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# Watershed-Scale Land Use Activities Influence the Physiological Condition of Stream Fish

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## ABSTRACT

Land use changes within watersheds can have large effects on stream ecosystems, but the mechanistic basis of those effects remains poorly understood. While changes to population size presumably reflect underlying variation in organismal health and condition, such individual-level metrics are rarely evaluated in the context of ecosystem disturbance. To address this deficiency, we combined physiological sampling with geographic information systems to quantify the effects of land use on physiological indicators of health in largemouth bass. More specifically, we first quantified blood metrics relating to nutrition, oxidative stress, and the glucocorticoid stress response from largemouth bass residing in eight watersheds. We then used Akaike's information criterion to define relationships between these blood metrics and land cover, including forests, agricultural areas, urban areas, and wetlands. The proportion of forest cover in a watershed was the best predictor of blood metrics representing recent feeding and resistance to oxidative stress, whereas the proportion of wetlands was the best predictor of glucocorticoid function; however, further investigation is needed, as the explanatory power of the models was relatively low. Patterns in energy reserves were not influenced by any land use practices. Interestingly, anthropogenic land use categories, such as urban and agricultural areas, were not the best predictor for any blood metrics. Together, our results

indicate that fish health is most related to natural features of a landscape rather than anthropogenic land uses. Furthermore, these findings suggest that physiological methods could supplement traditional population and community assessments to develop a more comprehensive understanding of ecosystem interactions and improve stream management.

*Keywords:* conservation physiology, landscape, macrophysiology, management, nutrition, oxidation, stress.

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## Introduction

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

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

Improving our understanding of the physiological consequences of watershed land use has the potential to improve conservation and restoration efforts. Physiological indexes, particularly in terms of stress and nutrition, offer a mechanistic understanding of many aspects of habitat quality, including food availability (Congleton and Wagner 2006), land use influences

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

Within this context, the objective of this study was to quantify the relationship between watershed-scale land cover and the physiological properties of resident fishes. To accomplish this goal, we collected *Micropterus salmoides* (largemouth bass) from streams that varied in their watershed land use practices and obtained blood samples to examine physiological indicators of health and condition. *Micropterus salmoides* was chosen as the model species for this study because their sedentary nature during the summer ensured residence in the streams where they were

collected (Winter 1977). This research can help define land use practices that have the largest influence on largemouth bass and tell us which physiological processes are affected. Together, this will enable better prediction of population responses before decline and potentially identify new indicators of aquatic ecosystem condition for use in future assessments of water quality and land use change.

## Material and Methods

### Site Selection

This study took place in a portion of the St. Lawrence Seaway near Cornwall, Ontario (lat. 45°01'17.5"N, long. 74°43'50.42"W; fig. 1). To quantify the effect of land use on the physiological properties of *Micropterus salmoides*, watersheds with various proportions of land use practices were selected. This area was chosen because of the available land use data, the abundance of watersheds in a small study area, and the variability in land use across watersheds.

Land use data were gathered by the Ontario Ministry of Natural Resources and compiled into the Southern Ontario

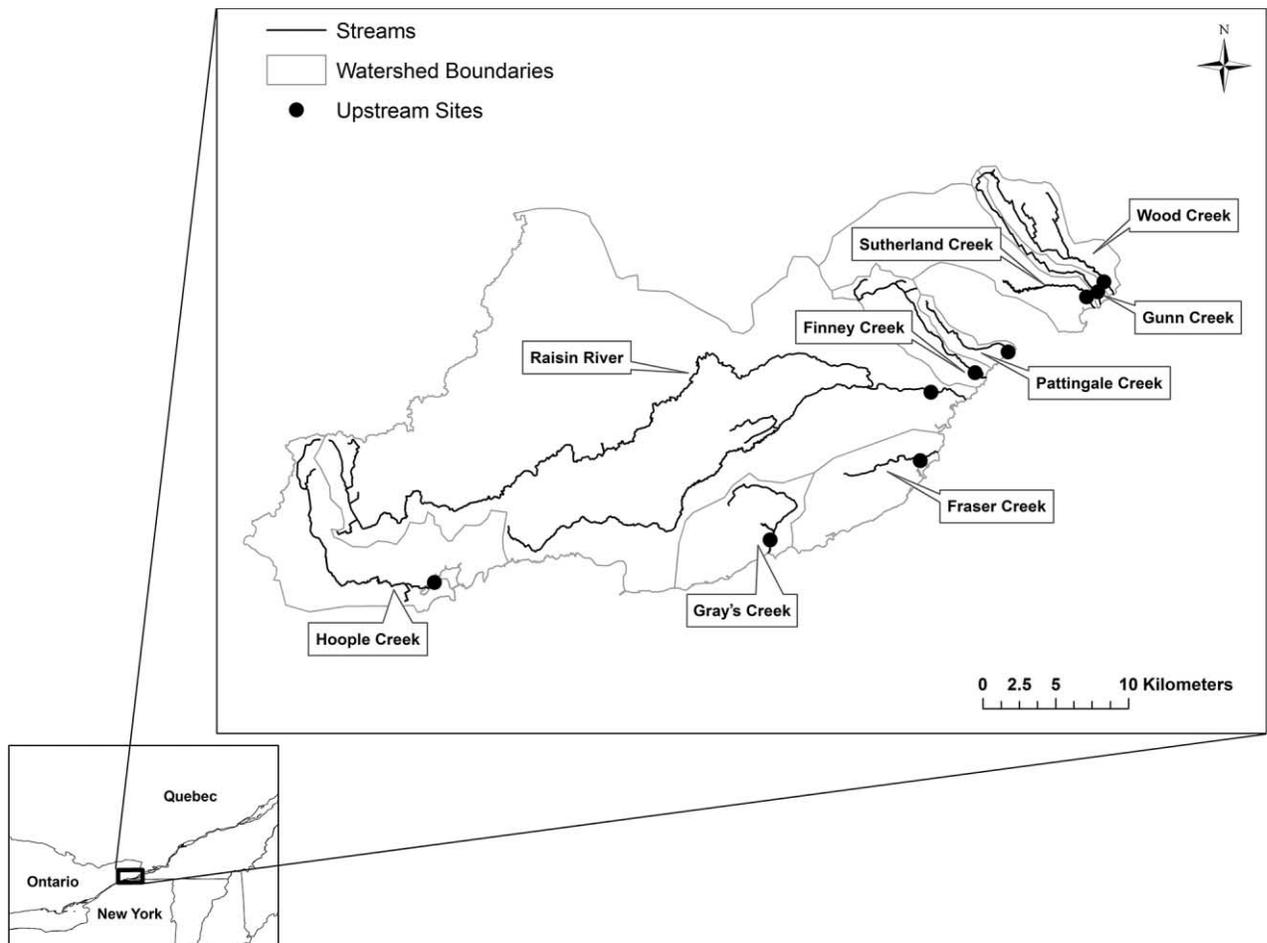


Figure 1. Map of the streams sampled in this study. Largemouth bass (*Micropterus salmoides*;  $n = 9-12$ ) were sampled from the confluence with the St. Lawrence Seaway to the upstream site labeled.

Land Resource Information System (SOLRIS) database (Ontario Ministry of Natural Resources 2008). Using Quantum GIS Lisboa software (ver. 1.8.0), this database was integrated with a geographic information system (GIS) layer developed by the Raisin Region Conservation Authority that delineated watershed boundaries within the study area. The SOLRIS database divides land use variables into many classes; for the purposes of this study, these classes were reduced to eight general land use categories (table 1). Once these general land use categories were established, the proportion each category occupied per watershed was determined (table 2). Using these data, eight watersheds representing a range of land use practices were chosen for field sampling.

