Reach-Scale Land Use Drives the Stress Responses of a Resident Stream Fish[*]

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ABSTRACT

To date, relatively few studies have tried to determine the practicality of using physiological information to help answer complex ecological questions and assist in conservation actions aimed at improving conditions for fish populations. In this study, the physiological stress responses of fish were evaluated in-stream between agricultural and forested stream reaches to determine whether differences in these responses can be used as tools to evaluate conservation actions. Creek chub *Semotilus atromaculatus* sampled directly from forested and agricultural stream segments did not show differences in a suite of physiological indicators. When given a thermal challenge in the laboratory, creek chub sampled from cooler forested stream reaches had higher cortisol levels and higher metabolic stress responses to thermal challenge than creek chub collected from warmer and more thermally variable agricultural reaches within the same stream. Despite fish from agricultural and forested stream segments having different primary and secondary stress responses, fish were able to maintain homeostasis of other physiological indicators to thermal challenge. These results demonstrate that local habitat conditions within discrete stream reaches may impact the stress responses of resident fish and provide insight into changes in community structure and the ability of tolerant fish species to persist in agricultural areas.

Introduction

An important aspect to consider when evaluating human impacts on ecosystem function and the health of stream fish populations is the scale at which negative impacts occur. Although watershed-level processes are important to consider because of their influence on habitat conditions within a stream, fish biodiversity is often most influenced by habitat quality and complexity within stream reaches (Allan 2004). For example, differences in flows, sediment, vegetative cover, and energy inputs create an ever-changing mosaic of habitats at the reach scale (Ward 1998) and are largely responsible for the high faunal biodiversity of these systems (Schlosser 1991; Robinson et al. 2002). In addition, the fish community composition within discrete reaches often changes within a stream depending on habitat variability that is maintained by numerous processes, such as those that influence stream bank sediment, vegetative cover, stream flows, and energy dissipation (Wesche and Isaak 1999). Human land use activities often disrupt these processes, and habitat heterogeneity and fish biodiversity typically decrease (Allan 2004). Many restoration activities occur at the scale of stream reaches rather than watersheds because of logistical and resource constraints (Bond and Lake 2003), which emphasizes the importance of understanding reach-scale dynamics for conservation activities (Imhof et al. 1996). Overall, understanding how human disturbance changes reach-level processes that determine ecosystem structure and function is important because of the large impact that such processes have on fish population health and community composition.

One overlooked element of ecosystem function is the physiological parameters (MacMahon and Holl 2001) of individual fish, which play a role in defining how individual organisms perceive their local environment (Busch and Hayward 2009). Changes in how organisms perceive stressors (Busch and Hayward 2009) and modify their physiology are directly responsible for the ability of an organism to adapt to new environmental conditions, such as changes due to degradation by humans (Adolph 1956; Ricklefs and Wikelski 2002). A particularly useful way of conceptualizing the stress response is that presented by McEwen and Wingfield (2003), in which organisms attempt to maintain allostasis, or maintenance of internal "set points" necessary for life, through environmental change or stressful situations. Essentially, the physiological response of an individual organism to a stressor acts as a series of events that attempt to maintain allostasis by eliciting a physiological response (McEwen and Wingfield 2003; Helmuth et al. 2005). By eliciting the appropriate physiological stress response, an organism is able to maintain allostasis (physiological performance) in the face of challenges (McEwen and Wingfield 2003), such as those

brought on by anthropogenic disturbance (Barton 2002). Reductions in the secondary stress response, such as hydromineral (Na+, K+, and water content) and circulatory systems (hematocrit), in a challenging environment are more likely to avoid allostatic overload and negative impacts on growth, reproduction, or immunocompetence (Wendelaar Bonga 1997; McEwen and Wingfield 2003; Shreck 2010). Furthermore, quantifying the physiological response to stress not only provides an understanding of how individuals cope with disturbance (Barton and Iwama 1991; Romero 2004) but also helps determine how individuals perceive stressors (and the allostatic load of these stressors) within their environment (McEwen and Wingfield 2003; Busch and Hayward 2009). Overall, it is essential to understand the physiological properties of resident organisms to determine how they perceive and cope with human-induced stressors to mitigate the impact of stressors on fish populations.

Recent syntheses have emphasized the use of physiological data in assisting with understanding complex ecological questions, including how restoration impacts resident fishes (Romero 2004; Cooke and Suski 2008; Busch and Hayward 2009; Cooke and O'Connor 2010; Adams and Ham 2011). Specifically, differences in the physiological response of animals residing in degraded habitats have helped define how organisms and populations cope with human-induced stressors (Cooke and Suski 2008). For example, European blackbirds *Terdus merula* (Linnaeus, 1758) from highly modified urban areas produced an attenuated physiological stress response (production of plasma corticosterone) to human noise stressors compared with birds from rural environments, and this reduced stress response may be important for birds to live successfully in urban areas (Partecke et al. 2006). In addition, black howler monkeys *Alouatta pigra* (Lacapede, 1799) living in highly fragmented forest patches displayed augmented fecal cortisol levels that were related to reduced food availability and quality, which could potentially cause long-term adverse health and population viability effects (Martinez-Mota et al. 2007). Unfortunately, there are very few examples of the effects of land use being evaluated in stream fish populations in which differences in physiological responses and fish health are likely to occur through variations in land use disturbance. Because human disturbance has been shown to cause terrestrial organisms to modify their physiological response to maintain performance and is detectable by physiological methods, it is likely that physiological techniques can also be used to determine whether resident fish species also modify their biochemical environment to cope with human-induced stressors. Overall, this information will help define the relationship between human-induced stressors and the physiological properties of fish and provide insight into how fish perceive and cope with human-induced stressors.

