



Cortisol modulates metabolism and energy mobilization in wild-caught pumpkinseed (*Lepomis gibbosus*)

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Abstract Acute elevation of cortisol via activation of the hypothalamic-pituitary-interrenal (HPI) axis aids the fish in dealing with a stressor. However, chronic elevation of cortisol has detrimental effects and has been studied extensively in lab settings. However, data pertaining to wild teleosts are lacking. Here, we characterized the metabolic consequences of prolonged cortisol elevation (96 h) in wild-caught pumpkinseed (*Lepomis gibbosus*). Pumpkinseed were implanted with cocoa butter alone (sham) or containing cortisol (25 mg kg⁻¹ body weight), and at 24, 48, 72, and 96 h,

tissue samples were collected, whole-body ammonia excretion was determined, and whole-organism metabolism was assessed using intermittent flow respirometry. Cortisol-treated pumpkinseed exhibited the highest plasma cortisol concentration at 24 h post-implantation, with levels decreasing over the subsequent time points although remaining higher than in sham-treated fish. Cortisol-treated fish exhibited higher standard and maximal metabolic rates than sham-treated fish, but the effect of cortisol treatment on aerobic scope was negligible. Indices of energy synthesis/mobilization, including blood glucose concentrations, hepatosomatic index, hepatic glycogen concentrations, and ammonia excretion rates, were higher in cortisol-treated fish compared with controls. Our work suggests that although aerobic scope was not diminished by prolonged elevation of cortisol levels, higher metabolic expenditures may be of detriment to the animal's performance in the longer term.

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Introduction

One of the central aims of the stress axis in vertebrates is to provide the necessary physiological adjustments to facilitate the reestablishment of internal homeostasis in response to a stressor. These adjustments include shifts in energy mobilization/allocation and/or the induction of relevant ion/acid-base regulatory systems (Sapolsky

et al. 2000; Romero et al. 2009; Schreck and Tort 2016). Over the last century, much research has focused on how stress influences the metabolism of vertebrates. Yet, the current literature relies heavily upon lab-based models with limited assessment of wild vertebrates (Hawlena and Schmitz 2010; Boonstra 2013; Breuner et al. 2013), particularly wild teleost fishes. In a large number of studies on wild fishes, cortisol's actions on energy metabolism are restricted to measurements of secondary responses (e.g., blood glucose, plasma ions, and hepatosomatic index; Cook et al. 2012; McConnachie et al. 2012; Zolderdo et al. 2016), with little investigation of the specific physiological pathways mediating these changes (Sopinka et al. 2015). In particular, the literature is deficient in descriptions of the specific metabolic pathways regulated by cortisol in wild-caught teleosts, in contrast to hatchery-raised fishes, as well as information on how various aspects of metabolic rate (e.g., standard and maximal metabolic rates, aerobic scope) are modulated by cortisol.

It has been well-established that exposure to a range of stressors elicits an increase in circulating cortisol titres in a diversity of teleosts (Wood et al. 1999; Jentoft et al. 2005; Cook et al. 2012; Lawrence et al. 2018; reviewed in Barton and Iwama 1991 and Barton 2002), although the magnitude and timing of the cortisol response are often context- and species-specific (Barton 2002; Cook et al. 2012; Winberg et al. 2016; Lawrence et al. 2018). The rise in circulating cortisol, which typically occurs over minutes to hours following a stressor, generally assists in the *de novo* synthesis of high energy substrates (i.e., gluconeogenesis), the reallocation of energetic reserves towards essential processes involved in stressor mitigation (e.g., suppression of growth and reproduction), and the reestablishment of hydromineral balance (reviewed in Mommsen et al. 1999, Aluru and Vijayan 2009, and Schreck and Tort 2016), responses that generally include increased plasma glucose levels (Pickering et al. 1982; Vijayan et al. 2003; McConnachie et al. 2012; Lawrence et al. 2017) as well as increased activity/expression of enzymes associated with gluconeogenesis (Aluru and Vijayan 2009; Momoda et al. 2007; Wiseman et al. 2007; reviewed in Mommsen et al. 1999). Additionally, cortisol is believed to have a role in the regulation of nitrogenous waste excretion in teleost fishes, possibly reflecting a role of cortisol in mediating proteolysis (Mommsen et al. 1999). Indeed, prior work has shown that cortisol treatment can enhance ammonia excretion

rates in teleosts (Chan and Woo 1978; Liew et al. 2013) while also potentially being an important regulatory hormone in enhancing activities/expression of critical proteins (see Nawata and Wood 2009; Tsui et al. 2009; Lawrence et al. 2015; Liew et al. 2015) involved in ammonia excretion (reviewed in Wright and Wood 2009). Cortisol thus exerts considerable influence over the regulation of the organism's energy metabolism. As stressor mitigation is generally considered to be energetically expensive (Barton and Schreck 1987; O'Connor et al. 2010; Schreck and Tort 2016), the actions of cortisol provide the animal with ample access to energetic reserves to facilitate homeostatic adjustments.

Acute activation of the glucocorticoid stress axis is generally considered to be beneficial to the organism in responding to a stressor (Schreck and Tort 2016). However, under the reactive scope model (Romero et al. 2009), sustained release of glucocorticoids can have a deleterious impact on the organism's physiological status because resources are diverted away from basic physiological requirements. This "homeostatic overload" (Romero et al. 2009) reflects the classical notion of "chronic" stress. In teleosts, the effects of homeostatic overload or chronic stress typically manifest as reduced growth (Sadoul and Vijayan 2016) and reproduction (Pankhurst 2016), as well as impaired immune function (Yada and Tort 2016). Homeostatic overload can also have consequences in a metabolic context. Aerobic scope (AS), or scope for activity, represents the energy available for non-maintenance activities and is the difference between the maximum metabolic rate (MMR) and the standard metabolic rate (SMR; i.e., aerobic scope = MMR – SMR; Fry 1947). Typically, available energy is allocated towards fitness-enhancing activities including growth, reproduction, immune function, and predator avoidance (reviewed in Guderley and Pörtner 2010 and Sokolova 2013). Given that cortisol elevation in teleosts generally results in increased routine (Barton and Schreck 1987; Chan and Woo 1978; Morgan and Iwama 1996; De Boeck et al. 2001; Herrera et al. 2012) and standard (O'Connor et al. 2010) metabolic rates, it may also reduce AS and therefore lower the allocation of energy towards fitness-enhancing activities. In an ecological context, this may have implications for predator evasion capacity (Mesa et al. 1994; Lawrence et al. 2017), growth dynamics (O'Connor et al. 2010), and reproductive output (Algera et al. 2017a, b). Despite the potential role of the hypothalamic-pituitary-interrenal

(HPI) axis in modulating metabolic rate in fishes, to date, there has been little research in this area. Indeed, the current literature lacks work addressing the influence of cortisol on maximal metabolic rate, aerobic scope, and recovery dynamics in teleost fishes despite these parameters being determinants of organismal performance (Guderley and Pörtner 2010; Eliason and Farrell 2016; Brownscombe et al. 2017). Furthermore, only a handful of studies have ascertained the impacts of cortisol on standard metabolic rates (O'Connor et al. 2010). To our knowledge, no studies have addressed the role of cortisol in affecting maximal metabolic rate. Maximal metabolic rate is typically set by cardiovascular performance (Norin and Clark 2016). However, some evidence suggests that cortisol has little bearing on cardiac performance in adult fish (Farrell et al. 1988). In some cases, though, cortisol can have negative impacts on the structural integrity of cardiac tissue in teleosts which could conceivably result in impaired cardiovascular performance (Johansen et al. 2011, 2017). Because cortisol-metabolism interactions have the potential to modulate an animal's fitness, understanding the underlying mechanisms is of critical importance in determining organismal responses to stressors, a significant consideration in the Anthropocene where human activity is having substantial impacts on the environment (Madliger et al. 2017).

The present study examined the role of cortisol in modulating standard and maximal metabolic rates, aerobic scope, and recovery dynamics in wild pumpkinseed (*Lepomis gibbosus*; Linnaeus 1758). To complement these whole-body metrics, a suite of tissue and blood energy metabolites was characterized. We hypothesized that prolonged cortisol elevation would increase standard metabolic rate, with no effect on maximal metabolic rate, thereby reducing the animal's aerobic scope.

Materials and methods

Animal collection, care, and implantation

Juvenile pumpkinseed ($N=285$; mass = 24.3 ± 0.7 g; total length = 110.6 ± 0.4 mm) were captured in Lake Opinicon (44.5590° N, 76.3280° W) in the months of July, August, and September 2016. Animals were collected (OMNRF permit no. 1082340) in shallow weedy bays using a seine net and were transported to Queen's

University Biological Station (Chaffey's Lock, ON, Canada). Fish were held in flow-through tanks (~ 435 L) supplied with natural lake water ($T=25.2 \pm 0.1$ °C; $\text{NH}_4^+ < 0.25$ mg L⁻¹; pH = 7.5) under natural photoperiod. During holding, fish were not fed. Pumpkinseed were allowed to acclimate to these holding conditions for 48 h prior to experimentation. Experimental protocols were approved by the Carleton University Animal Care Committee (AUP no. 104262) in compliance with the guidelines of the Canadian Council for Animal Care.