### Field Sampling

Before field sampling, our protocol (12058) was approved by the University of Illinois Institutional Animal Care and Use Committee. The field-sampling component of this study was performed between July 12 and 21, 2012, in the eight watersheds described above. Using pulsed direct current boat electrofishing gear (2.5 GPP Electrofisher System [01868]; Smith-Root, Vancouver, WA), *M. salmoides* ( $n = 9-12$ , totaling 84) were collected from the lower portions of each stream. Sampling occurred from the confluence of each tributary with the St. Lawrence Seaway to the most upstream portion accessible by boat, typically 1–2 km, except for Pattingale Creek, where only the lower ~150 m could be sampled by boat. This is similar to sample sizes used in other studies relating physiology to habitat quality (Homan et al. 2003; Martínez-Mota et al. 2007; Blevins et al. 2013). If 10 fish were not caught on the first pass of the stream, at least 48 h was allowed before returning to sample remaining fish. This delay allowed any potential cortisol elevations in disturbed but uncaptured fish to return to baseline levels (Mommsen et al. 1999).

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

To quantify cortisol and glucose responsiveness, we used a standardized challenge previously shown to induce maximal elevations of circulating cortisol in other *Micropterus* species (O'Connor et al. 2011). Each fish was subjected to 3 min of air exposure in a moist, padded, and covered container immediately following collection of the initial blood sample. *Micropterus salmoides* were then placed in a “fish bag” in the stream for 25 min to allow circulating cortisol concentrations to reach maximal elevations. The fish bag was a cylindrical sack (1 m long × 0.2 m diameter) constructed from hypalon rubber (chlorosulfonated polyethylene synthetic rubber) with

Table 1: Groupings of the Southern Ontario Land Resource Information System (SOLRIS) land use categories

Presented land use category	SOLRIS land use types included
Agriculture and other fields	All agricultural land, urban brown fields, forest clearings, etc.
Urban	Residential areas, industrial areas, commercial and civic areas, highways, roads
Forest	Coniferous forest, deciduous forest, forest, mixed forest, hedge row, plantations (tree cultivated)
Resource extraction	Pits, quarries
Wetland	Bogs, marshes, swamps
Recreation	Golf courses, playing fields, etc.

Note. The SOLRIS database provides specific information for many land use types. For the purposes of this study, these have been grouped into more broadly defined categories.

mesh on both ends to allow for continuous water exchange. After the 25-min confinement in the fish bag, *M. salmoides* were bled a second time in a manner identical to that of the first before being weighed, measured, fin-clipped to prevent resampling, and released. Immediately following withdrawal, extracted blood samples were spun in a centrifuge for 3 min at 6,000 rpm to separate plasma from erythrocytes (Liss et al. 2013). Following centrifugation, the plasma layer was removed and divided into two other microcentrifuge tubes. All three samples were placed in a dry shipper charged with liquid nitrogen for future laboratory analysis.

### Laboratory Analyses

We quantified cholesterol (mg dL<sup>-1</sup>) and triglycerides (mg dL<sup>-1</sup>) using the EnzyChrom Cholesterol Assay Kit (ECCH-100) and the EnzyChrom Triglyceride Assay Kit (ETGA-200), respectively (BioAssay Systems, Haywood, CA). Total antioxidant capacity (mM) and lipid peroxide levels (μM malondialdehyde) were quantified using the Antioxidant Assay Kit (709001) and the TBARS Assay Kit (10009055), respectively (Cayman Chemical, Ann Arbor, MI). Plasma cortisol (ng mL<sup>-1</sup>) was measured using the Cortisol EIA Kit (ADI-901-071; Enzo Life Sciences, Farmingdale, NY), previously validated for use in *M. salmoides* (Sink et al. 2008). Total protein (g dL<sup>-1</sup>) was measured using a handheld protein refractometer (AST model 1250; Thomas Scientific, Swedesboro, NJ), which has been certified for use in the range of 0–12 g dL<sup>-1</sup> (Wells and Pankhurst 1999).

### Statistical Analysis

To reduce the dimensionality of the physiological data and quantify relationships among metrics, a factor analysis (FA) on correlations was performed on the values from each of the 84 fish (Liss et al. 2013). Our ratio of samples to predictor

Table 2: Watershed size and land use

Watershed	Total watershed area (ha)	Agriculture and other fields (%)	Urban (%)	Forest (%)	Resource extraction (%)	Wetland (%)	Recreation (%)
Finney	3,182.0	77.9	4.7	13.0	.0	3.9	.2
Fraser	4,485.7	47.7	6.7	19.9	.0	24.5	1.1
Gray's	4,450.8	33.0	35.3	8.7	.0	16.5	6.1
Hoople	9,534.8	53.5	5.0	13.9	.3	26.3	.6
Pattingale	938.8	69.8	5.9	15.2	.0	4.9	4.0
Raisin	57,847.1	49.8	4.4	18.1	.3	26.6	.4
Sutherland	7,913.6	76.2	3.2	11.7	.4	8.2	.2
Wood	3,013.6	83.9	2.8	10.8	.0	2.1	.3

Note. Abundance of each land use category is displayed as the percentage of total watershed area. Watershed land use data were determined using Quantum GIS software to integrate land use data from the Southern Ontario Land Resource Information System and a layer delineating watershed boundaries produced by the Raisin Region Conservation Authority.

variables met those recommended by Grossman et al. (1991). First, a principal component analysis (PCA) was performed, and principal components (PCs) with eigenvalues  $>1$  were interpreted for analysis (Kaiser 1960). For the FA, these PCs were rotated using varimax rotation on the maximum likelihood solution (Kaiser 1960; Liss et al. 2013). Variables with factor loadings  $>|0.4|$  were considered maximal contributors to each factor (Kaiser 1960; Liss et al. 2013). Positive factor loadings indicate a positive correlation between the factor and raw data, while negative factor loadings indicate a negative correlation. Factors were used as response variables in all subsequent analyses (table 3). Once the factors were obtained, normality and homoscedasticity were assessed using visual inspection of fitted residuals (Williams 1959); all data met necessary assumptions, and no transformations were required.

Factors were fitted to biologically relevant models chosen a priori that sought to define the relationship between land use practices and physiological response variables (table 4). We did not include models with multiple land use types, as we were interested in determining the land use variables with the largest impact on *M. salmoides* physiological condition. While all fish were captured from the same stream, fish were kept as individual data points, as opposed to averaging stream values, to prevent artificially reducing the variance and retain information provided by each individual (Schank and Koehnle 2009). Watershed land use variables, considered treatments, were modeled against each factor using linear and nonlinear regression to determine the best predictor variable. For this, land use variables were also modeled with their square term, as biological responses to environmental conditions can often be nonlinear (Allan 2004; Mouillot et al. 2013). To account for the unequal size of our watersheds, we considered land use as a proportion of the total watershed area rather than the absolute area of each land use. Models were ranked using Akaike's information criterion adjusted for small sample size ( $AIC_c$ ) to determine the most parsimonious model (Hegyí and Garamszegi 2010). On ranking models, those with a  $\Delta_i AIC_c$  value  $<2$  of the best-fit model were deemed to be a competitor for drawing inference (Mazerolle 2006). Competitors for top model were

plotted to visualize the relationships between land use and physiological components. Using the mean PC score of each stream substantially improved the fit ( $R^2$ ) of these regressions; however, the regressions included were performed using the individual fish to maintain consistency with other analyses.

Model selection using  $AIC_c$  was performed without the use of stepwise multiple regression procedures because of the limitations and shortcomings of such techniques (e.g., model selection bias and that the stepwise method used and the order in which parameters are entered or removed can affect the selection of the "best" model; see Whittingham et al. 2006). The method of using the main contributor for each factor and dropping the collinear metrics to run analyses on raw data was also investigated; however, this did not improve the fit of our models or have a major impact on the overall results. Therefore, this was deemed a less preferable means of analysis, as it has been recognized that screening variables and dropping collinear ones is unlikely to increase fit and may actually lead to the loss of important variables from analysis (James and McCulloch 1990).

Relative weight ( $W_r$ ), a measure of fish condition that relates actual fish weight to the expected weight at a given length, was also compared between streams. This was done on all largemouth bass over the minimum recommended length of 150 mm with the  $W_r$  index using the regression-line-percentile equation developed and presented by Murphy et al. (1991). The equation for this  $W_r$  index is

$$W_r = 100 \frac{W}{W_s}$$

and

$$\log_{10} W_s = -5.528 + (3.273 \times \log_{10}(\text{total length})),$$

where  $W$  is the measured weight and  $W_s$  is the length-specific predicted weight of a healthy fish. An optimal  $W_r$  is near 95–105 (Murphy et al. 1990).