The goal of this study was to quantify the relationship between land cover properties in streams at the reach scale and the physiological properties of wild fish residing within these reaches. Specifically, this study tests the hypothesis that variation in land cover of streams at the reach scale will translate to altered capacities of resident fish to respond physiologically to environmental challenges. At present, no attention has been given to examining land use impacts on the physiological properties of stream fishes at scales smaller than entire watersheds (Blevins et al. 2013), and elucidating relationships at this scale would not only help us determine how physiological properties vary within streams as a result of land use practices and local environmental conditions but also provide foundational research for the potential use of physiological properties as bioindicators for the evaluation of land use restoration projects and conservation actions in stream reaches. In addition, it is important to point out that most conservation activities, restoration efforts, and remediation work in stream environments occur in individual stream reaches, which makes the stream reach a valuable conservation unit for restoration work. An improved understanding of how organisms respond to stressful conditions related to anthropogenic activity may improve our ability to mitigate negative outcomes, such as changes in fish community structure related to anthropogenic disturbance and restoration (Cooke and Suski 2008; Cooke and O'Connor 2010).

Material and Methods

Site Selection

To quantify how forest and agricultural land cover at the reach scale affected the physiological responses of resident fish, reaches within a stream were selected using ArcView GIS 9.1 (ESRI 2005) with remote-sensing layers based on the Land Cover of Illinois 1999–2000 classification online database with a 30 × 30-m ground spatial resolution (Illinois Department of Agriculture 2001). This study used a total of four streams for sampling: Bear Creek, Cottonwood Creek, Spring Point Creek, and Hurricane Creek (table 1). Each of the four streams contained two distinct reaches used in this study; one reach from each stream contained primarily row crop agriculture along the riparian area within the reach, and one reach from each stream contained primarily forest along the riparian area within the reach (table 1). In this way, the study design consisted of four replicate streams that each contained one forested reach and one agriculture reach separated by ∼1.1 km. In addition, two of the streams (Spring Point Creek and Hurricane Creek) had land cover oriented such that agricultural reaches were ∼1.1 km upstream of forested reaches, whereas for the other two streams (Bear Creek and Cottonwood Creek), agricultural reaches were ∼1.1 km downstream of forested reaches. By selecting streams that had both forested and agricultural land cover, it was possible to compare the impacts of land cover on physiological characteristics of resident fishes within streams, which controls for interstream variability in habitat and fish characteristics.

To classify a reach as either forested or agricultural, land use percentages (i.e., overall percentage of agriculture including row

Table 1: Reach-scale riparian land use percentages within each stream reach selected for this study

Stream name (county)	Reach coordinates (lat., long.)	Stream reach classification	Reach length (m)	Riparian land cover, %	
				Forested	Agriculture
Bear Creek (Cumberland)	39°17′N, 88°16′W	Agriculture	600	15	85
Cottonwood Creek (Cumberland)	39°19′N, 88°13′W	Agriculture	600	5	95
Spring Point Creek (Cumberland)	39°12′N, 88°19′W	Agriculture	600	20	80
Hurricane Creek (Cumberland)	39°21′N, 88°05′W	Agriculture	600	0	100
Bear Creek (Cumberland)	39°18′N, 88°17′W	Forested	600	73	27
Cottonwood Creek (Cumberland)	39°18′N, 88°13′W	Forested	600	77	23
Spring Point Creek (Cumberland)	39°12′N, 88°20′W	Forested	600	86	14
Hurricane Creek (Coles)	39°22′N, 88°05′W	Forested	600	93	7

Note. Percentage of riparian land use for streams was determined by GIS analysis of land cover in the riparian zone (30 m from the stream bank) for the watershed using the Land Cover of Illinois database (Illinois Department of Agriculture 2001).

crops and nonrow crops along with upland and lowland wooded forest) were calculated for the riparian area (land use within 30 m of the stream at the reach-level scale) along each 600-m stream reach. A riparian width of 30 m was selected for this study on the basis of the resolution of land cover data (30-m pixels) and because 30 m is the minimum riparian buffer size recommended by the US Department of Agriculture and by other studies for protection of water quality and stream biota (Welch 1991; Kiffney et al. 2003). Length of stream reaches were selected on the basis of the resolution of land cover data (30-m pixels) and also because previous research indicated that creek chub tend to associate with and complete their entire life cycle in stream segments <600 m in length (Fitzgerald et al. 1999; Belica and Rahel 2008). Forested reaches were classified as having >73% forest and <27% agriculture in the riparian area, whereas agricultural reaches were classified as having >80% agriculture and <20% forest in the riparian area. These agricultural and forested classifications were consistent with land use categories developed through the land use land cover classification system (Anderson et al. 1975). Second- and third-order streams were used for this study to hold morphometric characteristics, such as stream depth, width, and flow, constant. Land cover within each stream reach is further summarized in table 1.

Habitat Characteristics

Stream temperatures were collected for the eight stream reaches using submersible temperature-logging devices (DS1921G Thermochron iButton, Maxim Integrated Products, Sunnyvale, CA) over a period of 57 d during the months of July and August 2009. Two temperature loggers were used at each site placed at a depth of 1 m the middle of the stream and set to record stream temperature every 30 min. Stream reach canopy cover was calculated using a densiometer along five equally spaced transects at three locations, next to the banks and at midstream (Fitzpatrick et al. 1998). Point concentrations of dissolved oxygen were measured at each of the selected reaches using a portable meter (YSI, 550A Yellow Springs Instruments, Irvine, CA) twice during the months of June and July 2009.

