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PRIMARY RESEARCH PAPER

Health, condition, and survival of creek chub (*Semotilus atromaculatus*) across a gradient of stream habitat quality following an experimental cortisol challenge

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Abstract Urbanization and agricultural practices can lead to alterations in stream habitat quality. However, there have been few attempts to understand if these alterations influence the health and condition of fish, or their ability to respond to multiple stressors. This study examines a sentinel fish in streams adjacent to different types of land-use practices and how they respond to an experimental stressor. Creek chub (*Semotilus atromaculatus*) from replicate agricultural, urban, and reference streams were subjected to an experimental manipulation of cortisol, the primary stress hormone in fish. A single intraperitoneal injection raised circulating plasma cortisol for ~3 days, mimicking the physiological effects of a prolonged stressor. We compared the survival, health, and condition of cortisol-treated, sham-treated, and control fish across the different land-use types. While marginally non-significant ($P = 0.06$), cortisol-treated fish displayed an ~50% increase in mortality in streams adjacent to agricultural areas. We did not observe differences in blood glucose, condition factor, splenic index, and gonadosomatic index, or parasite burden among the treatment groups or relative to land-use type. However, within the agricultural watersheds, the hepatosomatic index value of fish receiving a sham treatment was ~20% greater than fish in the control treatment, a significant result that appears to be spurious given that a similar effects was not observed in other land-use types. Overall, these results suggest that in the wild, there are apparently compensatory mechanisms that enable creek chub to persist despite being exposed to a significant challenge with little evidence that the outcome is modulated by variation in habitat quality, at least among the three types of sites (i.e., urban, agricultural, and reference) studied here. Nonetheless, we encourage additional field-based studies with larger sample sizes or better recapture rates to improve statistical power and provide more clarity on how variation in habitat quality influences how fish respond to other challenges.

Keywords Body condition · Stress response · Corticosterone · Habitat quality · Creek chub · Environmental stress

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Introduction

The habitat and biological diversity of stream ecosystems are strongly influenced by the surrounding terrestrial landscapes through which they flow (reviewed in Allan, 2004). In particular, the riparian zone, defined as the interface between terrestrial and aquatic ecosystems (Gregory et al., 1991), plays an important role in controlling the effects that catchment activities have on neighboring aquatic ecosystems (Quinn et al., 2001). Human development promotes the transition from undisturbed to more human-dominated landscapes which has prompted research on the effects of various land-use practices on stream conditions (e.g., Lenat & Crawford, 1994; Niyogi et al., 2003; Roy et al., 2003). For example, anthropogenic alterations to terrestrial landscapes have been found to influence sediment loads (Henley et al., 2000), alter watershed hydrology (Allan et al., 1997), increase water temperature (LeBlanc, 1997), create imbalances in nutrient inputs (Mainstone & Parr, 2002), and increase pollution (Clements et al., 2000) within adjacent aquatic ecosystems. The extent that anthropogenic activities influence natural landscapes varies according to the type and severity of disturbance (e.g., agriculture, forestry, and urbanization). In general, anthropogenic alterations and associated changes in water quality and stream habitat expose the organisms that reside in these systems to more extreme environmental conditions.

For a species to persist in a particular environment, essential habitat requirements need to be satisfied. Although species can be found to inhabit a range of different environments, life-history traits and associated physiological tolerances and capacities may limit the extent to which an organism can adapt to anthropogenic disturbances. At some point, responses to unsuitable environments will represent a significant allostatic load (i.e., energetic, immune and other biological costs associated with maintaining homeostasis in the face of various challenges; McEwen, 1998; Wingfield, 2005), and an organism to be at a competitive disadvantage (Piersma & Drent, 2003). When faced with a stressor, species inhabiting sub-optimal environments may be less capable of adequately responding to the challenge (Wingfield, 2005) and may experience greater negative consequences (e.g., reduced food intake and immune function) than when exposed to a stressor in an optimal environment. For example, research evaluating the stress response of birds across different land use patterns (i.e., urban, suburban, and industrial) revealed that while all birds had a large physiological capacity to respond to stressors, individuals from industrial areas could become compromised if frequently exposed to high intensity stressors (Chávez-Zichinelli et al., 2010). In a conceptually similar study evaluating the stress response of salamanders within areas of varying habitat quality, Homan et al. (2003) was able to detect differences in corticosterone concentrations among individuals inhabiting sites with varying degrees of deforestation. Homan et al. (2003) found that individuals inhabiting more disturbed areas had lower baseline and stress-induced corticosterone concentrations then individuals from less disturbed sites. As proposed by these authors, a lower stress response among individuals within disturbed sites may indicate a significant reduction in the ability of an individual to cope with additional stressors in the future (Homan et al., 2003). Similar research as it pertains to fish is uncommon, with an exception being a long-term research project on contaminated and reference streams in Tennessee where a variety of biochemical, physiological, condition, growth, bioenergetic, and nutritional responses were monitored in sentinel fish (Adams & Ham, 2011). The application of physiological approaches for better understanding animal–environment relationships could prove valuable as more stream habitats become impacted by anthropogenic disturbances (Walsh et al., 2005). Furthermore, as freshwater fish are among the most threatened vertebrates on Earth (Burton, 1995; Saunders et al., 2002), research coupling fish stress response to a range of land-use patterns could help inform management decisions on how to mitigate the impacts of human activities on aquatic landscapes.