FA was performed using JMP software (ver. 12.0; SAS Institute, Cary, NC), while regression models and  $AIC_c$  values

Table 3: Nutrition and stress characteristics

Metric	Finney	Fraser	Gray's	Hoople	Pattingale	Raisin	Sutherland	Wood	Grand mean
Triglycerides (mg dL <sup>-1</sup> )	3.0 (.4, 11)	1.7 (.4, 10)	1.4 (.3, 10)	3.3 (.6, 10)	3.9 (.6, 10)	2.7 (.5, 10)	2.7 (.4, 12)	2.8 (.8, 11)	2.7 (.2, 84)
Total protein (g dL <sup>-1</sup> )	4.4 (.2, 11)	4.6 (.1, 10)	4.5 (.1, 10)	4.4 (.1, 10)	4.7 (.2, 10)	5.2 (.1, 9)	4.7 (.1, 12)	4.9 (.1, 11)	4.7 (.1, 83)
Cholesterol (mg dL <sup>-1</sup> )	337.8 (23.2, 11)	403.8 (30.1, 10)	367.7 (17.3, 10)	320.1 (30.1, 10)	351.4 (19.9, 10)	323.5 (27.7, 10)	321.1 (32.7, 12)	390.7 (13.8, 11)	351.6 (9.2, 84)
Total antioxidant capacity (mM)	1.5 (.1, 11)	1.4 (.1, 10)	1.1 (.1, 10)	.8 (.1, 10)	.7 (.1, 10)	1.6 (.1, 10)	1.1 (.1, 12)	1.3 (0, 11)	1.2 (.1, 84)
Lipid peroxide levels (µM MDA)	30.6 (4.6, 8)	14.5 (1.7, 9)	21.9 (1.9, 9)	37 (3.5, 8)	22.6 (2.4, 9)	12.2 (1.9, 9)	26.1 (2.1, 11)	23.2 (2, 10)	23.3 (1.2, 73)
Baseline cortisol (ng mL <sup>-1</sup> )	4.3 (.5, 11)	6.0 (1.5, 10)	5.8 (.9, 10)	9.6 (2.6, 8)	3.6 (.4, 9)	8.3 (1.2, 9)	7.5 (1.6, 12)	4.4 (.7, 9)	6.1 (.5, 78)
Maximal cortisol (ng mL <sup>-1</sup> )	85.6 (12.2, 11)	81.2 (13.6, 10)	130.9 (17.7, 10)	90.2 (20.2, 8)	94.4 (13.5, 9)	73.8 (14.8, 9)	76.4 (15.9, 12)	71.6 (15.8, 9)	88 (5.6, 78)
Baseline glucose (mg dL <sup>-1</sup> )	3.3 (.4, 11)	2.4 (.2, 9)	2.2 (.3, 9)	3.4 (.4, 7)	4.3 (.8, 8)	2.9 (.5, 8)	3.5 (.4, 12)	3.1 (.5, 9)	3.1 (.2, 73)
Maximal glucose (mg dL <sup>-1</sup> )	9.4 (.7, 11)	9.7 (1.2, 10)	7.1 (.7, 10)	7.1 (1.1, 8)	7.7 (.7, 8)	9.8 (1.3, 8)	8.7 (1.1, 12)	7.5 (.7, 9)	8.4 (.4, 77)
Cortisol responsiveness (ng mL <sup>-1</sup> )	81.4 (12.3, 11)	75.2 (14.3, 10)	125.1 (17.2, 10)	80.6 (20.4, 8)	90.8 (13.5, 9)	65.5 (15.1, 9)	68.9 (14.9, 12)	67.2 (15.5, 9)	81.8 (5.6, 78)
Glucose responsiveness (mg dL <sup>-1</sup> )	6.1 (.9, 11)	7.0 (1.4, 9)	5.0 (.8, 9)	3.8 (1.7, 7)	3.6 (.5, 8)	6.9 (1.3, 8)	5.2 (1, 12)	4.4 (.7, 9)	5.3 (.4, 73)
Relative weight (W <sub>r</sub> )	102.0 (1.4, 3)	102.1 (.9, 8)	102.7 (1.0, 6)	104.7 (1.1, 5)	101.8 (1.0, 6)	101.9 (.9, 7)	102.2 (.7, 11)	102.7 (.9, 8)	102.5 (.3, 54)

Note. Blood-based nutrition and stress parameters were measured in *Micropterus salmoides* ( $n = 9-12$ ) caught in streams in the vicinity of Cornwall, Ontario, Canada, in July 2012. Parenthetical data are standard error of the mean followed by sample size. Maximal cortisol and glucose elevations were determined by subjecting *M. salmoides* to 3-min air exposure. MDA = malondialdehyde.

Table 4: Results of the factor analysis summarizing stress and nutritional characteristics

Variable	F1	F2	F3	F4
Baseline cortisol (ng mL <sup>-1</sup> )	-.11	.31	-.03	-.08
Total protein (g dL <sup>-1</sup> )	.36	<b>.85</b>	.34	-.15
Triglycerides (mg dL <sup>-1</sup> )	<b>.99</b>	-.12	-.01	-.12
Total antioxidant capacity (mM)	.00	.07	<b>.74</b>	.16
Lipid peroxide levels (μM MDA)	.37	-.06	-.51	-.10
Cholesterol (mg dL <sup>-1</sup> )	-.13	<b>.64</b>	-.04	.35
Baseline glucose (mg dL <sup>-1</sup> )	<b>.56</b>	.01	-.24	-.58
Cortisol responsiveness (ng mL <sup>-1</sup> )	-.17	.13	-.38	<b>.40</b>
Glucose responsiveness (mg dL <sup>-1</sup> )	-.03	-.06	.12	<b>.49</b>
Variance explained	18	14.1	12.7	10.5
Eigenvalue	2.4	1.7	1.4	1.1

Note. Largemouth bass (*Micropterus salmoides*) were sampled from eight watersheds in the vicinity of Cornwall, Ontario, Canada, in July 2012. Variables were loaded into four factors, and factor loadings  $>|0.4|$  following varimax rotation were considered maximal contributors to each factor and are indicated in bold. MDA = malondialdehyde.

were obtained using maximum likelihood estimation in PROC MIXED with SAS software (ver. 9.3; SAS Institute). Rejection of the null hypothesis ( $\alpha$ ) for all tests was  $P \leq 0.05$ , and all values are reported as means  $\pm$  SE where appropriate. See the appendix for reasoning for using FA and an additional analysis of the data using PCA.

## Results

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

Physiological metrics were highly variable across streams (table 3). Lipid peroxide level, a measure of cellular oxidative damage, was the most variable metric, with a threefold increase from the lowest- to the highest-ranking watershed. The second most variable physiological metric was triglycerides, with watershed means ranging from 1.36 to 3.92 mg dL<sup>-1</sup>. It is also worth noting that total antioxidant capacity was the third most variable metric, with the highest watershed mean being 2.5 times greater than the lowest. The two least variable metrics were total protein and cholesterol, with a respective 20% and 26% increase from the lowest to the highest watershed means. One fish displayed a low cortisol and glucose response, with the prestressor and responsiveness values interacting to generate a negative scope. We chose to not exclude this individual from analyses because both the prestressor and the poststress values were within the range of prestressor levels observed in this study and attributed the negative value to a muted stress response, recovery in the fish bag, or inherent error ( $\pm 8\%$ ) in the cortisol assay (Enzo Life Sciences 2013). Both total length (ANOVA,  $F_{7,56} = 1.7098$ ,  $P = 0.2481$ ) and weight (ANOVA,  $F_{7,56} =$

1.7833,  $P = 0.2351$ ) of sampled *Micropterus salmoides* did not differ across streams.

