Behavioral and physiological responses of yellow perch (*Perca flavescens*) to moderate hypoxia

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Running title: Yellow perch tolerate moderate hypoxia

ms. has 53 pages, 4 figures, 2 tables, 4 suppl. file

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Abstract

While severe hypoxia can be lethal and is usually avoided by mobile aquatic organisms, moderate hypoxic conditions are likely more prevalent and may affect organisms, such as fishes, in a variety of systems. However, fishes have the potential to adjust physiologically and behaviorally and thus reduce the negative effects of hypoxia. Quantifying such physiological responses may shed light on the ability of fishes to tolerate reduced oxygen concentrations. This study assessed how two different hatchery populations of yellow perch *Perca flavescens*, a fish that is likely to encounter moderate hypoxic conditions in a variety of systems, responded to moderate hypoxic exposure through three experiments: 1) a behavioral foraging experiment, 2) an acute exposure experiment, and 3) a chronic exposure experiment. No marked behavioral or physiological adjustments were observed in response to hypoxia (e.g., hemoglobin, feeding rate, movement frequency, gene expression did not change to a significant degree), possibly indicating a high tolerance level in this species. This may allow yellow perch to utilize areas of moderate hypoxia to continue foraging while avoiding predators that may be more sensitive to moderately low oxygen.

Key words: acute, chronic, qPCR, HIF-1 α , IGF-1 β , hemoglobin, feeding, Bayesian, oxygen, gene expression

1. Introduction

Hypoxia, or the depletion of oxygen to relatively low concentrations (Vaquer-Sunyer and Duarte, 2008) can occur naturally in aquatic systems. Due to changing climate and continued increasing anthropogenic nutrient loading, the number of hypoxic zones has increased worldwide and will likely continue to increase in the future (Diaz, 2001; Diaz and Rosenberg, 2008; Zhang et al., 2010). Hypoxic zones are most commonly defined by oxygen concentrations that cause lethality (often dissolved oxygen, DO, concentrations < 2.0 mg/L). However, fishes and other aquatic species are likely affected sub-lethally by low oxygen concentrations that exceed 2.0 mg/L (see Hrycik et al., 2017; Vanderplanke et al., 2014; Vaquer-Sunyer and Duarte, 2008). Further, most exposure to hypoxia experienced by aquatic organisms is likely moderate in intensity or duration, such as during foraging forays into hypoxic waters (Roberts et al., 2012), diurnal oxygen depletion in small aquatic systems (Whitney, 1942), and conditions at the spatio-temporal edges of more extreme hypoxic events (Kraus et al., 2015). Despite the prevalence of moderate hypoxia, experiments to evaluate effects on fishes and other aquatic organisms are often performed at extreme hypoxia (≤ 2 mg/L). Analyzing the responses of fishes to moderate hypoxia may provide a more realistic understanding of the behaviors and physiological responses of fish to common, less severe oxygen limitations.

A variety of behavioral and physiological responses allow fishes to survive and minimize the potential negative effects of hypoxia. In general, the first response of fishes to severe hypoxia is avoidance. Several field studies show an inverse relationship between DO levels and fish densities (Hughes et al., 2015; Pollock et al., 2007; Suthers and Gee, 1986). Some exceptions to this common behavior include brief forays into the hypoxic zone to consume preferred prey items (see Rahel & Nutzman, 1994; Roberts *et al.*, 2012). Fishes which are unable to avoid hypoxic conditions or choose to remain in hypoxic areas may experience a variety of sub-lethal

organism-level consequences including reductions in activity (Schurmann and Steffensen, 1994), food consumption (Roberts et al., 2011; Sun et al., 2012), growth (Chabot and Dutil, 1999), and reproductive output (Wu et al., 2003), and may even become more vulnerable to contaminants (Hattink et al., 2005).

Organism-level responses by fishes can be indicated by changes in the expression of various proteins or gene transcripts. Specifically, hypoxia-inducible factor 1 α (HIF-1 α) plays a significant role, and many studies have examined its potential use as a biomarker for exposure to acute or chronic hypoxia in fishes (Terova *et al.*, 2008; Rimoldi *et al.*, 2012; Froehlich *et al.*, 2015). There is some evidence that other proteins involved in growth and stress responses, such as insulin-like growth factor-1 β (IGF-1 β), heat shock protein 70 (HSP70), and suppressor of cytokine signaling 3 (SOCS3) may be related to the hypoxic response and can be activated by HIF-1 α (Delaney and Klesius, 2004; Gracey et al., 2001; Zhang et al., 2012). Under acute and chronic hypoxic stress, blood hemoglobin (Hb) concentrations generally increase (Robb & Abrahams, 2003; Sun *et al.*, 2012; Tran-Duy *et al.*, 2008). Other proteins involved with reproduction and growth, such as aromatase (CYP19), may also be adjusted during the down-regulation of processes not necessary for survival, although, to our knowledge, this has not been explicitly examined for fishes. Given that fishes do respond to moderate hypoxia, a finer examination of the changes in protein and gene expression at less extreme oxygen concentrations could provide a link between molecular changes and physiological and behavioral responses.

In the present study, we focus on the effects of moderate hypoxia on yellow perch, a species which is exposed to depleted oxygen conditions in a variety of systems (Hubert and Sandheinrich, 1983; Petrosky and Magnuson, 1973; Roberts et al., 2009). The yellow perch has been called relatively tolerant to hypoxia (Petrosky and Magnuson, 1973), with a critical oxygen

tension (P_{crit}), i.e., the point below which the metabolic rate becomes limited by the amount of oxygen available, of around 3 mg/L for yellow perch (Cnaani et al., 2014) and 1.6-2.2 mg/L for the Eurasian perch (*Perca fluviatilis*) at 16-20 °C (Bilberg et al., 2010; Thuy et al., 2010). Although P_{crit} is not always a good indicator of hypoxia tolerance (Speers-Roesch et al., 2013), the observed values indicate that yellow perch may still have the ability to perform aerobic activity during moderate hypoxia (~2 mg/L).

We tested the hypothesis that yellow perch are resilient to moderate hypoxia using 1) a readily available hatchery population originating from North Carolina (henceforth, “North Carolina”) and 2) a population originating from Lake Erie (henceforth, “Lake Erie”). Lake Erie experiences hypolimnetic hypoxia on a regular basis and yellow perch from this system are known to experience low oxygen conditions (Roberts et al., 2012). While multiple generations in a hatchery setting (~20 generations) may have led to some divergence in traits from the founding populations, we assumed that the fish originating from Lake Erie were more similar to current Lake Erie populations than were the fish originating from North Carolina. If these populations exhibit differences in their responses to moderate hypoxia, this may partially reflect evolutionary adaptation by the parent populations.

We quantified population differences in behavior, blood parameters, and gene expression from yellow perch maintained in moderate DO concentrations across three experiments. Given that wild yellow perch frequently encounter these DO concentrations, we predicted that moderate hypoxia would cause only minor effects. Specifically we hypothesized: 1) movement between hypoxia and normoxia would be more common in treatments with more severe hypoxia, but overall time foraging in treatment waters would be positively related to DO (Experiment 1: Willingness to forage in hypoxic water); 2) hemoglobin and gene expression would adjust

slightly in acute exposure to moderate hypoxia (Experiment 2: Physiological response to acute hypoxia); and 3) consumption, growth, hemoglobin concentration, and gene expression would adjust slightly under chronic low exposure DO (Experiment 3: Physiological response to chronic hypoxia). Across populations and experiments, we predicted that responses would be strongest under the lowest tested DO concentration, however all of our responses should be less severe than documented in previous studies with severely low DO.

