>5.5</b>
Hoople (A)	48.8	<i>Actinocleidus incus</i>	6.31	14	<b>20.3</b>
Gray's (B)		<i>Posthodiplostomum ssp.</i>	0.31	7.63	<b>16.9</b>
		<i>Apatemon ssp.</i>	2.13	1.88	<b>7</b>
		<i>Onchocleidus ferox</i>	0.8125	1.1875	<b>3.5</b>
		<i>Diplostomum ssp.</i>	1.44	0.56	<b>3.5</b>

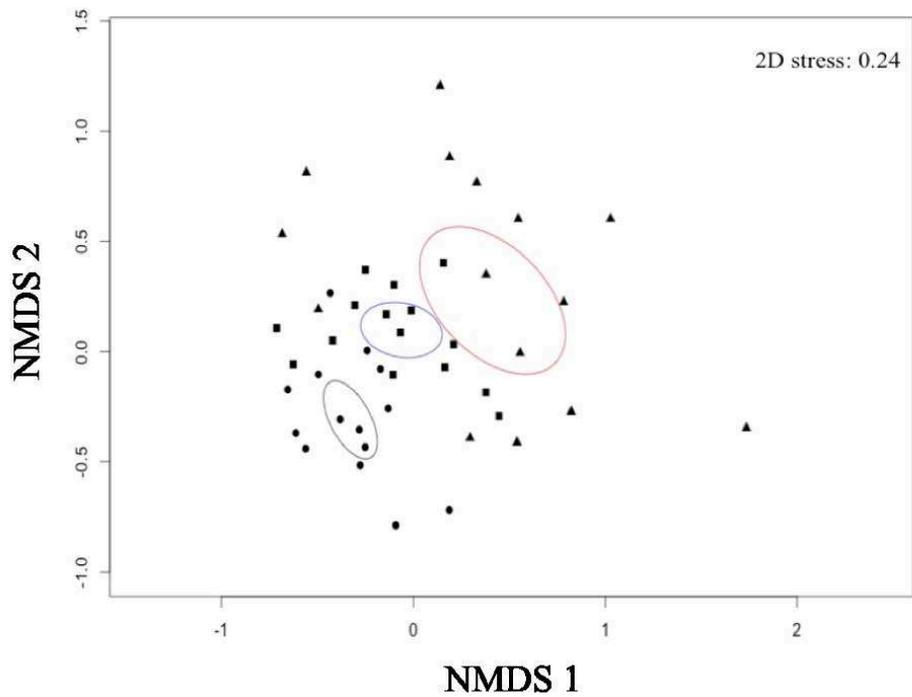
## Figures



**Figure 3-1:** Species accumulation curves with extrapolation calculated for the parasite fauna of overwinter juvenile pumpkinseed *Lepomis gibbosus* collected from three streams in Eastern Ontario. Arrows indicate the number of hosts examined from each stream.



**Figure 3-2.** Total abundance, Shannon-Weiner diversity ( $H'$ ), and health assessment index (HAI) for parasite component communities of *Lepomis gibbosus* collected in streams in Eastern Ontario. Values that are significantly different are not connected by the same letters.



**Figure 3-3:** Non-metric multidimensional scaling (NMDS) output with 95% confidence intervals of parasite species assemblage from overwintered YOY pumpkinseed *Lepomis gibbosus* collected in streams in Eastern Ontario. Hoople: red, triangles; Gray's: blue, squares; Gunn: black, circles.

