Land use drives the physiological properties of a stream fish

Z.W. Blevins [a,b,∗], E.L. Effert [a,b], D.H. Wahl [b], C.D. Suski [a]

[a] Department of Natural Resources and Environmental Science, University of Illinois, Turner Hall, 1102 South Goodwin Ave, Urbana, IL 61801, USA
[b] Illinois Natural History Survey, University of Illinois, 1816 S. Oak St., Champaign, IL 61820, USA

ABSTRACT

Human activities within the riparian zone can alter abiotic properties of a watershed, potentially resulting in abiotic conditions that are stressful for resident fishes. The inability of fish to cope physiologically with stressful abiotic conditions can have deleterious effects on individuals, and could potentially lead to population declines or changes to community structure (biodiversity). Defining links between landscape-level processes and performance of individual stream fishes can therefore improve our ability to predict how land use changes can impact stream communities, which has relevance for management activities. This study tested the hypothesis that land use at the watershed scale influences the physiological stress response of resident fishes. For this, replicate streams in agricultural watersheds and forested watersheds were identified; sampling demonstrated that streams in agricultural watersheds were warmer and more thermally variable than streams from agricultural areas. Creek chub from each land use type were sampled for blood and muscle in the field, following exposure to thermal and oxygen stressors in the laboratory, and after prolonged holding at elevated temperatures that replicated field conditions. No differences in baseline physiological parameters were found in fish sampled directly from streams. However, when exposed to low oxygen and high temperature conditions in the laboratory, creek chub from streams within agricultural areas maintained physiological performance with a reduced stress response relative to creek chubs from streams within forested watersheds. In addition, prolonged holding at high temperature removed landscape-level differences in stress responses, resulting in improved physiological performance for all fishes after a heat challenge. Results indicate that creek chub have the ability to adjust physiological responses to improve performance in disturbed environments, and also provide a novel mechanism by which landscape-level processes can influence biodiversity.

© 2012 Published by Elsevier Ltd.

1. Introduction

Changes in terrestrial land use and increasing human populations are two of the greatest threats to biodiversity in the United States (Czech et al., 2000). Land use changes, such as urbanization and agriculture, can negatively impact terrestrial biodiversity by consuming riparian habitat, altering riparian habitat properties, and increasing habitat fragmentation. Furthermore, recent research has demonstrated that alterations to terrestrial landscapes can exert pronounced negative effects on adjacent aquatic ecosystems (Allan, 2004). For example, destruction of riparian zones through urbanization and agriculture has been shown to impact watershed hydrology, sediment loads, inputs of nutrients, dissolved oxygen concentrations, and temperature regimes in adjacent aquatic ecosystems, which, in turn, can negatively impact biodiversity in aquatic ecosystems (Hayes et al., 1996; Jones

et al., 1996; Stauffer et al., 2000; Meador and Goldstein, 2003). More importantly, research has demonstrated strong links between destruction of riparian habitat, increased stream temperatures, and concomitant reductions in dissolved oxygen, all of which can impact community structure and biodiversity (Schlosser, 1991; Gergel et al., 2002; Allan, 2004).

Currently, the mechanisms that translate habitat alterations into changes in biodiversity have not been well defined. Through a cascade of biotic and abiotic interactions, alterations to terrestrial and aquatic habitat can negatively impact fish communities by modifying environmental parameters that can change predation risk, predator abundance, growth patterns, reproductive characteristics, and possibly mortality rates of fish (Hayes et al., 1996; Jones et al., 1996; Stauffer et al., 2000; Meador and Goldstein, 2003; Albanese et al., 2004). In addition, anthropogenic changes in land cover that alter the thermal, oxygen, and habitat properties in many aquatic systems have caused, or are suspected to cause, the declines of numerous North American inland fish taxa. One of the major contributing factors in the decline of brook trout (*Salvelinus fontinalis* Mitchill, 1814) in aquatic ecosystems, for example, is human landscape alterations that lead to warming

∗ Corresponding author. Present address: 4235 N. Winchester, Chicago, IL 60613, USA. Tel.: +1 773 414 5456; fax: +1 773 687 8371.
E-mail address: zblevs@gmail.com (Z.W. Blevins).

past the fish's physiological ability to cope, causing direct mortality and reproductive failure (Robinson et al., 2010). Furthermore, fish such as rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), bull trout (*Salvelinus confluentus* Suckley, 1859), and endangered topeka shiner (*Notropis Topeka* Gilbert, 1884) are all suspected to be experiencing declines in abundance through part of their range partially due to increases in temperatures and decreases in dissolved oxygen levels in streams brought about by land use alterations (Menzel et al., 1984; Matthews and Berg, 1997; Selong et al., 2001). Therefore, it is of critical importance to define the mechanism(s) that influence the ability of individual fish to cope with human alterations of aquatic systems (thermal and oxygen stressors in particular) that can translate to changes at the population level and impact biodiversity.

Physiological indicators have been identified as a useful tool in providing a mechanistic understanding of how human disturbances influence individual animals. Physiological indicators such as blood-based stress metrics are sensitive to varying levels of stressors associated with anthropogenic activity, and respond quickly (hours to days) to environmental change (Barton, 2002; Cooke and Suski, 2008). Glucocorticoids, for example, are stress hormones that have been used in management to mitigate the impacts of human activity in Rocky Mountain elk (*Cervus Canadensis* Linnaeus, 1758), grey wolves (*Canis lupis* Linnaeus, 1758), and black howler monkeys (*Alouatta* spp. Lacapede, 1799) (Millspaugh and Washburn, 2004; Creel et al., 2002; Homan et al., 2003; Martinez-Mota et al., 2007). Similarly, metabolic rate (i.e., oxygen consumption) represents a quantitative measure of the total activity of all physiological mechanisms and, for fish, includes processes such as ion regulation, maintenance costs (e.g., heart rate, opercular beats, etc.), and waste generation (Hill et al., 2008). In aquatic systems, riparian forest destruction can alter the thermal properties of streams from the range of temperatures to which aquatic organisms are accustomed and may be perceived by the organism as a stressor, resulting in physiological and/or metabolic consequences. Organisms that can successfully preserve homeostasis (physiological performance) of secondary stress indicators, such as hydromineral (Na^+, K^+, Water Content) and circulatory systems (Hematocrit), in a challenging environment are able to maintain normal physiological function, and likely growth and reproduction (Barton, 2002). Together, the sum of these individual physiological responses can change population growth rates, birth rates, and death rates and determine whether certain fish species persist or decline in the face of human alterations.

The goal of this study is to quantify the relationship between riparian land use at the watershed scale and the capacity of resident fishes to respond physiologically to environmental challenges. To achieve this goal we conducted a complimentary set of field and laboratory studies to ascertain differences in baseline and stress-induced physiological parameters of creek chub (*Semotilus atromaculatus* Mitchill, 1818) from streams within replicate disturbed (agricultural riparian zone) and undisturbed (forested riparian zone) watersheds following acute thermal and hypoxia challenges. Additionally, creek chub from replicate disturbed (agricultural riparian zone) and undisturbed (forested riparian zone) watersheds were acclimated to two different thermal conditions and given a thermal challenge to test how acclimation to different thermal regimes can impact the magnitude and scope of physiological responses. The hypothesis being tested with these experiments is that within-stream environmental variation (i.e., water temperature and dissolved oxygen concentration) caused by differences in riparian land use at the watershed scale will alter physiological properties of creek chub and permit improved performance during environmental challenges. Together, these experiments establish mechanistic links between altered terrestrial landscapes and their potential impacts on aquatic ecosystems.

2. Material and methods

To obtain fish from both disturbed (agricultural riparian zone) and undisturbed (forested riparian zone) streams, it was necessary to use a candidate fish species that could be found in adequate numbers across a range of habitat conditions. Creek chub were selected because they are one of the most common and abundant stream fishes in the eastern United States, are relatively easy to collect, and can tolerate a wide range of oxygen concentrations and thermal regimes (Pflieger, 1997; Fitzgerald et al., 1999). Creek chub living in streams within agricultural and urban areas (i.e., disturbed habitats) display reduced longevity, lower growth rate, and increased levels of reproductive anomalies compared to creek chub living in less-disturbed habitats (Fitzgerald et al., 1999). Creek chub also display high site fidelity, typically moving < 400 m in a year, providing opportunity for acclimatization to local habitat conditions (Fitzgerald et al., 1999; Belica and Rahel, 2008).