As aquatic ecosystems continue to be affected by human land-use practices, there is an increasing need to assess the degree in which anthropogenic activities impact natural riverscapes and their resident fish populations. The application of in situ, effects-based assessments provides an opportunity to broadly monitor the biological responses of resident fish, when exposed to site-specific conditions characterizing different land use patterns (e.g., Fitzgerald et al., 1999; Doherty et al., 2005; Gray & Munkittrick, 2005). Effects-driven assessments are valuable when examining the consequences of inhabiting particular environments as the effects of all stressors, within the system, are pooled together, generating an “accumulated environmental state” rather than evaluating the impacts of stressors individually.

Within effects-based assessments, monitoring physiological indicators (e.g., energy transfer, nutritional condition, and metabolism; see Adams & Ham, 2011) has been identified as an appropriate method to assess the performance of an organism within its environment. Indeed, physiology is often regarded as the driving force behind organismal responses to environmental change (Wikelski & Cooke, 2006; Cooke & O'Connor, 2010). In particular, studies concerned with the consequences of stress on fish (e.g., Pickering et al., 1987; Fevolden et al., 1993) commonly use circulating plasma cortisol concentrations as a means to quantitatively evaluate individual stress responses (Wendelaar-Bonga, 1997; Barton, 2002). However, much of that work has occurred in the laboratory and/or in response to environmental toxicants and pollutants (e.g., Adams & Greely, 2000; Almeida et al., 2005; Camargo & Martinez, 2006) rather than in natural environments in response to a suite of natural and anthropogenic stressors. The stress response can extend to whole-organism changes such as those related to condition, growth, behaviour, locomotion, reproductive output, and survival (Schreck, 1990). These whole-organism changes presumably underlies variation in commonly measured population characteristics (e.g., abundance, size structure) or community structure (e.g., Munkittrick & Dixon, 1989; Gibbons & Munkittrick, 1994; Fitzgerald et al., 1999, Helms et al., 2005). However, few studies address stress in natural systems, or do so using endpoints that transcend levels of biological organization.

An alternative approach to studying stress involves the experimental manipulation of cortisol titers to raise circulating cortisol to levels observed in stressed fish (Gamperl et al., 1994). While many studies have found exogenous manipulation of cortisol titers useful in assessing the consequences of stress on fish, few experiments have applied this methodology outside the confines of a laboratory (e.g., Vijayan et al., 1996; DiBattista et al., 2005; O'Connor et al., 2010). Of the existing field research using exogenous cortisol manipulations, none have assessed whether the consequences of experimental challenges vary across systems of differing habitat quality. Studies of this nature can contribute to the understanding of the ecological consequences of stress on fish and provide insight as to whether alterations in habitat quality can influence the ability of fish to respond to multiple stressors—a fundamental question in ecology for some time (Huey, 1991). Moreover, such an experimental approach can help to reveal mechanistic relationships rather than simply identifying correlations.

In this study, we compared the health, condition, and survival of control, cortisol- and sham-treated fish inhabiting streams within reference, agricultural, and urban landscapes. Specifically, cortisol-treated fish received a single exogenous, intra-peritoneal injection of cortisol suspended in cocoa butter, which has been found to be an effective method in experimentally studying how chronic levels of plasma cortisol in teleost fish influence various biological endpoints (reviewed in Gamperl et al., 1994). Moreover, an implant of cortisol/coconut butter functions by slowly releasing hormone over an extended period ($\sim$3 to 5 days). The experimental elevation of cortisol challenges fish with an ecologically and physiologically relevant cortisol dosage and provides a controlled means of examining how fish respond to stress across different land use patterns. Creek chub (*Semotilus atromaculatus*) were used as our sentinel species as they occur in high numbers, inhibit streams of varying habitat quality, lack major fishing pressures, exhibit rapid growth, and maturation (Scott & Crossman, 1973; Fitzgerald et al., 1999), and have previously been shown to vary in condition relative to a variety of chemical pollutant stressors (e.g., Dubé et al., 2006).