2. Materials and methods

2.1 Ethical procedures

All animal handling and experimental procedures were approved by the Purdue Animal Care and Use Committee, following the American Association for Laboratory Animal Science (AALAS) guidelines.

2.2 Acquisition and rearing of fish

Details on the acquisition and rearing of yellow perch fry to juveniles are described in Supplement A. At the initiation of each experiment, fish in the North Carolina (NC) population were 7.1 ± 1.6 g and 91.0 ± 5.9 mm (mean $\pm$ SD). Fish in the Erie population were 9.0 ± 2.0 g and 97.5 ± 7.3 mm (mean $\pm$ SD).

2.3.1 Experiment 1: Willingness to Forage in Hypoxic Water

A balanced 2×3 experimental design was used to assess the willingness of individuals from both populations of yellow perch to forage under three DO treatments: low (~ 2.5 mg/L),

moderate (~4.5 mg/L), and control (~9.0 mg/L). For each treatment and population combination, 20 replicates (experimental units) were included without reusing fish over the course of four trials (five replicates per DO treatment × population per trial). The duration of each trial was 7 days (2 experimental unit acclimation days, 1 fasting day, 3 hypoxic treatment acclimation days, and 1 filming day), and all four trials were completed over the course of 4.5 consecutive weeks.

At the beginning of each trial, four fish were placed in each experimental unit which consisted of two 10 L tanks, connected to each other with a PVC pipe 5 cm in diameter and 13 cm long (Fig. 1, details about flow and water conditions are described in Supplement A). One tank of the experimental unit was randomly selected using a random number table as the “Refuge” tank, where the DO always remained normoxic (actual 9.0 mg/L ± 2.6 SD, measured with a YSI, Yellow Springs, Ohio, USA, Pro ODO meter). During the 4 hypoxic treatment days, the other tank, the “Treatment” tank, was adjusted to the treatment DO (i.e., low, moderate, control) 20 to 40 minutes prior to feeding and maintained for approximately 20 minutes while food was available. Ambient air was bubbled into both the Refuge and Treatment tanks via diffusers until experimental adjustment of DO in the Treatment tank, at which point the diffuser in the Treatment tank was replaced with a diffuser that bubbled nitrogen gas (>99.99% N₂). In the low and moderate hypoxia treatments, the Treatment tank DO was adjusted to 2.5 mg/L (actual 2.7 mg/L ± 0.5 SD) and about 4.5 mg/L (actual 4.5 mg/L ± 0.6 SD), respectively. While DO in the Treatment tank for the control treatment was not adjusted (maintained at 9.0 mg/L ± 0.5 SD), the diffuser was removed and replaced to replicate the replacement of the air diffuser with the N₂ diffuser in the hypoxic treatments. At the end of the 20 minutes of feeding, the N₂ diffuser was replaced with the air diffuser in the Treatment tanks.

The fish were provided with food (20 pellets of Zeigler, Gardners, Pennsylvania, USA 2.5 mm slow sink feed) once daily in the Treatment tank except for on the fasting day. During the hypoxic treatment days, fish were only fed after target DO levels had been achieved. At the time of the feeding, the temperatures of the Treatment and Refuge tanks were recorded. In addition, the DO in the Treatment and Refuge tanks and the number of fish in the Treatment tank 0, 10, and 20 minutes after the food was added were recorded. The Treatment tank and the 20 minute feeding protocol were video recorded on day 7 with a Canon Vixia HF G20 portable camcorder (Canon, Tokyo, Japan). During video recording, people were only visible to fish during three time periods: 0, 10, and 20 minutes after feeding when temperature and DO were recorded. All fish were removed from tanks and euthanized with MS-222 (200 mg/L MS-222 buffered with 200 mg/L NaHCO₃) at the end of filming on day seven.

2.3.2 Video Analysis

Two metrics were recorded from videos: a binary value of whether or not any fish moved into or out of the Treatment tank and the average time spent in the Treatment tank per fish. In total, 12 replicates were removed from the analyses due to poor visibility. The metric ‘average time in Treatment’ only included replicate tanks in which at least one fish moved in or out of the Treatment tank (i.e., binary movement = 1) to remove the effect of replicates which did not move. A total of 31 replicates were used for the average time analysis. Statistical analyses are described for all experiments below.

2.4 Experiment 2: Physiological Response to Acute Hypoxia

Yellow perch from both populations were briefly exposed to severe hypoxia by gradually decreasing the DO concentration in a tank from normoxic (actual $9.6 \text{ mg/L} \pm 0.5 \text{ SD}$) to approximately 2.0 mg/L (actual $2.1 \text{ mg/L} \pm 0.4 \text{ SD}$) over 30 minutes and maintaining the target concentration for 30 minutes, similar to the experiment by Rimoldi *et al.* (2012). Over 2 consecutive days (trials), there were a total of 6 replicate tanks ($40 \times 18 \times 15.25 \text{ cm}$) with 3 fish in each tank for each treatment (hypoxic exposure and control) and population ($n = 6$; details about flow and temperature described in Supplement A).

Fish were acclimated to their tanks for 1 day before the trial began. At the start of the trial, a diffuser bubbling N_2 gas was placed into the hypoxic treatment tanks and a diffuser bubbling ambient air was placed into the control tanks. The DO was monitored with a YSI DO probe every 10 minutes, except in the first 10 minutes when it was monitored every 5 minutes (exact DO levels in Supplement A). Immediately after the hour-long trial, fish were euthanized with MS-222. Blood samples were collected with a heparinized capillary tube after cutting into the anterior spinal artery and immediately transferred to tubes containing Drabkin's reagent (Sigma-Aldrich, Saint Louis, Missouri, part № D5941). Samples of gill and muscle and whole brains and livers were collected from each fish. For each organ, samples of similar mass from all fish in a tank were pooled together to reduce variation and frozen immediately in liquid nitrogen ($n = 24$ final combined samples per tissue type) and stored at -80°C for up to 6 months until RNA extraction and quantitative real-time polymerase chain reaction (PCR) (details describing RNA extraction and PCR are below). Blood samples ($n = 72$) were immediately processed for quantification of Hb concentrations using the cyanmethemoglobin method according to the manufacturer's instructions (Sigma-Aldrich part № D5941).

2.5 Experiment 3: Physiological Response to Chronic Hypoxia

After 5 days of acclimation, yellow perch from each population were exposed to one of three DO treatments: low (actual $2.5 \text{ mg/L} \pm 0.8 \text{ SD}$), moderate ($3.7 \text{ mg/L} \pm 0.5 \text{ SD}$), and control ($9.0 \text{ mg/L} \pm 0.5 \text{ SD}$) for 10 days. Previous studies indicated that 5 days of acclimation to experimental chambers allowed yellow perch to adjust to a normal feeding regime (Roberts et al., 2012), and 5 treatment days were sufficient to observe differences in consumption between hypoxic treatments 2.0 mg/L and 5.0 mg/L (Roberts et al., 2011). Exposure for 10 days to hypoxia was assumed to be sufficient to observe changes at the levels of hypoxia we were testing. During acclimation and experiment, fish were fed in excess and food was available for 6 to 8 hours daily except for the day the DO was altered to experimental levels, at which time they were fasted. Each treatment $\times$ population combination was replicated in 4 tanks (i.e., 24 tanks total), with 3 fish in each tank (water quality and acclimation information can be found in Supplement A).