## **Chapter 4: General Discussion**

### **Findings and Implications**

The consequences of habitat degradation on aquatic ecosystems are well studied. To date, research on the influence of anthropogenic land use on freshwater fish has been largely limited to characterizing community structure (e.g. Wang et al. 1997; Richards et al. 1997; Allan 2004). Through these studies, urban and agricultural development has been identified as primary drivers of decreases in fish diversity in stream ecosystems (Walser and Bart 1999; Wang et al. 2003). While these studies remain informative, they do not explore any potential mechanistic explanations for observed declines in species diversity in response to land use or the associated impacts of habitat degradation on the health of resident fish. Throughout this thesis I evaluated the effects of land use on individual fish in an effort to identify relationships between fish health and watershed scale human development. In Chapter 2, proximate body composition and health indices of central mudminnow were quantified across a gradient of watershed land use to assess the influence land use has on fish energy stores. Differences in male and females and their unique overwintering strategies were also considered. In Chapter 3, the influence of overall habitat quality resultant from watershed land use and the response of parasite infection dynamics in juvenile pumpkinseed was investigated in three streams representing a gradient of habitat quality within the study area. Health assessments were conducted on all examined hosts to determine if overall fish condition was related to parasite communities, parasite abundance, habitat quality, or a combination of these factors.

Understanding the health of fish residing in impacted habitats is important to elucidate mechanisms driving previously observed trends in community dynamics (Wikelski and Cooke 2006). Results of this study indicate that watershed land use has differential effects on fish health dependent on the species and health measurement in question. In Chapter 2, central mudminnow *Umbra limi* principal components summarizing energy stores and condition were significantly influenced by sex and season, varied among streams, yet no relationship was found between these measurements and land use. For males, condition in spring was found to be significantly lower than fall, corresponding with an overall decrease in the proportion of protein and lipids. This response was opposite to that of females, who were found to increase in their lipid and protein stores over winter, likely as a result of gonadogenesis. Interestingly, it appears that females of this species are able to maintain feeding more effectively than males throughout the winter, as females increased in condition, protein, and lipids while undergoing gonadogenesis, while males decreased in these values while no gonadogenesis was apparent. In Chapter 3, I quantified parasite infection dynamics across a gradient of disturbed habitat. The abundance of two species increased in response to decreasing habitat quality, perhaps indicating changes in transmission pathways or host immune functions. As the two species identified as major drivers of between stream community dissimilarity represented different parasite guilds – direct transfer monoxenous ectoparasites and indirect transfer heteroxenous endoparasites – one simple explanation for the observed trends cannot be postulated. It is again likely that the ecosystem dynamics, reflected in invertebrate, fish, and parasite communities, are variably influenced by anthropogenic degradation.

Landscapes such as the historically disturbed area of Cornwall, Ontario are impaired to an extent where it is generally accepted that systems will not return to their natural states within the foreseeable future (Palmer et al. 2010). Consequently, resources have been allocated towards restoration focused on targets set in the Remedial Action Plan (e.g. lower total phosphorous in stream effluent, reestablish spawning habitat). Progress for these restoration initiatives with respect to fish is measured through community surveys with focus placed on the presence or absence of sensitive indicator species (Karr 1981). In heavily degraded systems, communities that are present are often composed of generalist species such as those studied in both chapters of this thesis. The likelihood of sensitive species re-establishing within these heavily degraded watersheds is low within the timeframe of a typical monitoring program. This is particularly true for stream systems that discharge into equally disturbed water bodies such as the St. Lawrence River, as a source population of sensitive species is likely far removed. Consequently, data from community surveys in extremely degraded systems may not indicate how changes in habitat quality are affecting resident fishes. In such cases, the use of individual health metrics may provide early indication of positive or negative changes to resident fishes, before changes at the community level are apparent.

Individual measurements related to organismal physiology, condition and health may be particularly useful for resource managers attempting to quantify the success of restoration efforts targeted at a specific habitat characteristic, focal species, or land use. In areas where variation in community level response is unlikely to change or be detected (i.e., areas with high levels of degradation populated by generalist species) individual health measurements may be the most effective way to address changes in fish health.