Our research question centred on understanding if sentinel fish occupying streams associated with different types of land use responded differently to an experimental cortisol challenge. An experimental approach was used to evaluate fish response to cortisol elevations because past research (i.e., Blevins et al., 2013) has established that while baseline physiological parameters of creek chub are consistent across different land use patterns (i.e., agricultural and forested), fish originating from agricultural areas maintained physiological performance to ecologically relevant thermal and hypoxia challenges relative to fish within forested watersheds. However, results of this study also suggested that creek chub exhibit the ability to improve physiological performance within suboptimal environments as prolonged holding at high temperature removed landscape-level differences (Blevins et al., 2013). Here, we tested the null hypothesis that there is no difference in the health, condition, and survival of control, sham- and cortisol-treated fish across reference, agricultural, and urban landscapes. Since past studies have found creek chub behaviour and growth to be influenced by pollution

(Katz & Howard, 1955; Dubé et al., 2006) and turbidity levels (Gradall & Swenson, 1982), we predict that the health, condition, and survival would vary among habitat types, with fish at reference sites faring better than fish in agricultural or urban streams. Moreover, we anticipate that the response to the experimental cortisol challenge would result in negligible changes in health, condition, and survival in reference sites but comparatively more extreme negative consequences in agricultural and urban sites.

Materials and methods

Experimental animals and cortisol treatments

All fish were sampled under an Ontario Ministry of Natural Resources Scientific Collection Permit (Licence No.: 1061994; Granted to S.J.C) and were processed in adherence to the guidelines set out by the Canadian Council on Animal Care, as issued by Carleton University (B10-9). As creek chub generally spawn in the spring beginning at temperatures of 12.8°C (Scott & Crossman, 1973), all sampling occurred outside this range to avoid confounding effects of the reproductive period. Captured fish were individually anaesthetized in an induction bath of 70 ppm clove oil emulsified in ethanol (1 part clove oil to 10 parts ethanol; Anderson et al., 1997). Once unresponsive, fish were randomly distributed across the following three groups: cortisol-, sham-treated and control. Cortisol-treated fish received a single intra-peritoneal injection of 10 mg ml^{-1} of cortisol (hydrocortisone; Sigma H2882, Sigma-Aldrich) suspended in cocoa butter (*Cocos nucifera*; Sigma C1758, Sigma-Aldrich, St. Louis, MO) at 0.005 ml g^{-1} body weight (reviewed in Gamperl et al., 1994; but see O'Connor et al., 2010). Sham-treated fish received a single intra-peritoneal injection of pure cocoa butter at 0.005 ml g^{-1} body weight (i.e., without cortisol to evaluate the effects of the carrier and the injection procedure), while control fish were handled in a manner identical to treatment fish, but received no injections. This method of experimentally raising circulating plasma cortisol concentrations to physiologically and ecologically relevant level is an established technique used to study the effects of stressors on fish (Gamperl et al., 1994). The dosage administered was confirmed by Nagrodski et al. (unpublished data) to experimentally raise cortisol levels in creek chub to 753 ± 256 ng/ml, for ~3 days relative to control values of 203 ± 60 ng/ml. This cortisol dosage was intended to emulate the stress that might be encountered during ecologically relevant events such a hypoxic event (e.g., Herbert & Steffensen, 2005), a short-term starvation (e.g., McConnachie, 2010), heat shock (e.g., McConnachie et al., 2012), exposure to supercooling and frazil ice (e.g., Brown et al., 1999), droughts and floods (e.g., Flodmark et al., 2002). For context, wild creek chub exposed to heat shock in a laboratory experienced an 11× increase in cortisol titers relative to baseline values, reaching over 1,200 ng/ml (Blevins et al., 2013), validating that the experimental manipulations achieved in this study were realistic and ecologically relevant. After treatment, fish were placed in separate, aerated recovery bins (~52 l). All fish were also identified individually using fluorescent orange visual implant (VI) Alpha Tags (Northwest Marine Technology, Inc., Tumwater, WA) and collectively, according to treatment, by small partial caudal fin clips (Rounsefell & Kask, 1945). While cortisol- and sham-treated fish received upper and lower fin clips, respectively, control fish received both upper and lower fin clips. Prior to field application, a laboratory pilot study found that VI Alpha Tag had an ~88% retention rate after 14 days when implanted in the cheek epidermis of creek chub in an outdoor holding tank (~4290 l) at Queen's University Biological Field Station (QUBS; 44°31′N, 76°20′W).