Pumpkinseed were randomly selected and given an intraperitoneal implant of cocoa butter (5 mL kg⁻¹ body weight (BW)) either alone as a control (sham) or containing cortisol (hydrocortisone 21-hemisuccinate; 25 mg kg⁻¹ BW). This method of elevating circulating cortisol has been validated for use in teleost fishes (Gamperl et al. 1994). Cocoa butter containing cortisol was prepared as described in Hoogenboom et al. (2011). Briefly, the hydrocortisone 21-hemisuccinate salt was dissolved in ethanol to distribute the cortisol evenly throughout the cocoa butter (i.e., avoid clumping of the cortisol). Pre-warmed cocoa butter was then added to the mixture and heated to 75 °C to evaporate the ethanol. Handling procedures associated with implantation were not likely to have substantially affected the variables measured here as prior work with bluegill sunfish, a close relative of the pumpkinseed sunfish, demonstrated comparable stress-related parameters between no-treatment controls and sham-treated fish (see McConnachie et al. 2012).

The use of cortisol-containing cocoa butter implants for elevating plasma cortisol concentrations in teleosts is well-known to yield variable results in plasma cortisol titres (reviewed in Gamperl et al. 1994; Sopinka et al. 2015; Crossin et al. 2016). Such cortisol treatment can produce supraphysiological levels of cortisol in the blood that are not reflective of an endogenous cortisol response (e.g., see McConnachie et al. 2012 and Lawrence et al. 2017), or individuals may clear the hormone rapidly (i.e., in a few hours), failing to meet the criterion for chronic exposure and making interpretation of treatment effects difficult (see Foster and Moon 1986). Thus, to avoid confounding effects associated with fish in which the implant was ineffective (plasma cortisol concentration not elevated at the sampling time) or too effective (plasma cortisol concentration elevated beyond

the desired maximum of 140 ng mL^{-1}), individuals that exhibited plasma cortisol concentration outside of the desired range of 36 to 140 ng mL^{-1} within the first 24 h of implantation were excluded from analyses of blood or tissue variables. This approach was not possible for individuals used in respirometry or ammonia excretion trials because plasma samples could only be collected post-experiment, when circulating cortisol levels would have been vulnerable to stress associated with the experiment and handling, and the implants would have been past their effective period. Following implantation, all fish of a single treatment group (25 individuals) were transferred to a holding tank ($\sim 211 \text{ L}$) under holding conditions as described above. However, pumpkinseed used in 24 h respirometry and metabolite flux experiments (see below) were transferred immediately to respirometry chambers following implantation. All fish were maintained in a fasted state throughout the experiments.

Experiment 1: Tissue level effects of exogenous cortisol elevation

Shoals of cortisol- or sham-treated pumpkinseed were maintained in holding tanks for 96 h. Starting at 24 h post-implantation, four fish per treatment group per day were selected haphazardly from the shoal and blood and liver tissues were collected for analysis. Individual fish were captured with a small dip net, taking great care to minimize disturbance of conspecifics. A blood sample ($\sim 300 \mu\text{L}$) was collected immediately via caudal venipuncture using a 23 G needle and a chilled, heparinized (Na^+ heparin, $10,000 \text{ USP units mL}^{-1}$; Sandoz Canada Inc., Boucherville, QC, Canada) 1-mL syringe. Blood glucose concentration was immediately determined using a portable, medical-grade glucose meter (Accu-Chek Compact Plus, Hoffman-La Roche Limited, Mississauga, ON, Canada) that was previously validated for use with teleost fishes (Wells and Pankhurst 1999; Serra-Llinares and Tveiten 2012; Stoot et al. 2014). The remaining blood was centrifuged for 2 min ($2000g$; Mandel Scientific, Guelph, ON, Canada). Plasma was decanted, flash frozen in liquid nitrogen, and stored at $-80 \text{ }^\circ\text{C}$ for subsequent analysis of concentrations of total ammonia (T_{amm} ; T_{amm} indicates total ammonia concentration, whereas NH_4^+ and NH_3 refer to the ammonium ion and non-ionic ammonia, respectively) and cortisol. Following blood sampling, the fish was quickly euthanized via cerebral percussion and wet mass

and total length were measured. The liver was excised, weighed for determination of hepatosomatic index (HSI; see below), freeze clamped in liquid nitrogen, and stored at $-80 \text{ }^\circ\text{C}$ for later determination of hepatic glycogen and ammonia concentrations (see below). This process was repeated every 24 to 96 h, the final time at which samples were collected. This sampling protocol was carried out at a total of 5 times (total $N=20/\text{day}/\text{treatment}$) with the final sample size for blood/tissues selected based on the aforementioned procedure (i.e., individual fish within a physiologically relevant range of cortisol; see “[Animal collection, care, and implantation](#)” for further details).

Plasma T_{amm} concentrations were assessed using a commercially available enzyme-linked assay kit (Raichem, Cliniqua, San Marcos, CA, USA) and microplate reader as in Lawrence et al. (2015). A commercial radioimmunoassay kit (ImmuChem Cortisol Coated Tube RIA Kit, MP Biomedicals, Solon, OH, USA) was used to measure plasma cortisol concentration. This assay was previously validated for use in teleost fishes (Gamperl et al. 1994). Intra- and inter-assay coefficients of variation were 8.7 and 4.7%, respectively.

Hepatic glycogen content was determined as described by Keppler and Decker (1974). Liver tissue was sonicated ($\sim 20 \text{ s}$ on an ice-water slurry) in a perchloric acid (PCA; 6%) solution and centrifuged (5 min at $10,000g$). The resulting supernatant was pH balanced ($\text{pH} = 5.0$) with K_2HCO_3 and then incubated with a 1% amyloglucosidase solution for 2 h at $37 \text{ }^\circ\text{C}$. The reaction was terminated using 25 μL of 70% PCA. Hydrolyzed glycogen samples were then assessed for total glucose content using the hexokinase-linked glucose assay described by Bergmeyer (1974). Hepatic ammonia concentration was determined using a commercially available enzyme-linked assay kit (Raichem, Cliniqua, San Marcos, CA, USA) as described in Lawrence et al. (2015).

Experiment 2: Effects of exogenous cortisol elevation on ammonia excretion

To investigate the effect of exogenous cortisol elevation on whole-body ammonia excretion and, consequently, metabolic functioning, T_{amm} excretion was determined using a simple flux chamber. As in previous studies (Wilson et al. 1994; Zimmer et al. 2010; Lawrence et al. 2015), flux chambers consisted of small ($\sim 1.5 \text{ L}$), blacked-out flow-through boxes supplied with

aerated, natural lake water. Immediately following injection of the cocoa butter implant, fish ($N = 25$ total; 14 cortisol-treated and 11 sham) were transferred to individual flux chambers; this point constituted time $t = 0$ h. Unlike the respirometry trials (see “[Experiment 2: Effects of exogenous cortisol elevation on ammonia excretion](#)”), fish were allowed to freely move within the container. Fish were assessed over a 96 h period with fluxes carried out every 24 h to match blood/tissue (“[Experiment 1: Tissue level effects of exogenous cortisol elevation](#)”) and respirometry trials (“[Experiment 3: Characterization of metabolic rate under elevated cortisol](#)”). To carry out a flux, water flow to the chamber was stopped, while maintaining aeration, for the 5 h period leading up to each experimental time point (i.e., 19–24 h, 43–48 h, 67–72 h, 91–96 h post-implant). Water samples (720 μL) were collected at the beginning and end of the 5 h flux period and were immediately frozen and stored at -20 °C for later analysis of T_{amm} concentration. At 96 h, fish were euthanized and weighed. Assessments of control (sham) and cortisol-treated fish were conducted simultaneously.

Water T_{amm} concentrations were determined using the colorimetric salicylate assay of Verdouw et al. (1978).

Experiment 3: Characterization of metabolic rate under elevated cortisol

Oxygen uptake rates ($\dot{M}\text{O}_2$) were assessed at either 24 h or 96 h post-implant. For each time point, three fish from each treatment group ($N = 6$ fish in total) were assessed simultaneously, and this procedure was repeated four times for both time points (for a total of $N = 12$ per treatment per time point). This approach was adopted because pilot studies revealed that continuous confinement of pumpkinseed in a respirometer for 96 h resulted in confinement stress and mortality. For each assessment of oxygen uptake, three fish from the same treatment group were placed into individual respirometry chambers held in a reservoir; separate reservoirs were used for different treatment groups. Treatment groups were assigned to reservoirs in a randomized fashion to avoid reservoir-induced biases. After transfer into the respirometry chambers, the animals were left undisturbed for ~ 29 – 35 h during which $\dot{M}\text{O}_2$ was continuously assessed by intermittent flow respirometry. Maximum metabolic rate (MMR) was then assessed for each fish by removing an individual from its

chamber, manually chasing it for 3 min and then exposing it to air for 1 min as per the recommendations of Norin and Clark (2016). Animals were then returned to their chamber, and $\dot{M}\text{O}_2$ measurements resumed immediately to capture MMR. Fish were allowed to recover for ~ 15 h. At the end of the trial, fish were euthanized via cerebral percussion, weighed, and measured for total length.

Intermittent flow respirometry as described in Norin et al. (2014) and Clark et al. (2011) was used to measure $\dot{M}\text{O}_2$. Respirometers were held in a reservoir (~ 435 L) supplied with fresh lake water that was well-aerated and thermostatted to 25 °C using submersible aquarium heaters. The water was replaced after every experimental series. Each respirometer consisted of a rectangular, polypropylene plastic box (~ 1.3 L) fitted with a recirculation loop that had its own independent flow (~ 5 L min^{-1} ; Eheim Universal 300 model 1046, Germany). A set of baffles on either end smoothed flow through respirometer and restricted the movement of the fish. The oxygen concentration of the water in the recirculation loop was measured continuously (0.5 Hz), under the control of Pyro Oxygen Logger software (V2.312; Pyroscience, Germany), using an oxygen sensor spot (Pyroscience, Aachen, Germany) coupled to an optical oxygen meter (Firesting O_2 , Pyroscience). The flush loop of each chamber consisted of a port attached to a large water pump (flush pump; ~ 40 L min^{-1} ; Atman PH 2000, China) that was under the control of an automatic timer. Based on previous research (Crans et al. 2015) and pilot work, the cycle used for these experiments consisted of a 7-min flush period (i.e., flush pump is running) followed by a 3-min closed (i.e., flush pump is off), measurement period. The slope of the decline in oxygen concentration during the measurement period was used to determine $\dot{M}\text{O}_2$. This analysis was conducted in Labchart (V 7.0.2; ADInstruments, Dunedin, New Zealand). Background rates of oxygen consumption (i.e., no fish in chamber) were determined before and after each experimental trial and were subtracted from the fish’s $\dot{M}\text{O}_2$. Chambers were cleaned routinely to avoid elevated background $\dot{M}\text{O}_2$.