2.1. Site selection

To quantify how forest and agricultural land use affects the physiological properties of resident fish, replicate watersheds with similar proportions of forest and agriculture were identified. Both replicate watersheds, as well as individual study sites within each watershed, were selected using ArcView GIS 9.1 (ESRI, 2005) based on the Land Cover of Illinois 1999–2000 Classification on-line database compiled by the Illinois Department of Natural Resources and the Illinois State Geological Survey (IDOA, 2001). Using this database, a total of 4 headwater (2nd order) streams were chosen. Two were located within watersheds with forested riparian areas; Big (N39°4′, W87°49′) and Brushy Creeks (N38°52′, W87°39′) in Crawford County, IL. Two streams were located within watersheds with agricultural riparian areas: Bear (N39°18′, W88°17′) and Cottonwood Creeks (N39°19′, W88°13′) in Cumberland County, IL. Land cover proportions for each stream were calculated for the riparian zone scale (land cover within 30 m of the stream bank) and the watershed level scale (the entire area upstream of each site). The riparian zone scale was selected based on the resolution of available land cover data (30 m pixels) and because a riparian width of 30 m is the minimum size recommended by the United States Department of Agriculture (USDA) for maintenance of water quality (Welch, 1991). Land cover categories were created based on the overall proportion of agriculture (row crop + non-row crops), forest (upland + lowland wooded forest), and urban development (residential + commercial). Agricultural streams had >65% agriculture and <26% forest in the riparian area of the watershed above sampling locations. Forested streams had >70% forest and <23% agriculture in the riparian area in the watershed above the sampling sites. All watersheds had very little (<1%) urban development upstream of sampling sites, so urban land use was not included as a category. Agricultural and forested classifications are consentient with land cover categories developed through the Land Use Land Cover classification system (Anderson et al., 1975).

2.2. Habitat characteristics

Water temperatures were collected for the 4 streams using a temperature logging device (DS1921G Thermochron iButton, Maxim Integrated Products, Sunnyvale, CA, USA) over a period of 57 days during the months of July and August, 2009. Two temperature loggers were placed at a depth of 1 m in the middle of the stream at each site and programmed to record temperature every 30 min (minutes). Dissolved oxygen concentrations were also collected using oxygen probes (YSI Model 600XL, Yellow Springs, Ohio, USA) for two separate 48 h (hour) time periods on two consecutive days during the months of June and July 2009. Additional

stream characteristics such as depth, width, flow, and canopy cover were collected twice for each stream on two separate dates during the study period according to the transect methods developed by Fitzpatrick (1998). Mean reach width, depth, and flow were estimated based on measurements taken at 10 equally spaced cross-stream transects. Stream depth and flow velocity (FLOW-MATE, Marsh-McBirney, Fredrick, MD, USA) were measured at five equally spaced points along each transect. Stream channel canopy cover were measured along each transect at three locations, next to the banks and at midstream, using a densiometer.

2.3. In-field fish sampling

To quantify baseline concentrations of physiological parameters for resident fish, free-swimming creek chub ($n = 6$–10) were collected from the four sites identified above using backpack electroshocking gear [200–250 V, 60 HZ, 6 ms pulsed direct current (DC)]. 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 m L^{-1} NaCO$_3$], a process typically requiring < 120 s (seconds). A blood sampling time under 120 s ensured that blood was drawn prior to the onset of any primary stress responses that may occur due to electroshocking and handling (Romero and Reed, 2005). Following cessation of ventilation, fish were weighed to the nearest g (gram) and measured to the nearest mm [total length (TL)]. Blood was then drawn from the caudal vessel posterior to the anal fin using a 25-gauge needle and 1 mL syringe rinsed with lithium heparin. Hematocrit values for whole blood (% packed cell volume, PCV) were determined on-site by inducing a small amount of whole blood into two heparinized microcapillary tubes (about 20 μL each) that were then puttied and centrifuged at 15,800 rpm [13,700 × gravity (g)] for 2 min using a hematocrit centrifuge (CritSpin Models CS22 and CSD2, Iris International Inc., Chatsworth, CA). The remaining whole blood was transferred to a 1.5 mL microcentrifuge tube and spun at 2000 × 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 labeled 1.5 mL microcentrifuge tubes that were immediately flash frozen in liquid nitrogen. Following collection of plasma, a 3–5 g portion of white epaxial musculature anterior to the operculum was excised using a razorblade, freeze-clamped in aluminum tongs pre-cooled in liquid nitrogen, wrapped in pre-labeled aluminum foil, and flash frozen in liquid nitrogen (Suski et al., 2006). Following field collection, all plasma and muscle samples were transferred to an ultra-cold freezer (< −75 °C) daily after sampling.

To obtain fish for thermal and hypoxia challenges in the laboratory, creek chub were collected from the same 4 streams described above using identical electrofishing techniques. After being stunned by electroshocking gear, creek chub were transferred to aerated hauling tanks filled with stream water and transported (typically < 4 h). Creek chub from each stream were placed in one of four outdoor 960 L tanks. Tanks received water from a 0.04 ha earthen pond using a submersible pump (McMaster-Carr 42945K29, Atlanta, GA, USA) 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 = 20.3 ± 2 °C standard error, SE) and dissolved oxygen (mean = 8.09 ± 1 mg O$_2$ L^{-1}) with a portable meter (YSI, 550A Yellow Springs Instruments, Irvine, CA, USA). Ammonia concentrations in outdoor tanks remained < 0.25 ppm throughout the study as confirmed by a commercially available assay kit (Aquarium Pharmaceuticals Inc, LR8600, Chalfont, PA, USA), 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

prior to being used in experiments. All tanks were siphoned every two days to remove accumulated solid wastes, and fish were held in outdoor tanks for at least 3 days prior to use in experiments to allow for recovery from capture and transport as has been used successfully in other species of fish (Donelson et al., 2011; Cook et al., 2012).

2.4. Laboratory study

To generate resting control values for the suite of physiological parameters measured, six creek chub from each of the 4 streams were transferred from outdoor tanks to individual, darkened chambers supplied with aerated, recirculating pond water at 20 ± 0.2 °C, with a dissolved oxygen concentration of 8.0 ± 0.3 mg L^{-1}. Each of these individual, darkened chambers were small and appropriately sized to limit creek chub movement and activity. Pond water, pumped from a central basin, was allowed to overflow from the individual chambers and then drain back to the central basin forming a closed system similar to Suski et al. (2006). Following 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 field sampling described above. These methods of generating stress-free control values have been used successfully for many species of fish (Gingerich and Suski, 2010; Shultz et al., 2011).

To quantify differences in physiological disturbances following thermal and hypoxia challenges, six creek chub from each of the 4 streams were transferred from 960 L outdoor tanks to small, 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 ± 0.2 °C and 8.0 ± 0.3 mg O$_2$ L^{-1}. Following this acclimation period, nitrogen gas (99.5% nitrogen, 0.5% helium) was bubbled into the central basin to generate dissolved oxygen concentrations of 3.5 ± 0.3 mg O$_2$ L^{-1} for fish in the hypoxia treatment, and temperature was maintained at 20 ± 0.2 °C. Dissolved oxygen stabilized at the lower concentration in less than 5 min. After a 4 h exposure to this dissolved oxygen concentration, fish were lethally anesthetized in their individual containers, and sampled for blood and muscle as described above.

The thermal challenge experiment again used 6 fish from each of the 4 streams, and fish received 24 h to acclimate to the individual chambers. A submersible heater (1000 W, SCSUB10, Clepco, Cleveland, OH, USA) in the central basin was used to increase water temperature to 30.0 ± 0.2 °C, while air stones in each chamber maintained dissolved oxygen concentrations at 7.5 ± 0.3 mg O$_2$ L^{-1}. The elevated water temperature was reached in less than 5 min. Following 4 h exposure to this novel temperature, creek chub were lethally anesthetized and sampled for blood and muscle as described above.

2.5. Resting metabolic rates (RMR)

The impacts of thermal and hypoxia challenges on metabolic rate were determined using computerized, intermittent-flow respirometry (LoligoSystems, Hobro, Denmark) (Steffensen, 1989). The system consisted of 4 glass chambers (200 mm long × 62 mm inner diameter; 0.57 L) immersed in a 140 L tank of aerated pond water maintained at 20.0 °C (±0.52 °C) with a temperature-controlled heater. Change in oxygen concentration (α) for each chamber was calculated as slope ($\Delta O_{2saturation}/\Delta t$), and oxygen consumption rate ($\dot{M}O_2$, mg O$_2$ kg^{-1} h^{-1}) for each fish was calculated by:

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

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) prior to placing in 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 quantified 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 prior to 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, 8 fish from each of the 4 streams were transferred to individual chambers during the day (approximately 4:00 p.m.), acclimated to chambers for 4 h, and left 12 h (8:00 p.m.–8:00 a.m.) overnight with exposure to the same high temperature and low oxygen conditions described above; a group of fish from each site was also left overnight with no temperature or oxygen change to serve as a control. Resting metabolic rate for each fish was determined by selecting the six lowest 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 fish and chamber for 4 h prior to commencing experiments.