In-Field Fish Sampling

To quantify baseline physiological parameters for free-swimming fish, creek chub *Semotilus atromaculatus* (Mitchill, 1818; $n = 6$–10) were collected from each of the eight stream reaches listed above using standard backpack electroshocking gear (200–250 V, 60 Hz, 6 ms pulsed direct current) from July 12, 2009, to August 4, 2009. Creek chub are one of the most common and abundant stream fishes in the eastern United States, are relatively easy to collect, can tolerate a wide range of oxygen concentrations and thermal regimes, and typically reside within a single stream section throughout their entire life cycle (Scott and Crossman 1973; Pflieger and Smith 1997; Fitzgerald et al. 1999). For collection, fish were stunned by electroshocking gear in the stream and immediately transferred to a vessel containing a lethal dose of anesthetic (250 mg L^{-1} of 3-aminobenzoic acid ethyl ester methanesulphonate [MS-222] buffered with 500 mg NaCO$_3$ L^{-1}). After cessation of ventilation, fish were weighed to the nearest gram and measured to the nearest millimeter (total length). Blood was then drawn directly from the caudal vessel posterior to the anal fin using a 25-gauge needle and 1-mL syringe rinsed with lithium heparin, and blood was typically removed in <180 s. Sampling times <180 s ensured that blood was drawn before the onset of any primary stress responses that may occur as a result of electroshock or handling (Romero and Reed 2005). Hematocrit values for whole blood (percentage packed cell volume) were determined on site by inducing a small amount of whole blood into two heparinized microcapillary tubes (∼20 μL each) that were centrifuged at 15,800 rpm (13,700 g) for 120 s using a hematocrit centrifuge and read using a digital reader (CritSpin Models CS22 and CSD2, Iris International, Chatsworth, CA). The remaining whole blood was transferred to a 1.5-mL microcentrifuge tube and spun at 2,000 g for 120 s to separate red cells from plasma. Plasma was separated from erythrocytes using a disposable transfer pipette

and divided into two 100-μL aliquots in 1.5-mL microcentrifuge tubes that were immediately frozen in liquid nitrogen. After collection of plasma, a 3–5-g portion of white epaxial musculature anterior to the operculum was excised using a razor blade, freeze clamped in aluminum tongs precooled in liquid nitrogen, wrapped in prelabeled aluminum foil, and flash frozen in liquid nitrogen (Suski et al. 2006). After field collection, all plasma and muscle samples were transferred to an ultracold freezer ($<-75°$C) after sampling.

To obtain fish for thermal challenges in the laboratory, creek chub were again collected from each of the eight stream reaches described above using the same electrofishing methods mentioned above. After immobilization by electroshock gear, fish were transferred to aerated hauling tanks filled with creek water and transported (typically taking <4 h). After arrival at the aquatic research facility, creek chub from each site were placed in one of four outdoor 960-L tanks. Tanks were connected to a 0.04-ha earthen pond using a submersible pump (McMaster-Carr 42945K29, Atlanta, GA), and water supplied to the tanks was returned to the pond for filtration and removal of waste products. Tanks were measured for daily temperature (mean ± SE = 20.4° ± 2°C) and dissolved oxygen (mean ± SE = 8.1 ± 1 mgO$_2$ L^{-1}) with a portable meter (YSI 550A). Ammonia concentrations in outdoor tanks remained <0.25 ppm throughout the study as confirmed by a commercially available assay kit (Aquarium Pharmaceuticals, LR8600, Chalfont, PA), and fish were fed daily with commercially available feed (TetraMin Goldfish Flakes, Tetra Werke, Melle, Germany) but were starved for a minimum of 48 h before being used in experiments. All fish were held in outdoor tanks for at least 3 d to allow for recovery from capture and transport, as has been done successfully in other species of fish (Donelson et al. 2011; Cook et al. 2012).

Laboratory Sampling

To generate resting control values for the suite of physiological parameters measured, six creek chub from each of the eight reaches were transferred from outdoor tanks to individual darkened chambers provided with aerated recirculating pond water

maintained at 20° ± 0.3°C, with a mean (±SE) dissolved oxygen concentration of 8.0 ± 0.3 mg O$_2$ L^{-1}. Individual chambers were sized appropriately to hold fish with a minimum of room for movement and were supplied with pond water pumped from a central basin that was allowed to overflow and drain back to the central basin, forming a closed system, similar to Suski et al. (2006). After 28-h acclimation to individual chambers, the flow of water to each chamber was terminated, and fish were euthanized by adding buffered anesthetic directly to the individual container. Creek chub were then sampled for blood and muscle in a manner identical to the field sampling described above. These methods of generating stress-free control values have been used successfully in the past for many species of fish (Gingerich and Suski 2011; Shultz et al. 2011).

To quantify differences in physiological disturbances after an acute thermal challenge, six creek chub from each of the eight stream reaches were transferred from 960-L outdoor tanks to individual darkened chambers provided with aerated recirculating pond water. Fish were allowed 24 h to acclimate to these individual chambers, which were maintained at 20° ± 0.3°C and 8.0 ± 0.3 mg O$_2$ L^{-1}. After this acclimation period, six fish from each of the eight stream reaches received a thermal challenge using a submersible heater (1000 W, SCSUB10, Clepco, Cleveland, OH) in a central basin that increased water temperature to 30.0° ± 0.2°C, while air stones in each chamber maintained dissolved oxygen concentrations at 7.5 ± 0.4 mg O$_2$ L^{-1}. After 4-h exposure to this novel temperature, creek chub were lethally anesthetized and sampled for blood and muscle as described above. A portable meter (YSI, 550A) confirmed that water conditions changed in <5 min.