Measurements of $\dot{M}\text{O}_2$ were derived during closed respirometry phases where the relationship between water O_2 concentration and time had $r^2 > 0.9$. Standard metabolic rate was calculated as the average of the lowest 10th percentile of all $\dot{M}\text{O}_2$ values for an individual fish (Chabot et al. 2016). Routine metabolic rate (RMR) was considered to be the average of all $\dot{M}\text{O}_2$

values over the 12 h period from midnight to noon (6 h D: 6 h L) prior to measurement of MMR. Maximum metabolic rate was calculated as the single highest value following the chase/air exposure event (Norin and Clark 2016). Aerobic scope was calculated as both absolute scope ($AS_a = MMR - SMR$) and as factorial scope ($AS_f = MMR - SMR^{-1}$). Recovery time was taken to be the period from chasing/air exposure to the time when a fish's $\dot{M}O_2$ returned to within 10% of its RMR (RMR_{10} ; Lee et al. 2003) over three consecutive time points. Excess post-exercise oxygen consumption (EPOC) was determined by integrating the area under the $\dot{M}O_2$ curve and subtracting RMR_{10} from it (Lee et al. 2003).

Calculations and statistical analyses

Hepatosomatic index (HSI) was calculated as in Busacker et al. (1990) using $HSI = (m_L/m_f) \times 100\%$ where m_L is the wet mass of the liver (g) and m_f corresponds to the total mass of the fish (g). T_{amm} flux (J_{amm}) was calculated as the difference in water T_{amm} concentrations between the initial and final water samples (M_{diff}), taking chamber volume (V), fish mass (m), and flux time (t) into account such that $J_{amm} = (M_{diff} \times V)/(t \times m)$.

All statistical analyses were conducted using SigmaPlot v11.0 (Systat Software Inc., San Jose, CA, USA). Unless otherwise noted, all data are presented as mean \pm 1 SEM (N) with statistical significance being accepted at $\alpha = 0.05$. A two-way analysis of variance (ANOVA) was employed to compare treatment and time effects for all blood, tissue, and metabolic parameters. Ammonia excretion was analyzed using a two-way repeated measures ANOVA with time and treatment group as the two factors. When statistical significance was detected, Tukey's HSD post hoc test was used.

Results

Plasma cortisol

Plasma cortisol concentrations were higher in cortisol-treated fish across all sampling times, relative to sham-treated individuals (Fig. 1). The time \times treatment interaction was found to be significant ($F = 6.097$; $df = 3$; $P = 0.001$). In cortisol-treated fish, plasma cortisol concentrations decreased by over 70% from 24 to 48 h, remaining relatively constant thereafter.

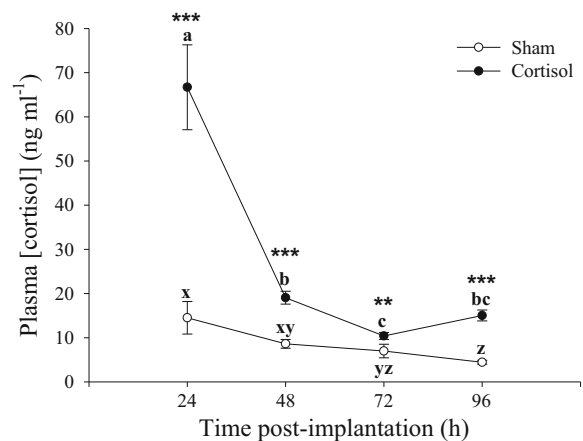


Fig. 1 Plasma [cortisol] of sham- (5 mL kg⁻¹ BW cocoa butter) and cortisol-treated (25 mg kg⁻¹ BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) over a 96 h sampling period for only those fish used in tissue assays ($N = 9$). Values are shown as means \pm 1 SEM. Statistical significance was accepted at $\alpha = 0.05$ with differences between treatment groups represented by an asterisk (** $P \leq 0.01$, *** $P \leq 0.001$) whereas different letters designate differences within a treatment group

Metabolic rate parameters

Cortisol-treated fish exhibited significantly higher SMR relative to shams ($F = 21.678$; $df = 1$; $P < 0.001$; Fig. 2a). In cortisol-treated fish, SMR was $\sim 20\%$ and 12% greater than values for the corresponding shams for pumpkinseed assessed at 24 h and 96 h, respectively. Cortisol treatment also resulted in a significant elevation of RMR relative to sham-treated fish ($F = 18.536$; $df = 1$; $P < 0.001$; Fig. 2b). For both SMR and RMR, neither the effect of time (SMR : $F = 2.340$; $P = 0.134$; $df = 1$; RMR : $F = 1.457$; $P = 0.234$; $df = 1$) nor the interaction of time and treatment group (SMR : $F = 1.368$; $P = 0.249$; $df = 1$; RMR : $F = 2.034$; $P = 0.162$; $df = 1$) was found to be significant.

MMR was also significantly higher in cortisol-treated pumpkinseed relative to sham-treated fish ($F = 7.240$; $df = 1$; $P = 0.010$; Fig. 2c). Measurement time was also found to have a significant influence on MMR ($P = 0.017$), with fish measured at 24 h post-implant having significantly higher MMR than fish measured at 96 h (Fig. 2c). However, the interaction of time and treatment group was not significant ($P = 0.182$). The similar rises in both SMR and MMR with cortisol treatment resulted in comparable AS_A ($F = 2.159$; $df = 1$; $P = 0.150$; Fig. 2d) and AS_F ($F = 1.901$; $df = 1$; $P = 0.176$; Table 1) between sham- and cortisol-treated fish. Time was found to have a significant

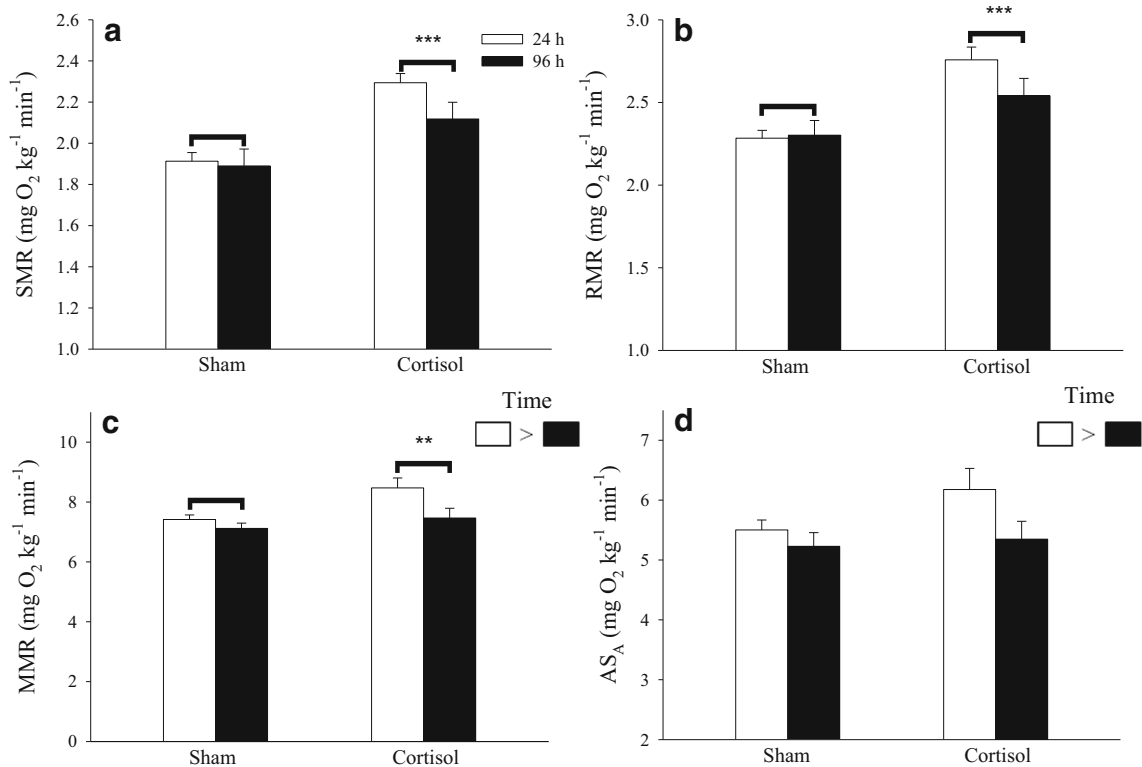


Fig. 2 The influence of sham- (5 mL kg⁻¹ BW cocoa butter) and cortisol-treatment (25 mg kg⁻¹ BW cortisol in cocoa butter) on pumpkinseed (*Lepomis gibbosus*) metabolic parameters over a 96 h sampling period. The metabolic parameters measured included (a) standard metabolic rate (SMR; $N = 11$), (b) routine metabolic rate (RMR; $N = 11$), (c) maximal metabolic rate (MMR; $N =$

11), and (d) absolute aerobic scope (AS_A ; $N = 11$). Values are shown as means \pm 1 SEM. Statistical significance was accepted at $\alpha = 0.05$ with differences between treatment groups represented by an asterisk (** $P \leq 0.01$, *** $P \leq 0.001$). Significant effects of time ($P < 0.05$) are indicated on the plot by the open box being greater than the filled boxed symbol

influence on AS_A ($P = 0.048$; Fig. 2d), with neither AS_A nor AS_F displaying a significant interaction term ($P > 0.05$).