Site selections

To quantify how reference, agricultural, and urban land-use patterns affect the health, condition, and survival of resident fish when exposed to an experimental cortisol challenge, three replicate streams for each land-use pattern were selected. Representative stream types (i.e., reference, urban, and agricultural) were selected using an extensive government database on all stream crossings in eastern Ontario. To select candidate sites a 100-m buffer was generated around each catchment using ArcView GIS (version 10, Environmental Systems Research Institute, Redlands, CA). Additional geospatial data mapping protocols were then used to characterize land use patterns for the various catchments associated with stream crossings. Replicate study streams based on the following predetermined parameters: reference streams had to be surrounded by: <5% developed area, <30% cropland, and <25 km of road running through the catchment, agricultural streams had to be surrounded by: >40% cropland, <20% developed area, and <25 km of road running through the catchment, and urban streams had to be surrounded by >30 developed area, and >30 km of road running through the catchment. Although these criteria were somewhat subjective, the choice of reference system sites (i.e., those deemed to be the least disturbed and essentially acting as controls) was consistent with the approach used by provincial and regional governments for the purposes of environmental monitoring. Reference sites included Stevens Creek (45°05'N, 75°47'W), Keelers Creek (44°48'N, 75°34'W), and Hobbs Drainage (45°10'N, 75°56'W), agricultural sites included the Middle Castor (45°12'N, 75°34'W), East Castor (45°09'N, 75°19'W), and a tributary of the Jock River (45°12'N, 75°52'W), while urban sites included Sawmill Creek (45°20'N, 75°37'W), Shields Creek (45°15'N, 75°33'W), and a stream off the Castor River (45°14'N, 75°28'W; Table 1, adapted from database provided by the Ministry of the Environment).

In-field setup

Standard battery-powered backpack electrofishing (Halltech Aquatic Research Inc., Guelph, ON; model Ht-2000) was used to collect creek chub at each of the above study sites. Pulsed-DC electrofishing is considered a safe tool for studying stream fish, and results by Gatz & Linder (2008) suggest this practice is unlikely to have meaningful biological effects on creek chub condition, growth, or movements. Also, all treatment groups were handled in an identical manner, so the results should not be influenced. Streams were sampled individually at approximately the same time everyday (~09:00). In an effort to reduce sampling biases across habitat types, creeks within different land-use patterns were sampled alternately (i.e., urban–agricultural–reference; Table 2). However, on one occasion the sampling order of a reference stream and an agricultural stream (i.e., Hobbs Drainage and a Tributary of the Jock River) were reversed. Before electrofishing at each site, a sampling reach of approximately 100–150 m was chosen, and on every sampling occasion the reach was lengthened to increase sample size (reach lengths reported in Table 1; electrofishing efforts reported in Table 2). While electrofishing, creek chub were collected and held in multiple bait pails that were periodically left submerged in the stream, with efforts made to minimize crowding. Once electrofishing was completed, all fish were gathered at a centralized location along the reach and held in aerated bins. Once treated and allowed to recover (as above), all fish were released in a centralized location that offered some protection/cover for fish.

In-field sampling

Electrofishing techniques were used to recapture study fish from each site ~25 days after injections. In addition, many streams were sampled twice in an effort to increase recapture rates. At this time, electrofishing efforts were extended further than the area where fish were collected for tagging (i.e.,

Table 1 Summary of riparian land-cover and associated classifications for study sites where reach length is the total length of stream sampled and road length represents the length of road through the catchment

Stream	Classification	Reach length (m)	Road length (km)	%Riparian land cover		
				Crop	Forest	Urban
Sawmill creek	Urban	336.6	30.1	3	55.9	32
Shields creek	Urban	501.5	35.9	10	13.1	54.8
Castor stream	Urban	510.5	32.8	19	34.5	38.3
Middle castor	Agricultural	534.7	4.6	40.1	30.5	16.5
East castor	Agricultural	610	14.4	74.1	25.1	0
Tributary of jock	Agricultural	739.3	0.4	86.9	13.1	0
Stevens creek	Reference	437.6	2	22	62	0
Keelers creek	Reference	260	8.1	26.6	67.9	0
Hobbs drainage	Reference	348.5	21.1	29.1	60	3.3

Values adapted from Eastern Ontario Reference Condition & Biocriteria Project database provide by the Ministry of the Environment

Table 2 The classification, date sampled, electrofishing effort for initial fish collection, and date re-visited for fish recapture at each of the study streams

Stream	Classification	Catch per unit effort	Dates (in 2011)		Days in-between sampling
			Injections	Recap	
Sawmill creek	Urban	0.016	June 07	July 02 and 04	25, 27
Middle castor	Agricultural	0.024	June 09	July 05 and 06	26, 27
Stevens creek	Reference	0.032	June 13	July 07 and 08	24, 25
Shields creek	Urban	0.053	June 15	July 10	25
East castor	Agricultural	0.037	June 20	July 14 and 15	24, 25
Keelers creek	Reference	0.074	June 21	July 16	25
Castor stream	Urban	0.033	June 24	July 19 and 20	25, 26
Hobbs drainage	Reference	0.032	June 29	July 21	22
Tributary of jock	Agricultural	0.016	June 30	July 25 and 26	25