2.6. Acclimation experiment

To obtain fish for thermal acclimation in the laboratory, creek chub were again collected from each of the four streams and returned to the aquatic holding facility where they were held in a manner identical to that described above. Following 36 h recovery from transport, creek chub were given a site-specific fin clip and transferred to two aerated, indoor 960 L tanks for long-term thermal acclimation. One acclimation tank was maintained at $19.9 \pm 1\,^\circ\text{C}$ for six weeks and served as a control temperature, while the high temperature acclimation tank was maintained at $24.9 \pm 1\,^\circ\text{C}$ for the same six week period. In this way, fish from each stream with watersheds comprised of riparian agriculture or forest were held at both high temperatures and control temperatures for 6 weeks in a reciprocal experimental design. Temperatures in both tanks were recorded every 30 min over the six week period using a temperature logging device, and dissolved oxygen was checked daily with a portable meter (mean = $8.2 \pm 1\,\text{mg}\,\text{O}_2\,\text{L}^{-1}$). Following acclimation to these temperatures for 6 weeks, creek chub were subjected to the same laboratory thermal challenge experiment described above and were sampled for blood and muscle.

2.7. Laboratory analysis

Concentrations of cortisol in plasma were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Assay Designs, Kit #900-071, Ann Arbor, Michigan) recommended by Sink et al. (2008) due to 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 Company, Model 2655-00, Chicago, IL, USA), while plasma chloride (Cl^-) concentrations were generated using a digital chloridometer (Labconco, Model 4425000, Kansas City, MO, USA). Muscle lactate and glucose concentrations were determined enzymatically following the methods of Lowry and Passonneau (1972) using a microplate spectrophotometer (Molecular Devices, Spectra Max Plus 384, Model #05362, Union City, CA, USA). For the acclimation experiment, glutathione in plasma was determined using a commercially available assay kit (BioAssay Systems, Catalog # DIGT-250). Muscle water content was determined by drying tissue at $80\,^\circ\text{C}$ for 48 h and comparing wet mass to dry mass.

2.8. Statistical analysis

Daily minimum, mean, and maximum stream temperatures at each stream were calculated using data downloaded from the in-stream temperature logging devices. Daily temperature change was calculated by subtracting daily minimum temperature from daily maximum temperature. Stream temperature and dissolved oxygen concentrations were compared across streams using repeated measures analysis of variance (ANOVA) with land type (forested or agricultural) entered as a fixed effect and both date and sampling stream (nested within land type) entered as random effects (Bennington and Thayne, 1994; Zar, 1984; Ott and Longnecker, 2010; Wagner et al., 2006). Differences in stream depth, flow rate, and canopy cover were assessed across sites using a mixed model ANOVA. A mixed model ANOVA consists of fixed (levels set by 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). The mixed model ANOVA that was used here incorporates land type (forested or agricultural) entered as a fixed effect, and both date and sampling stream (nested within land type) entered as random effects. 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 to ascertain health and condition) was calculated using the weight length relationship ($W_r = -3.39611 \times L^{2.92494}$) developed by Carlander (1969) and compared across streams using the mixed-model ANOVA approach described above. Differences in baseline and stress-induced physiological parameters in creek chub following thermal and heat challenges were detected using a mixed model ANOVA with land type, treatment (heat or hypoxia) entered into the model as fixed effects and stream (nested within land type) entered as a random effect. This approach allows the variability of fish samples taken within stream to be included in the model and the error term (Wagner et al., 2006). All means are reported ± SE where appropriate, and all statistical analyses were performed using JMP version 8.2 (SAS Institute, Cary, NC, USA). The level of significance (α) for all tests was 0.05.

3. Results

3.1. Site characteristics

Streams with watersheds dominated by forested and agricultural riparian zones did not differ significantly in mean depth, width, dissolved oxygen concentration or flow rate ($P > 0.05$). Daily temperature fluctuations, however, were 41% greater and the daily maximum temperature averaged $3\,^\circ\text{C}$ higher for agricultural streams relative to streams with watersheds comprised of riparian forest ($P < 0.05$). Agricultural streams (13%) also had significantly lower canopy cover than streams with watersheds dominated by riparian forest (93%, $P < 0.05$). The range of temperatures that creek chub experienced in agricultural streams during the study period was $18–32\,^\circ\text{C}$ and $18–27\,^\circ\text{C}$ in forested streams. The range of dissolved oxygen concentrations that creek chub experienced in agricultural streams were $4.75–11.45\,\text{mg}\,\text{O}_2/\text{L}$ and $7.42–8.96\,\text{mg}\,\text{O}_2/\text{L}$ in forested streams.

3.2. In-field sampling

Creek chub collected and sampled in the field exhibited few differences in physiological parameters across land use types. There were no statistical differences in baseline concentrations of plasma cortisol, chloride, plasma sodium, potassium, muscle lactate, muscle water content, or hematocrit for fish from either land use type ($P > 0.05$). Baseline plasma glucose concentrations were approximately 30% higher in fish collected from forested streams compared to fish from streams with watersheds dominated by riparian agricultural ($P < 0.05$). Fish from agricultural streams averaged 13.8 mm longer than fish from forested streams but there were no differences in weight or condition for either land use type ($P > 0.05$).

3.3. Laboratory sampling and resting metabolic rates – thermal challenge

Following a 4 h exposure to high temperature (30 °C, 7.5 mg O$_2$ L^{-1}), creek chub collected from both forested and agricultural streams displayed a significant increase in plasma cortisol and glucose concentration ($P < 0.05$, Table 1, Fig. 1a and b). Plasma cortisol concentration for creek chub increased approximately 12-fold relative to control levels but differences were not detected across land use types (Table 1, Fig. 1a). Similarly, plasma glucose concentrations increased by approximately 50% following thermal challenge but there were no differences in the response of fish within or across treatment groups ($P > 0.05$, Table 1, Fig. 1b). Heat treatments increased the RMR of creek chub an average of 2.3 times relative to controls for fish from both forested and agricultural streams ($P < 0.05$, Table 1, Fig. 1c). Creek chub from forested streams displayed oxygen consumption rates that were 15% greater than fish from streams with watersheds comprised of riparian agricultural following the thermal challenge ($P < 0.05$, Table 1, Fig. 1c).

No significant changes within or across land use types were observed for concentrations of plasma chloride, plasma sodium, plasma potassium or muscle lactate following heat exposure ($P > 0.05$, Table 1, Fig. 2a). Whole blood hematocrit and muscle water content values were significantly higher for fish from both forested and agricultural streams for thermal challenges relative to controls but differences were not detected across land use types ($P > 0.05$, Table 1, Fig. 2b and c). No differences were detected in creek chub total length, weight, or condition within or across treatment groups ($P > 0.05$, Table 1).

3.4. Laboratory sampling and resting metabolic rates – low oxygen

Following a 4 h exposure to low oxygen conditions (20 °C, 3.5 mg O$_2$ L^{-1}), creek chub from forested streams showed significant increases in plasma cortisol concentrations compared to fish from streams with watersheds dominated by riparian agriculture ($P < 0.05$, Table 2, Fig. 3a). Plasma glucose concentrations following an oxygen challenge did not differ statistically across fish from both land use types ($P > 0.05$, Table 2, Fig. 3b). Resting metabolic rates were 15% lower following low oxygen exposure for fish from both forested and agricultural streams relative to controls, although differences across land use types were not significant ($P < 0.05$, Table 2, Fig. 3c). No differences in plasma chloride, plasma sodium, muscle lactate, blood hematocrit, or water content were detected for fish from both forested and agricultural streams within or across treatments ($P > 0.05$, Table 2, Fig. 4a and b). Creek chub from forested streams, however, lost 16% more potassium than fish from streams with watersheds comprised riparian agriculture following low oxygen exposure ($P < 0.05$, Table 2, Fig. 4c). Creek chub total length,
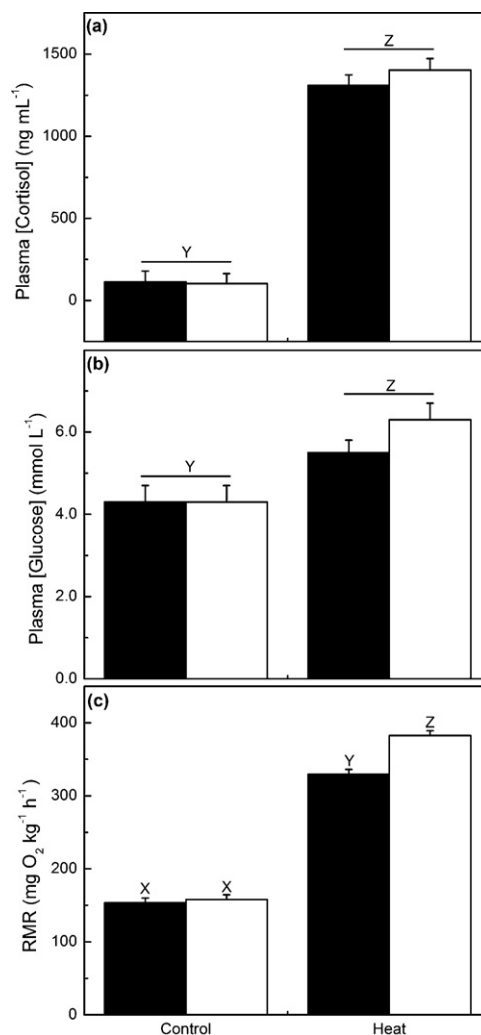