Resting Metabolic Rates (RMRs)

The impacts of a thermal challenge on metabolic rate were determined using computerized, intermittent-flow respirometry (Loligo Systems, Hobro, Denmark; Steffensen 1989). The system consisted of four glass chambers (200 mm long with an inner diameter of 62 mm; 0.57 L) immersed in a 140-L tank of aerated pond water maintained at 20.0° ± 0.5°C with a temperature-controlled heater. Change in oxygen concentration (α)

Table 2: Mixed-model ANOVAs examining differences in temperature, dissolved oxygen, and canopy characteristics of replicate agricultural ($n = 4$) and forested ($n = 4$) stream reaches

Variable	Mean (±SE)		F	P
	Agricultural reaches	Forested reaches		
Dissolved oxygen (mg O$_2$ L^{-1})	8.3 (.8)	8.0 (.7)	<.1	.77
Daily minimum temperature (°C)	20.3 (.2)	20.4 (.2)	<.1	.79
Daily mean temperature (°C)	23.5 (.2)	21.5 (.2)	22.4	**.01**
Daily maximum temperature (°C)	26.0 (.3)	22.5 (.3)	113.8	**.01**
Daily temperature change (Δ°C)	5.7 (.3)	2.5 (.3)	65.1	**.01**
Canopy cover (%)	12.7 (5)	90.0 (7)	172.3	**.01**

Note. Proportions of land cover characteristics used to define streams as agricultural or forested are provided in table 1. Boldface type indicates statistical significance.

Table 3: Mixed-model ANOVAs examining differences in baseline, field-sampled physiological, and morphometric characteristics of creek chub

Variable	Mean ($\pm$SE)		F	P
	Agricultural reaches	Forested reaches		
Plasma:				
Cortisol (ng mL^{-1})	169.2 (35.2)	173.6 (29.4)	.9	.92
Glucose (mmol L^{-1})	4.3 (.4)	4.2 (.3)	<.1	.88
Chloride (meq L^{-1})	96.5 (6.0)	92.1 (4.9)	.3	.58
Sodium (meq L^{-1})	88.1 (4.4)	84.6 (3.7)	.4	.56
Potassium (meq L^{-1})	6.4 (.3)	6.4 (.3)	<.1	.87
Glutathione (mmol L^{-1})	149.7 (14.3)	130.0 (12.2)	1.1	.29
Muscle lactate (mmol g^{-1})	1.7 (.06)	1.5 (.07)	.1	.74
Water content (%)	89.7 (5.6)	93.5 (4.5)	.3	.61
Whole-blood hematocrit (%)	27.7 (1.2)	26.9 (.9)	.2	.65
Total length (mm)	146.9 (8.0)	129.9 (6.7)	2.6	.14
Weight (g)	144.3 (2.9)	137.4 (2.6)	3.1	.11
Relative weight (%)	32.5 (2.3)	30.3 (2.0)	.5	.50

Note. Fish were sampled immediately after electroshocking from replicate agricultural ($n = 4$) and forested ($n = 4$) stream reaches.

for each chamber was calculated as slope ($\Delta O_{2\,\text{saturation}}/\Delta t$), and oxygen consumption rate ($\dot{M}O_2$, mg $\times$ O_2 $\times$ kg^{-1} $\times$ h^{-1}) for each fish was calculated as

$$\dot{M}O_2 = \alpha V_{\text{resp}} \beta M_b^{-1},$$

where V_{resp} is the volume of each glass chamber minus the volume of the fish (L), β is oxygen solubility (adjusted nightly for both temperature and barometric pressure), and M_b is the fish mass (kg) before placement in the respirometry chamber. Oxygen was quantified with fiber-optic oxygen probes (calibrated with oxygen-free water and fully aerated water regularly throughout experiments), and data were recorded with AutoResp software, version 1.4 (Steffensen 1989; Schurmann and Steffensen 1997). Experiments were designed so that oxygen consumption in each individual chamber was determined with 13-min cycles that consisted of an 8-min measurement phase, a 4-min flush period to refill chambers with fresh water, and a 1-min wait period after each flushing before commencing measurements. During each measurement period, water from the chambers was continually recirculated across fiber-optic oxygen probes to ensure adequate mixing, and oxygen concentration was recorded every 2 s.

To generate RMR values for controls and treatments, eight fish from each of the eight stream reaches were transferred to individual chambers during the day (at approximately 4:00 p.m.), acclimated to chambers for 4 h, and left for 12 h (8:00 p.m. to 8:00 a.m.) overnight with exposure to the same high-temperature conditions described above; a second group of eight fish from each site was also left overnight with no temperature change to serve as a control. Temperature was manipulated using a thermostatically controlled heater (1,000 W, SCSUB10), and dissolved oxygen was maintained by bubbling oxygen into the 140-L tank. Resting metabolic rate for each fish was determined by selecting the six lowest consecutive

oxygen consumption values overnight (Steffensen 1989). During each trial, the coefficient of determination (r^2) for all slope measurements was >0.95, and all calculated dissolved oxygen values were corrected for background oxygen consumptions generated for each specific fish and chamber for 4 h before commencing experiments.

Laboratory Analysis

Concentrations of cortisol in plasma were determined using a commercially available enzyme-linked immunosorbent assay kit (Assay Designs, kit 900-071, Ann Arbor, MI), recommended by Sink et al. (2008) because of its high accuracy and low cross reactivity with other hormones in other species of fish. Plasma potassium (K^+) and sodium (Na^+) concentrations were quantified using a digital flame photometer (Cole-Parmer Instrument, model 2655-00, Chicago, IL), whereas plasma chloride (Cl^-) concentrations were generated using a digital chloridometer (Labconco, model 4425000, Kansas City, MO). Muscle lactate and plasma glucose concentrations were determined enzymatically following the methods of Lowry and Passonneau (1972) using a microplate spectrophotometer (Spectra Max Plus 384, model 05362, Molecular Devices, Union City, CA). Plasma glutathione concentrations were measured with a microplate spectrophotometer using a commercially available kit (BioAssay Systems, kit DIGT-250, Hayward, CA). Muscle water content was determined by drying tissue at 80°C for 48 h and comparing wet mass with dry mass.