Regular measurements were taken throughout the acclimation period and 10 day experiment. All fish total lengths and weights were measured at three times: before the experiment began, at the end of the acclimation period, and at the end of the experiment to obtain tank averages of size throughout the experiment. Dissolved oxygen, temperature, and consumption of food pellets were documented daily. Immediately after the experiment, fish were euthanized with MS-222 and blood and tissue samples from gill, brain, liver, and muscle were collected and processed as described above for the acute exposure experiment.

2.6.1 RNA Extraction and Quantitative Real-Time PCR for Experiments 2 and 3

Gene expression was quantified using quantitative polymerase chain reaction (qPCR) following standard procedures (e.g., Coulter et al., 2015). Liver and muscle samples were ground to a powder in liquid nitrogen using a mortar and pestle. Brain and gill samples, as well as powdered liver and muscle samples, were immersed in 1 ml QIAzol[®] Lysis Reagent (QIAGEN Sciences, Valencia, California, product №79306) and placed on ice, sonicated for 1 minute and placed on ice again. Samples were then frozen in QIAzol reagent at -20 °C for 12-48 hours. RNA was extracted from the homogenized samples according to the manufacturer's instructions. RNA concentration and purity was determined with a NanoDrop 2000c spectrophotometer (Thermo Scientific). Only samples with a A260/A280 ratio of 1.8 to 2.0 were carried into the next step; otherwise, they were cleaned via ethanol precipitation. RNA (2.0 µg) was treated with DNase I (Thermo Scientific part №EN0521) and 0.8 µg was then reverse-transcribed into cDNA (Applied Biosystems part №4368814) according to the manufacturer's instructions in a final volume of 20 µl. Samples of cDNA were stored at -20 °C.

If available for yellow perch, published sequences of forward and reverse primers for the target genes were used, otherwise, published sequences from a closely related species were used and verified. Primers targeting *β-actin* (used as reference), *igf-1β*, *cyp19a1a*, *hsp70*, and *socs3* were obtained from the literature for yellow perch, and primers targeting *hif-1α* were obtained for Eurasian perch (Table 1). To verify the *hif-1α* primers, the product of a PCR reaction was found to be a single band using agarose gel electrophoresis. It was then cloned into a pMD-20 T-vector (Clontech Laboratories Inc. product № 3270) according to the manufacturer's instructions and sequenced at the Purdue Genomics Core Facility on an ABI PRISM 3700 genetic analyzer. The DNA sequence that was amplified was submitted to GenBank

(<https://www.ncbi.nlm.nih.gov/genbank/>) with accession № **KX050166**, and was found to be identical to the Eurasian perch *hif-1α* sequence (GenBank accession № **EF100706**).

qPCR was performed with Bio-Rad iQ™ SYBR® Green Supermix using 1.6 ng of cDNA following the manufacturer's instructions in a total reaction volume of 20 µl. Thermocycling and fluorescence measurement was performed on a CFX Connect™ Real-Time System with the following protocol: polymerase activation and DNA denaturing at 95 °C for 3 minutes and amplification of 40 cycles of 10 seconds of denaturation at 95 °C followed by 60 seconds of annealing/extension and fluorescence measurement at 53 °C. The melt curve analysis occurred between 55 and 95 °C in 0.5 °C increments with 5 seconds per step. Only a single melting peak was observed for each qPCR target. Primer efficiency was determined using the method of Pfaffl (2001). Efficiencies were between 1.73 and 2.10, and the linear range of the assay coincided with cDNA dilutions of 1:10 to 1:6250.

2.6.2 Gene expression analysis

Because a significant effect of population was identified in the expression of the reference gene *β-actin* in brain for the acute experiment ($F_{1,21} = 4.3$, $p = 0.05$), any population results from the acute experiment brain tissue were interpreted with caution. However, *β-actin* was determined to be a reasonable reference gene in all other cases. Attempts to use the gene *rplp0* as a second reference gene were hampered by the appearance of multiple PCR products in gill, brain, and muscle tissues, but not liver tissues; therefore, it was abandoned. The quantitation cycle (Cq) values obtained from the RT-qPCR were converted to fold change according to the $\Delta\Delta Cq$ method of Pfaffl (2001). Values were then log transformed before statistical analysis.

2.7 Statistical Analyses

Bayesian analyses were used to compare responses among populations and treatments using JAGS software v. 4.1.0 (Plummer, 2003) and R (R Development Core Team, 2013) package ‘jagsUI’ (Kellner, 2015). This method allowed for estimation of the posterior distributions based on prior information and observations (McCarthy, 2007). Bayesian-based analyses were appropriate because they allowed for the assignment of degrees of belief to our hypotheses and for the computation of 95% credible intervals. Differences between treatment $\times$ population groups were identified if the 95% credible intervals (CI) of respective means did not overlap. In some analyses (Experiments 2 and 3), moderate differences between groups were additionally identified if the 80% CI of means did not overlap. This more liberal test was performed to identify potential marginal differences not evident through comparison of 95% CI. One advantage of using Bayesian analyses is the ability to detect potential differences with varying degrees of credibility. Given the hypothesized minor responses under moderate hypoxia, we used the 80% CI to determine if marginal differences were apparent.

In Experiment 1, binary movement was analyzed assuming a Bernoulli distribution and a generalized linear mixed model. Average time in Treatment was analyzed assuming normally distributed data in a linear mixed model. In Experiment 2, both hemoglobin concentration (mg/ml) and log fold change data were analyzed assuming a normal distribution in a linear mixed model. And in Experiment 3, tank average consumption ($\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$), tank average growth in length (mm), and log fold change data were analyzed assuming normal distributions in linear models and hemoglobin concentration (mg/ml) was analyzed with a normal distribution in a linear mixed model. Consumption was arcsine transformed prior to analysis to fit a normal distribution. For all of these analyses, treatment and population were fixed, additive factors. Trial was a random factor for both analyses in Experiment 1 and for hemoglobin and log fold change

in Experiment 2 (also listed as “day”). Tank was a random factor for hemoglobin concentration in Experiments 2 and 3.

All priors were uninformed (Table 2). Markov chain Monte Carlo (MCMC) methods were used to obtain parameter estimates with 3 sample chains and 20,000 iterations with a burn-in of 10,000 to remove any effects of initial parameter values. All models converged based on Gelman-Rubin statistic (Rhat) values less than 1.1 (Gelman and Rubin, 1992).

3. Results

3.1 Experiment 1: Willingness to Forage in Hypoxic Water

The number of tanks in which at least one fish moved between the Treatment and Refuge tanks was higher in the NC population (low = 9, moderate = 5, control = 8) than the Lake Erie population (low = 4, moderate = 3, control = 2); however, there was no clear effect of population on the likelihood of movement (i.e., 95% CI of β_{Erie} and β_{NC} overlap with zero, Table C1). Treatment also did not markedly affect the likelihood of movement between tanks (Table C1). Similarly, there was no great effect of DO treatment or population on the time spent in the Treatment tank for the replicates that did move between tanks ($\mu_{\text{NC-L}} = 9.4 \pm 3.6$ min, $\mu_{\text{NC-M}} = 7.7 \pm 4.4$ min, $\mu_{\text{NC-C}} = 9.5 \pm 3.9$ min, $\mu_{\text{Erie-L}} = 7.4 \pm 4.4$ min, $\mu_{\text{Erie-M}} = 5.8 \pm 5.0$ min, $\mu_{\text{Erie-C}} = 7.5 \pm 5.3$ min; Table B1, C1).

3.2 Experiment 2: Acute Exposure

Hemoglobin concentration was not notably affected by population or hypoxic treatment (Fig. 2a, Table C2). With exception of brain *socs3*, which was moderately up-regulated in both

populations exposed to hypoxia (Fig. D1), no large differences in gene expression were identified (Fig. 3).