Fig. 1. Plasma cortisol (a) plasma glucose (b) and resting metabolic rate (RMR) (c) of creek chub exposed 4 h to control conditions (20 °C, 8 mg O$_2$ L^{-1}) or high temperature conditions (30 °C, 7.5 mg O$_2$ L^{-1}) from two agricultural streams (black) and two forested streams (white). Letters (Y and Z) above horizontal lines represent a significant difference from control values across treatment groups (mixed model ANOVA, Tukey HSD). Letters (X, Y, and Z) directly above bars represent significant differences between agricultural and forested streams within a treatment (significant interaction, mixed model ANOVA and LSMEANS Tukey HSD, $P < 0.05$).

weight, and relative weight were not different for site groupings within or across treatments ($P > 0.05$, Table 2).

3.5. Acclimation experiment

Following a 4 h exposure to high temperature, creek chub acclimated to low temperatures for six weeks from both agricultural and forested streams displayed a significant increase in plasma cortisol and glucose concentration ($P < 0.05$, Table 3, Fig. 5a). Plasma cortisol concentration for creek chub increased approximately 11-fold relative to control levels following a thermal challenge and cortisol concentrations for fish from forested streams were 15% higher than fish from streams with watersheds comprised of riparian agriculture ($P < 0.05$, Table 3, Fig. 5a). Similarly, plasma glucose concentrations increased by approximately 19% following a thermal challenge but there were no statistical differences in the glucose response of fish within treatment groups ($P > 0.05$, Table 3, Fig. 5b). No significant changes within or across land use types were observed for concentrations of plasma chloride, plasma sodium, plasma potassium, muscle water content, or glutathione

Table 1
Mixed model ANOVAs examining the impact of high temperature on the physiological responses of creek chub. Creek chub were collected from replicate agricultural ($n = 2$) and forested ($n = 2$) streams, given a 48 h recovery period, and then exposed to high temperatures (30 °C) for 4 h before morphometric and blood sampling.

Variable	Source	SS	df	F	P
Plasma [Cortisol] (ng mL^{-1})	Land type	42,345.8	1	0.4	0.60
	Treatment	16,835,744	1	645.7	**<0.0001***
	Type × Treatment	80,061.1	1	3.1	0.08
	Error	18,336,724.0	47		
Plasma [Glucose] (mmol L^{-1})	Land type	0.7	1	0.2	0.73
	Treatment	24.5	1	25.0	**<0.0001***
	Type × Treatment	0.2	1	0.2	0.68
	Error	37.3	43		
RMR (mg O$_2$ g^{-1} h^{-1})	Land type	13,185.9	1	16.0	0.06
	Treatment	642,239.0	1	953.4	**<0.0001***
	Type × Treatment	9400.2	1	13.9	**0.0004***
	Error	705,543.7	63		
Plasma [Chloride] (meq L^{-1})	Land type	235.2	1	0.2	0.70
	Treatment	373.8	1	2.0	0.16
	Type × Treatment	17.1	1	0.1	0.77
	Error	9727.7	35		
Plasma [Sodium] (meq L^{-1})	Land type	0.2	1	<0.1	0.98
	Treatment	13.1	1	0.2	0.69
	Type × Treatment	161.3	1	1.5	0.23
	Error	4503.4	43		
Plasma [Potassium] (meq L^{-1})	Land type	3.7	1	4.3	0.19
	Treatment	1.3	1	0.4	0.51
	Type × Treatment	0.6	1	0.2	0.67
	Error	108.8	46		
Muscle [Lactate] (mmol g^{-1})	Land type	0.1	1	0.8	0.47
	Treatment	0.1	1	1.2	0.28
	Type × Treatment	<0.1	1	<0.1	0.99
	Error	4.3	47		
Water content (%)	Land type	62.2	1	0.8	0.46
	Treatment	371.9	1	4.4	**0.041***
	Type × Treatment	7.4	1	0.1	0.77
	Error	4116.7	47		
Blood hematocrit (%)	Land type	0.8	1	<0.1	0.96
	Treatment	241.3	1	9.3	**0.004***
	Type × Treatment	42.5	1	1.6	0.21
	Error	1602.1	43		
Total length (mm)	Land type	0.2	1	<0.1	0.98
	Treatment	13.1	1	0.2	0.69
	Type × Treatment	161.3	1	1.5	0.23
	Error	4503.4	43		
Total weight (mm)	Land type	3.7	1	4.3	0.19
	Treatment	1.3	1	0.4	0.51
	Type × Treatment	0.6	1	0.2	0.67
	Error	108.8	46		
Relative weight	Land type	0.1	1	0.8	0.47
	Treatment	0.1	1	1.2	0.28
	Type × Treatment	<0.1	1	<0.1	0.99
	Error	4.3	47		

The bold values in table highlight significance of a term in the model.

for fish acclimated to control temperatures following heat exposure ($P > 0.05$, Table 3). Whole blood hematocrit values were significantly higher for fish from both forested and agricultural streams following thermal challenges relative to controls but differences were not detected across land use types ($P < 0.05$, Table 3). No differences were detected in creek chub total length, weight, or condition within or across treatment groups ($P > 0.05$, Table 3).

Following a 4 h exposure to high temperature (30 °C, 7.5 mg O$_2$ L^{-1}), creek chub acclimated to high temperatures for six weeks from both forested and agricultural streams displayed significantly elevated plasma cortisol and glucose concentration relative to controls ($P < 0.05$, Table 3, Fig. 5a and b) but differences were not detected across land use types ($P > 0.05$, Table 3, Fig. 5a and b). Plasma cortisol concentration for creek chub acclimated to high temperature and given a thermal challenge increased approximately 1.3-fold relative to creek chub acclimated to high temperature under control temperatures but differences were not detected in heat acclimated fish across land use types ($P < 0.05$, Table 3, Fig. 5a).

No significant changes within or across land use types were observed for concentrations of plasma chloride, plasma sodium, plasma potassium, plasma glutathione, or muscle water content for fish acclimated to high temperatures following heat exposure ($P > 0.05$, Table 3). Whole blood hematocrit and muscle water content values were significantly higher for fish from both forested and agricultural streams acclimated to high temperatures following thermal challenges relative to controls but no differences were detected across land use types ($P > 0.05$, Table 3). No differences

Table 2
Mixed model ANOVAs examining the impact of low oxygen on physiological responses of creek chub. Creek chub were collected from replicate agricultural ($n=2$) and forested ($n=2$) streams, given a 48 h recovery period, and exposed to low oxygen (3.5 mg O_2/L) for 4 h before morphometric and blood sampling.