Statistical Analysis

Daily minimum, mean, and maximum temperatures from each reach were calculated using data downloaded from in-stream temperature-logging devices. Daily temperature change was cal-

Table 4: Mixed-model ANOVAs comparing the impact of high temperature on physiological responses of creek chub

Variable, source	SS	df	F	P
Plasma cortisol (ng mL^{-1}):				
Land type	120,217.2	1	3.9	**.08**
Treatment	37,645,831.0	1	1,827.6	**<.0001**
Land type × treatment	121,607.4	1	5.9	**.02**
Error	42,777,790.0	118		
Plasma glucose (mmol L^{-1}):				
Land type	1.2	1	.2	.64
Treatment	17.0	1	9.2	**.0030**
Land type × treatment	2.4	1	1.3	.25
Error	250.5	115		
RMR (mg O$_2$ g^{-1} h^{-1}):				
Land type	35,991.9	1	8.8	**.02**
Treatment	1,107,272.0	1	989.8	**<.0001**
Land type × treatment	36,800.9	1	33.1	**<.0001**
Error	1,500,366.6	151		
Plasma chloride (meq L^{-1}):				
Land type	801.3	1	.4	.52
Treatment	3.5	1	<.1	.87
Land type × treatment	47.7	1	.4	.55
Error	28,910.9	112		
Plasma sodium (meq L^{-1}):				
Land type	115.1	1	<.1	.80
Treatment	214.1	1	1.0	.31
Land type × treatment	265.1	1	1.3	.26
Error	34,609.2	111		
Plasma potassium (meq L^{-1}):				
Land type	16.6	1	1.2	.31
Treatment	8.7	1	3.1	.08
Land type × treatment	.9	1	.3	.57
Error	434.8	118		
Muscle lactate (mmol g^{-1}):				
Land type	.2	1	4.1	.07
Treatment	<.1	1	.9	.33
Land type × treatment	<.1	1	<.1	.98
Error	6.0	119		
Wave content (%):				
Land type	.2	1	<.1	.98
Treatment	33.5	1	.3	.61
Land type × treatment	100.6	1	.8	.39
Error	18,216.3	119		
Blood hematocrit (%):				
Land type	.8	1	<.1	.92
Treatment	225.5	1	13.0	**.0005**
Type × treatment	25.3	1	1.5	.24
Error	2,568.1	108		
Plasma glutathione (mmol L^{-1}):				
Land type	12,609.4	1	2.5	.15
Treatment	1,939.0	1	.2	.81
Type × treatment	47.1	1	<.1	1.00
Error	414,285.0	119		
Total length (mm):				
Land type	3.3	1	<.1	.96
Treatment	4,016.8	1	13.7	**.0006**

Table 4 (*Continued*)

Variable, source	SS	df	F	P
Land type × treatment	325.6	1	1.1	.29
Error	43,731.4	117		
Total weight (mm):				
Land type	20.6	1	<.1	.74
Treatment	1,175.4	1	12.6	**.0003**
Land type × treatment	92.5	1	.9	.32
Error	12,706.5	117		
Relative weight:				
Land type	11.6	1	.3	.60
Treatment	4.5	1	.2	.68
Land type × treatment	10.6	1	.4	.52
Error	3,145.5	117		

Note. Creek chub were collected from replicate agricultural ($n = 4$) and forested ($n = 4$) stream reaches, given a 3-d recovery period, and then exposed to high temperatures (30°C) for 4 h before morphometric and blood sampling. Boldface type indicates statistical significance. RMR = resting metabolic rate; SS = sum of squares.

culated for each reach by subtracting daily minimum temperature from daily maximum temperature. Stream temperature and dissolved oxygen differences were compared across reaches using a repeated-measures ANOVA with riparian land cover type (forested or agricultural) entered as a fixed effect, and both date and stream (nested within land type) entered as random effects (Zar 1984; Bennington and Thayne 1994; Wagner et al. 2006; Ott and Longnecker 2010). Relative weight (W_r) of creek chub (a metric that relates the actual weight of the fish to an "ideal" weight based on broad-scale surveys) was calculated using the weight-length relationship ($W_r = -3.39611 \times L^{2.92494}$) developed by Carlander (1969) and compared across reaches using a mixed-model ANOVA. A mixed-model ANOVA consists of fixed (levels set by the experimenter) and random (or random samples within a larger population of samples) effects and is an ideal analytical tool for improved estimation of error terms without violating assumptions in hierarchical stream data (Wagner et al. 2006). Differences in baseline and stress-induced physiological parameters in creek chub after thermal challenges were detected using a mixed-model, two-factor ANOVA, with land cover type, treatment (heat), and land cover type × treatment entered into the model as fixed effects and both stream (nested within land cover type) and individual (nested within site) fish entered as random effects. Size and weight were run as covariates in all models but then removed when it was shown that they had no influence on response variables ($P > 0.05$; Engqvist 2005). All values are reported as means ($\pm$SE) where appropriate, and all statistical analyses were performed using JMP, version 8.2 (SAS Institute, Cary, NC). The level of significance (α) for all tests was 0.05.

Results

Reach Characteristics

Forested stream reaches had 72.5% more riparian forest and 78% more canopy coverage than did agricultural stream reaches

(table 1). There were no statistical differences detected in dissolved oxygen concentrations or minimum daily stream temperatures between stream reaches across land cover types ($P > 0.05$; table 2). Agricultural stream reaches, however, had significantly higher ($+3.5$°C) daily mean and maximum temperatures as well as 43% higher daily temperature changes (table 2). The range of temperatures that creek chub experienced in agricultural stream reaches during the study period was 17°–33°C and 18°–28°C in forested streams. The range of dissolved oxygen concentrations that creek chub experienced was 4.97–10.76 mg O_2/L in agricultural streams and 6.01–9.96 mg O_2 L^{-1} in forested streams.