Recovery dynamics were generally unaffected by cortisol treatment. Both recovery time ($P = 0.404$; Table 1) and effort (i.e., EPOC; $P = 0.506$; Table 1) were similar between treatment groups, with fish taking approximately 5 h to return to RMR_{10} .

Blood and tissue metabolites

Blood glucose concentrations were significantly higher in cortisol-treated fish compared with sham-treated fish ($F = 52.836$; $df = 1$; $P < 0.001$; Fig. 3a). Although there was a significant influence of time on blood glucose concentrations ($F = 3.376$; $df = 3$; $P = 0.024$), no significant interaction was detected between time and treatment group ($F = 2.480$; $df = 3$; $P = 0.069$). Plasma T_{amm} concentrations were not significantly affected by either

measurement time ($F = 0.474$; $df = 3$; $P = 0.702$) or treatment group ($F = 0.887$; $df = 1$; $P = 0.351$) (Fig. 3b).

Cortisol treatment in pumpkinseed resulted in elevation of both HSI ($F = 20.994$; $df = 1$; $P < 0.001$; Fig. 4a)

Table 1 The effect of cortisol implantation (25 mg kg⁻¹ BW) on factorial scope, excess post-exercise oxygen consumption (EPOC), and recovery time in pumpkinseed (*Lepomis gibbosus*) 24 h and 96 h post-implantation. No significant differences were observed between treatment groups or sampling time points (two-way ANOVA, $P > 0.05$). Values are shown as means \pm 1 SEM with $N = 11$ in all cases

Parameter	Sham		Cortisol	
	24 h	96 h	24 h	96 h
Factorial scope	3.9 \pm 0.1	3.8 \pm 0.2	3.7 \pm 0.2	3.6 \pm 0.2
EPOC (mg O ₂ kg ⁻¹)	277 \pm 16	327 \pm 42	300 \pm 55	271 \pm 19
Recovery time (h)	5.0 \pm 0.5	5.2 \pm 0.5	4.8 \pm 0.7	4.8 \pm 0.7

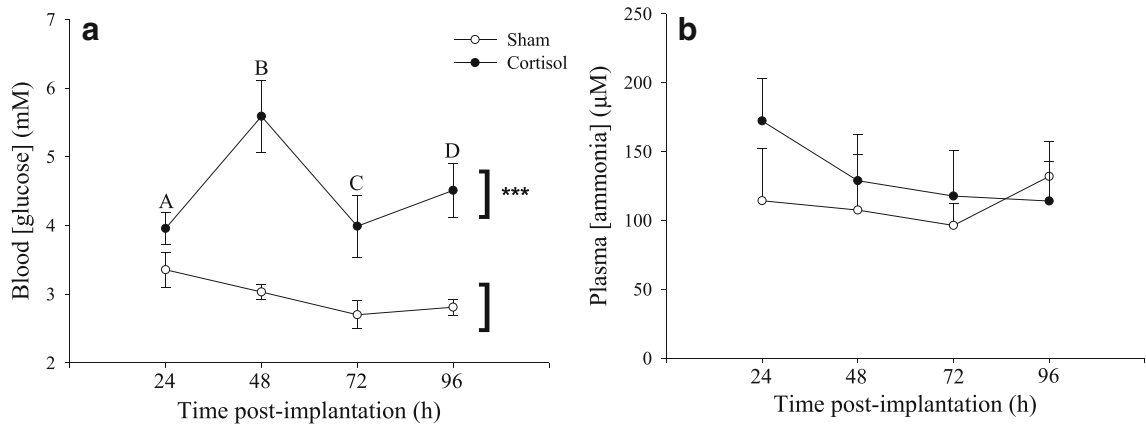


Fig. 3 Blood [glucose] (**a**; $N=9$) and plasma [ammonia] (**b**; $N\leq 9$) for sham- (5 mL kg^{-1} BW cocoa butter) and cortisol-treated (25 mg kg^{-1} BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) over a 96 h sampling period. Values are shown as means

± 1 SEM. Statistical significance was accepted at $\alpha=0.05$ with differences between treatment groups represented by an asterisk ($***P<0.001$). Capital letters denote a statistically significant effect of time ($P<0.05$)

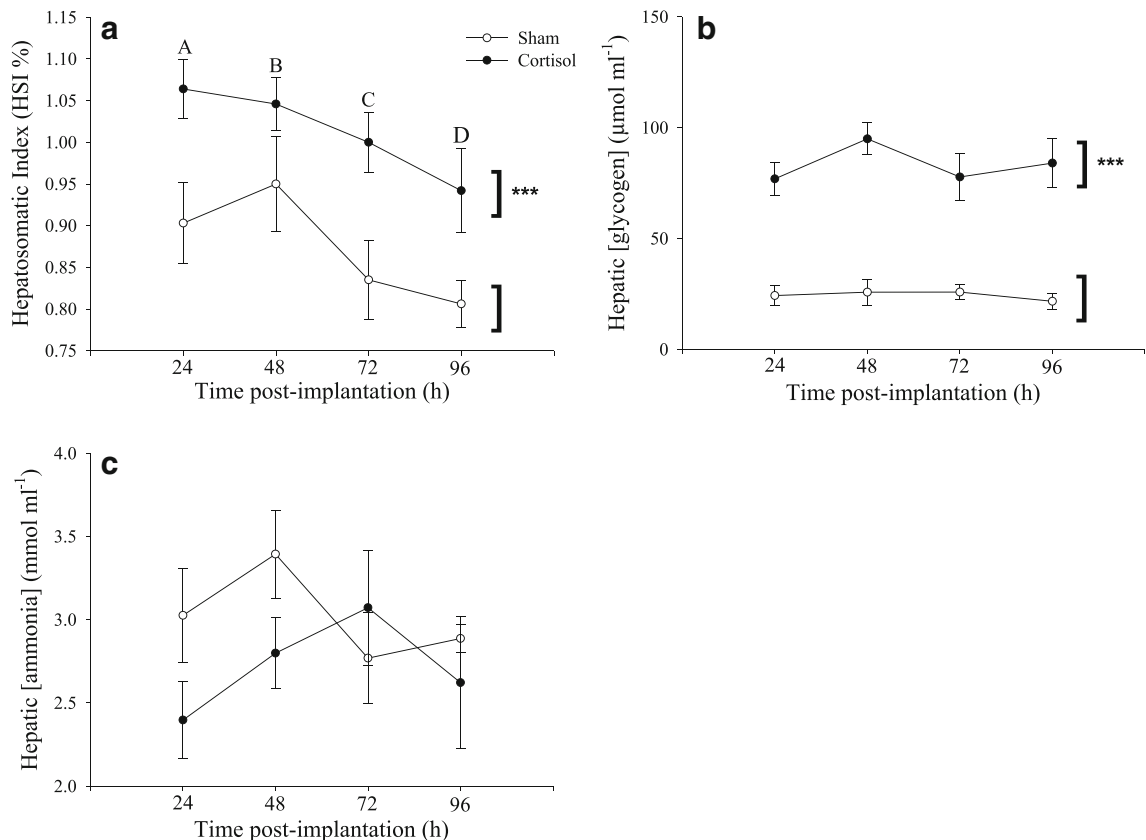


Fig. 4 Hepatosomatic index (**a**; $N\leq 9$), hepatic [glycogen] (**b**; $N\leq 9$), and total hepatic [ammonia] (T_{amm} ; **c**; $N\leq 6$) for sham- (5 mL kg^{-1} BW cocoa butter) and cortisol-treated (25 mg kg^{-1} BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) over a 96 h sampling period. Values are shown as means ± 1 SEM.

Statistical significance was accepted at $\alpha=0.05$ with differences between treatment groups represented by an asterisk ($***P\leq 0.001$). Capital letters denote a statistically significant effect of time ($P<0.05$)

and liver glycogen content ($F = 120.163$ $df = 1$; $P < 0.001$; Fig. 4b). However, unlike hepatic glycogen concentrations ($F = 0.504$; $df = 3$; $P = 0.681$), HSI was found to decrease across sampling times ($F = 3.559$; $df = 3$; $P = 0.019$; Fig. 4b); in neither case was the interaction of treatment group and sampling time significant. Hepatic T_{amm} concentrations were unaffected by sampling time ($F = 0.763$; $df = 3$; $P = 0.524$), treatment ($F = 1.783$; $df = 1$; $P = 0.192$; Fig. 4c), or the interaction of these two factors ($F = 1.443$; $df = 3$; $P = 0.251$).

Ammonia excretion

An interaction between effects of treatment group and flux time ($F = 7.249$; $df = 3$; $P < 0.001$; Fig. 5) was detected for ammonia excretion. Cortisol-treated fish exhibited a higher ammonia excretion rate over the first 48 h, relative to sham-treated fish, returning to sham levels by 72 h post-implant (Fig. 5). The ammonia excretion rate peaked at 24 h in cortisol-treated fish and was $\sim 1.9\times$ higher than the corresponding sham value.