Variable	Source	SS	df	F	P
Plasma [Cortisol] (ng mL^{-1})	Land type	20,626.1	1	1.3	0.37
	Treatment	10,197.4	1	1.4	0.24
	Type × Treatment	49,151.3	1	6.9	**0.01***
	Error	297,235.3	42		
Plasma [Glucose] (mmol L^{-1})	Land type	<0.1	1	<0.1	0.94
	Treatment	<0.1	1	<0.1	0.82
	Type × Treatment	1.3	1	0.1	0.16
	Error	25.8	42		
RMR (mg O_2 g^{-1} h^{-1})	Land type	1297.9	1	0.6	0.51
	Treatment	8036.6	1	25.4	**<0.0001***
	Type × Treatment	329.5	1	1.0	0.31
	Error	32,123.5	63		
Plasma [Chloride] (meq L^{-1})	Land type	485.1	1	0.6	0.53
	Treatment	443.3	1	2.4	0.13
	Type × Treatment	17.1	1	0.1	0.74
	Error	6354.9	34		
Plasma [Sodium] (meq L^{-1})	Land type	57.9	1	0.2	0.72
	Treatment	398.5	1	2.6	0.12
	Type × Treatment	435.1	1	3.0	0.09
	Error	7419.1	45		
Plasma [Potassium] (meq L^{-1})	Land type	0.3	1	0.1	0.77
	Treatment	10.9	1	5.7	**0.02***
	Type × Treatment	10.6	1	5.5	**0.02***
	Error	106.3	47		
Muscle [Lactate] (mmol g^{-1})	Land type	0.1	1	1.1	0.41
	Treatment	<0.1	1	<0.1	0.82
	Type × Treatment	<0.1	1	<0.1	0.87
	Error	4.4	47		
Water content (%)	Land type	195.8	1	0.5	0.53
	Treatment	25.5	1	2.4	0.13
	Type × Treatment	77.9	1	0.1	0.74
	Error	3841.6	47		
Blood hematocrit (%)	Land type	74.9	1	2.8	0.24
	Treatment	45.0	1	1.8	0.18
	Type × Treatment	0.7	1	<0.1	0.84
	Error	998.6			
Total length (mm)	Land type	507.0	1	0.2	0.71
	Treatment	300.0	1	0.9	0.35
	Type × Treatment	2.1	1	<0.1	0.94
	Error	20,717.3	47		
Total weight (mm)	Land type	117.2	1	0.2	0.70
	Treatment	72.5	1	0.7	0.42
	Type × Treatment	42.2	1	0.4	0.54
	Error	4670.5	42		
Relative weight	Land type	30.6	1	0.4	0.59
	Treatment	6.6	1	0.2	0.65
	Type × Treatment	95.9	1	2.9	0.09
	Error	1639.0	42		

The bold values in table highlight significance of a term in the model.

were detected in creek chub total length, weight, or condition within or across treatment groups or acclimation conditions ($P > 0.05$, Table 3).

4. Discussion

Variation in riparian land use at the watershed scale had a significant influence on the stress responses of creek chub following exposure to hypoxia. Specifically, following an acute hypoxia challenge, circulating concentrations of cortisol were 76% lower and loss of potassium ions from plasma was almost 20% lower in fish from agricultural streams compared to fish from streams with watersheds comprised of riparian forest, indicating a reduced stress response for fish from agricultural streams. Cortisol is an integral part of the primary stress response of fish that liberates energy and initiates a host of secondary stress pathways essential for the maintenance of homeostasis following the onset of a stressor (Wendelaar Bonga, 1997; Barton, 2002). Increased concentrations of cortisol, however, can also negatively impact individual behavior, inhibit growth, impair reproduction, and reduce the effectiveness of the immune system, highlighting physiological benefits to reduced concentrations of cortisol for individuals (Romero and Butler, 2007). Elevated plasma cortisol also plays a role in increasing gill surface area to maximize oxygen uptake during low oxygen conditions, leading to a concomitant loss of ions through the gills (McDonald et al., 1991). More importantly, circulating levels of cortisol, as well as the magnitude of ion losses from plasma, correlate positively with the magnitude of stress perceived by

Table 3

Mixed model ANOVAs examining the impact of high temperature on the physiological responses of creek chub following laboratory acclimation. Creek chub were collected from replicate agricultural ($n = 2$) and forested ($n = 2$) streams, acclimated to 20 °C and 25 °C for six weeks in the laboratory, and exposed to a 4 h high temperature challenge (30 °C) before sampling.

Variable	Source	SS	df	F	P
Plasma [Cortisol] (ng mL^{-1})	Land type	2,644,338.0	3	150.2	**0.0001***
	Treatment	11,300,000.0	1	1620.0	**<0.0001***
	Type × Treatment	8,054,182.0	3	386.5	**<0.0001***
	Error	23,451,818.0	95		
Plasma [Glucose] (mmol L^{-1})	Land type	6.6	3	0.79	0.56
	Treatment	13.1	1	8.62	**0.0043***
	Type × Treatment	2.2	3	0.48	0.70
	Error	160.8	95		
Plasma [Chloride] (meq L^{-1})	Land type	29,142.4	3	2.6	0.19
	Treatment	3633.3	1	0.6	0.43
	Type × Treatment	5048.3	3	0.3	0.83
	Error	6890.9	95		
Plasma [Sodium] (meq L^{-1})	Land type	1180.2	3	1.3	0.4
	Treatment	68.0	1	68.0	0.5
	Type × Treatment	813.2	3	813.2	0.1
	Error	13,493.3	95		
Plasma [Potassium] (meq L^{-1})	Land type	2275.6	3	2.2	0.23
	Treatment	810.5	1	1.6	0.21
	Type × Treatment	1991.5	3	1.3	0.28
	Error	48,872.2	95		
Plasma [Glutathione] (mmol L^{-1})	Land type	5.9	3	3.0	0.16
	Treatment	1.7	1	1.3	0.26
	Type × Treatment	1.0	3	0.2	0.86
	Error	123.8	95		
Water content (%)	Land type	97.7	3	0.2	0.89
	Treatment	321.0	1	3.2	0.08
	Type × Treatment	85.8	3	0.9	0.46
	Error	9684.1	95		
Blood hematocrit (%)	Land type	24.1	3	1.1	0.44
	Treatment	114.8	1	9.0	**<0.1***
	Type × Treatment	55.4	3	1.5	0.23
	Error	1066.2	95		
Total length (mm)	Land type	96.5	3	0.3	0.86
	Treatment	45.4	1	0.1	0.72
	Type × Treatment	2648.2	3	2.5	0.06
	Error	32,953.9	95		
Total weight (mm)	Land type	158.8	3	0.8	0.54
	Treatment	3.7	1	<0.1	0.85
	Type × Treatment	715.2	3	2.4	0.08
	Error	9579.5	95		
Relative weight	Land type	348.4	3	1.4	0.36
	Treatment	109.2	1	1.6	0.21
	Type × Treatment	318.1	3	1.5	0.21
	Error	6890.9	95		

The bold values in table highlight significance of a term in the model.

an organism (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Hontela, 1998).

Previous research has demonstrated that the environment of an organism can impact physiological characteristics and cause differences in the magnitude of their stress response. Both spotted salamanders (*Ambystoma maculatum* Shaw, 1802) and tree lizards (*Urosaurus ornatus* Baird and Girard, 1852) residing in highly disturbed (urban) areas exhibited an attenuated stress response following a common challenge relative to individuals from less disturbed (natural) areas (Homan et al., 2003; French et al., 2008). The reduced stress response in organisms from disturbed environments was attributed to either chronic activation of the stress response or decreased resource availability in disturbed sites resulting in an impaired stress response (Homan et al., 2003; French et al., 2008). Physiological responses beyond cortisol concentration were not measured, however, and it was not possible to distinguish between these explanations or assess the overall stress response of resident