~500 m upstream and downstream from initial study reach) and sampling was restricted to days when water clarity was sufficient to enable effective netting and thus not bias recapture rates. Although we did not measure turbidity, anecdotally there were no obvious visual differences in turbidity among streams/stream types during recapture electrofishing periods. When recaptured, ~0.2–0.4 ml of blood was collected via caudal puncture, using a combination of sodium heparinized 1 ml syringes with 25 gauge, 38 mm needles and sodium heparinized 0.5 ml insulin syringes with 28.5 gauge, 13 mm needles (Becton–Dickinson & Co. Tuberculin Slip Tip Syringes and 1/2 cc LO-DOSE U-100 Insulin Syringe, Franklin Lakes, NJ). Blood glucose levels were assessed using ~10 µl of whole blood with a hand-held glucose meter (ACCU-CHEK glucose meter; Roche Diagnostics, Basel, Switzerland) as previously validated by Cooke et al. (2008). Directly after blood sampling, fish were euthanized using cerebral percussion. Fish were placed individually into labeled Ziploc bags and transferred to a cooler of ice before being transported to an indoor facility for necropsy-based condition assessments. Euthanized fish were assessed for hepato-somatic index (HSI), condition factor (K), splenic somatic index (SSI), gonadosomatic index (GSI), and a modified health assessment index (HAI) ~6–8 h post-capture using wet weights (to 0.001 g; as described in Adams et al., 1993 and Barton et al., 2002 and using the refinements in McConnachie, 2010). More specifically, based on liver measurements, a major glycogen reserve in fish, the hepato-somatic index (HSI = (liver mass/body mass) × 100) was used as an indicator of individual energy and nutritional status (Chellappa et al., 1995). Similarly, condition factor (K = mass × length^{-3}) was used as an alternative, all-encompassing means of assessing nutrimental state (i.e., energy storage and feeding activity) as variations in length-weight measurements were monitored between treatment groups (Bolger & Connolly, 1989; Barton et al., 2002). The splenic index [SSI = (spleen mass/body mass) × 100] is commonly employed to assess immune function and disease resistance (e.g., Hadidi et al., 2008) and was therefore included to determine whether cortisol treatment impaired fish immune resilience compared to sham-treated and control groups. The gonadosomatic index (GSI = (testis or ovarian mass/body mass) × 100) was used to assess alterations in reproductive endocrine function to indicate a potential consequence of reproductive impairment of a treatment group (Thomas, 1988). Lastly, a modified HAI was employed to quantitatively assess the effects that elevated cortisol levels have on fish health, across different landscape patterns. To this end, index variables suspected to differ among treatment groups were assessed, based on necropsy observations, and were assigned numerical values according to the condition of the organs or tissues (sensu Adams et al., 1993). The variables chosen included: total parasite load (each organ [i.e., gills, liver, kidney, and spleen] affected was given a score of 0 = none, 5 = low, 10 = moderate, 15 = high), gill condition (fraying was given a score of 0 = none, 5 = low, 10 = moderate, 15 = high), and dermal fungus (cover was scored based on 0 = none, 5 = low, 10 = moderate, 15 = high) as previously outlined in McConnachie (2010). All assessments were conducted by the same individual to insure standardization.

Statistical analysis

Recapture rates of cortisol-, sham-treated and control fish were compared across land use types by using a 3 × 3 χ^2 test. A two-way mixed analysis of variance (ANOVA), with land type (i.e., reference, agricultural, or urban) entered as a fixed effect, and stream (nested within land type) entered as a random effect, was used to test for differences among responses variables across land-use types (i.e., blood glucose concentrations, HSI, K, SSI, GSI, and HAI; Zar, 1984). Levene's and Shapiro–Wilk tests were used to insure the assumptions of normality of the sampling distribution and homogeneity of variance were met (Zar, 1984). In the event that one of these assumptions was violated, square-root or log-transformations were employed. When a significant main effect or interaction was present, a Tukey–Kramer HSD post hoc test was employed to separate means. All statistical analyses were performed using JMP version 4 (SAS Institute, Cary, NC, USA), with exception to the χ^2 test which was performed using IBM SPSS Statistics 19.0 (IBM Corporation, Armonk, NY). The level of significance for all tests was set a priori as $\alpha = 0.05$.

Results

Contingency table analysis showed that over the course of the study there was no difference in recapture rates among land use types (Table 3; χ^2, $df = 2$, $P = 0.51$) or treatments (χ^2, $df = 2$, $P = 0.95$). Nevertheless, it is noteworthy that within agricultural streams, cortisol-treated fish were recaptured at a rate of almost half that of the control and sham-treated fish, a differences that was marginally non-significant (c^2, $df = 2$, $P = 0.060$).