3.3 Experiment 3: Chronic Exposure

Dissolved oxygen levels were maintained within the expected bounds over the course of the 10 day experiment, except for days nine and 11 when DO from the lowest (~2.5 mg/L) and moderate (~4.0 mg/L) hypoxic treatments rose above 5.0 mg/L. The longest deviation from target DO levels was less than 24 hours.

Consumption, growth, and Hb concentration did not differ markedly between populations or treatments (Fig. 2b, Table C3). Gene expression did not differ much between groups for any tissue or gene (95% CI, Fig. 4). However, for both populations there were indications of moderate up-regulation of liver *hif-1α* in the Moderate DO treatment versus Control (Fig. D2).

4. Discussion

Yellow perch responses to acute and chronic moderate hypoxia indicate that moderate hypoxia may have minimal effects on foraging behavior and physiological responses in this species. In all three experiments, yellow perch responded mildly or not at all to the hypoxic conditions tested. Further, responses did not differ between the two populations tested. Effect sizes observed in similar studies that detect significant differences in fold change (e.g., Hedge's $g = 6.87$ in Terrova et al. 2008, 2.22 – 8.43 in Rimoldi et al. 2012) were far greater than our null results (largest Hedge's $g = 1.79$). However, these studies which documented significant differences in fold change (e.g., Terrova et al., 2008, Rimoldi et al. 2012) had a comparable number of replicates as our study. Thus, we interpret our results to suggest that yellow perch may

be able to utilize a wide range of habitats during the onset of hypoxia and in areas near severe hypoxic zones.

Yellow perch forage in hypoxic habitats, and, in conjunction with previous research, the results of this study indicate that they may do so without strong negative physiological consequences. Behavioral results demonstrated that DO did not significantly influence the willingness of yellow perch to move in or out of hypoxia, nor did it influence the amount of time perch spent foraging. Physiological results demonstrated no change in consumption, growth, and Hb concentration after exposure to hypoxic conditions. However, we did observe some indication of moderate differences and trends in gene expression, potentially indicating that the yellow perch response is tempered at higher than lethal DO concentrations. Fluctuating DO, which fish would experience during brief forays into hypoxic water, has been found to not affect yellow perch consumption and growth, particularly with moderate changes in DO (Carlson et al., 1980; Roberts et al., 2012).

Collectively, these results suggest that yellow perch responses to moderate hypoxia may require limited physiological adjustment. Perhaps yellow perch can satisfy their oxygen needs for these durations of moderate hypoxia through other responses, such as hyperventilation or the use of glycogen stores. Increasing ventilation rate (a known response of fish to hypoxia; Gerald & Cech, 1970) is postulated as a low-energy means for relatively hypoxia-tolerant fishes to maintain oxygen uptake without necessitating activation of further physiological responses (Tran-Duy et al., 2008). Yellow perch have been seen to alter their ventilation rate over a range of hypoxic levels (<2.0 – 3.5 mg/L) without accompanying changes in hematocrit or hemoglobin concentration (Robb and Abrahams, 2003). Another hypoxia-tolerant fish, the moonlight gourami *Trichogaster microlepis*, was found to both alter behavior and utilize glycogen stores to

maintain homeostasis in hypoxia (Huang et al., 2015). Yellow perch may be using similar mechanisms to respond to moderate hypoxic conditions via behavioral and physiological responses other than those that we tested.

Results from our study conflict with those from previous research on the effects of constant, moderate hypoxia on yellow perch, but this may be due to differences in the temperatures at which studies were conducted. In previous studies, yellow perch consumption decreased under chronic hypoxia at constant moderate (3.5 mg/L) and low (2.0 mg/L) concentrations (Carlson et al., 1980; Roberts et al., 2012, 2011). However, water temperatures in these studies were much higher (20 and 26 °C) compared with the present study (~15 °C). Consistent with our results, experiments performed at cooler temperatures (11 °C), similar to temperatures yellow perch and other fish species would experience in the hypolimnion of a lake such as Lake Erie (Roberts et al., 2011) or in the upper part of a smaller lake during an under-ice hypoxic event (Rahel and Nutzman, 1994), demonstrate that some fishes can tolerate exposure to moderate hypoxia or brief exposure to severe hypoxia in cooler temperatures. The experiments reported in the present study may be more indicative of potential responses in vertically stratified hypoxic systems or in ice-covered lakes, as the experiment temperatures are more similar to the temperatures in these systems.

In addition to temperature, past studies demonstrate that the duration of hypoxia exposure and severity of hypoxia can greatly affect fish responses. Gene expression for *hif-1α* is species-specific and quite sensitive to duration and severity of exposure. In Pacific herring (*Clupea pallasii*) liver tissue, expression of *hif-1α* reached a peak after 2 to 8 hours of exposure to 2.3 mg/L DO (Froehlich et al., 2015). Similarly, sea bass (*Dicentrarchus labrax*) exposed to 1.9 mg/L DO for 4 hours increased liver *hif-1α* expression (Terova et al., 2008). However, sea bass

liver *hif-1 α* expression also continuously increased after exposure to moderate hypoxia (4.3 mg/L) after 48 hours, 5 days, and 15 days (Terova et al., 2008). Studies on Eurasian perch have demonstrated a significant increase in *hif-1 α* in brain and liver after exposure to DO of 0.4 mg/L for approximately 40 minutes, but a decrease in muscle *hif-1 α* expression and no change in brain or liver *hif-1 α* expression after DO exposure of 2.8 mg/L for 15 days (Rimoldi et al., 2012). The durations and DO levels tested in the current study were estimations of environmental conditions yellow perch are most likely to encounter. Therefore, it may be reasonable to conclude that yellow perch are fairly tolerant in their response to moderate hypoxia. However, by not examining the effects of more extreme hypoxia or a broader range of exposure times, our interpretation is limited to the oxygen concentrations and durations tested. Furthermore, the way in which yellow perch responses differ between moderate and severe hypoxia is not fully known.

In light of individual-level tolerances and response mechanisms, hypoxia is known to alter species distributions and affect trophic interactions (Breitburg, 2002; Howell and Simpson, 1994; Ludsin et al., 2009; Pollock et al., 2007; Pothoven et al., 2012). Moderate hypoxia is more pervasive than severe hypoxia in aquatic systems, therefore, yellow perch and similarly tolerant fish may be able to utilize a greater amount of habitat given their mild response to moderate hypoxia. Juvenile yellow perch may be able to use moderate hypoxia as refuges from more sensitive predators, while continuing to forage on preferred prey (Ludsin et al., 2009; Vanderploeg et al., 2009). However, if yellow perch selectively utilize areas of moderate hypoxia, such aggregation may have density-dependent effects (Eby et al., 2005) and increase their vulnerability to fisheries harvest (Kraus et al., 2015).

Hypoxia can affect fish behavior (Chapman and McKenzie, 2009; Roberts et al., 2012; Wannamaker and Rice, 2000) and can initiate a physiological response (Chabot and Dutil, 1999;

Rimoldi et al., 2012; Tran-Duy et al., 2012), but the DO concentrations at which these responses occur depend on a variety of factors such as species-specific physiology (He et al., 2015), acclimation (Carlson et al., 1980), and temperature (He et al., 2015; Roberts et al., 2011). Effects of moderate levels of hypoxia on yellow perch appear to be minimal. However, indirect effects of hypoxia on yellow perch and similarly tolerant fish, such as redistributed competitors and altered predator-prey relationships, may lead to various effects on yellow perch in natural environments.