organisms (Homan et al., 2003; French et al., 2008). A third potential explanation for a reduced stress response from organisms residing in disturbed habitats is that these organisms have become acclimatized to repeated stressors via phenotypic plasticity, thereby requiring a smaller stress response to maintain homeostasis in the face of challenges (Homan et al., 2003; French et al., 2008). For creek chub from both forested and agricultural streams, no differences in ion loss, glucose release or anaerobic metabolism were observed across land use types despite differences in cortisol concentrations, indicating maintenance of homeostasis through a lower production of cortisol, possibly as a result of acclimation. Additionally, there were no differences in weight or condition across land use types, indicating that the energetic status and nutritional properties of wild fish were likely similar. Although no differences in oxygen concentrations were detected across land use types during field sampling, it is likely that the higher water temperatures observed in streams with watersheds comprised of riparian
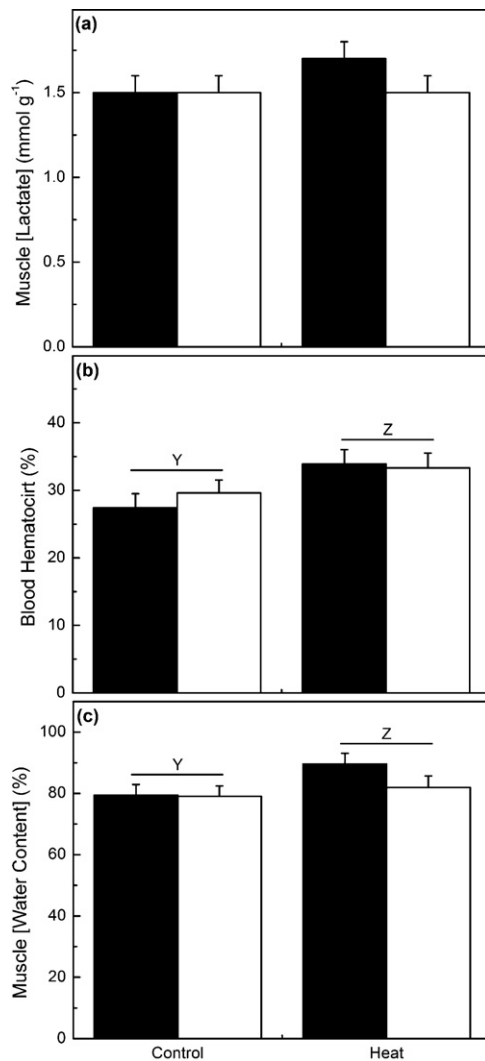
Fig. 2. Muscle lactate (a) water content (c) and blood hematocrit (b) of creek chub exposed 4 h to control conditions (20 °C, 8 mg O_2 L^{-1}) or high temperature conditions (30 °C, 7.5 mg O_2 L^{-1}) from two agricultural streams (black) and two forested streams (white). Letters (A and B) above horizontal lines represent a significant difference from control values across treatment groups (significant fixed effect for treatment, mixed model ANOVA and LSMEANS Tukey HSD, $P < 0.05$).

Fig. 3. Plasma cortisol (a) plasma glucose (b) and resting metabolic rate (RMR MO_2) (c) of creek chub exposed 4 h to control conditions (20 °C, 8 mg O_2 L^{-1}) or low oxygen conditions (20 °C, 3.5 mg O_2 L^{-1}) from two agricultural streams (black) and two forested streams (white). 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 and LSMEANS Tukey HSD, $P < 0.05$). Letters (X, Y, and Z) directly above bars represent significant differences between agricultural and forested streams within a treatment (significant interaction, mixed model ANOVA and LSMEANS Tukey HSD, $P < 0.05$).

agricultural caused reductions in oxygen levels outside of our sampling, potentially impacting the physiological properties of resident fishes. Together, these results demonstrate that a reduction in forest in the riparian area at the watershed scale causes an attenuation of the stress response and improved physiological performance following hypoxia challenges for creek chub relative to individuals from less disturbed watersheds.

Similar to the response to hypoxia, creek chub from streams with watersheds comprised of agriculture in the riparian area consumed 15% less energy as part of their response to an environmentally relevant acute thermal challenge, while still maintaining homeostasis in the face of the thermal challenge. Metabolic rate is a measure of oxygen consumption and represents the sum total of energy expenditure for a number of processes for fish that include ion regulation, digestion, and thermoregulation (Jobling, 1981). More importantly, metabolic rate is sensitive to environmental stressors, and is therefore commonly used as an indicator of disturbance for fish (Barton et al., 1986; Dalhoff and Menge, 1996; Blank et al., 2007; Bonier et al., 2007). Following an acute thermal challenge, creek chub demonstrated an overall elevation in metabolic rate not only because they are poikilothermic, but also
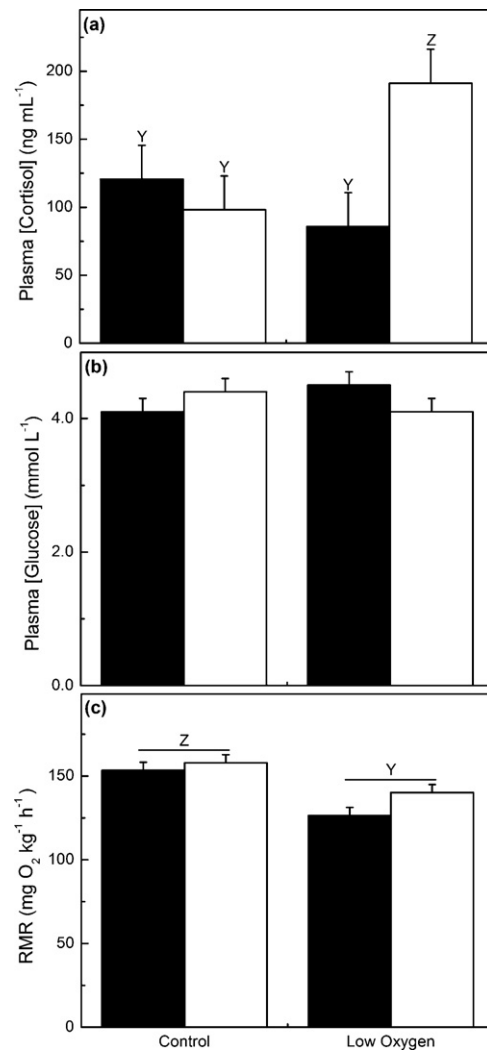
because of an elevation in biological activity required to cope with stressful conditions, such as increased regulation of ions and mobilization of energy substrates for potential physiological or behavioral adjustments to stress (Dahlhoff, 2004). Creek chub residing in agricultural streams, which were warmer and more thermally variable than forested streams, consumed 15% less energy as part of their response to an acute thermal challenge, indicating a reduction in energetic expenditure. Reduction in metabolic rate during a thermal challenge means lower energy consumption for creek chubs from agricultural streams, allowing for a redistribution of energy to other activities such as foraging and may translate to an advantage in the wild following thermal challenges of similar magnitude. This 15% reduction represents a 52 mg O_2 kg^{-1} h^{-1} lower oxygen consumption rate for fish from agricultural streams on average as compared with fish from forested sites and is much larger difference than that found in related studies of other fish species. In a similar study, the tropical reef fish spiny chromis (*Acanthochromis polyacanthus* Bleeker, 1855) reared in elevated water temperatures showed reduced resting metabolic rates
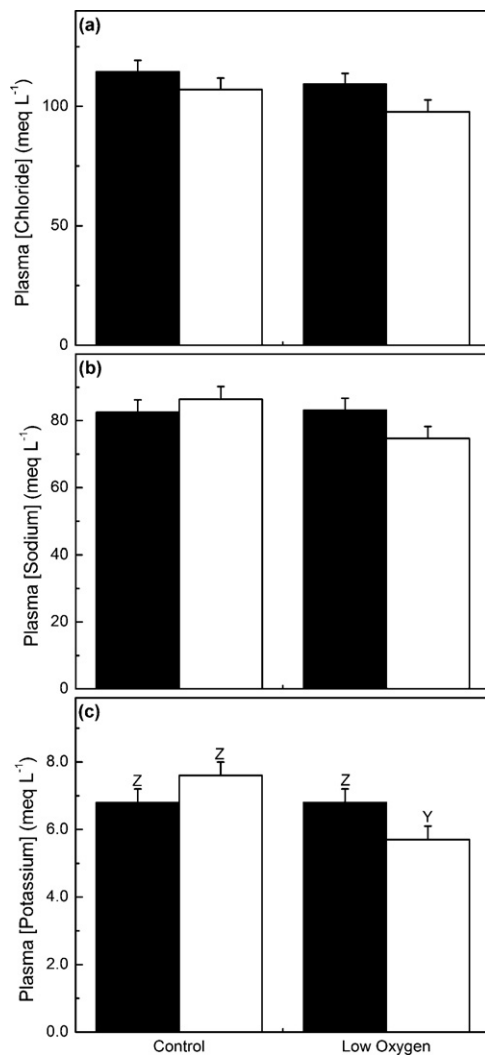
Fig. 4. Plasma chloride (a) sodium (b) and potassium (c) of creek chub exposed 4 h to control conditions (20 °C, 8 mg O_2 L^{-1}) or low oxygen conditions (20 °C, 3.5 mg O_2 L^{-1}) f from two agricultural streams (black) and two forested streams (white). Letters (Y and Z) above horizontal lines represent a significant difference from control values across treatment groups (significant fixed effect for treatment, mixed model ANOVA and LSMEANS Tukey HSD, $P < 0.05$). Letters (X and Y) directly above bars represent significant differences between agricultural and forested streams within a treatment (significant interaction, mixed model ANOVA and LSMEANS Tukey HSD, $P < 0.05$).