Discussion

Overview

Cortisol treatment of wild-caught pumpkinseed using intraperitoneal implants revealed effects of prolonged cortisol elevation on both SMR and MMR. Higher

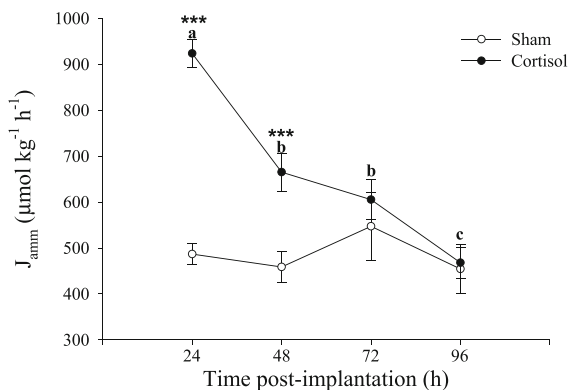


Fig. 5 Whole-body ammonia excretion rate for sham- (5 mL kg^{-1} BW cocoa butter) and cortisol-treated (25 mg kg^{-1} BW cortisol in cocoa butter) pumpkinseed (*Lepomis gibbosus*) in individual pumpkinseed monitored over a 96 h period. Values are shown as means \pm 1 SEM. Statistical significance was accepted at $\alpha = 0.05$ with differences between treatment groups represented by an asterisk (***) ($P \leq 0.001$), whereas different letters designate differences within a treatment group

SMR likely reflects increased maintenance costs under cortisol elevation whereas higher MMR could be the result of several factors, including adrenergic sensitization, enhanced mitochondrial capacity, and/or increased branchial/cutaneous O_2 uptake. Neither AS_A nor recovery was affected by prolonged cortisol elevation, suggesting that physiological performance would not be impaired. Cortisol treatment appeared to modulate carbohydrate metabolism by increasing blood glucose levels and hepatic glycogen content. Despite the absence of changes in plasma and hepatic ammonia concentrations, cortisol treatment elicited higher rates of ammonia excretion in pumpkinseed, which was likely a result of either increased turnover of proteins or enhanced ammonia transport capacity. This work contributes to our knowledge of cortisol effects on metabolism by focusing on SMR, MMR, and AS_A , and the specific metabolic pathways that are impacted by prolonged cortisol elevation, in a wild-caught teleost fish, a group that is underrepresented in the literature.

Validation of cortisol implants

Cortisol implants have been widely used as a mechanism of elevating plasma cortisol levels over sustained durations in a number of teleost species (Basu et al. 2001; DiBattista et al. 2005; Lawrence et al. 2017; reviewed in Gamperl et al. 1994 and Sopinka et al. 2015), including wild centrarchids (O'Connor et al. 2009; McConachie et al. 2012; Zolderdo et al. 2015; Algera et al. 2017b). This method attempts to mimic the sustained cortisol titres in blood that would be reflective of a semi-chronically stressed state (Carmichael et al. 1984; Pickering and Pottinger 1989; Sloman et al. 2001; Lankford et al. 2005). However, it is important to realize that cortisol represents only one component of the stress response, which also includes neuroendocrine inputs that were not manipulated in this work (Sopinka et al. 2015, 2016). By excluding individuals in which the cortisol-cocoa butter implant was either not effective or elevated cortisol above physiologically relevant levels, the pumpkinseed used in the present study exhibited plasma cortisol titres that were typical of those observed under a natural, acute stressor (e.g., 66 ng mL^{-1} peak here; Davis and Parker 1986; Cook et al. 2012; Lawrence et al. 2019; reviewed in Barton and Iwama 1991). The sharp decrease in plasma cortisol concentrations from 24 to 48 h post-implant is typical of this methodology, with plasma cortisol titres stabilizing

at an elevated level over more chronic timeframes (Vijayan et al. 1991; Gamperl et al. 1994; McConnachie et al. 2012; i.e., to 96 h in the present study). It is possible, although unlikely, that repeated netting of fish in the shoal for blood/tissue sampling may have influenced some of the physiological metrics observed here.

Cortisol's influence on whole-body metabolism

As predicted, both standard and routine metabolic rates were higher in wild-caught cortisol-treated pumpkinseed relative to shams. Similarly, previous studies in teleost fishes reported that cortisol treatment can increase routine (Barton and Schreck 1987; Chan and Woo 1978; Morgan and Iwama 1996; De Boeck et al. 2001; Liew et al. 2013) and standard metabolic rates (O'Connor et al. 2010), as can chronic stress (24 h social stress; Sloman et al. 2000). Even longer, 28 days, exposure to stress elevated SMR to approximately 30% higher than the control value in green sturgeon (*Acipenser medirostris*; Lankford et al. 2005). The higher SMR observed in the present study likely stems from increased maintenance costs associated with cortisol's regulatory actions (e.g., impacts on protein synthesis, energy substrate formation, and ionoregulation; reviewed in Wendelaar Bonga 1997 and Schreck and Tort 2016). Supporting this notion are the higher blood glucose and hepatic glycogen concentrations, as well as the higher whole-body ammonia excretion rate observed in cortisol-treated pumpkinseed. Interestingly, prolonged elevation of circulating cortisol titres has been shown to reduce locomotory activity in teleosts (Overli et al. 2002; Algera et al. 2017a), suggesting that the changes in routine metabolism observed here likely reflected alterations in internal metabolic processes rather than behavior.

Cortisol treatment also resulted in higher MMR relative to sham-treated pumpkinseed. Information pertaining to the influence of cortisol on MMR in fishes appears to be lacking in the literature. In green sturgeon, exposure to a randomized, chronic stressor had no influence on MMR but elevated SMR and lowered AS (Lankford et al. 2005). In common carp (*Cyprinus carpio*), cortisol treatment via an implant resulted in higher $\dot{M}O_2$ during active swimming when compared with sham-implanted and control fish (Liew et al. 2013), but MMR was not assessed. The present work appears to be the first to directly investigate the role of cortisol

on MMR. In teleosts, MMR is dictated primarily through the animal's ability to deliver oxygen to its tissues (i.e., cardiac performance) and oxygen extraction by the tissues (Fry and Hart 1948; Clark et al. 2011; Eliason et al. 2011; reviewed in Farrell et al. 2009 and Norin and Clark 2016). Cortisol may sensitize adrenergic responsiveness of the cardiovascular system, thereby enhancing oxygen delivery to tissues (e.g., Reid et al. 1992; Perry and Reid 1993; Reid et al. 1996; reviewed in Perry and Capaldo 2011). Alternatively, citrate synthase activity has been found to be higher under elevated blood cortisol in teleosts (Foster and Moon 1986; Tripathi and Verma 2003), and this enzyme is considered to be a proxy for mitochondrial density and aerobic capacity (Johnston 1981; Torres and Somero 1988). Another possibility is that cutaneous and gill oxygen uptake may increase in fish with elevated plasma cortisol levels, which would increase MMR (Farrell et al. 2014) but this idea remains highly speculative and unproven. Clearly, the mechanisms underlying changes in MMR with cortisol treatment require further study.

In teleost fishes, AS represents the available aerobic energy that can be allocated towards fitness-related activities (e.g., growth, reproduction, and swimming) and is calculated as the difference between SMR and MMR (Fry 1947; Guderley and Pörtner 2010; Sokolova 2013). Our results (AS unaffected by cortisol treatment) contrast with our prediction that cortisol would constrain AS of pumpkinseed by increasing SMR. To our knowledge, the present study is the first investigating whether cortisol plays a direct role in modulating AS. However, using repeated stress in green sturgeon over 28 days, Lankford et al. (2005) reported increased SMR and reduced AS_A relative to control fish. Because organismal performance is thought to reflect AS (e.g., swimming capacity, reproduction, and growth; Fry 1947; Guderley and Pörtner 2010; Sokolova 2013), our results suggest that performance, at the physiological level, should not be impacted by semi-chronic (i.e., 24–96 h) cortisol elevation in pumpkinseed. However, we remain cautious in this interpretation because the greater SMR under cortisol treatment likely would require increased food intake (Brett and Groves 1979; Metcalfe 1986; Gregory and Wood 1999). At the same time, cortisol reduces food conversion efficiency (Gregory and Wood 1999; Bernier et al. 2004) and acts as an anorexigenic agent at high doses (Gregory and Wood 1999; Bernier et al. 2004; Madison et al. 2015). Thus, if pumpkinseed

are unable to meet their basic nutritional demands, there may be physiological consequences of elevated SMR such as reduced growth. Furthermore, increased time spent foraging to meet dietary requirements could have implications for predator avoidance, thereby impacting organismal fitness (reviewed in Lima and Dill 1990 and Godin 1997).

Regulation of carbohydrate metabolism by cortisol

In teleost fishes, cortisol acts as the primary glucocorticoid promoting increased gluconeogenic capacity and the diversion of energy resources away from non-essential processes (reviewed in Wendelaar Bonga 1997 and Schreck and Tort 2016). Elevation of circulating cortisol increases blood glucose levels in a variety of teleost fishes under various environmental settings (e.g., Soivio and Oikari 1976; Pickering et al. 1982; Vijayan et al. 1997; Suski et al. 2007; McConnachie et al. 2012; Lawrence et al. 2017; reviewed in Mommsen et al. 1999). In centrarchids, this effect has been well-characterized following exposure to stressors (Carmichael et al. 1984; Gustavson et al. 1991; Suski et al. 2003; Cook et al. 2012; Lawrence et al. 2018) or cortisol implants (McConnachie et al. 2012; Zolderdo et al. 2016). In line with the literature, our cortisol-treated pumpkinseed demonstrated significantly higher blood glucose concentrations.