Acknowledgements

The authors appreciate the helpful comments from three anonymous reviewers that improved the manuscript. Thanks to Robert Rode at the Purdue Aquaculture Research Laboratory, Tyler Krieg, and Timothy Malinich for help with experimental design and collection and rearing of yellow perch. Thanks to Ahmed Abdel-moneim for assistance in cloning and sequencing, Jiejun Gao for training on RNA extraction, and Zach Feiner for guidance with Bayesian analyses. Additional thanks to Stuart Ludsin and members of the Höök lab for help with design and implementation of the experiment, analysis, and comments on the manuscript. Funding was provided by Purdue University's Department of Forestry and Natural Resources, a Knox Fellowship from Purdue University, and a National Science Foundation Graduate Research Fellowship (awarded to Leah Zoe Almeida) under Grant №1333468.

References

- Bilberg, K., Malte, H., Wang, T., Baatrup, E., 2010. Silver nanoparticles and silver nitrate cause respiratory stress in Eurasian perch (*Perca fluviatilis*). *Aquat. Toxicol.* 96, 159–165. doi:10.1016/j.aquatox.2009.10.019
- Breitburg, D., 2002. Effects of hypoxia, and the balance between hypoxia and enrichment, on

438 coastal fishes and fisheries. *Estuaries* 25, 767–781. doi:10.1007/BF02804904

439 Carlson, A.R., Blocher, J., Herman, L.J., 1980. Growth and survival of channel catfish and
 440 yellow perch exposed to lowered constant and diurnally fluctuating dissolved oxygen
 441 concentrations. *Progress. Fish-Culturist* 42, 73–78. doi:10.1577/1548-8659(1980)42

442 Chabot, D., Dutil, J.D., 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions.
 443 *J. Fish Biol.* 55, 472–491. doi:10.1006/jfbi.1999.1005

444 Chapman, L.J., McKenzie, D.J., 2009. Behavioral responses and ecological consequences, *Fish*
 445 *Physiology: Hypoxia*, Fish Physiology. Elsevier. doi:10.1016/S1546-5098(08)00002-2

446 Cnaani, A., Hallerman, E.M., McLean, E., 2014. Physiological responses of yellow perch to
 447 hypoxia, air exposure, and bleeding. *N. Am. J. Aquac.* 76, 423–429.
 448 doi:10.1080/15222055.2014.933750

449 Coulter, D.P., Höök, T.O., Mahapatra, C.T., Guffey, S.C., Sepúlveda, M.S., 2015. Fluctuating
 450 water temperatures affect development, physiological responses and cause sex reversal in
 451 fathead minnows. *Environ. Sci. Technol.* 49, 1921–1928. doi:10.1021/es5057159

452 Delaney, M.A., Klesius, P.H., 2004. Hypoxic conditions induce Hsp70 production in blood,
 453 brain and head kidney of juvenile Nile tilapia *Oreochromis niloticus* (L.). *Aquaculture* 236,
 454 633–644. doi:10.1016/j.aquaculture.2004.02.025

455 Diaz, R.J., 2001. Overview of hypoxia around the world. *J. Environ. Qual.* 30, 275–281.
 456 doi:10.2134/jeq2001.302275x

457 Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems.
 458 *Science* (80-.). 321, 926–929. doi:10.1126/science.1156401

459 Eby, L.A., Crowder, L.B., McClellan, C.M., Peterson, C.H., Powers, M.J., 2005. Habitat
 460 degradation from intermittent hypoxia: Impacts on demersal fishes. *Mar. Ecol. Prog. Ser.*
 461 291, 249–261.

462 Froehlich, H.E., Roberts, S.B., Essington, T.E., 2015. Evaluating hypoxia-inducible factor-1 α
 463 mRNA expression in a pelagic fish, Pacific herring *Clupea pallasii*, as a biomarker for
 464 hypoxia exposure. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 189, 58–66.
 465 doi:10.1016/j.cbpa.2015.07.016

466 Gelman, A., Rubin, D.B., 1992. Inference from iterative simulation using multiple sequences.
 467 *Stat. Sci.* 7, 457–472.

468 Gerald, J.W., Cech, J.J., 1970. Respiratory responses of juvenile catfish (*Ictalurus punctatus*) to
 469 hypoxic conditions. *Physiol. Zool.* 43, 47–54.

470 Gracey, A.Y., Troll, J.V., Somero, G.N., 2001. Hypoxia-induced gene expression profiling in the
 471 euryoxic fish *Gillichthys mirabilis*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1993–1998.
 472 doi:10.1073/pnas.98.4.1993

473 Hattink, J., De Boeck, G., Blust, R., 2005. The toxicokinetics of cadmium in carp under
 474 normoxic and hypoxic conditions. *Aquat. Toxicol.* 75, 1–15.
 475 doi:10.1016/j.aquatox.2005.06.007

476 He, W., Cao, Z.D., Fu, S.J., 2015. Effect of temperature on hypoxia tolerance and its underlying
 477 biochemical mechanism in two juvenile cyprinids exhibiting distinct hypoxia sensitivities.
 478 Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 187, 232–241.
 479 doi:10.1016/j.cbpa.2014.05.004

480 Howell, P., Simpson, D., 1994. Abundance of marine resources in relation to dissolved oxygen
 481 in Long Island Sound. Estuaries 17, 394. doi:10.2307/1352672

482 Hrycik, A.R., Almeida, L.Z., Höök, T.O., 2017. Sub-lethal effects on fish provide insight into a
 483 biologically-relevant threshold of hypoxia. Oikos 126, 307–317. doi:10.1111/oik.03678

484 Huang, C.Y., Lin, H.C., Lin, C.H., 2015. Effects of hypoxia on ionic regulation, glycogen
 485 utilization and antioxidative ability in the gills and liver of the aquatic air-breathing fish
 486 *Trichogaster microlepis*. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 179, 25–34.
 487 doi:10.1016/j.cbpa.2014.09.001

488 Hubert, W.A., Sandheinrich, M.B., 1983. Patterns of variation in gill-net catch and diet of yellow
 489 perch in a stratified Iowa lake. North Am. J. Fish. Manag. 3, 156–162. doi:10.1577/1548-
 490 8659(1983)3<156:POVIGC>2.0.CO;2

491 Hughes, B.B., Levey, M.D., Fountain, M.C., Carlisle, A.B., Chavez, F.P., Gleason, M.G., 2015.
 492 Climate mediates hypoxic stress on fish diversity and nursery function at the land–sea
 493 interface. Proc. Natl. Acad. Sci. 112, 8025–8030. doi:10.1073/pnas.1505815112

494 Kellner, K., 2015. jagsUI: A wrapper around rjags to streamline JAGS analyses. R Packag.
 495 version 1.

496 Kraus, R.T., Knight, C.T., Farmer, T.M., Gorman, A.M., Collingsworth, P.D., Warren, G.J.,
 497 Kocovsky, P.M., Conroy, J.D., 2015. Dynamic hypoxic zones in Lake Erie compress fish
 498 habitat, altering vulnerability to fishing gears. Can. J. Fish. Aquat. Sci. 72, 797–806.
 499 doi:10.1139/cjfas-2014-0517

500 Ludsin, S.A., Zhang, X., Brandt, S.B., Roman, M.R., Boicourt, W.C., Mason, D.M., Costantini,
 501 M., 2009. Hypoxia-avoidance by planktivorous fish in Chesapeake Bay: Implications for
 502 food web interactions and fish recruitment. J. Exp. Mar. Bio. Ecol. 381, 121–131.
 503 doi:10.1016/j.jembe.2009.07.016