FA produced four factors with eigenvalues  $>1$ , which explained 55% of the total variation in physiological metrics measured (table 4). Factor 1 (F1) explained 18% of the total variation and was characterized by positive factor loadings for prestressor glucose and triglycerides, which are known to respond in the short term to feeding events (Wagner and Congleton 2004; Congleton and Wagner 2006; German 2011; Liss et al. 2013; table 4). F2 explained 14.1% of total variation and was characterized by positive factor loadings for total protein and cholesterol, indicating energy reserves (Wagner and Congleton 2004; German 2011; table 4). F3 explained 12.7% of the total variation and was characterized by a positive factor loading for total antioxidant capacity and a negative factor loading for lipid peroxide levels, therefore representative of oxidative stress resistance (Beckman and Ames 1998; table 5). Finally, F4 explained 10.5% of the total variation and was characterized by a negative factor loading for prestressor glucose and positive factor loadings for cortisol and glucose responsiveness, indicating both current nutritional status and glucocorticoid responsiveness (Romero 2004).

Variation in F1 (recent feeding) was best explained by the model containing the quadratic term for the proportion of forested area in a watershed (table 5). No other models received a  $\Delta_i$  AIC<sub>c</sub> value  $<2$  and were therefore not considered competitive. The greatest F1 scores occurred in watersheds with an intermediate proportion of forested area (between 13% and 15%), while *M. salmoides* residing in watersheds with greater or less than this intermediate proportion displayed lower F1 scores (fig. 2).

Variation in F2 (energy reserves) did not receive substantial support from any model examined. Of the 12 models examined, nine were competitors for best-fit model receiving a  $\Delta_i$  AIC<sub>c</sub> value  $<2$ . The top six were the linear models for each land use category, and the next three also contained the quadratic term

Table 5: AIC<sub>c</sub> model selection results describing variation in stress and nutritional characteristics of individual largemouth bass (see table 4) in relation to the percentage of land use in the watershed

Factor, model <sup>a</sup>	K <sup>b</sup>	-2 log likelihood	AIC <sub>c</sub>	Δ <sub>i</sub> AIC <sub>c</sub>	w <sub>i</sub>	R <sup>2</sup>
F1:						
%Forest <sup>2</sup>	4	173.2	181.9	0	.53	.15
%Agriculture and other fields	3	178.7	185.1	3.2	.11	.08
%Agriculture and other fields <sup>2</sup>	4	176.5	185.2	3.3	.10	.11
%Urban	3	178.9	185.3	3.4	.10	.08
%Wetland <sup>2</sup>	4	177.3	186	4.1	.07	.10
%Urban <sup>2</sup>	4	178.6	187.3	5.4	.04	.08
%Wetland	3	181.5	187.9	6	.03	.04
%Recreation	3	182.8	189.2	7.3	.01	.02
%Recreation <sup>2</sup>	4	181.2	189.9	8	.01	.04
%Forest	3	183.9	190.3	8.4	.01	.00
%Resource extraction	4	181.7	190.4	8.5	.01	.01
%Resource extraction <sup>2</sup>	4	182.9	191.5	9.6	.00	.02
F2:						
%Resource extraction	3	179.3	185.7	0	.12	.01
%Recreation	3	179.3	185.7	0	.12	.00
%Agriculture and other fields	3	179.3	185.7	0	.12	.00
%Wetland	3	179.4	185.8	.1	.11	.00
%Urban	3	179.4	185.8	.1	.11	.00
%Forest	3	179.5	185.9	.2	.11	.00
%Forest <sup>2</sup>	4	178.1	186.8	1.1	.07	.02
%Urban <sup>2</sup>	4	178.1	186.8	1.1	.07	.02
%Agriculture and other fields <sup>2</sup>	4	178.8	187.5	1.8	.05	.01
%Resource extraction <sup>2</sup>	4	179.1	187.8	2.1	.04	.00
%Recreation <sup>2</sup>	4	179.3	188	2.3	.04	.01
%Wetland <sup>2</sup>	4	179.4	188.1	2.4	.04	.00
F3:						
%Forest <sup>2</sup>	4	152	160.7	0	.26	.11
%Forest	3	155	161.4	.7	.18	.06
%Recreation	3	155.5	161.9	1.2	.14	.06
%Wetland <sup>2</sup>	4	153.4	162.1	1.4	.13	.09
%Wetland	3	156.3	162.7	2	.09	.04
%Recreation <sup>2</sup>	4	154.5	163.2	2.5	.07	.07
%Resource extraction	3	158.3	164.7	4	.03	.01
%Urban	3	158.4	164.8	4.1	.03	.01
%Agriculture and other fields	3	158.8	165.2	4.5	.03	.01
%Urban <sup>2</sup>	4	157.8	166.5	5.8	.01	.02
%Resource extraction <sup>2</sup>	4	158.1	166.8	6.1	.01	.02
%Agriculture and other fields <sup>2</sup>	4	158.8	167.5	6.8	.01	.01
F4:						
%Wetland	3	133.7	140.1	0	.28	.06
%Agriculture and other fields	3	134.2	140.6	.5	.22	.06
%Wetland <sup>2</sup>	4	133.6	142.3	2.2	.09	.06
%Forest <sup>2</sup>	4	133.9	142.6	2.5	.08	.06
%Agriculture and other fields <sup>2</sup>	4	134.2	142.9	2.8	.07	.06
%Forest	3	137	143.4	3.3	.05	.01
%Urban	3	137	143.4	3.3	.05	.01
%Resource extraction	3	137.6	144	3.9	.04	.00
%Recreation	3	137.8	144.2	4.1	.04	.00

Table 5 (Continued)

Factor, model <sup>a</sup>	$K^b$	$-2 \log$ likelihood	$AIC_c$	$\Delta_i AIC_c$	$w_i$	$R^2$
%Resource extraction <sup>2</sup>	4	135.7	144.4	4.3	.03	.03
%Urban <sup>2</sup>	4	136.1	144.8	4.7	.03	.03
%Recreation <sup>2</sup>	4	137.6	146.3	6.2	.01	.00

Note. For each dependent variable (factor), the best model is listed first. *Micropterus salmoides* were captured from streams near Cornwall, Ontario, Canada, in July 2012. Factors are as follows: F1 corresponds to recent feeding, F2 corresponds to energy reserves, F3 corresponds to resistance to oxidative stress, and F4 corresponds to current nutritional status and glucocorticoid responsiveness. Factors (dependent variable) were modeled against each land use type (independent variable). Models were then ranked using Akaike's information criterion adjusted for small sample sizes ( $AIC_c$ ); the model with the lowest  $AIC_c$  value has the best fit with the data. Also presented are Akaike weights ( $w_i$ ) for the models. This is the "weight of evidence," or probability, that a given model is actually the best model of those tested (Burnham and Anderson 2004).

<sup>a</sup>Models indicated with a square term (<sup>2</sup>) analyzed effects of both linear and quadratic variation.

<sup>b</sup>Parameter count ( $K$ ) includes intercept and variance.

for the proportion of forested area, urban area, and agricultural area and other fields (table 5).

Variation in F3 (resistance to oxidative stress) was explained by several competing models. The top-ranked model contained the quadratic term for the proportion of forested area in a watershed (table 5). More specifically, the lowest F3 scores occurred in watersheds with an intermediate proportion of forested area (between 12% and 16%), and watersheds with proportions of forested area greater or less than this intermediate amount showed higher F3 scores for resident *M. salmoides* (fig. 3a). The first competing model contained the linear term for the proportion of forested area in a watershed, demonstrating a positive relationship between the proportion of forested area in the watershed and F3 scores (fig. 3a). The next competing model contained the linear term for the proportion of recreational land, demonstrating a negative relationship between F3 scores and the amount of recreational land in the watershed (fig. 3b). The final competing model contained the quadratic term for the proportion of wetlands in a watershed. Similar to the relationship between F3 and forests, the lowest F3 scores occurred in watersheds with an intermediate proportion of wetlands, and watersheds greater or less than this intermediate proportion showed higher F3 scores for resident *M. salmoides* (fig. 3c).

Variation in F4 (prestessor glucose and glucocorticoid responsiveness) was explained by two competing models: proportion of wetlands and proportion of agricultural area and other fields in the watershed. The top model overall contained the proportion of wetlands in the watershed (table 5), which demonstrated a positive relationship between F4 scores and the proportion of wetlands in the watershed (fig. 4a). The competing model for F4 contained the proportion of agricultural area and other fields in a watershed. This model received a  $\Delta_i AIC_c$  value of 0.5, and F4 scores demonstrated a negative relationship with the proportion of agricultural area and other fields in a watershed (fig. 4b).