In-Field Sampling

Differences in land cover at the stream reach scale did not result in significant differences in physiological parameters of resident creek chub sampled in the field from each stream (table 3). Plasma cortisol and glucose concentrations were statistically similar across all fish collected from forested and agricultural stream reaches ($P > 0.05$; table 3). There were also no statistical differences in the baseline concentrations of plasma chloride, plasma sodium, or plasma potassium and no differences in baseline muscle lactate, muscle water content, or hematocrit values for fish from either site grouping ($P > 0.05$; table 3). Plasma glutathione also did not differ between the two stream reach classifications ($P > 0.05$; table 3). Furthermore, there were no differences in the length, weight, or condition of creek chub collected from any stream segment ($P > 0.05$; table 3).

Laboratory Sampling and Metabolic Rates

After a 4-h exposure to high temperature (30°C, 7.5 mg O_2 L^{-1}), creek chub from both forested and agricultural reaches displayed a significant increase in plasma cortisol and glucose concentration ($P < 0.05$; table 4; fig. 1*a*, 1*b*). Plasma cortisol
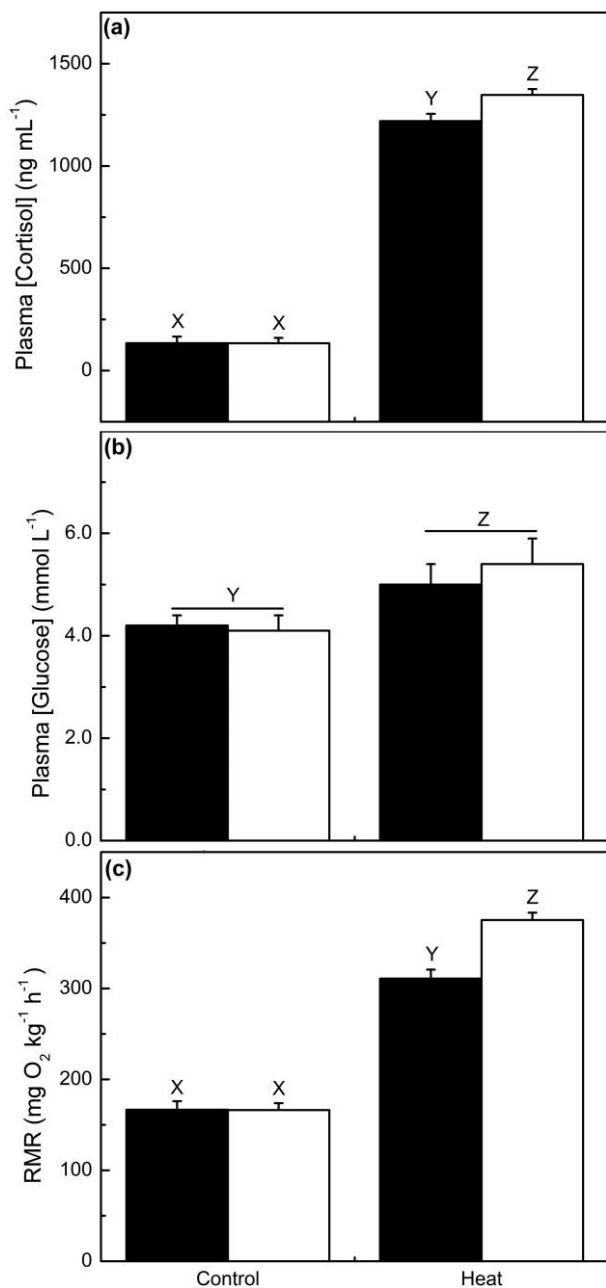
Figure 1. Plasma cortisol (*a*), plasma glucose (*b*), and resting metabolic rate (RMR; *c*) of creek chub from four agricultural (black) and four forested (white) stream reaches exposed for 4 h to control conditions (20°C, 8 mg O_2 L^{-1}) or high-temperature conditions (30°C, 7.5 mg O_2 L^{-1}). Letters (Y and Z) above the horizontal lines represent a significant difference from control values across treatment groups (mixed-model ANOVA). Letters (X, Y, and Z) directly above bars represent significant differences between agricultural and forested reaches within a treatment (significant interaction, mixed-model ANOVA, $P < 0.05$).

concentrations for creek chub increased ~10-fold relative to control levels after a thermal challenge, and fish from forested stream reaches had plasma cortisol concentrations that were 10% greater than those in fish from agricultural stream reaches.

Similarly, plasma glucose concentrations increased by ~20% after a thermal challenge ($P < 0.05$; fig. 1*b*), but there were no statistical differences across land cover types ($P > 0.05$; table 4; fig. 1*b*). An acute thermal challenge increased the RMR of creek chub an average of 2.1 times relative to control fish for both agricultural and forested land cover types ($P < 0.05$; table 4; fig. 1*c*). Creek chub from forested reaches displayed metabolic rates that were 17% greater than those in fish from agricultural reaches after the thermal challenge ($P < 0.05$; table 4; fig. 1*c*).

No significant changes within or across land use types were observed for concentrations of plasma chloride, plasma sodium, plasma potassium, muscle water content, muscle lactate, or glutathione after heat exposure ($P > 0.05$; table 4; fig. 2*a*, 2*b*). Whole-blood hematocrit values were significantly higher for both agricultural and forested reaches after an acute thermal challenge relative to control values, but differences were not detected across land cover types ($P < 0.05$; table 4; fig. 2*b*). Creek chub total length and weight were 13 mm and 8 g higher for fish from both land use types for controls compared with fish used in high-temperature treatments ($P < 0.05$; table 4). Because size differences were detected, length and weight were initially run as a covariate in all statistical models but were removed when they were not shown to be a significant parameter in any model (Engqvist 2005). However, fish condition and muscle water content were similar within or across treatment groups ($P > 0.05$; table 4).