504 Lynn, S.G., Powell, K.A., Westneat, D.F., Shepherd, B.S., 2009. Seasonal and sex-specific
 505 mRNA levels of key endocrine genes in adult yellow perch (*Perca flavescens*) from Lake
 506 Erie. Mar. Biotechnol. 11, 210–222. doi:10.1007/s10126-008-9136-3

507 McCarthy, M.A., 2007. Bayesian methods for ecology. Cambridge University Press, New York.

508 Petrosky, B.R., Magnuson, J.J., 1973. Behavioral responses of northern pike, yellow perch and
 509 bluegill to oxygen concentrations under simulated winterkill conditions. Copeia 1973, 124–
 510 133. doi:10.2307/1442367

511 Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR.
 512 Nucleic Acids Res. 29, 2002–2007. doi:10.1093/nar/29.9.e45

513 Pierron, F., Bourret, V., St-Cyr, J., Campbell, P.G.C., Bernatchez, L., Couture, P., 2009.
 514 Transcriptional responses to environmental metal exposure in wild yellow perch (*Perca*

515 *flavescens*) collected in lakes with differing environmental metal concentrations (Cd, Cu,
516 Ni). *Ecotoxicology* 18, 620–631. doi:10.1007/s10646-009-0320-7

517 Plummer, M., 2003. JAGS: A program for analysis of Bayesian graphical models using gibbs
518 sampling JAGS: Just another gibbs sampler. *Proc. 3rd Int. Work. Distrib. Stat. Comput.*
519 (DSC 2003) March 20–22, Vienna, Austria. ISSN 1609-395X. doi:10.1.1.13.3406

520 Pollock, M.S., Clarke, L.M.J., Dubé, M.G., 2007. The effects of hypoxia on fishes: From
521 ecological relevance to physiological effects. *Environ. Rev.* 15, 1–14. doi:10.1139/a06-006

522 Pothoven, S.A., Vanderploeg, H.A., Höök, T.O., Ludsin, S.A., Sprules, G., 2012. Hypoxia
523 modifies planktivore–zooplankton interactions in Lake Erie. *Can. J. Fish. Aquat. Sci.* 69,
524 2018–2028. doi:10.1139/cjfas-2012-0144

525 R Development Core Team, R., 2013. R: A Language and Environment for Statistical
526 Computing. R Found. Stat. Comput., R Foundation for Statistical Computing.
527 doi:10.1007/978-3-540-74686-7

528 Rahel, F.J., Nutzman, J.W., 1994. Foraging in a lethal environment: Fish predation in hypoxic
529 waters of a stratified lake. *Ecology* 75, 1246–1253.

530 Rimoldi, S., Terova, G., Ceccuzzi, P., Marelli, S., Antonini, M., Saroglia, M., 2012. HIF-1 α
531 mRNA levels in Eurasian perch (*Perca fluviatilis*) exposed to acute and chronic hypoxia.
532 *Mol. Biol. Rep.* 39, 4009–4015. doi:10.1007/s11033-011-1181-8

533 Robb, T., Abrahams, M. V., 2003. Variation in tolerance to hypoxia in a predator and prey
534 species: An ecological advantage of being small? *J. Fish Biol.* 62, 1067–1081.
535 doi:10.1046/j.1095-8649.2003.00097.x

536 Roberts, J.J., Brandt, S.B., Fanslow, D., Ludsin, S.A., Pothoven, S.A., Scavia, D., Höök, T.O.,
537 2011. Effects of hypoxia on consumption, growth, and RNA:DNA ratios of young yellow
538 perch. *Trans. Am. Fish. Soc.* 140, 1574–1586. doi:10.1080/00028487.2011.638576

539 Roberts, J.J., Greco, P.A., Ludsin, S.A., Pothoven, S.A., Vanderploeg, H.A., Höök, T.O., 2012.
540 Evidence of hypoxic foraging forays by yellow perch (*Perca flavescens*) and potential
541 consequences for prey consumption. *Freshw. Biol.* 57, 922–937. doi:10.1111/j.1365-
542 2427.2012.02753.x

543 Roberts, J.J., Höök, T.O., Ludsin, S.A., Pothoven, S.A., Vanderploeg, H.A., Brandt, S.B., 2009.
544 Effects of hypolimnetic hypoxia on foraging and distributions of Lake Erie yellow perch. *J.*
545 *Exp. Mar. Bio. Ecol.* 381. doi:10.1016/j.jembe.2009.07.017

546 Schurmann, H., Steffensen, J.F., 1994. Spontaneous swimming activity of Atlantic cod *Gadus*
547 *morhua* exposed to graded hypoxia at three temperatures. *J. Exp. Biol.* 197, 129–42.

548 Shepherd, B.S., Rees, C.B., Binkowski, F.P., Goetz, F.W., 2012. Characterization and evaluation
549 of sex-specific expression of suppressors of cytokine signaling (SOCS)-1 and -3 in juvenile
550 yellow perch (*Perca flavescens*) treated with lipopolysaccharide. *Fish Shellfish Immunol.*
551 33, 468–481. doi:10.1016/j.fsi.2012.05.026

552 Speers-Roesch, B., Mandic, M., Groom, D.J.E., Richards, J.G., 2013. Critical oxygen tensions as
553 predictors of hypoxia tolerance and tissue metabolic responses during hypoxia exposure in

554 fishes. J. Exp. Mar. Bio. Ecol. 449, 239–249. doi:10.1016/j.jembe.2013.10.006

555 Sun, H., Li, J., Tang, L., Yang, Z., 2012. Responses of crucian carp *Carassius auratus* to long-
556 term exposure to nitrite and low dissolved oxygen levels. Biochem. Syst. Ecol. 44, 224–
557 232. doi:10.1016/j.bse.2012.06.011

558 Suthers, I.M., Gee, J.H., 1986. Role of hypoxia in limiting diel spring and summer distribution of
559 juvenile yellow perch (*Perca flavescens*) in a prairie marsh. Can. J. Fish. Aquat. Sci. 43,
560 1562–1570. doi:10.1139/f86-194

561 Terova, G., Rimoldi, S., Corà, S., Bernardini, G., Gornati, R., Saroglia, M., 2008. Acute and
562 chronic hypoxia affects HIF-1 α mRNA levels in sea bass (*Dicentrarchus labrax*).
563 Aquaculture 279, 150–159. doi:10.1016/j.aquaculture.2008.03.041

564 Thuy, N.H., Tien, L.A., Tuyet, P.N., Huong, D.T.T., Cong, N.V., Bayley, M., Wang, T., Lefevre,
565 S., 2010. Critical oxygen tension increases during digestion in the perch *Perca fluviatilis*. J.
566 Fish Biol. 76, 1025–1031. doi:10.1111/j.1095-8649.2009.02533.x

567 Tran-Duy, A., Schrama, J.W., van Dam, A.A., Verreth, J.A.J., 2008. Effects of oxygen
568 concentration and body weight on maximum feed intake, growth and hematological
569 parameters of Nile tilapia, *Oreochromis niloticus*. Aquaculture 275, 152–162.
570 doi:10.1016/j.aquaculture.2007.12.024

571 Tran-Duy, A., van Dam, A.A., Schrama, J.W., 2012. Feed intake, growth and metabolism of Nile
572 tilapia (*Oreochromis niloticus*) in relation to dissolved oxygen concentration. Aquac. Res.
573 43, 730–744. doi:10.1111/j.1365-2109.2011.02882.x

574 Vanderplanke, G., Claireaux, G., Quazuguel, P., Huelvan, C., Corporeau, C., Mazurais, D.,
575 Zambonino-Infante, J.L., 2014. Exposure to chronic moderate hypoxia impacts
576 physiological and developmental traits of European sea bass (*Dicentrarchus labrax*) larvae.
577 Fish Physiol. Biochem. 41, 233–242. doi:10.1007/s10695-014-0019-4

578 Vanderploeg, H.A., Ludsins, S.A., Cavaletto, J.F., Höök, T.O., Pothoven, S.A., Brandt, S.B.,
579 Liebig, J.R., Lang, G.A., 2009. Hypoxic zones as habitat for zooplankton in Lake Erie:
580 Refuges from predation or exclusion zones? J. Exp. Mar. Bio. Ecol. 381, S108–S120.