Although cortisol can have variable effects on hepatic glycogen content in teleost fishes (Storer 1967; Foster and Moon 1986), in the present study, as in other works (Butler 1968; Inui and Yokote 1975; Chan and Woo 1978; De Boeck et al. 2001; Laiz-Carrión et al. 2002, 2003; Vijayan et al. 2003), cortisol elicited higher hepatic glycogen content. Teleost hepatic glycogen content is the net product of glycogen synthesis (catalyzed by glycogen synthase) and catabolism (i.e., glycogen phosphorylase; reviewed in Faught and Vijayan 2016). Cortisol treatment in teleosts has been found to lower glycogen phosphorylase activity (Laiz-Carrión et al. 2002, 2003; Milligan 2003) and increase glycogen synthase transcripts/activity (Milligan 2003; Leung and Woo 2010), effects that could account for the increase in glycogen content noted in cortisol-treated pumpkinseed. In contrast, however, cortisol has also been shown to increase the expression of glycogen phosphorylase (Baltzegar et al. 2014), highlighting the variation within the literature (Mommsen et al. 1999).

Nitrogenous waste metabolism

Whole-body ammonia excretion was higher under cortisol treatment, suggesting that amino acid turnover was likely elevated (Wood et al. 1999; Lawrence et al. 2015). In teleosts, ammonia is the primary nitrogenous waste product and is formed from the transdeamination of amino acids (reviewed in Wright 1995 and Wright and Wood 2009). Thus, heightened protein turnover is often associated with elevated ammonia production and excretion (Smith 1929; Wood et al. 1999; Lim et al. 2001; Wood et al. 2007; Zimmer et al. 2010; Lawrence et al. 2015). Because cortisol regulates protein turnover and amino acid metabolism (reviewed in Mommsen et al. 1999), a rise in cortisol is expected to be accompanied by increased ammonia production/excretion (Storer 1967; Chan and Woo 1978; Hopkins et al. 1995; Wood et al. 1999; Liew et al. 2013, 2015; Lawrence et al. 2015), which is consistent with the pumpkinseed data presented here. However, this effect may not be ubiquitous as prior studies have also failed to detect an effect of cortisol treatment on ammonia excretion rates in teleosts (De Boeck et al. 2001; McDonald and Wood 2004). In addition to modifying rates of ammonia synthesis, cortisol could also enhance ammonia excretion through regulation of Rhesus (Rh) glycoproteins (Wright and Wood 2009). While evidence remains sparse, a few studies have reported that expression of Rh glycoproteins coincides with elevated cortisol titres in teleosts (Nawata and Wood 2009; Tsui et al. 2009; Lawrence et al. 2015; Liew et al. 2015). An upregulation of Rh glycoproteins in cortisol-treated pumpkinseed could account for the observation of higher ammonia excretion rates in the absence of net change in plasma ammonia concentrations. Clearly, additional research is required to investigate the role of cortisol in relation to ammonia production and excretion in teleosts especially given the important role of nitrogenous waste excretion on the metabolic operation of teleost fishes (Wright 1995). Together, these data suggest that cortisol is an important mediator of protein metabolism in pumpkinseed.

Conclusions

Despite higher SMR with cortisol treatment, AS was unaffected in pumpkinseed, owing to concomitantly higher MMR in cortisol-treated fish. Thus, aerobic

performance is unlikely to be impaired under prolonged cortisol elevation. Additionally, cortisol treatment resulted in hyperglycemia paired with increased stores of glycogen in the liver and increased whole-body ammonia excretion rates. Thus, energy mobilization and storage appeared to be enhanced under cortisol treatment. Our results are among the first characterizations of the direct role of the HPI axis, specifically cortisol, in mediating metabolic dynamics in a wild-caught teleost fish. While providing insight into the specific physiological mechanisms by which cortisol exerts an effect on the metabolic operation of a teleost, this work is also relevant to understanding the “ecology of stress” (Boonstra 2013) in wild-caught animals—an important consideration in the ever-changing Anthropocene (Madliger et al. 2017).

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References

- Algera DA, Brownscombe JW, Gilmour KM, Lawrence MJ, Zolderdo AJ, Cooke SJ (2017a) Cortisol treatment affects locomotor activity and swimming behaviour of male smallmouth bass engaged in paternal care: a field study using acceleration biologgers. *Physiol Behav* 181:59–68
- Algera DA, Gutowsky LF, Zolderdo AJ, Cooke SJ (2017b) Parental care in a stressful world: experimentally elevated cortisol and brood size manipulation influence nest success probability and nest-tending behavior in a wild teleost. *Fish Physiol Biochem Zool* 90(1):85–95
- Aluru N, Vijayan MM (2009) Stress transcriptomics in fish: a role for genomic cortisol signaling. *Gen Comp Endocrinol* 164(2):142–150
- Baltzegar DA, Reading BJ, Douros JD, Borski RJ (2014) Role for leptin in promoting glucose mobilization during acute hyperosmotic stress in teleost fishes. *J Endocrinol* 220(1): 61–72
- Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr Comp Biol* 42(3):517–525
- Barton BA, Iwama GK (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 BA, Schreck CB (1987) Metabolic cost of acute physical stress in juvenile steelhead. *Trans Am Fish Soc* 116(2):257–263
- Basu N, Nakano T, Grau EG, Iwama GK (2001) The effects of cortisol on heat shock protein 70 levels in two fish species. *Gen Comp Endocrinol* 124(1):97–105
- Bergmeyer H (1974) Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, Gawehn K (eds) *Methods of enzymatic analysis*, vol 3. Academic Press, Cambridge, pp 1196–1201
- Bernier NJ, Bedard N, Peter RE (2004) Effects of cortisol on food intake, growth, and forebrain neuropeptide Y and corticotropin-releasing factor gene expression in goldfish. *Gen Comp Endocrinol* 135(2):230–240
- Boonstra R (2013) The ecology of stress: a marriage of disciplines. *Funct Ecol* 27(1):7–10
- Borowiec BG, Darcy KL, Gillette DM, Scott GR (2015) Distinct physiological strategies are used to cope with constant hypoxia and intermittent hypoxia in killifish (*Fundulus heteroclitus*). *J Exp Biol* 218(8):1198–1211
- Brett JR, Groves TDD (1979) Physiological energetics. In: Hoar W, Randall DJ, Brett JR (eds) *Fish physiology*, vol VIII. Academic Press, Cambridge, pp 280–352
- Breuner CW, Delehanty B, Boonstra R (2013) Evaluating stress in natural populations of vertebrates: total CORT is not good enough. *Funct Ecol* 27(1):24–36
- Brownscombe JW, Cooke SJ, Algera DA, Hanson KC, Eliason EJ, Burnett NJ, Danylchuk AJ, Hinch SG, Farrell AP (2017) Ecology of exercise in wild fish: integrating concepts of individual physiological capacity, behavior, and fitness through diverse case studies. *Integr Comp Biol* 57(2):281–292
- Bucking C (2017) A broader look at ammonia production, excretion, and transport in fish: a review of impacts of feeding and the environment. *J Comp Physiol B* 187(1):1–18
- Busacker GP, Adelman IR, Goolish EM (1990) Growth. In: Schreck CB, Moyle PB (eds) *Methods for fish biology*. American Fisheries Society, Bethesda, pp 363–387
- Butler DG (1968) Hormonal control of gluconeogenesis in the North American eel (*Anguilla rostrata*). *Gen Comp Endocrinol* 10(1):85–91
- Carmichael GJ, Tomasso JR, Simco BA, Davis KB (1984) Characterization and alleviation of stress associated with hauling largemouth bass. *Trans Am Fish Soc* 113(6):778–785
- Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. *J Fish Biol* 88(1):81–121
- Chan DK, Woo NY (1978) Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. *Gen Comp Endocrinol* 35(3):205–215
- Chrousos GP (2009) Stress and disorders of the stress system. *Nature Rev* 5(7):374
- Clark TD, Jeffries KM, Hinch SG, Farrell AP (2011) Exceptional aerobic scope and cardiovascular performance of pink

- salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *J Exp Biol* 214(18):3074–3081
- Cook KV, O'Connor CM, McConnachie SH, Gilmour KM, Cooke SJ (2012) Condition dependent intra-individual repeatability of stress-induced cortisol in a freshwater fish. *Comp Biochem Physiol A* 161(3):337–343
- Crans, K. D., Prancevicus, N. A., & Scott, G. R. (2015). Physiological tradeoffs may underlie the evolution of hypoxia tolerance and exercise performance in sunfish (Centrarchidae). *J Exp Biol*, 218(20): 3264–3275
- Crossin GT, Love OP, Cooke SJ, Williams TD (2016) Glucocorticoid manipulations in free-living animals: considerations of dose delivery, life-history context and reproductive state. *Funct Ecol* 30(1):116–125
- Davis KB, Parker NC (1986) Plasma corticosteroid stress response of fourteen species of warmwater fish to transportation. *Trans Am Fish Soc* 115(3):495–499
- De Boeck G, Alsop D, Wood C (2001) Cortisol effects on aerobic and anaerobic metabolism, nitrogen excretion, and whole-body composition in juvenile rainbow trout. *Physiol Biochem Zool* 74(6):858–868
- DiBattista JD, Anisman H, Whitehead M, Gilmour KM (2005) The effects of cortisol administration on social status and brain monoaminergic activity in rainbow trout *Oncorhynchus mykiss*. *J Exp Biol* 208(14):2707–2718
- Eliason EJ, Farrell AP (2016) Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. *J Fish Biol* 88(1):359–388
- Eliason EJ, Clark TD, Hague MJ, Hanson LM, Gallagher ZS, Jeffries KM, Gale MK, Patterson DA, Hinch SG, Farrell AP (2011) Differences in thermal tolerance among sockeye salmon populations. *Science* 332(6025):109–112
- Farrell AP, Eliason EJ, Sandblom E, Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Can J Zool* 87(10):835–851
- Farrell AP, Eliason EJ, Clark TD, Steinhausen MF (2014) Oxygen removal from water versus arterial oxygen delivery: calibrating the Fick equation in Pacific salmon. *J Comp Physiol B* 184:855–864
- Farrell, A. P., MacLeod, K. R., & Scott, C. (1988). Cardiac performance of the trout (*Salmo gairdneri*) heart during acidosis: effects of low bicarbonate, lactate and cortisol. *Comp Biochem Physiol A Physiol* 91(2):271–277
- Faught E, Vijayan MM (2016) Mechanisms of cortisol action in fish hepatocytes. *Comp Biochem Physiol B* 199:136–145
- Foster GD, Moon TW (1986) Cortisol and liver metabolism of immature American eels, *Anguilla rostrata* (LeSueur). *Fish Physiol Biochem* 1(2):113–124
- Fry FEJ (1947) Effects of the environment on animal activity. *Publ Ontario Fish Res Lab* 68:1–52
- Fry F, Hart JS (1948) The relation of temperature to oxygen consumption in the goldfish. *Biol Bull* 94(1):66–77
- Gamperl AK, Vijayan MM, Boutilier RG (1994) Experimental control of stress hormone levels in fishes: techniques and applications. *Rev Fish Biol Fish* 4(2):215–255
- Godin JGJ (1997) Evading predators. In: Godin JGJ (ed) Behavioural ecology of teleost fishes. Oxford University Press, Oxford, pp 191–236
- Gregory TR, Wood CM (1999) The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiol Biochem Zool* 72(3):286–295
- Guderley H, Pörtner HO (2010) Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. *Can J Zool* 88(8):753–763
- Gustaverson AW, Wydoski RS, Wedemeyer GA (1991) Physiological response of largemouth bass to angling stress. *Trans Am Fish Soc* 120(5):629–636
- Hawlena D, Schmitz OJ (2010) Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *Am Nat* 176(5):537–556
- Herrera M, Aragão A, Hachero I, Ruiz-Jarabo I, Vargas-Chacoff L, Mancera JM, Conceição L (2012) Physiological short-term response to sudden salinity change in the Senegalese sole (*Solea senegalensis*). *Fish Physiol Biochem* 38:1741–1751
- Herrera M, Ruiz-Jarabo I, Vargas-Chacoff L, De La Roca E, Mancera JM (2015) Metabolic enzyme activities in relation to crowding stress in the wedge sole (*Dicologlossa cuneata*). *Aquac Res* 46:2808–2818
- Hoogenboom MO, Armstrong JD, Miles MS, Burton T, Groothuis TG, Metcalfe NB (2011) Implantation of cocoa butter reduces egg and hatchling size in *Salmo trutta*. *J Fish Biol* 79(3):587–596
- Hopkins TE, Wood CM, Walsh PJ (1995) Interactions of cortisol and nitrogen metabolism in the ureogenic gulf toadfish *Opsanus beta*. *J Exp Biol* 198(10):2229–2235
- Inui Y, Yokote M (1975) Gluconeogenesis in the eel-IV gluconeogenesis in the hydrocortisone-administered eel. *Bull Jap Soc Scient Fish* 41:973–981
- Jentoft S, Aastveit AH, Torjesen PA, Andersen Ø (2005) Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol A* 141(3):353–358
- Johansen IB, Lunde IG, Røsjø H, Christensen G, Nilsson GE, Bakken M, Øverli Ø (2011) Cortisol response to stress is associated with myocardial remodeling in salmonid fishes. *J Exp Biol* 214(8):1313–1321
- Johansen IB, Sandblom E, Skov PV, Gräns A, Ekström A, Lunde IG et al (2017) Bigger is not better: cortisol-induced cardiac growth and dysfunction in salmonids. *J Exp Biol* 220(14): 2545–2553
- Johnston, I. A. (1981). Structure and function of fish muscles. *Symp Zool Soc Lond* 48:71–113.
- Keppeler D, Decker K (1974) Glycogen: determination with amyloglucosidase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, pp 1127–1131
- Laiz-Carrión R, Sangiao-Alvarellos S, Guzmán JM, Del Río MPM, Míguez JM, Soengas JL, Mancera JM (2002) Energy metabolism in fish tissues related to osmoregulation and cortisol action. *Fish Physiol Biochem* 27(3–4):179–188
- Laiz-Carrión R, Del Río MPM, Míguez JM, Mancera JM, Soengas JL (2003) Influence of cortisol on osmoregulation and energy metabolism in gilthead seabream *Sparus aurata*. *J Exp Zool A* 298(2):105–118
- Lankford SE, Adams TE, Miller RA, Cech JJ Jr (2005) The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. *Physiol Biochem Zool* 78(4):599–609
- Lawrence MJ, Wright PA, Wood CM (2015) Physiological and molecular responses of the goldfish (*Carassius auratus*)

- kidney to metabolic acidosis, and potential mechanisms of renal ammonia transport. *J Exp Biol* 218(13):2124–2135
- Lawrence MJ, Eliason EJ, Brownscombe JW, Gilmour KM, Mandelman JW, Cooke SJ (2017) An experimental evaluation of the role of the stress axis in mediating predator-prey interactions in wild marine fish. *Comp Biochem Physiol A* 207:21–29
- Lawrence M, Jain-Schlaepfer S, Zolderdo A, Algera D, Gilmour K, Gallagher A, Cooke SJ (2018) Are 3-minutes good enough for obtaining baseline physiological samples from teleost fish. *Can J Zool* 96:774–786
- Lawrence MJ, Zolderdo AJ, Godin JGJ, Mandelman JW, Gilmour KM, Cooke SJ (2019) Cortisol does not increase risk of mortality to predation in juvenile bluegill sunfish: a manipulative experimental field study. *J Exp Zool A* 331:253–261
- Lee CG, Farrell AP, Lotto A, Hinch SG, Healey MC (2003) Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O kisutch*) salmon following critical speed swimming. *J Exp Biol* 206(18):3253–3260
- Leung LY, Woo NY (2010) Effects of growth hormone, insulin-like growth factor I, triiodothyronine, thyroxine, and cortisol on gene expression of carbohydrate metabolic enzymes in sea bream hepatocytes. *Comp Biochem Physiol A* 157(3):272–282
- Liew HJ, Chiarella D, Pelle A, Faggio C, Blust R, De Boeck G (2013) Cortisol emphasizes the metabolic strategies employed by common carp, *Cyprinus carpio* at different feeding and swimming regimes. *Comp Biochem Physiol A* 166(3):449–464
- Liew HJ, Fazio A, Faggio C, Blust R, De Boeck G (2015) Cortisol affects metabolic and ionoregulatory responses to a different extent depending on feeding ration in common carp, *Cyprinus carpio*. *Comp Biochem Physiol A* 189:45–57
- Lim CB, Chew SF, Anderson PM, Ip YK (2001) Reduction in the rates of protein and amino acid catabolism to slow down the accumulation of endogenous ammonia: a strategy potentially adopted by mudskippers (*Periophthalmodon schlosseri* and *Boleophthalmus boddarti*) during aerial exposure in constant darkness. *J Exp Biol* 204(9):1605–1614
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68(4): 619–640
- Madison BN, Tavakoli S, Kramer S, Bernier NJ (2015) Chronic cortisol and the regulation of food intake and the endocrine growth axis in rainbow trout. *J Endocrinol* 226(2):103–119
- Madliger CL, Franklin CE, Hultine KR, van Kleunen M, Lennox RJ, Love OP, Rummer JL, Cooke SJ (2017) Conservation physiology and the quest for a “good” Anthropocene. *Conserv Physiol*. <https://doi.org/10.1093/conphys/cox003>
- McConnachie SH, O'Connor CM, Gilmour KM, Iwama GK, Cooke SJ (2012) Supraphysiological cortisol elevation alters the response of wild bluegill sunfish to subsequent stressors. *J Exp Zool A* 317(5):321–332
- McDonald MD, Wood CM (2004) The effect of chronic cortisol elevation on urea metabolism and excretion in the rainbow trout (*Oncorhynchus mykiss*). *J Comp Physiol B* 174(1):71–81
- Mesa MG, Poe TP, Gadomski DM, Petersen J (1994) Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. *J Fish Biol* 45:81–96
- Metcalf NB (1986) Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. *J Fish Biol* 28(5):525–531
- Milligan CL (2003) A regulatory role for cortisol in muscle glycogen metabolism in rainbow trout *Oncorhynchus mykiss*. *Walbaum J Exp Biol* 206(18):3167–3173
- Mommsen TP, French CJ, Hochachka PW (1980) Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. *Can J Zool* 58(10): 1785–1799
- Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Rev Fish Biol Fish* 9(3):211–268
- Momodata TS, Schwindt AR, Feist GW, Gerwick L, Bayne CJ, Schreck CB (2007) Gene expression in the liver of rainbow trout, *Oncorhynchus mykiss*, during the stress response. *Comp Biochem Physiol D* 2(4):303–315
- Morgan JD, Iwama GK (1996) Cortisol-induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout (*Oncorhynchus clarki clarki*) parr. *Fish Physiol Biochem* 15(5):385–394
- Nawata CM, Wood CM (2009) mRNA expression analysis of the physiological responses to ammonia infusion in rainbow trout. *J Comp Physiol* 179(7):799–810
- Norin T, Clark TD (2016) Measurement and relevance of maximum metabolic rate in fishes. *J Fish Biol* 88(1):122–151
- Norin T, Malte H, Clark TD (2014) Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *J Exp Biol* 217(2):244–251
- O'Connor CM, Gilmour KM, Arlinghaus R, Van Der Kraak G, Cooke SJ (2009) Stress and parental care in a wild teleost fish: insights from exogenous supraphysiological cortisol implants. *Physiol Biochem Zool* 82(6):709–719
- O'Connor CM, Gilmour KM, Arlinghaus R, Matsumura S, Suski CD, Philipp DP, Cooke SJ (2010) The consequences of short-term cortisol elevation on individual physiology and growth rate in wild largemouth bass (*Micropterus salmoides*). *Can J Fish Aquat Sci* 68(4):693–705
- Overli O, Kotzian S, Winberg S (2002) Effects of cortisol on aggression and locomotor activity in rainbow trout. *Horm Behav* 42(1):53–61
- Pankhurst NW (2016) Reproduction and development. In: Schreck CB, Tort L, Farrell AP, Brauner CJ (eds) *Fish physiology*, vol 35. Academic Press, Cambridge, pp 295–331
- Perry SF, Capaldo A (2011) The autonomic nervous system and chromaffin tissue: neuroendocrine regulation of catecholamine secretion in non-mammalian vertebrates. *Auton Neurosci* 165(1):54–66
- Perry SF, Reid SD (1993) β -Adrenergic signal transduction in fish: interactive effects of catecholamines and cortisol. *Fish Physiol Biochem* 11:195–203
- Pickering AD, Pottinger TG (1989) Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. *Fish Physiol Biochem* 7(1):253–258
- Pickering AD, Pottinger TG, Christie P (1982) Recovery of the brown trout, *Salmo trutta* L, from acute handling stress: a time-course study. *J Fish Biol* 20(2):229–244
- Reid SD, Moon TW, Perry SF (1992) Rainbow trout hepatocyte beta-adrenoceptors, catecholamine responsiveness, and effects of cortisol. *Am J Phys* 262(5):R794–R799