Discussion

The physiological responses of creek chub to an acute thermal challenge were influenced by surrounding riparian land cover in stream reaches. More specifically, creek chub collected from stream reaches dominated by agriculture in the riparian zone exhibited a plasma cortisol response that was 10% lower in magnitude relative to fish residing in forested reaches after an acute thermal challenge. Fish from agricultural reaches also showed a 17% lower metabolic rate after thermal challenges compared with fish collected from forested stream reaches after a heat exposure. Cortisol is a hormone that is an integral part of the primary stress response of fish, and its production initiates a host of secondary stress pathways essential for the maintenance of homeostasis after the onset of a stressor (Wendelaar Bonga 1997). Specifically, cortisol regulates protein production, glucose mobilization, and fat metabolism, thus liberating energy stores required to meet the increased energy demands of fish coping with stressful situations (Barton 2002). Similar to cortisol, increases in metabolic rate help fish meet the energy demands of increased biological activity required to cope with stressful conditions, in which increased production of aerobic energy allows for potential physiological or behavioral adjustments to stress (Dalhoff 2004). Results from the current study are consistent with previous work that shows that the cortisol and metabolic stress response of organisms can be influenced by local habitat quality and conditions. For example, spotted salamanders *Ambystoma maculatum* (Shaw, 1802) that live in ponds surrounded by habitat disturbed by human activities
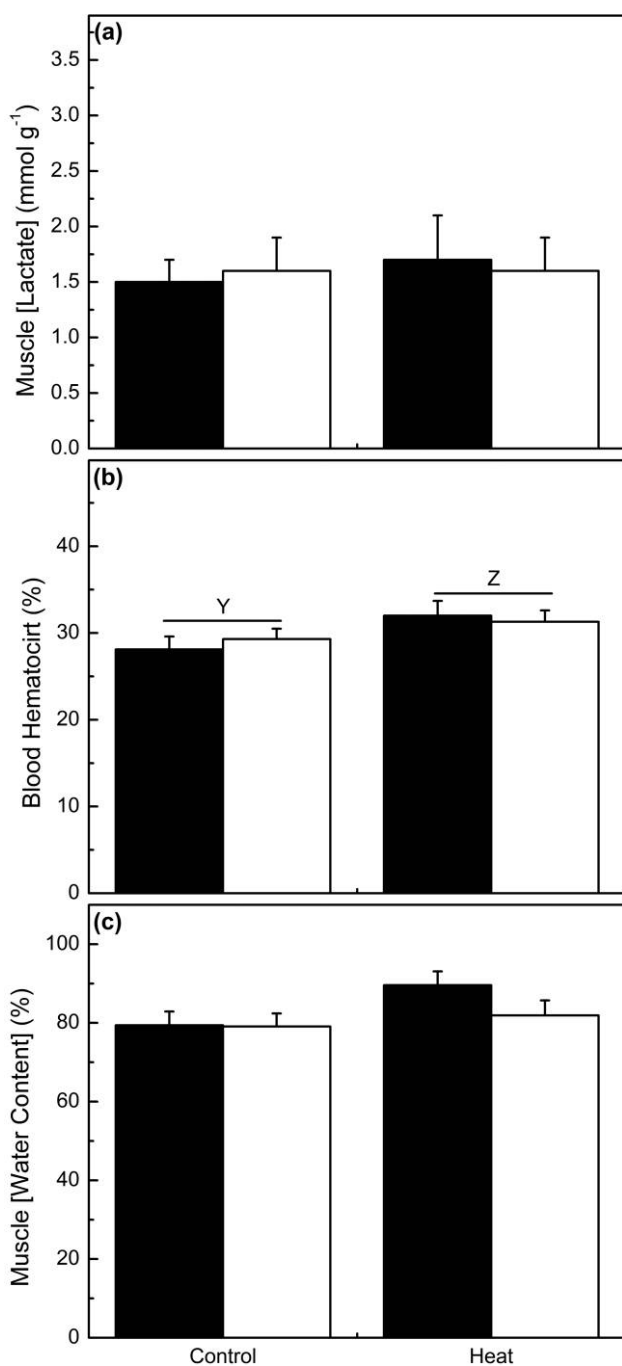
Figure 2. Muscle lactate (*a*), blood hematocrit (*b*), and water content (*c*) of creek chub exposed for 4 h to control conditions (20°C, 8 mg O_2 L^{-1}) or high-temperature conditions (30°C, 7.5 mg O_2 L^{-1}) from four agricultural (black) and four forested (white) stream reaches. Letters (Y and Z) above horizontal lines represent a significant difference from control values across treatment groups (significant fixed effect for treatment, mixed-model ANOVA, $P < 0.05$).

33% more calories over an entire year than do salamanders found within unharvested controls (Homyack et al. 2011). We found that exposure to a more disturbed environment (i.e., streams with riparian areas dominated by agriculture) can lead to an attenuation in the stress response of individuals and also demonstrate that land use at the reach scale has an impact on the primary and metabolic stress responses of resident creek chub.