581 Vaquer-Sunyer, R., Duarte, C.M., 2008. Thresholds of hypoxia for marine biodiversity. Proc.
582 Natl. Acad. Sci. U. S. A. 105, 15452–15457. doi:10.1073/pnas.0803833105

583 Wannamaker, C.M., Rice, J.A., 2000. Effects of hypoxia on movements and behavior of selected
584 estuarine organisms from the southeastern United States. J. Exp. Mar. Bio. Ecol. 249, 145–
585 163. doi:10.1016/S0022-0981(00)00160-X

586 Whitney, R.J., 1942. Diurnal fluctuations of oxygen and pH in two small ponds and a stream. J.
587 Exp. Biol. 19, 92–99.

588 Wu, R.S.S., Zhou, B.S., Randall, D.J., Woo, N.Y.S., Lam, P.K.S., 2003. Aquatic hypoxia is an
589 endocrine disruptor and impairs fish reproduction. Environ. Sci. Technol. 37, 1137–1141.
590 doi:10.1021/es0258327

591 Zhang, J., Gilbert, D., Gooday, A.J., Levin, L., Naqvi, S.W.A., Middelburg, J.J., Scranton, M.,
592 Ekau, W., Peña, A., Dewitte, B., Oguz, T., Monteiro, P.M.S., Urban, E., Rabalais, N.N.,

Ittekkot, V., Kemp, W.M., Ulloa, O., Elmgren, R., Escobar-Briones, E., Van Der Plas, A.K., 2010. Natural and human-induced hypoxia and consequences for coastal areas: Synthesis and future development. *Biogeosciences* 7, 1443–1467. doi:10.5194/bg-7-1443-2010

Zhang, Z., Wells, M.C., Boswell, M.G., Beldorth, I., Kirk, L.M., Wang, Y., Wang, S., Savage, M., Walter, R.B., Booth, R.E., 2012. Identification of robust hypoxia biomarker candidates from fin of medaka (*Oryzias latipes*). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 155, 11–17. doi:10.1016/j.cbpc.2011.05.015

Legends

Figure 1. Schematic of experiment unit for Experiment 1

Figure 2. Mean hemoglobin concentration (mg/ml) for Experiment 2: Acute (a) and Experiment 3: Chronic (b). ‘Erie’ is the Erie population and ‘NC’ is the North Carolina population. Bars around mean indicate 80% credible interval, stars outside bars indicate 95% credible interval.

Figure 3. Mean fold change $\pm$ 95% CI for all genes examined in Experiment 2: Acute exposure, including brain (a), gill (b), liver (c), and muscle (d) tissues. ‘Erie’ is the Erie population and ‘NC’ is the North Carolina population.

Figure 4. Mean fold change $\pm$ 95% CI for all genes examined in Experiment 3: Chronic exposure, including brain (a), gill (b), liver (c), and muscle (d) tissues. ‘Erie’ is the Erie population and ‘NC’ is the North Carolina population.

Legends to supplementary files

Supplementary file 1. Methodological details

Supplementary file 2. Original data

620 **Table B1.** Experiment 1 movement and time in treatment data

621 **Table B2.** Experiment 2 fold data

622 **Table B3.** Experiment 3 growth, consumption, and [Hb] data

623 **Table B4.** Experiment 3 fold data

624

625 **Supplementary file 3.** Bayesian analysis parameter estimates

626 **Table C1.** Parameter estimates from Bayesian analysis of Experiment 1: Foraging

627 **Table C2.** Parameter estimates from Bayesian analysis of Experiment 2: Acute exposure

628 **Table C3.** Parameter estimates from Bayesian analysis of Experiment 3: Chronic

629 exposure

630

631 **Supplementary file 4.** 80% CI for Experiments 2 and 3

632 **Figure D1.** Mean fold change $\pm$ 80% CI for all genes examined in Experiment 2: Acute

633 exposure

634 **Figure D2.** Mean fold change $\pm$ 80% CI for all genes examined in Experiment 3: Chronic

635 exposure

636

Figure 1. Schematic of experiment unit for Experiment 1

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653 **Table 1.** Sequences of specific primer pairs used in quantitative PCR analyses

Gene	Accession	No	Primer Sequence
<i>β-actin</i> ¹	<u>AY332493</u>	F	GCCTCTCTGTCCACCTTCCA
		R	GGGCCGGACTCATCGTACT
<i>hif-1α</i>	<u>KX050166</u>	F	ATGGACACAGGAATTGTACC
		R	TGCATCCCTTGACTTCTCCTT
<i>igf-1β</i> ¹	<u>AY332492</u>	F	CGCAGGGCACAAGTGGAC
		R	CCCAGTGTTGCCTCGACTTG
<i>cyp19a1a</i> ²	<u>DQ984126</u>	F	TCTGGGTTTGGGGCCACTTC
		R	ACCGCTGATGCTCTGCTGAG
<i>hsp70</i>	<u>KX050165</u>	F	TGTTGGTCGGTGGCTCAA
		R	TTGAAGAAGTCCTGAAGCAGCTT
<i>socs3</i> ³	<u>HQ696607</u>	F	ACGAGCGAGAAGGTGTTTCAG
		R	CTCATTGCGGAGTCAAACCTTGC

654 ¹Pierron *et al.* (2009)

655 ²Lynn *et al.* (2009)

656 ³Shepherd *et al.* (2012)

657

658 **Table 2.** Prior and hyperprior distributions with mean and variance used in Bayesian analyses.

659 All priors were uninformative.

Experiment	Response	Parameter	Prior distribution	Values
1 Foraging	Binary movement, average time	Trial mean	Normal (μ , τ)	0, 0.01
		Trial SD	Uniform (a, b)	0, 100
		α ($\times$ 4 random intercepts, trials)	Normal (μ , τ)	Trial mean, trial SD^{-2}
		β ($\times$ 2 populations)	Normal (μ , τ)	0, 0.001
		β ($\times$ 3 treatments)	Normal (μ , τ)	0, 0.001
		Standard deviation	Uniform (a, b)	0, 100
2 Acute	Hb concentration	Day mean	Normal (μ , τ)	0, 0.01
		Day SD	Uniform (a, b)	0, 100
		Tank mean	Normal (μ , τ)	0, 0.01
		Tank SD	Uniform (a, b)	0, 100
		α ($\times$ 2 random intercepts, days)	Normal (μ , τ)	Day mean, day SD^{-2}
		α ($\times$ 24 random intercepts, tanks)	Normal (μ , τ)	Tank mean, tank SD^{-2}
		β ($\times$ 2 populations)	Normal (μ , τ)	0, 0.001
		β ($\times$ 2 treatments)	Normal (μ , τ)	0, 0.001
		Standard deviation	Uniform (a, b)	0, 100
	Gene expression ($\times$ 5 genes)	Day mean	Normal (μ , τ)	0, 0.01
		Day SD	Uniform (a, b)	0, 100
		α ($\times$ 2 random intercepts, days)	Normal (μ , τ)	Day mean, day SD^{-2}
		β ($\times$ 2 populations)	Normal (μ , τ)	0, 0.001
		β ($\times$ 2 treatments)	Normal (μ , τ)	0, 0.001
		Standard deviation	Uniform (a, b)	0, 100
3 Chronic	Consumption, Growth	α	Normal (μ , τ)	0, 0.01
		β ($\times$ 2 populations)	Normal (μ , τ)	0, 0.001
		β ($\times$ 3 treatments)	Normal (μ , τ)	0, 0.001
		Standard deviation	Uniform (a, b)	0, 100
	Hb concentration	Tank mean	Normal (μ , τ)	0, 0.01
		Tank SD	Uniform (a, b)	0, 100
		α ($\times$ 24 random intercepts, tanks)	Normal (μ , τ)	Tank mean, tank SD^{-2}
		β ($\times$ 2 populations)	Normal (μ , τ)	0, 0.001
		β ($\times$ 3 treatments)	Normal (μ , τ)	0, 0.001
		Standard deviation	Uniform (a, b)	0, 100
	Gene expression ($\times$ 5 genes)	α	Normal (μ , τ)	0, 0.01