Fig. 5. Plasma cortisol (a) and plasma glucose (b) of creek chub acclimated to control temperatures (20 °C) for six weeks and then exposed 4 h to control conditions (20 °C, 8 mg O_2 L^{-1}) from two agricultural streams (black) and two forested streams (white). The additional bars represent creek chub acclimated to high temperatures (25 °C) and then exposed 4 h to high temperature conditions (30 °C, 7.5 mg O_2 L^{-1}) from two agricultural streams (horizontal stripes) and two forested streams (vertical stripes). Letters (Y and Z) above horizontal lines represent a significant difference from control values across treatment groups (mixed model ANOVA, Tukey HSD). Letters (W, X, Y, and Z) directly above bars represent significant differences between agricultural and forested streams within a treatment (significant interaction, mixed model ANOVA and LSMEANS Tukey HSD, $P < 0.05$).

when exposed to high temperatures compared to individuals reared in cooler water temperatures (Donelson et al., 2011). This reduction in metabolic rate was attributed to thermal acclimation of fish reared in warm environments, and highlights the energetic benefits for warm-acclimated fish that would be accrued in the wild. In addition, common killifish (*Fundulus heteroclitus* Linnaeus, 1766) living in warmer and more thermally variable southern estuaries have a lower onset temperature and reduced magnitude of heat shock protein expression following a thermal challenge relative to conspecifics from northern estuaries, demonstrating a reduced sensitivity to thermal stressors and an avoidance of production of costly heat shock proteins to maintain metabolic homeostasis (Somero, 2002; Fangue et al., 2006). Repeated exposure to higher temperatures and more thermally variable conditions in agricultural streams caused an attenuation of creek chub metabolic stress responses and allows fish from these streams to use less energy to maintain physiological performance during thermal challenges.
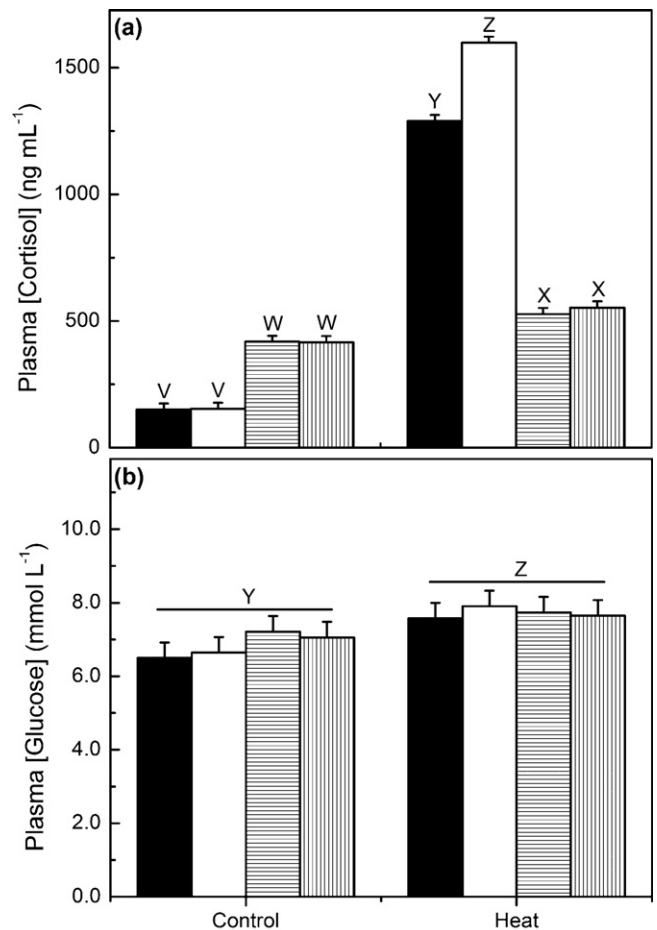
We induced an attenuated stress response and improved physiological performance in fish held at environmentally relevant high temperatures in the laboratory, regardless of land use of origin. Following six weeks of holding at control (cool) conditions, the cortisol response of creek chub following acute thermal challenges were representative of animals challenged immediately after removal from their home environment; fish residing in warmer conditions demonstrated an improved performance relative to fish from cooler environments by maintaining homeostasis despite reductions in cortisol production. However, six weeks of holding at high temperatures removed these landscape-level differences and fish acclimated to high temperatures did not differ in their response to thermal challenges based on riparian watershed land cover. More importantly, fish acclimated to high temperatures exhibited lower plasma cortisol concentrations following both control and thermal challenge relative to cool-acclimated individuals while plasma glucose, ion, and muscle water concentrations did not vary from control values. Previous studies have demonstrated that many physiological properties of organisms are quite plastic and can change due to environmental conditions. The Antarctic fish bald notothen (*Pagothenia borchgrevinki* Boulenger, 1902) acclimated to

−1 °C for 4–5 weeks maintained cardiac output by changing heart pumping strategy when tested at higher temperatures (Franklin et al., 2007). Similarly, acclimation of crucian carp (*Carassius carassius* Linnaeus, 1758) to 10, 15, 20 and 25 °C for 8 weeks resulted in the rapid formation of protruding gill lamellae at higher acclimation temperatures relative to cooler holding temperatures (Sollid et al., 2005). We found that prolonged holding of creek chub at high temperatures for six weeks induced physiological changes that lead to plastic reductions in primary stress responses and allowed for homeostasis of many secondary stress pathways following a subsequent thermal challenge.

Physiological responses are directly responsible for the ability of an organism to cope with stressors in its environment and to maintain growth and reproduction (Adolph, 1956). In the current study, higher temperatures, thermal variability, and likely thermally limited oxygen capacity within streams impacted by agriculture and deforestation affected creek chub physiological responses and improved physiological performance to better cope with environmental challenges. In addition, improved physiological tolerances occurred in fish from both forested and agricultural streams following prolonged holding and acclimation to high temperatures in the laboratory. These results suggest that the stress response of creek chub is plastic and can be adjusted based on perceived environmental stressors and thermal conditions, providing this species with a mechanism to cope with a range of environmental conditions. Plastic responses likely allow populations to persist across a wide array of thermal and oxygen conditions in the wild, potentially providing a coping mechanism to withstand future global change. In contrast, species that are negatively impacted at the population-level by elevated temperatures may lack the physiological capacity for beneficial acclimation to environmental stressors, thus necessitating either movement/redistribution or negative consequences for individuals and populations. The current study also demonstrates the potential of beneficial, plastic changes in physiological responses to be used as indicators to evaluate the impacts of disturbance on the physiology of organisms within aquatic environments, potentially allowing managers to form proactive strategies to mitigate these stressors before they can cause population declines (Cooke and Suski, 2008). Studies such as this are crucial for establishing the basic connections between organisms and their environment (Falk et al., 2006) which allows for an improved ability to predict the impacts of anthropogenic alteration and proactively form protection strategies. In addition, this study illustrates anthropogenic activity and associated landscape modifications can be detected using physiological techniques at temporal scales useful for natural resource managers at relatively low costs (Cooke and O'Connor, 2010). Changes in the physiological responses of creek chub were detected in as few as six weeks, which could conceivably provide restoration activities with information to mitigate the source of stress before aquatic organism populations start to decline and aid in short-term evaluation of protection strategies.

Acknowledgements

References

Adolph, E.F., 1956. General and specific characteristics of physiological adaptations. Am. J. Physiol. 184, 18–28.

Allan, J.D., 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annu. Rev. Ecol. Syst. 35, 257–284.

Albanese, B., Angermeier, P.L., Dorai-Raj, S., 2004. Ecological correlates of fish movement in a network of Virginia streams. Can. J. Fish. Aquat. Sci. 61, 857–869.

Anderson, J.R., Harvey, E.H., Roach, J.T., Witmer, R.E., 1975. A land use and land cover classification system for use with remote sensor data. Geological Survey Professional Paper 964. U.S. Government Printing Office, Washington, DC, 27 pp.

Barton, B.A., Shreck, C.B., Sigismondi, L.A., 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile Chinook salmon. Trans. Am. Fish. Soc. 115, 245–251.

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish. Dis. 1, 3–26.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integ. Comp. Biol. 42, 517–525.

Belica, L.A., Rahel, F.J., 2008. Movements of creek chubs, *Semotilus atromaculatus*, among habitat patches in a plains stream. Ecol. Freshw. Fish. 17, 258–272.

Bennington, C.C., Thayne, W.V., 1994. Use and misuse of mixed-model analysis of variance in ecological-studies. Ecology 75, 717–722.

Blank, J.M., Monissette, J.M., Farwell, C.J., Price, M., Schallert, J., Block, B.A., 2007. Temperature effects on metabolic rate of juvenile Pacific bluefin tuna *Thunnus orientalis*. J. Exp. Biol. 210, 4254–4261.