None of the land type (ANOVA, $F = 0.6$, $df = 2$, 6, $P = 0.56$), treatment (ANOVA, $F = 0.5$, $df = 2$, 241, $P = 0.61$) or their interaction effects (ANOVA, $F = 1.5$, $df = 4$, 241, $P = 0.12$) had any influence on fish glucose concentrations. Similarly, condition factor did not vary by land use type (ANOVA, $F = 1.7$, $df = 2$, 6, $P = 0.26$), treatment (ANOVA, $F = 0.61$, $df = 2$, 255, $P = 0.54$) or the interaction between treatment and land use type (ANOVA, $F = 0.09$, $df = 4$, 255, $P = 0.98$).

While HSI did not significantly differ across land types (ANOVA, $F = 0.7$, $df = 2$, 6, $P = 0.53$), treatment (ANOVA, $F = 8.8$, $df = 2$, 254, $P < 0.01$) and the interaction between treatment and land use (ANOVA, $F = 3.8$, $df = 4$, 254, $P < 0.01$) had an effect. More specifically, within the agricultural watersheds, the HSI of fish receiving a sham treatment was ~20% greater than fish in the control treatment (Tukey HSD, $P < 0.05$). Conversely, SSI was not found to be significantly different across land types (ANOVA, $F = 5.1$, $df = 2$, 6, $P = 0.051$), treatments (ANOVA, $F = 1.1$, $df = 2$, 254, $P = 0.33$), or between their interactions (ANOVA, $F = 0.6$, $df = 4$, 254, $P = 0.66$). Likewise, no land type (ANOVA, $F = 0.3$, $df = 2$, 6, $P = 0.75$), treatment (ANOVA, $F = 0.48$, $df = 2$, 253, $P = 0.62$) or interaction effect (ANOVA, $F = 1.1$, $df = 4$, 253, $P = 0.35$) was found to effect GSI values.

No difference in total parasite load was found across land use (ANOVA, $F = 0.2$, $df = 2$, 6, $P = 0.81$), treatment (ANOVA, $F = 2.8$, $df = 2$, 205, $P = 0.61$) or their interactions (ANOVA, $F = 0.6$, $df = 4$, 205, $P = 0.65$). Gill fraying was also unaffected by land use (ANOVA, $F = 0.3$, $df = 2$, 6, $P = 0.73$), treatment (ANOVA, $F = 1.9$, $df = 2$, 254, $P = 0.15$) and their interaction (ANOVA, $F = 0.16$, $df = 4$, 254, $P = 0.96$).

Discussion

Our approach involved identifying streams that were broadly characterized as either reference, urban, or agricultural, and then experimentally manipulating the cortisol concentration of creek chub in replicates of each stream type. Generally speaking, agricultural streams were relatively shallow, contained little foliage within the riparian zone, and possessed few forms of structure within the watercourse, which can provide cover and protection to resident fish. Agricultural activity requires clearing of foliage, which can lead to increases in water temperature, sediment load, and nutrient inputs (Muscutt et al., 1993; Matson et al., 1997). Of our chosen study sites, agricultural streams were found to contain the lowest percentage of forest habitat, which has been found to decrease the overall abundance of fish as the length of non-forested

Table 3 Total number of creek chub captured, released and recaptured ~25 days posttreatment per land-use type and means (±SE) of response variables, ~25 days after treatment, across reference, agriculture, and urban streams

Variable	Averages ± SE								
	Reference streams			Agricultural streams			Urban streams		
	Control	Cortisol	Sham	Control	Cortisol	Sham	Control	Cortisol	Sham
Total fish captured	135	124	124	99	101	102	109	108	107
Fish recaptured	35	36	37	26	16	30	27	32	21
Percent	25.9	29	29.8	26.3	15.8	29.4	24.8	29.6	19.6
Recaptured (%)									
Glucose (mmol l^{-1})[a]	2.3 ± 0.07	2.3 ± 0.1	2.3 ± 0.1	2.7 ± 0.07	2.5 ± 0.2	2.6 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.4 ± 0.1
K ($10^{-5} \pm 10^{-7}$)	1.02 ± 1.3	1.03 ± 1.4	1.03 ± 1.6	1.01 ± 1.6	1.02 ± 1.9	1.03 ± 1.7	1.05 ± 1.9	1.07 ± 1.7	1.07 ± 2.2
HSI[a]	2.2 ± 0.1	2.8 ± 0.2	2.6 ± 0.1	1.9 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	3.0 ± 0.1
SSI[b]	0.3 ± 0.01	0.3 ± 0.02	0.3 ± 0.02	0.3 ± 0.05	0.4 ± 0.05	0.4 ± 0.03	0.4 ± 0.02	0.4 ± 0.02	0.4 ± 0.02
GSI	0.8 ± 0.08	0.8 ± 0.09	0.6 ± 0.09	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.8 ± 0.1
Total parasite load (out of 60)[b]	16.4 ± 2.1	18.4 ± 2.2	15.6 ± 2.1	6.7 ± 1.0	12.6 ± 2.5	10.2 ± 2.0	15.2 ± 2.2	16.2 ± 1.9	15.7 ± 2.1
Gill fraying (out of 15)	5 ± 0.8	6.4 ± 0.8	5.6 ± 0.8	5.3 ± 0.9	6.2 ± 1.5	6.2 ± 0.9	4.2 ± 0.8	6.4 ± 1.0	4.8 ± 0.9