Reductions in forested riparian areas in agricultural reaches, coupled with a concomitant increase in stream temperatures and thermal variability, likely led to modifications in the stress responses of fish from agricultural stream reaches to thermal challenge. Creek chub residing in stream reaches dominated by agriculture in the riparian area were exposed to stream conditions that were (on average) 3.5°C warmer and 44% more variable, because of less canopy coverage and less riparian forest, than conditions in forested stream reaches. Thermal regime has long been recognized as one of the primary factors that dictate the physiological makeup of individuals (Helmuth and Hofmann 2001; Pörtner 2001; Somero 2002). One example of the relationship between thermal properties and physiological makeup is that of intertidal organisms that inhabit areas of vertical zonation that receive exposure to different thermal regimes. Two species of intertidal gastropods, *Tegula brunnea* (Philippi, 1848) and *Tegula montereyi* (Kiener, 1850), that occupy the relatively stenothermic subintertidal zone had heat shock protein stress responses that made them unable to tolerate the wide range of thermal challenges that eurythermal *Tegula funebralis* (Adams, A., 1855) and *Tegula rugosa* (Adams, A., 1853) face in the midintertidal zone (Tomanek and Helmuth 2002). Differences in the physiological stress responses, such as heat shock proteins, result in different thermal tolerances among species and limit species ranges. In addition, a 3°C increase in rearing temperature caused a 10% reduction in the metabolic rate of *Acanthochromis polyacanthus* (Bleeker, 1855), which resulted in a significant benefit to daily energy expenditure in response to stressful thermal challenges (Donelson et al. 2011). Similarly, we found that variations in temperature, likely brought about by agricultural land use in the riparian area of stream reaches, may be associated with changes in the overall primary endocrine and metabolic stress responses of resident creek chub.

The attenuation of the cortisol and metabolic stress response of creek chub in agricultural stream reaches was not accompanied by changes in other osmoregulatory (Na^+, K^+, Cl^-, and water content) or circulatory (hematocrit) stress indicators and likely indicates improved physiological performance (maintenance of secondary stress indicators). Specifically, creek chub from agricultural stream reaches were able to maintain allostasis of crucial physiological systems, such as those that regulate hydromineral balance and oxygen levels, even though they produced a lower-magnitude cortisol and metabolic response. These results are consistent with other studies that demonstrate that chronic or frequent exposure to a stressor, such as elevated temperatures and thermal variability, can cause the downregulation of stress responses (Shrimpton and Randall 1994; Dhab-

have lower stress-induced cortisol concentrations than those that are surrounded by high-quality forest habitat (Homan et al. 2003). Eastern redbacked salamanders *Plethodon cinereus* (Green, 1818) from experimentally harvested forests require

har et al. 1997; Fowler 1999; Walker et al. 2005). In addition, previous works suggests that the attenuation of metabolic and cortisol stress responses of organisms in disturbed environments is likely beneficial, because severe and chronic elevations in stress cortisol are known to be deleterious to growth, reproduction, and immunocompetence (Wendelaar Bonga 1997; Sapolsky et al. 2000; Moore and Jessop 2003; Romero 2004). For example, common killifish *Fundulus heteroclitus* (Linnaeus, 1766) that live in southern estuaries at higher temperatures with greater thermal variability display a modified heat shock protein response to anticipate changes in temperature to protect physiological performance of proteins (Helmuth and Hoffman 2001). With respect to creek chub specifically, previous work has demonstrated that watershed-level degradation (i.e., deforestation) results in attenuation of the stress response, likely through phenotypically plastic modifications to the stress response in an effort to minimize energetic expenditure during thermal challenges (Blevins et al. 2013). In addition, a recent study on free-swimming creek chub showed that individuals from streams dominated by agriculture showed greater mortality rates after a chronic stress than individuals monitored in streams surrounded by forests (Nagrodski et al. 2012). Together, results from these two studies suggest that attenuation in the stress response of creek chub is likely driven by land use patterns in individual stream reaches, which may act in concert with or in lieu of factors acting at larger, watershed-level scales. More importantly, the ability of creek chub from disturbed environments (i.e., streams dominated by agriculture) to consume less energy during stressful events means that they likely have to redistribute and acquire less energy after a stressor to allow for recovery from thermal stress, which may have beneficial implications on growth and reproduction (Shreck 2010; Donelson et al. 2011).

Data from this study demonstrate how local habitat conditions caused by variations in terrestrial land cover affect physiological responses, which should prove useful in conservation and restoration by better explaining changes in fish community structure and the persistence of tolerant fish species, such as creek chub, in agricultural environments. For example, stress responses over short timescales can alter energy metabolism, which can cause fish to modify fish foraging rates and feeding behaviors (McEwen and Wingfield 2003). At longer timescales, prolonged activation of the stress response can cause organisms to have chronically elevated energy consumption, which leads to energy imbalance (allostatic overload) and an allostatic state that results in impaired immune function, reduced growth, and comprised reproduction (McEwen and Wingfield 2003; Busch and Hayward 2009). It is conceivable that creek chub from agricultural areas may have an attenuated cortisol and metabolic stress response to high temperature to limit the potential costs of these stress responses to frequent thermal challenge in disturbed areas. In addition, this reduction of the stress response may be important to creek chub persistence in disturbed areas and cause a shift in community composition that favors creek chub in reaches with disturbed habitat over more sensitive species. Understanding differences, such as the ability of creek chub or other fish to cope with stress and maintain allostasis (physiological performance) despite disturbances operating at the reach scale, could help explain the patch dynamics of fish populations and help to define stream reaches that meet the habitat requirements of different fish species, thereby aiding in their potential recovery. Conservation practitioners have long known of the importance of longitudinal impacts of stressors operating upstream of restoration areas (Allan 2004), but data from our study highlight the importance of disturbances at the reach scale for influencing energy use, and likely community composition, in streams. In addition, monitoring of changes in the allostatic state of individuals compared with reference organisms (Adams and Ham 2011; this study) at the reach scale could demonstrate recovery of stream reaches after the restoration actions. Overall, studies that describe reach-scale differences in stream fish populations and the use of fish physiological responses as a bioindicator may be important in describing patterns in fish community structure over larger scales, provide greater resolution to watershed-scale models, and assist with conservation actions (Fausch et. al 2002; Cooke and Suski 2008).

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