Bonier, F., Martin, P.R., Sheldon, K.S., Jensen, J.P., Foltz, S.L., Wingfield, J.C., 2007. Sex-specific consequences of life in the city. Behav. Ecol. 18, 121–129.

Carlander, K.D., 1969. Handbook of Freshwater Fishery Biology. Iowa State University Press, Ames, IA.

Cook, K.V., O'Connor, C.M., McConnachie, S.H., Gilmour, K.M., Cooke, S.J., 2012. Condition dependent intra-individual repeatability of stress-induced cortisol in a freshwater fish. Comp. Biochem. Phys. A 161, 337–343.

Cooke, S.J., Suski, C.D., 2008. Ecological restoration and physiology: an overdue integration. Bioscience 58, 957–968.

Cooke, S.J., O'Connor, C.M., 2010. Making conservation physiology relevant to policy managers and conservation practitioners. Conserv. Lett. 3, 159–166.

Creel, S., Fox, J.E., Hardy, A., Sands, J., Garrott, B., Peterson, R.O., 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. Conserv. Biol. 16, 809–814.

Czech, B., Krausman, P.R., Devers, P.K., 2000. Economic associations among causes of species endangerment in the United States. Bioscience 50, 593–601.

Dalhoff, E.P., Menge, B.A., 1996. Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. Mar. Ecol. Prog. Ser. 144, 97–107.

Dahlhoff, E.P., 2004. Biochemical indicators of stress and metabolism: applications for marine ecological studies. Annu. Rev. Physiol. 66, 183–207.

Donelson, J.M., Munday, P.L., McCormick, M.I., Nilsson, G.E., 2011. Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. Glob. Change Biol. 17, 1712–1719.

E.S.R.I., 2005. ArcView GIS 9.1. ESRI Redlands, CA.

Falk, D.A., Palmer, M.A., Zedler, J.B., 2006. In: Falk, D.A., Palmer, M.A., Zedler, J.B. (Eds.), Foundations of Restoration Ecology. Island Press, Washington, DC.

Fangue, N.A., Hofmeister, M., Schulte, P.M., 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. J. Exp. Biol. 209, 2859–2872.

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

Fitzpatrick, F.A., U.S.G.S., 1998. Revised Methods for Characterizing Stream Habitat in the National Water-Quality Assessment Program. U.S. Geological Survey, Branch of Information Services, Raleigh, NC.

Franklin, C.E., Davison, W., Seebacher, F., 2007. Antarctic fish can compensate for rising temperatures: thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. J. Exp. Biol. 210, 3068–3074.

French, S., Fokidis, H., Moore, M., 2008. Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban-rural gradient. J. Comp. Physiol. B 178, 997–1005.

Gergel, S.E., Turner, M.G., Miller, J.R., Melack, J.M., Stanley, E.H., 2002. Landscape indicators of human impacts to riverine systems. Aquat. Sci. 64, 118–128.

Gingerich, A.J., Suski, C.D., 2010. The role of progeny quality and male size in the nesting success of smallmouth bass: integrating field and laboratory studies. Aquat. Ecol. 45, 505–515.

Hayes, D.B., Ferreri, C.P., Taylor, W.W., 1996. Linking fish habitat to their population dynamics. Can. J. Fish. Aquat. Sci. 53, 383–390.

Hill, R.W., Wyse, G.A., Anderson, M., 2008. Thermal relations. Animal Physiology, vol. 2. Sinauer Associates, Inc., Sunderland, MA, pp. 205–252.

Homan, R.N., Reed, J.M., Romero, L.M., 2003. Corticosterone concentrations in free-living spotted salamanders (*Ambystoma maculatum*). Gen. Comp. Endocrinol. 130, 11–18.

Hontela, A., 1998. Interrenal dysfunction in fish from contaminated sites: in vivo and in vitro assessment. Environ. Toxicol. Chem. 17, 44–48.

I.D.O.A., 2001. Land Cover of Illinois 1999–2000: GIS Database. State of Illinois Department of Agriculture, Springfield, IL.

Jobling, M., 1981. The influence(s) of feeding on the metabolic rate of fishes: a short review. J. Fish. Biol. 18, 385–400.

Jones, M.L., Randall, R.G., Hayes, D., Dunlop, W., Imhof, J., Lacroix, G., Ward, N.J.R., 1996. Assessing the ecological effects of habitat change: moving beyond productive capacity. Can. J. Fish. Aquat. Sci. 53, 446–457.

Lowry, O.H., Passonneau, J.V., 1972. A Flexible System of Enzymatic Analysis. Academic Press, New York.

Martinez-Mota, R., Valdespino, C., Sanchez-Ramos, M.A., Serio-Silva, J.C., 2007. Effects of forest fragmentation on the physiological stress response of black howler monkeys. Anim. Conserv. 10, 374–379.

Matthews, K.R., Berg, N.H., 1997. Rainbow trout responses to water temperature and dissolved oxygen in two southern California stream pools. J. Fish. Biol. 50, 50–67.

McDonald, D.G., Cavdek, V., Ellis, R., 1991. Gill design in freshwater fishes: interrelationships among gas exchange, ion regulation, and acid-base regulation. Physiol. Zool. 64, 103–123.

Meador, M.R., Goldstein, R.M., 2003. Assessing water quality at large geographic scales: relations among land use, water physicochemistry, riparian condition, and fish community structure. Environ. Manage. 31, 504–517.

Menzel, B.W., Barnum, J.B., Antosch, L.M., 1984. Ecological alterations of Iowa prairie-agricultural streams. Iowa State J. Res. 59, 5–30.

Millspaugh, J.J., Washburn, B.E., 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen. Comp. Endocrinol. 138, 189–199.

Ott, L., Longnecker, M., 2010. An Introduction to Statistical Methods and Data Analysis. Brooks/Cole Cengage Learning, Belmont, CA.

Pflieger, W.L., Missouri Dept. of Conservation, 1997. The Fishes of Missouri. Department of Conservation, Missouri, Jefferson City, MO.

Robinson, J.M., Josephson, D.C., Weidel, B.C., Kraft, C.E., 2010. Influence of variable interannual summer water temperatures on brook trout growth, consumption, reproduction, and mortality in an unstratified Adirondack Lake. Trans. Am. Fish. Soc. 139, 685–699.

Romero, L.M., Butler, L.K., 2007. Endocrinology of stress. Integ. J. Comp. Physiol. 20, 89–95.

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. A 140, 73–79.

Schlosser, I.J., 1991. Stream fish ecology: a landscape perspective. Bioscience 41, 704–712.

Schurmann, H., Steffensen, J.F., 1997. Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. J. Fish. Biol. 50, 1166–1180.

Selong, J.H., McMahon, T.E., Zale, A.V., Barrows, F.T., 2001. Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance for fishes. Trans. Am. Fish. Soc. 130, 1026–1037.

Shultz, A.D., Murchie, K.J., Griffith, C., Cooke, S.J., Danylchuk, A.J., Goldberg, T.L., Suski, C.D., 2011. Impacts of dissolved oxygen on the behavior and physiology of bonefish: implications for live release angling tournaments. J. Exp. Mar. Biol. Ecol. 402, 19–26.

Sink, T.D., Lochmann, R.T., Fecteau, K.A., 2008. Validation, use and disadvantages of enzyme-linkedimmunosorbent assay kits for detection of cortisol inchannel catfish, largemouth bass, red pacu and golden shiners. Fish. Physiol. Biochem. 34, 95–101.

Sollid, J., Weber, R.E., Nilsson, G.E., 2005. Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. J. Exp. Biol. 208, 1109–1116.

Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integ. Comp. Biol. 42, 780–789.

Stauffer, J.C., Goldstein, R.M., Newman, R.M., 2000. Relationship of wooded riparian zones and runoff potential to fish community composition in agricultural. Can. J. Fish. Aquat. Sci. 57, 307–316.

Steffensen, J.F., 1989. Some errors in respirometry of aquatic breathers – how to avoid and correct for them. Fish. Physiol. Biochem. 6, 49–59.

Suski, C.D., Killen, S.S., Kieffer, J.D., Tufts, B.L., 2006. The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: implications for live-release angling tournaments. J. Fish. Biol. 68, 120–136.

Wagner, T., Hayes, D.B., Bremigan, M.T., 2006. Accounting for multilevel data structures in fisheries data using mixed models. Fisheries 31, 180–187.

Welch, D.J., 1991. Riparian forest buffers – functional and design protection and enhancement of water resources. Technical Report NA-PR-07-91. USDA Forest Service, Radnor, PA.

Wendelaar Bonga, S.E.W., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.