Fish condition did not vary between the streams sampled in this study. Across all streams, 54 fish exceeded 150 mm ( $n = 3-11$ ), with  $W_r$  ranging from 97.9 to 108.3 (table 3). When compared across streams, no difference was found in resident largemouth bass  $W_r$  ( $F_{7,46} = 0.77$ ,  $P = 0.62$ ).

## Discussion

The proportion of forested area and the proportion of wetlands were found to be the strongest watershed-scale drivers of physiological condition of resident *Micropterus salmoides*. The importance of these variables in our study is likely the result of forests and wetlands creating better habitat by generating more stable hydrologic regimes (Richardson 1994); reducing agricultural runoff, pollutants, and sedimentation (Allan 2004); and increasing forage availability and quality (Theodoropoulos and Iliopoulou-Georgudaki 2010; Miserendino et al. 2011). The relationship between the extent of these natural areas and *M. salmoides* physiology is particularly informative, as our analysis did not discern the location of the land use practices within the watershed, meaning that these natural areas have a large impact on streams even though they were not necessarily riparian. Taken together, our results indicate that the pro-

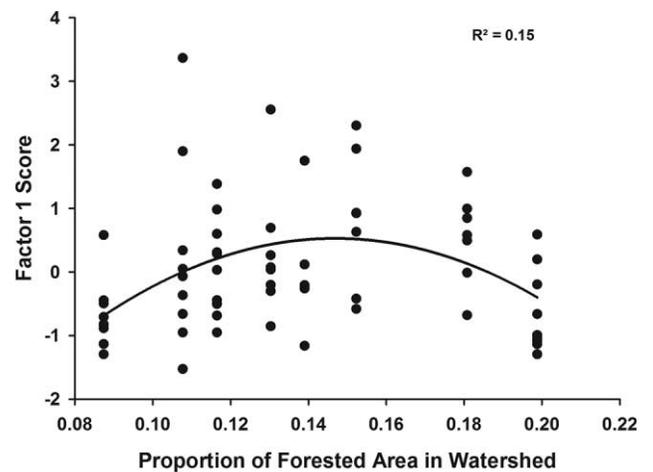


Figure 2. Relationship between the proportion of forested area in a watershed and factor 1 (F1) scores (recent feeding) for largemouth bass (*Micropterus salmoides*). *Micropterus salmoides* ( $n = 9-12$ ) were collected from a total of eight watersheds in the vicinity of Cornwall, Ontario, Canada, and sampled for blood in the field immediately after electroshocking. Information on F1 is given in table 4.

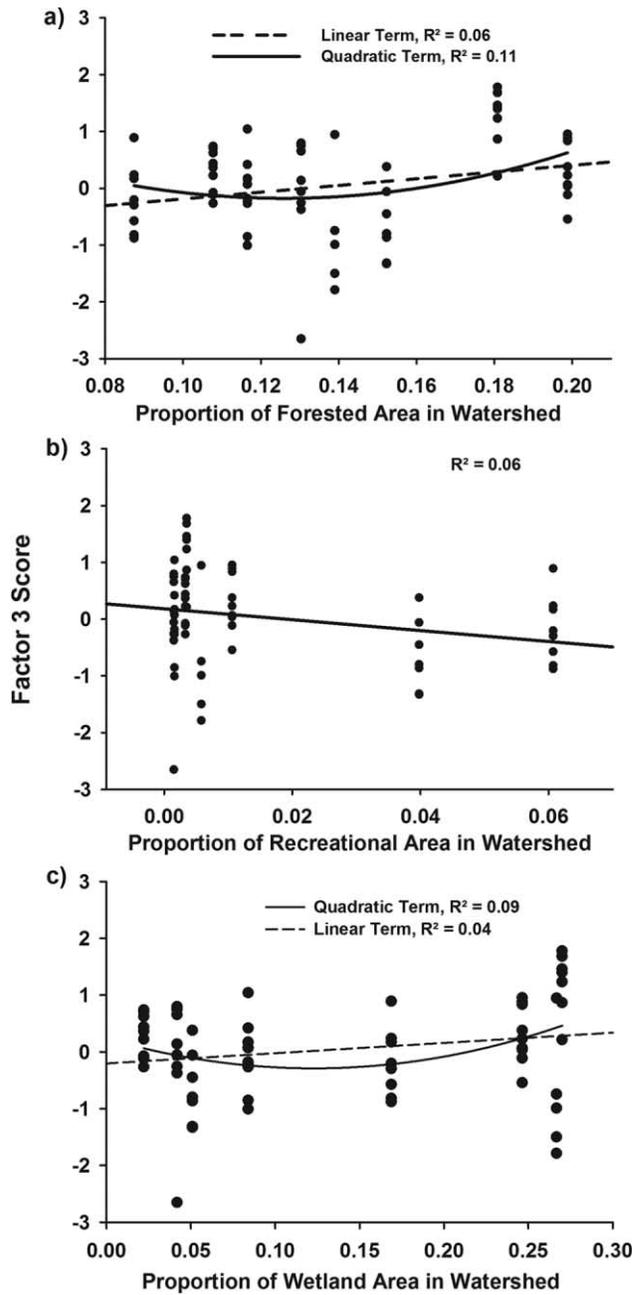


Figure 3. Relationship between the proportion of forested area (a), recreational area (b), or wetlands (c) in a watershed and factor 3 (F3) scores (resistance to oxidative stress) for largemouth bass (*Micropterus salmoides*). *Micropterus salmoides* ( $n = 9-12$ ) were collected from a total of eight watersheds in the vicinity of Cornwall, Ontario, Canada, and sampled for blood in the field immediately after electroshocking. Information on F3 is given in table 4.

portion of forested area and the proportion of wetlands within a watershed have the strongest relationship with physiological condition in resident *M. salmoides* in relation to other land use types, such as agricultural and urban areas.

Interestingly, for some of the metrics examined, the relationship between land use (forested area in particular) and physio-

logical metrics was parabolic rather than linear. More specifically, the best-fit model between land use type and F1 (recent feeding) peaked in watersheds with intermediate proportions (13%–15%) of forested area and decreased when proportions of forested area were higher or lower. F1 was explained primarily by the energy sources triglycerides and glucose, which have been demonstrated to decrease during periods of fasting and improve after feeding (Wagner and Congleton 2004; Congleton and Wagner 2006). As forests can increase water quality and optimize aquatic community structure and diversity (including taxa *M. salmoides* use for forage), increasing triglyceride and glucose concentrations from low to intermediate proportions of forested area is likely related to increasing water and forage quality and quantity (Miserendino and Masi 2010; Theodoropoulos and Iliopoulou-Georgiadaki 2010). Decreased F1 scores