Further, it may be beneficial to conduct research of this nature on systems that are not already heavily impacted, perhaps in areas where sensitive species are present in adequate numbers. As an example, Blevins et al. (2013) used creek chub (*Semotilus atromaculatus*) in an attempt to quantify the stress response of fishes inhabiting degraded environments, believing degraded habitat may influence baseline cortisol levels. The selection of this species as the model species was likely a result of their abundance in these systems; however, as creek chub are a hardy generalist species, cortisol levels did not vary between agriculturally dominated streams and forested streams. As such, species that are found in high abundance in degraded systems may represent inappropriate bioindicators for subtle variations in habitat quality, or analysis of physiological measurements that are subject to acclimation such as stress responses. My research has indicated the same may be true with respect to proximate body composition and health indices of central mudminnow. That is, that localized adaptation may allow these fish to remain physiologically unaffected by non-point source contamination. The reasons for variation in observed condition and energy measurements do not follow any patterns associated with land use that were addressed within the scope of this research. Future research would be required in order to identify factors that influence these variables in this species.

An important caveat demonstrated through this research is the need to assess the baseline levels for each variable being measured (Wileski and Cooke 2006). This is true even for trends that have been established in previous research. For example, using the proportion of water as a surrogate for lipids was suggested by Murchie et al. (2010) to ease the laboratory demands of proximate body composition analysis. While this may be

true for certain species, the relationship between water and lipids was weak for central mudminnow, and further varied significantly by sex and season. This research has demonstrated how variation in individual-level measurements may be influenced by both intrinsic biological and extrinsic environmental factors. For parasitological work, prior knowledge of the prevalence and spatial distribution of parasite species within a host species is necessary to evaluate changes, as community data on its own is seldom simple to interpret as a result of multiple potential confounding variables (MacKenzie et al. 1995; Marcogliese 2005). With this knowledge, the addition or loss of species from an ecosystem would be noteworthy, perhaps indicating changes in food-web dynamics, or the addition of a new host to the area (Marcogliese and Cone 1997).

### **Future directions**

This research has supported the outstanding and seminal paper by Hynes (1975), who stated, “in every respect, the valley rules the stream”. In Chapter 2, central mudminnow energy stores and condition were found to be influenced by watershed land use. Further research into this phenomena using more detailed scale of watershed land use, such as types of agriculture (e.g. monoculture, specific crops or fertilizer use) and urban development (e.g. presence or absence of storm water management systems), or proximity of particular land uses to water bodies, may guide future management and development initiatives. As winter is known to be challenging for fishes (Shuter et al. 2012), understanding such interactions alongside overwinter energy stores and condition may further illuminate the driving factors behind extirpation of non-migratory fishes in disturbed environments. Proximate body composition remains a potential tool in such

research, as previous literature indicates more sensitive species respond to changes in habitat (e.g. Murchie et al. 2010).

Chapter 3 provided support for studies that have demonstrated variation in the community structure of parasites in varying habitat qualities (Valtonen et al. 1996; Khan 2004; Marcogliese 2006; Zargar et al. 2012). As demonstrated by Huspeni and Lafferty (2004), the continued study of parasites over multiple years in remediated areas can effectively indicate changes in invertebrate and hosts densities. Further, if parasites found within resident hosts are show sensitivity to specific contaminants of concern (e.g. mercury), their presence or absence may indicate contamination. The research potential surrounding parasite community of fishes residing in impacted systems is outstanding, and while initially labour intensive such research can be conducted with minimal financial investment (Marcogliese and Cone 1997).

## **Conclusion**

While recent research using individual measurements has demonstrated how an organism's environment directly influences its condition, major knowledge gaps exist regarding the mechanisms that drive these important relationships. As natural systems are extremely complex and comprised of multiple interacting and synergistic pathways, pinpointing the causal mechanisms for physiological responses may prove to be difficult, if not impossible in some cases. Through my research I have demonstrated examples where such interactions may exist, though I cannot identify what factors cause the observed effects. This issue may be addressed through research that utilizes a combination of laboratory and in-situ experimentation to isolate specific independent

factors responsible for various changes in individual-level parameters related to organismal physiology, condition and health.

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