[a] Variables whose data was square root transformed

[b] Variables whose data was log-transformed

riparian patches increases (Jones et al., 1999). Indeed, anecdotally we observed the lowest abundance of creek chub in agricultural streams (Nagrodski, unpublished data). Comparatively, urban streams were found to have small buffered areas (ranging between 0 and 30 m), consisting of mostly trees and foliage, separating the surrounding residential areas from the watercourse. While sampling our study sites, urban streams were also found to have the highest number of culverts, storm water inputs, length of road through the catchment (>30 km), and in-stream litter (mainly in the form of residential dumping and litter). Consequences associated with high levels of urbanization surrounding streams include hydrographs with more frequent transitions between base flows and high flows, elevated concentrations of nutrients and contaminants, and altered channel morphology which can translate to effects on population dynamics (reviewed in Walsh et al., 2005). Although our reference sites contained the most forested land cover, some levels of development made it impossible to consider them as truly pristine environments. Nonetheless, land-use activities and their associated impacts on habitat quality were certainly reduced relative to the other two land-use types. Hence, we are confident that fish in the three stream types experienced differential challenges and environmental conditions prior to exposing a subset of them to experimental cortisol elevations. We are confident that when ranked from most to least degraded the order of stream types was agricultural > urban > reference.

Despite chronically elevating circulating plasma cortisol concentration for a ~3 day period, we did not observe a significant effect of land-use type or treatment on survival when all factors were incorporated into a contingency table analysis. However, if one looks only at the agricultural site, the cortisol-treated fish were recaptured ~50% less frequently than controls and sham-treated fish, albeit a difference that was not statistically significant (i.e., $P = 0.060$). Nonetheless it was consistent with our prediction in that agricultural streams were the most degraded and survival was reduced in cortisol-treated fish relative to control and sham-treated fish. Moreover, that finding is consistent with work on birds and amphibians where stress responses are mediated by habitat quality (Homan et al., 2003; Chávez-Zichinelli et al., 2010). Clearly additional research is needed with larger sample sizes to improve power and verify the finding or determine if it is spurious.

Overall, we failed to recapture ~75% of fish that we tagged in the experiment. We used recapture as a proxy for survival and there were several limitations with that approach. First, it assumes that our marks will be retained. However, as VI Alpha tags exhibited poor retention rates in the field, we relied largely on fin clips to differentiate between treatments groups. We have no reason to think that tag loss rates should vary among treatments or land-use types supporting our use of this proxy for survival. Our initial goal was to also measure growth of individuals but significant loss of VI Alpha tags meant that we could not track individuals through time and thus could only rely on condition factor to assess energy status. In particular, the loss of VI Alpha tags prohibited us from detecting whether growth depression occurred across treatment groups, which has been documented as a consequence of stress in fish (e.g., Ali et al., 2003). Another issue with mark recapture approaches is that, to evaluate survival, one must assume that animals are restricted to the study reach. That was not the case in the current study, and other work by our group (Nagrodski et al., Unpublished manuscript) has revealed using radio telemetry that creek chub are somewhat mobile and tend to exhibit leptokurtic movement distributions. Finally, a third assumption of mark-recapture studies is that gross behaviour is similar among treatments (i.e., cortisol, sham, and control) such that probability of recapture is equal among groups. Previous work by Nagrodski et al. (Unpublished manuscript) revealed that behaviour (i.e., activity levels, gross movements) was similar between control and cortisol-treated fish in a single stream (would equate to an urban stream in this study), suggesting that this third assumption was not violated. As such, we are confident in the relative differences among groups and systems is a proxy for survival, but suggest that future research quantify survival of individuals, possibly with passive integrated transponders or radio telemetry, such that fish survival can be evaluated over greater temporal and spatial scales.