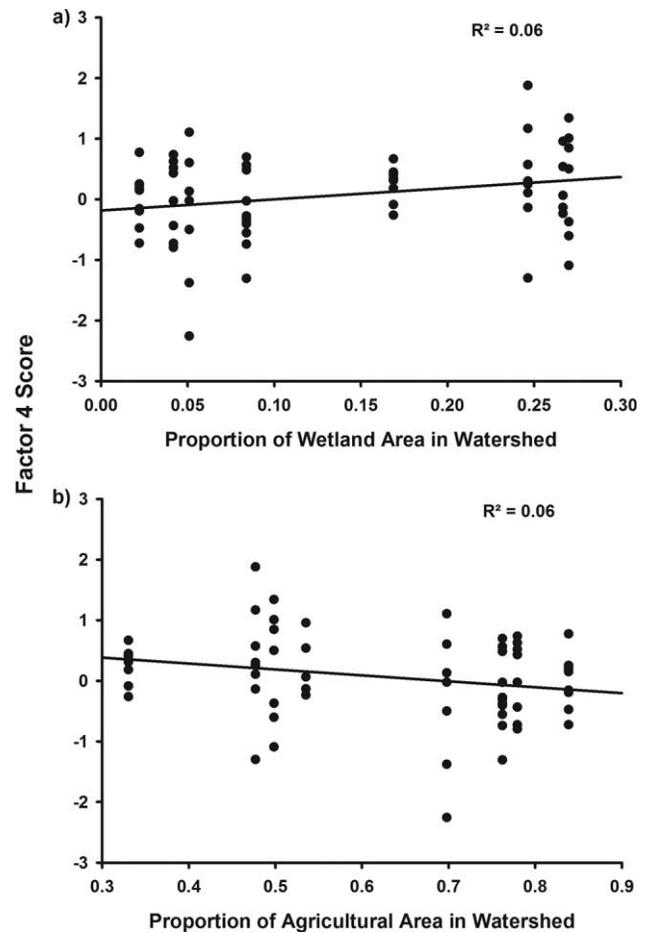


Figure 4. Relationship between the proportion of wetlands (a) or agricultural area and other fields (b) in a watershed and factor 4 (F4) scores (glucocorticoid function) for largemouth bass (*Micropterus salmoides*). *Micropterus salmoides* ( $n = 9-12$ ) were collected from a total of eight watersheds in the vicinity of Cornwall, Ontario, Canada, and sampled for blood in the field immediately after electroshocking and again after being subjected to a standardized stressor. Information on F4 is given in table 4.

at high proportions of forested area may indicate reduced feeding caused by changes in community and habitat structure, such as increased competition (Ward et al. 2006); however, other factors, such as stream order, would dictate the direct influence forests have on these streams (Vannote et al. 1980; Allan 2004). While the mechanism is unclear, the results clearly show that the proportion of forested area in a watershed influences feeding habits in *M. salmoides*.

The influence of land use on F3 scores (resistance to oxidative stress) received the most competition of our physiological components. Five models had a  $\Delta_i$  AIC<sub>c</sub> value  $<2$ ; however, four of these five models contained the natural land uses, forests and wetlands. Similar to F1 scores, F3 scores demonstrated a parabolic relationship with the proportion of forested area and wetlands in a watershed, declining in watersheds at intermediate proportions and increasing at high and low proportions. F3 was composed of a positive factor loading for total antioxidant capacity and a negative factor loading for lipid peroxide levels, therefore representing resistance to oxidative stress. Oxidation can damage proteins, lipids, and nucleic acids; increase susceptibility to disease; and speed the aging and death of cells (Beckman and Ames 1998). Because a substantial portion of antioxidants are exogenously derived from food, oxidative stress has been shown to relate to habitat quality, food availability, and food quality (Costantini 2008; van de Crommenacker et al. 2011). In the current study, decreased F3 scores at intermediate proportions of forested area and wetlands were likely related to poor-quality forage, while increased F3 scores at high proportions were likely related to higher-quality forage (Miserendino and Masi 2010; Theodoropoulos and Iliopoulou-Georgoudaki 2010). The elevated F3 scores from watersheds with low levels of forested area and wetlands in the watershed is not clear but may have resulted from the up-regulation of endogenously produced antioxidants in *M. salmoides* experiencing a mild but chronic shortage of exogenous antioxidants (Monaghan et al. 2009). This may not be the case, however, as the competing models of the proportion of forested area and wetlands demonstrate a positive linear relationship with resistance to oxidative stress, indicating that low levels of forested area and wetlands result in decreased resistance to oxidative stress. The high ranking of the model containing recreational land suggests that these areas (e.g., golf courses and playing fields) may have a large impact on stream fish even at very low densities. Regardless of the mechanism, results from the current study clearly indicate that natural land cover and recreational land are important drivers of *M. salmoides* oxidative stress.

F4, composed of prestressor glucose along with both cortisol and glucose responsiveness, was best predicted by the model containing the proportion of wetlands in a watershed, with which there was a weak positive linear relationship. Cortisol and glucose responsiveness represent the ability of *M. salmoides* to mount a glucocorticoid stress response (Romero 2004). Glucose is released during a stress event and was negatively associated with cortisol and glucose responsiveness in this component. This suggests that *M. salmoides* inhabiting streams with a low proportion of wetlands in the watershed had elevated prestressor

stress levels and a reduced ability to mount a stress response relative to those from streams with high proportions of wetlands. Natural areas have previously been shown to increase a fish's ability to mount a glucocorticoid stress response (Blevins et al. 2013), which is critical to the short-term survival of an animal (Romero 2004). In these watersheds, higher proportions of wetlands likely confer greater hydrological stability and reduced inputs from runoff, lowering the frequency of stress events caused by floods and chronic stress caused by pollutants (Hontela et al. 1992; Richardson 1994). The result of this factor clearly indicates that increased proportions of wetlands in a watershed increases the ability of *M. salmoides* to mount a proper glucocorticoid stress response, likely resulting in increased survival in these individuals.

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

Interestingly, F2, which was representative of energy reserves, did not appear to be influenced by any land use practices in this study. Examination of data revealed that nine models had a  $\Delta_i$  AIC<sub>c</sub> value  $<2$ , which indicates no strong support for any land use. This result is not too surprising, as total protein and cholesterol, which comprise F2, were the two least variable metrics, with a respective 20% and 26% increase across all streams. Total protein and cholesterol respond to changes in nutritional status (e.g., food consumption, growth, and body condition; Wagner and Congleton 2004). The lack of a land use effect on energy reserves could indicate that the land use practices within our study watersheds were not variable enough to result in any effects on body condition. Indeed,  $W_r$ , a measure of fish condition, agreed with this conclusion and was also consistent across streams. These results further demonstrate the importance of in-depth physiological assessments. Studies assessing condition factor would have concluded that there was no difference among streams within our study area; however, our results show that land cover within these watersheds is affecting the physiological condition of these fish. Although *M. salmoides* energy reserves show resilience to changes in watershed land use, the effects can still be seen in other metrics, which demonstrate the benefit of forests and wetlands.

Our results also indicate that the extent of anthropogenic land use had less impact on physiological properties than natural landscape features. Although land use characterized as recreational and as agriculture and other fields did result in compet-

itors for the best-fit model in two factors, no anthropogenic land use category resulted in a top model predicting physiological variables despite the large impact human land use has on stream ecosystems (see Meybeck 2004). This result is similar to those of other studies that found human land use to be a poor predictor of stream quality in areas of widespread anthropogenic influence (Allan 2004). Anthropogenic land use is indeed widespread in our study watersheds, with disturbed land ranging from 54% to 87%. As major degradation can occur at levels as low as 5%–15% (Paul and Meyer 2001), the lack of human land use resulting in top models predicting *M. salmoides* physiology suggests that disturbances may have little influence once a certain level is reached.

The physiological properties of resident stream fishes are driven by the proportion of forested area and wetlands in these watersheds. This finding demonstrates that physiological consequences of habitat use can be quantified (Huey 1991) and used to assess the health of a fish population to improve our understanding of the relationship between watershed-scale processes and stream quality. However, some considerations could be incorporated into future studies that could improve the predictive ability of these investigations and account for other factors that could be influencing the physiological responses of stream fishes to land use practices. For example, no data were available for flow/discharge in our streams, which has been shown to impact forage and habitat (Poff et al. 1997; Allan 2004). Additionally, we did not determine the sex of fish used in this study, and sex-specific differences in physiological indexes have been seen in fish (McCarthy et al. 1975), which could influence trends in hormone data (although, for largemouth bass, several studies have found there to be no sex-specific differences in many blood parameters, including cortisol, glucose, and protein concentrations; Clark et al. 1979; Carmichael et al. 1984). Future studies could also add a seasonal component to sampling and sample fish from the same stream across multiple seasons, as cortisol concentrations can vary seasonally due to such things as temperature and photoperiod (Pascoli et al. 2011) as well as reproduction (O'Connor et al. 2011). The improved mechanistic understanding offered by this study, along with future work, can increase the success rate of restoration projects by enabling them to target the most influential land use practices in their system (Cooke and Suski 2008). Determining that natural land use on the watershed scale created the best predictor models for the physiological condition of *M. salmoides* highlights its importance for resident stream fishes. Appreciating this relationship is critically important, as a poor understanding of watershed-scale effects has been found to be a major cause of restoration project failures worldwide (Roni et al. 2008). For these projects to be successful, practitioners must gain a mechanistic understanding by determining both the land use practices that affect the stream and the in-stream processes that are affected (Allan 2004). Our results show that, in these watersheds, it is the forests and wetlands that are likely affecting resident stream fish by influencing their feeding, resistance to oxidative stress, and ability to mount a stress response. This result improves our understanding of watershed-scale land use effects and can be

used by restoration practitioners to develop projects directed toward these natural areas as well as strategies that will most benefit the impaired processes.