- Reid SG, Vijayan MM, Perry SF (1996) Modulation of catecholamine storage and release by the pituitary-interrenal axis in the rainbow trout, *Oncorhynchus mykiss*. *J Comp Physiol B* 165(8):665–676
- Romero LM, Dickens MJ, Cyr NE (2009) The reactive scope model—a new model integrating homeostasis, allostasis, and stress. *Horm Behav* 55(3):375–389
- Sadoul B, Vijayan MM (2016) Stress and growth. In: Schreck CB, Tort L, Farrell AP, Brauner CJ (eds) *Fish physiology*, vol 35. Academic Press, Cambridge, pp 167–205
- Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 21(1):55–89
- Schreck CB, Tort L (2016) The concept of stress in fish. In: Schreck CB, Tort L, Farrell AP, Brauner CJ (eds) *Fish physiology*, vol 35. Academic Press, Cambridge, pp 1–34
- Serra-Llinares RM, Tveiten H (2012) Evaluation of a fast and simple method for measuring plasma lactate levels in Atlantic cod, *Gadus morhua* (L.). *Int J Fish Aquacult* 4(11):217–220
- Sloman KA, Motherwell G, O'Connor K, Taylor AC (2000) The effect of social stress on the standard metabolic rate (SMR) of brown trout, *Salmo trutta*. *Fish Physiol Biochem* 23(1):49–53
- Sloman KA, Desforges PR, Gilmour KM (2001) Evidence for a mineralocorticoid-like receptor linked to branchial chloride cell proliferation in freshwater rainbow trout. *J Exp Biol* 204(22):3953–3961
- Smith HW (1929) The excretion of ammonia and urea by the gills of fish. *J Biol Chem* 81(3):727–742
- Soivio A, Oikari A (1976) Haematological effects of stress on a teleost, *Esox lucius* L. *J Fish Biol* 8(5):397–411
- Sokolova IM (2013) Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr Comp Biol* 53(4):597–608
- Sopinka NM, Patterson LD, Redfern JC, Pleizier NK, Belanger CB, Midwood JD, Crossin GT, Cooke SJ (2015) Manipulating glucocorticoids in wild animals: basic and applied perspectives. *Conserv Physiol* 3(1). <https://doi.org/10.1093/conphys/cov031>
- Sopinka NM, Donaldson MR, O'Connor CM, Suski CD, Cooke SJ (2016) Stress indicators in fish. In: Schreck CB, Tort L, Farrell AP, Brauner CJ (eds) *Fish physiology*, vol 35. Academic Press, Cambridge, pp 405–462
- Stoot LJ, Cairns NA, Cull F, Taylor JJ, Jeffrey JD, Morin F, Mandelman JW, Clark TD, Cooke SJ (2014) Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conserv Physiol* 2(1). <https://doi.org/10.1093/conphys/cou011>
- Storer JH (1967) Starvation and the effects of cortisol in the goldfish (*Carassius auratus* L.). *Comp Biochem Physiol* 20(3):939–948
- Suski CD, Killen SS, Morrissey MB, Lund SG, Tufts BL (2003) Physiological changes in largemouth bass caused by live-release angling tournaments in southeastern Ontario. *N Am J Fish Manag* 23(3):760–769
- Suski CD, Cooke SJ, Danylchuk AJ, O'Connor CM, Gravel MA, Redpath T, Hanson KC, Gingerich AJ, Murchie KJ, Danylchuk SE, Koppelman JB, Goldberg TL (2007) Physiological disturbance and recovery dynamics of bonefish (*Albula vulpes*), a tropical marine fish, in response to variable exercise and exposure to air. *Comp Biochem Physiol A* 148(3):664–673
- Torres JJ, Somero GN (1988) Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. *Mar Biol* 98(2):169–180
- Tripathi G, Verma P (2003) Pathway-specific response to cortisol in the metabolism of catfish. *Comp Biochem Physiol B* 136(3):463–471
- Tsui TKN, Hung CYC, Nawata CM, Wilson JM, Wright PA, Wood CM (2009) Ammonia transport in cultured gill epithelium of freshwater rainbow trout: the importance of Rhesus glycoproteins and the presence of an apical Na⁺/NH₄⁺ exchange complex. *J Exp Biol* 212(6):878–892
- Verdouw H, Van Echteld CJA, Dekkers EMJ (1978) Ammonia determination based on indophenol formation with sodium salicylate. *Water Res* 12(6):399–402
- Vijayan MM, Ballantyne JS, Leatherland JF (1991) Cortisol-induced changes in some aspects of the intermediary metabolism of *Salvelinus fontinalis*. *Gen Comp Endocrinol* 82(3):476–486
- Vijayan MM, Pereira C, Grau EG, Iwama GK (1997) Metabolic responses associated with confinement stress in tilapia: the role of cortisol. *Comp Biochem Physiol C* 116(1):89–95
- Vijayan MM, Raptis S, Sathiyaa R (2003) Cortisol treatment affects glucocorticoid receptor and glucocorticoid-responsive genes in the liver of rainbow trout. *Gen Comp Endocrinol* 132(2):256–263
- Wells RM, Pankhurst NW (1999) Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. *J World Aquacult Soc* 30(2):276–284
- Wendelaar Bonga S (1997) The stress response in fish. *Physiol Rev* 77(3):591–625
- Wilson R, Wright P, Munger S, Wood C (1994) Ammonia excretion in freshwater rainbow trout (*Oncorhynchus mykiss*) and the importance of gill boundary layer acidification: lack of evidence for Na⁺/NH₄⁺ exchange. *J Exp Biol* 191(1):37–58
- Wilson AD, Binder TR, McGrath KP, Cooke SJ, Godin JGJ (2011) Capture technique and fish personality: angling targets timid bluegill sunfish, *Lepomis macrochirus*. *Can J Fish Aquat Sci* 68(5):749–757
- Winberg S, Höglund E, Overli O (2016) Variation in the neuroendocrine stress response. In: Schreck CB, Tort L, Farrell AP, Brauner CJ (eds) *Fish physiology*, vol 35. Academic Press, Cambridge, pp 35–74
- Wiseman S, Osachoff H, Bassett E, Malhotra J, Bruno J, VanAggelen G, Vijayan MM (2007) Gene expression pattern in the liver during recovery from an acute stressor in rainbow trout. *Comp Biochem Physiol D* 2(3):234–244
- Wood CM, Milligan CL, Walsh PJ (1999) Renal responses of trout to chronic respiratory and metabolic acidosis and metabolic alkalosis. *Am J Phys* 277(2):R482–R492
- Wood CM, Kajimura M, Sloman KA, Scott GR, Wals PJ, Almeida-Val VM, Val AL (2007) Rapid regulation of Na⁺ fluxes and ammonia excretion in response to acute environmental hypoxia in the Amazonian oscar, *Astronotus ocellatus*. *Am J Phys* 292(5):R2048–R2058
- Wright PA (1995) Nitrogen excretion: three end products, many physiological roles. *J Exp Biol* 198(2):273–281

- Wright PA, Wood CM (2009) A new paradigm for ammonia excretion in aquatic animals: role of Rhesus (Rh) glycoproteins. *J Exp Biol* 212(15):2303–2312
- Yada T, Tort L (2016) Stress and disease resistance: immune system and immunoendocrine interactions. In: Schreck CB, Tort L, Farrell AP, Brauner CJ (eds) *Fish physiology*, vol 35. Academic Press, Cambridge, pp 365–403
- Zimmer AM, Nawata CM, Wood CM (2010) Physiological and molecular analysis of the interactive effects of feeding and high environmental ammonia on branchial ammonia excretion and Na⁺ uptake in freshwater rainbow trout. *J Comp Physiol B* 180(8):1191–1204
- Zolderdo AJ, Algera DA, Lawrence MJ, Gilmour KM, Fast MD, Thuswaldner J, Willmore WG, Cooke SJ (2016) Stress, nutrition and parental care in a teleost fish: exploring mechanisms with supplemental feeding and cortisol manipulation. *J Exp Biol* 219(8):1237–1248

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