We did not observe any significant differences in health (i.e., parasite burden) or condition (i.e., condition factor, hepato-somatic, splenic, and gonado-somatic indices, blood glucose) of creek chub relative to land-use or experimental treatment. However, one exception occurred within the agricultural watersheds, where we observed that sham-treated fish (which were similar to cortisol-treated fish) had HSI values that were approximately 20% higher than control fish. Although an increase in HSI values can suggest exposure to contaminants (e.g., Fletcher et al., 1982), while a decrease is an indicator of chronic environmental stress (e.g., Lee et al., 1983), we believe this difference is more likely attributed to the relatively low number of recaptured fish within the agricultural watersheds. In light of the fact that all other response variables do not vary, and that both sham and control treatments have functionally very similar effects on fish, it is likely that this finding is spurious. It is also worth noting that the HSI values of control fish in agricultural streams were lower than other land-use types which may be the driver of this relationship. Nevertheless, the general lack of variation across treatments groups and land-use types is surprising as previous work has shown chronic elevation of cortisol levels can lead to negative consequences for fish, including reduce body weight and condition factor (Davis et al., 1985), hepatic glycogen content (Peters et al., 1980), and immune function (Maule et al., 1989). Moreover, as studies in the past have also been able to make connections between deleterious environmental conditions and reduced growth (Pickering, 1993), immune function (Bly & Clem, 1992), and gill morphology (Laurent & Perry, 1991) our results indicating that fish respond similarly to cortisol manipulation across land type is equally surprising.

There are a number of factors that could have contributed to our failure to detect sub-lethal impairments. For example, it is possible that given our inability to recapture ~75% of fish that were experimentally manipulated that our physiological and condition-based sampling focused on the healthiest fish that were still alive. However, given that recapture rates were rather consistent across most treatments; this would likely only be a reasonable scenario for the cortisol-treated fish in the agricultural treatment where we did observe evidence of treatment-induced mortality. Also possible is that there was selection for stress-resistant fish prior to our experimentation in the perturbed systems such that the animals that were manipulated represented different genotypes and phenotypes than in the reference system. Common garden studies would be needed to determine if that was the case. Another explanation is simply that the time-course used in this study was inappropriate for documenting organism-level changes in condition-related metrics. Experiments were conducted during the late spring and summer spanning the period of highest water temperatures which we regarded as a stressful period in which we might expect sublethal differences among land-use types and cortisol treatments to be maximal. Between initial treatment (including cortisol manipulation) and sampling we allowed ~25 days to pass. We did so based on the fact that a previous study (i.e., McConnachie et al., 2012) revealed that sublethal differences in fish health and response to stressors were evident some 30 days after initial injection of fish with cortisol in a similar manner to that done in this study. Moreover, as noted above, future studies would benefit from being able to track changes in individual condition, something not possible here due to high loss of individually numbered VI tags.

Results from this study suggest that, in the wild, there may be compensatory mechanisms that enable creek chub to persist despite exposure to significant challenge(s). We reason that the compensatory mechanisms employed by creek chub may include: (i) down-regulation of central nervous system or glucocorticoid receptors, (ii) blockage of the hypothalamic–pituitary–adrenal axis thus reducing further secretion of glucocorticoids and accentuating the consequences of the stress response, or (iii) stimulation of an alternate axis capable of counteracting the consequences associated with elevated glucocorticoids. However, as creek chub are regarded as a rather tolerant organism it is unknown if other species would respond differently, or if greater levels of experimental stress or habitat degradation would result in negative alterations in fish health, condition, and survival. Pottinger et al. (2000) has identified the European chub (*Leuciscus cephalus*), a close relative of the creek chub, as having high blood cortisol levels with low cortisol receptor affinity, which may offset some adverse effects associated with high circulating plasma cortisol concentrations. Although these results do not directly apply to creek chub, our result suggest that this species may possess a trait similar to the European chub, making them more tolerant as opposed to other traditional species studied (e.g., salmonids, centrarchids). As noted by Pottinger et al. (2000), similar strategies for glucocorticoid resistance has been documented in some species of rodents and New World primates which may also make them more resistant to stressful events (e.g., Reynolds et al., 1997; Taymans et al., 1997; Hastings et al., 1999). Although it is possible that the cortisol injection dosage or duration did not disrupt the fish from homeostasis to a degree that would be required to see changes in the sublethal variables that were measured or to induce mortality, we believe that to not be the case. As noted above, heat shock of creek chub in a laboratory environment (i.e., Blevins et al., 2013) elevated cortisol titers to a similar level as in our study confirming the ecological relevance of the dosage.

When encountering environmental stress associated with anthropogenic alternations to natural landscapes, animals can exhibit physiological and behavioural responses that can have population level effects on resident fish species (Ricklefs & Wikelski, 2002). Given that we may have observed reduced survival in cortisol-treated fish in what we regarded as the most degraded (i.e., agricultural streams), there was still remarkable resilience of creek chub to what we regard as a significant stressor in that health, condition, and physiology were not affected (i.e., only HSI significant but not for cortisol). While it is difficult for our results to be extrapolated to other fish species, any compensatory mechanisms that allowed creek chub to overcome our cortisol challenge potentially exist in other species. As such, further experimentation to determine whether fish native to reference steams have similar reactions to challenges, when transferred to urban or agricultural streams, may be of benefit as reference fish with reduced familiarity to anthropogenic stressors may have a lower capacity to cope with multiple imposed challenges.

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