### Acknowledgments

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## APPENDIX

### Techniques for Variable Reduction: Factor Analysis versus Principal Component Analysis

When many metrics are measured from each individual in a study, it is often desirable to reduce the dimensionality of the data. One way to accomplish this is to consider only a subset of the original metrics (Jolliffe 1990). For example, by examining correlations in the data set, highly correlated metrics may be discovered, making it possible to consider only one of these. An alternative approach is to use principal component analysis (PCA) or factor analysis (FA). Both of these techniques are useful because they reduce the dimensionality of data without eliminating variables. While PCA and FA create new variables that are different from the original metrics, using them can be more effective than using only a subset of variables, as they generally retain a greater amount of the original variation or the same amount of variation with a greater reduction in dimensionality (Jolliffe 1990; Jolliffe and Morgan 1992).

For the analyses in our article, we chose to perform FA as opposed to PCA to simplify the data and look for trends and/or relationships. In any FA there are two steps: first, reduction of the dimensionality of data; and second, rotation. The main purpose of rotation is to increase the interpretability of results (Abdi 2003). Rotation can also be performed on principal components (PCs) to increase interpretability without being considered "full-blown" FA (Jolliffe and Morgan 1992; Jolliffe 1993). In fact, a number of problems can occur with unrotated PCs, and some authors believe that it is often preferable to use rotation (Richman 1986).

In PCA, there are several restrictions that are imposed on the resulting PCs, namely, that the PCs are unrelated to every other factor and that the first PC explains the maximum

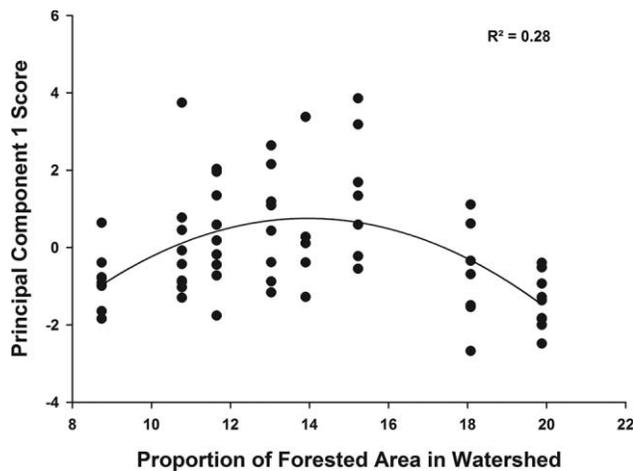


Figure A1. Relationship between the proportion of forested area in a watershed and principal component 1 (PC1) scores for largemouth bass (*Micropterus salmoides*). *Micropterus salmoides* ( $n = 9-12$ ) were collected from a total of eight watersheds in the vicinity of Cornwall, Ontario, Canada, and sampled for blood in the field immediately after electroshocking. Information on PC1 is given in table A1.

amount of variation possible, the second explains the maximum amount of variation while remaining uncorrelated to the first PC, the third explains the maximum amount possible while remaining uncorrelated to the first and second PCs, and so on (Kim and Mueller 1978; Thompson 1984). This will continue until there is the same number of PCs as original variables; however, much of the variation in the original variables can be accounted for in far fewer PCs. Therefore, only a subset of PCs is kept, based on eigenvalues or a chosen amount of explained variation (Jolliffe and Morgan 1992). The mathematical restrictions are arbitrary in a biological sense and can result in PCs that are not necessarily interpretable (Thompson 1984). One or both restrictions are removed when PCs are rotated, which can allow for a more meaningful, interpretable, and reliable (i.e., replicable) factorial structure (Thurstone 1947; Thompson 1984; Jolliffe 1993; Abdi 2003). Using FA, there will still be as many components as variables before rotation, and similar to PCA the number of factors to keep and rotate is usually chosen on the basis of eigenvalues and variation explained (Humphreys 1964; Jolliffe and Morgan 1992; Preacher et al. 2013). In FA, the first factor still explains more variation than the other factors and the factors are still orthogonal and explain correlations among the variables, but the variance is distributed among the retained factors (Jolliffe and Morgan 1992; Abdi 2003). Because the variance is distributed among retained factors, the decision of how many to keep is more important in FA than PCA (Jolliffe and Morgan 1992; Preacher et al. 2013).

For the analyses in our article, we chose to perform FA with varimax rotation to simplify our data and look for trends and/or relationships (Kaiser 1960). Varimax is an orthogonal rotation that is very commonly used (Abdi 2003; Browne 2010).

As an orthogonal rotation, varimax keeps factors uncorrelated, making them easier to interpret, and likely makes them more replicable (Gorsuch 1983; Kieffer 1998). Below, we use our data to illustrate differences in analyses and show how the use of PCA would change the interpretation of our results.

## Results

### Factor Analysis

Results obtained from FA are presented in detail in the article. Briefly, FA explained 55% of the variation in the data and generated reasonable factor groupings. For example, factor 1 (F1) was composed of metrics related to energy and feeding (Wagner and Congleton 2004; Congleton and Wagner 2006), F3 was composed of metrics related to oxidation (Beckman and Ames 1998), and F4 was composed of metrics related to stress (Mommsen et al. 1999; Cousineau et al. 2014). While the variation explained is not extremely high, the factor groupings allow for meaningful interpretation of the results.

### Principal Component Analysis

Analyses with unrotated PCs produced four components with eigenvalues  $>1$ , which explained 73.1% of the total variation in the physiological metrics measured (table 4). PC1 was characterized by positive factor loadings for triglycerides, lipid peroxide levels, and baseline glucose levels (table A1). PC1 is therefore composed of one of the indicators for short-term feeding and cellular oxidative damage. PC2 was characterized by positive factor loadings for total protein and total antioxidant capacity (table A1), meaning that PC2 is composed of an indicator for more long-term feeding (energy reserves) and resistance to oxidative stress. PC3 was characterized by

Table A1: Unrotated principal components (PCs)

Variable	PC1	PC2	PC3	PC4
Baseline cortisol (ng mL <sup>-1</sup> )	-.08	.29	.38	-.39
Total protein (g dL <sup>-1</sup> )	.04	<b>.67</b>	.19	.19
Triglycerides (mg dL <sup>-1</sup> )	<b>.49</b>	.19	-.07	<b>.43</b>
Total antioxidant capacity (mM)	-.24	<b>.41</b>	-. <b>43</b>	.19
Lipid peroxide levels (μM MDA)	<b>.40</b>	-.19	.37	.14
Cholesterol (mg dL <sup>-1</sup> )	-.30	.32	<b>.50</b>	.10
Baseline glucose (mg dL <sup>-1</sup> )	<b>.55</b>	.13	.07	-.05
Cortisol responsiveness (ng mL <sup>-1</sup> )	-.21	-.31	<b>.49</b>	.33
Glucose responsiveness (mg dL <sup>-1</sup> )	-.29	.13	-.07	<b>.67</b>
Variance explained	26.3	18.8	15.9	12
Eigenvalue	2.4	1.7	1.4	1.1

Note. Largemouth bass (*Micropterus salmoides*) were sampled from eight watersheds in the vicinity of Cornwall, Ontario, Canada, in July 2012. Variables were loaded into four factors, and factor loadings  $>|0.4|$  were considered maximal contributors to each factor and are indicated in bold. MDA = malondialdehyde.

Table A2: AIC<sub>c</sub> model selection results

Principal component, model <sup>a</sup>	K <sup>b</sup>	-2 log likelihood	AIC <sub>c</sub>	Δ <sub>i</sub> AIC <sub>c</sub>	w <sub>i</sub>	R <sup>2</sup>
PC1:						
%Forest <sup>2</sup>	4	210.03	218.70	.00	.96	.28
%Agriculture and other fields	3	220.90	227.30	8.60	.01	.15
%Wetland	3	220.96	227.36	8.65	.01	.15
%Agriculture and other fields <sup>2</sup>	4	220.23	228.91	10.20	.01	.16
%Wetland <sup>2</sup>	4	220.39	229.06	10.36	.01	.16
%Urban	3	228.40	234.80	16.10	.00	.05
%Forest	3	229.24	235.64	16.94	.00	.03
%Urban <sup>2</sup>	4	227.69	236.37	17.66	.00	.06
%Resource extraction	3	231.30	237.70	19.00	.00	.00
%Recreation	3	231.33	237.73	19.02	.00	.00
%Recreation <sup>2</sup>	4	230.20	238.88	20.17	.00	.02
%Resource extraction <sup>2</sup>	4	231.19	239.87	21.16	.00	.00
PC2:						
%Recreation	3	213.79	220.19	.00	.20	.04
%Urban	3	214.58	220.98	.79	.14	.03
%Forest	3	214.59	220.99	.80	.14	.03
%Wetland <sup>2</sup>	4	213.00	221.68	1.49	.10	.06
%Resource extraction	3	215.64	222.04	1.85	.08	.02
%Urban <sup>2</sup>	4	213.55	222.23	2.03	.07	.05
%Recreation <sup>2</sup>	4	213.79	222.46	2.27	.07	.05
%Wetland	3	216.30	222.70	2.50	.06	.01
%Agriculture and other fields	3	216.58	222.98	2.79	.05	.00
%Forest <sup>2</sup>	4	214.31	222.99	2.80	.05	.04
%Resource extraction <sup>2</sup>	4	215.63	224.31	4.12	.03	.02
%Agriculture and other fields <sup>2</sup>	4	216.45	225.13	4.94	.02	.00
PC3:						
%Recreation	3	206.39	212.79	.00	.20	.03
%Forest	3	206.57	212.97	.19	.18	.03
%Urban	3	207.34	213.74	.95	.12	.02
%Agriculture and other fields	3	208.19	214.59	1.81	.08	.00
%Wetland	3	208.36	214.76	1.97	.07	.00
%Resource extraction	3	208.37	214.77	1.99	.07	.00
%Recreation <sup>2</sup>	4	206.31	214.99	2.21	.07	.03
%Forest <sup>2</sup>	4	206.47	215.15	2.36	.06	.03
%Wetland <sup>2</sup>	4	207.00	215.68	2.89	.05	.02
%Urban <sup>2</sup>	4	207.34	216.02	3.23	.04	.02
%Agriculture and other fields <sup>2</sup>	4	208.14	216.82	4.03	.03	.00
%Resource extraction <sup>2</sup>	4	208.36	217.04	4.25	.02	.00
PC4:						
%Forest	3	180.67	187.07	.00	.14	.01
%Urban	3	180.68	187.08	.01	.14	.01
%Recreation	3	180.92	187.32	.25	.12	.01
%Agriculture and other fields	3	181.23	187.63	.55	.10	.00
%Resource extraction	3	181.45	187.85	.77	.09	.00
%Wetland	3	181.46	187.86	.78	.09	.00
%Wetland <sup>2</sup>	4	179.47	188.15	1.07	.08	.03
%Forest <sup>2</sup>	4	179.86	188.53	1.46	.07	.02
%Urban <sup>2</sup>	4	180.61	189.29	2.22	.05	.01
%Resource extraction <sup>2</sup>	4	180.63	189.31	2.23	.04	.01
%Agriculture and other fields <sup>2</sup>	4	180.80	189.47	2.40	.04	.01
%Recreation <sup>2</sup>	4	180.85	189.53	2.45	.04	.01

Note. *Micropterus salmoides* were captured from streams near Cornwall, Ontario, Canada, in July 2012. Principal components (PCs) were modeled against each land use type. Models were then ranked using Akaike's information criterion adjusted for small sample sizes (AIC<sub>c</sub>); the model with the lowest AIC<sub>c</sub> value has the best fit with the data. Also presented are Akaike weights (w<sub>i</sub>) for the models. This is the "weight of evidence," or probability, that a given model is actually the best model of those tested (Burnham and Anderson 2004).

<sup>a</sup>Models indicated with a square term (²) analyzed effects of both linear and quadratic variation.

<sup>b</sup>Parameter count (K) includes intercept and variance.

a negative factor loading for total antioxidant capacity and positive factor loadings for cholesterol and cortisol responsiveness to a stressor (table A1). Therefore, as PC3 scores increase, resistance to oxidative stress decrease and cholesterol and the stress response increase. Finally, PC4 was characterized by positive factor loadings for triglycerides and glucose responsiveness (table A1). PC4 is therefore composed of an indicator for short-term feeding and the ability to mount a stress response.

The PCs were then modeled against watershed land use practices. Variation in PC1 (triglycerides, baseline glucose, and lipid peroxide levels) was best explained by the model containing the quadratic term for the proportion of forested area in a watershed (table 5). No other models received a  $\Delta_i AIC_c$  value  $<2$  and were therefore not considered competitive. Similar to our FA, the greatest PC1 scores occurred in watersheds with an intermediate proportion of forested area (between 13% and 15%), while *Micropterus salmoides* residing in watersheds with greater or less than this intermediate proportion displayed lower PC1 scores.

There were many competing models for PC2, PC3, and PC4. For PC2, five models received a  $\Delta_i AIC_c$  value  $<2$ . These were the models examining the effect of recreational areas, urban areas, forests, resource extraction, and the quadratic term for the proportion of wetlands. For PC3, six land use models received a  $\Delta_i AIC_c$  value  $<2$ , and for PC4, eight models received a  $\Delta_i AIC_c$  value  $<2$ . All analyses were performed using JMP software (ver. 12.0).

## Discussion

While PCA did explain 18% more of the variation in the data than FA, these components were more difficult to interpret than factors generated through FA. For example, cortisol responsiveness and glucose responsiveness (measures of stress response) were split into different components, as were metrics related to oxidation and energy reserves. This is important because many authors point out that these physiological metrics should not be viewed alone but with other, related metrics (Wagner and Congleton 2004; Monaghan et al. 2009). After rotation with FA, metrics related to feeding grouped together and those related to oxidative stress grouped together (see table 4), making interpretation easier and more reliable. However, PCA increased the number of metrics in PC1 and PC3 and separated many of the related metrics into different components (table A2). For our data set, the use of PCs made it more difficult to label the PCs as representing a given physiological characteristic, making interpretation more difficult.

When PCs were modeled against land use practices, PC1 was the only component to have a single best-fit model. This was the quadratic model for the proportion of forested area in the watershed, which peaked in watersheds with intermediate proportions (13%–15%) of forested area and decreased when proportions of forested area were higher or lower (fig. A1). This finding is similar to trends with F1 (table 5; fig. 2), except that along with recent feeding PC1 also represents cellular

oxidative damage. Therefore, these analyses would suggest that intermediate proportions of forested area maximized both short-term feeding and cellular oxidative damage for resident largemouth bass. Again, it is difficult to draw definitive conclusions viewing trends in cellular oxidative damage alone without also knowing what is happening with an animal's antioxidant capacity (Monaghan et al. 2009), and this variable loaded into PC2. The other models had many competitors, showing that  $AIC_c$  ranked them about equally. Moreover, none of the models performed well for PC3 and PC4. The main separator was the penalty  $AIC_c$  gives for adding extra predictor variables into models, making all the quadratic models perform worse than the linear models (table A2). With these results, it appears that land use has a limited effect on largemouth bass physiology. The effects were limited to forested areas having the largest impact on PC1; however, this component was difficult to interpret, so any conclusions would be tenuous.

In summary, PCs explained a greater amount of variation in our data set, but the resulting components were more challenging to interpret. In contrast, the increase in biological interpretability and reliability gained from FA improved our ability to analyze our data and resulted in biologically meaningful groupings of metrics that were easy to relate to land use practices.

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