β ($\times$ 2 populations)	Normal (μ , τ)	0, 0.001
β ($\times$ 2 treatments)	Normal (μ , τ)	0, 0.001
Standard deviation	Uniform (a, b)	0, 100

Supplemental file 1. Methodological details

A.1 Obtainment and rearing of yellow perch fry

During the first week of June 2014, yellow perch fry from two populations were obtained from Saint Mary's State Fish Hatchery in Ohio, which originated from Lake Erie (Erie), and from the Purdue Aquaculture Research Lab (ARL), which originated from North Carolina (NC). Both populations were subsequently reared at the ARL in separate flow-through 3200 L tanks with temperatures initially around 26 °C to spur growth and gradually adjusted to 15 °C throughout the four months of rearing to reach target experimental temperatures. Experimental temperatures were planned to be near 15 °C to mimic the temperature of the hypolimnion of a stratified lake in late summer (Roberts et al., 2012, 2009). Initial fish densities were variable (roughly between 0.6 fish/L and 1.5 fish/L). At the end of August, all densities were adjusted to approximately 0.6 fish/L. Temperature and DO were recorded weekly until the last month of rearing when both were recorded daily. pH was recorded weekly for the extent of rearing and experimentation. The light period followed natural light and dark periods, adjusting throughout rearing with sunset and sunrise times.

Yellow perch were initially fed to satiation twice daily with a combination of Otohime (Marubeni Nisshin Feed Co., Tokyo, Japan) 200 micron larval diet for marine fish and Zeigler (Gardners, Pennsylvania, USA) 0.6 – 0.85 mm crumble, transitioning throughout the first two months to Zeigler 1.5 mm crumble. At the end of July, fish were adjusted to once daily satiation

681 feedings, and they were gradually transitioned to Zeigler 2.5 mm slow sink crumble in the last
682 month of rearing. The 2.5 mm slow sink feed was used for feeding in all three experiments.

684 *A.2 Experiment 1 water quality values*

685 Water flowed into experimental tanks by gravity ($100 \text{ mL} \cdot \text{min}^{-1}$) from three head tanks,
686 temperature was 16.8 ± 1.2 (mean $\pm$ SD of all experimental units in trial), 18.2 ± 0.6 , 15.2 ± 0.5 ,
687 and 14.9 ± 0.6 °C for trials one through four respectively, and light was maintained at a 12h:12h
688 light:dark regime for all trials. Prior to starting the experiment, the amount of water mixing
689 between conjoined tanks was examined. The change in DO within each conjoined tank was less
690 than 1.0 mg/L. At least once during acclimation and hypoxic exposure, both Treatment and
691 Refuge tank pH was documented. Trial pH values were 8.2 ± 0.1 (mean $\pm$ SD), 8.3 ± 0.1 , $8.7 \pm$
692 0.1 , and 8.7 ± 0.1 , respectively.

694 *A.3 Experiment 2 water quality, flow, and average DO levels*

695 Water flowed into the tanks by gravity ($100 \text{ mL} \cdot \text{min}^{-1}$) from 3 head tanks, and
696 temperature was maintained at 15.3 ± 0.1 °C. Air was bubbled into the head tanks with a
697 diffuser, maintaining normoxic levels in the replicate tanks while fish acclimated. Dissolved
698 oxygen levels were maintained within the expected bounds over the course of the 60 minute
699 experiment. Initial DO levels in the hypoxic exposure and control tanks averaged 7.9 mg/L ($\pm$
700 0.1 SE) and 8.1 mg/L (± 0.1 SE) respectively. After 30 minutes, DO averaged 2.3 mg/L (± 0.2
701 SE) and 9.7 mg/L (± 0.2 SE) in the exposure and control tanks, respectively. After 60 minutes,
702 DO was stable around 2.0 mg/L (± 0.1 SE) and 9.7 mg/L (± 0.1 SE).

704 *A.4 Experiment 3 water quality and acclimation methods*

The aquarium system described above under Experiment 2 was reused. Temperature was maintained at 14.7 ± 0.4 °C, and pH was maintained between 8.2 and 8.7. Each of the 3 head tanks flowed to replicate tanks of the 3 different DO treatments. Dissolved oxygen treatments were maintained by bubbling in ambient air and/or N₂ gas with a diffuser into the respective head tank. The fish were fed in excess during a five day acclimation period prior to beginning the experiment. Dissolved oxygen was maintained at normoxic levels during acclimation (actual $9.0 \text{ mg/L} \pm 0.2 \text{ SD}$). On day six, the fish were starved and tank DO levels were gradually dropped throughout the day to treatment levels. Feeding resumed and treatment DO levels were maintained for the subsequent 10 days.

Supplemental file 2. Original data

Table B1. Experiment 1 movement and time in treatment data

Trial	Tank	Population	Treatment	Movement	Time in Treatment
1	1	Erie	Control	0	0.0
1	2	NC	Control	0	20.0
1	3	Erie	Moderate	0	0.0
1	4	NC	Control	1	6.6
1	5	Erie	Low	1	9.0
1	6	Erie	Low	1	1.9
1	7	Erie	Low	1	13.8
1	8	Erie	Low	0	15.0
1	10	NC	Moderate	0	10.0
1	11	Erie	Moderate	0	0.0
1	12	NC	Low	1	16.7
1	13	Erie	Moderate	0	20.0
1	14	NC	Control	1	0.7
1	15	NC	Low	1	7.6
1	16	NC	Moderate	1	2.3
1	17	NC	Control	1	19.1
1	18	NC	Moderate	1	14.0
1	19	NC	Low	1	13.0
1	20	Erie	Control	0	0.0
1	21	Erie	Control	0	0.0

1	22	Erie	Low	0	10.0
1	23	Erie	Moderate	0	0.0
1	24	Erie	Control	0	0.0
1	25	Erie	Control	1	1.5
1	27	NC	Low	1	1.2
1	28	Erie	Moderate	1	3.1
1	29	NC	Low	1	19.7
1	30	NC	Moderate	1	8.6
2	2	Erie	Control	0	0.0
2	3	Erie	Moderate	0	5.0
2	4	Erie	Low	0	0.0
2	5	Erie	Low	0	5.0
2	7	Erie	Low	0	5.0
2	8	Erie	Control	0	5.0
2	9	NC	Control	1	14.1
2	10	NC	Moderate	0	10.0
2	11	NC	Moderate	0	5.0
2	13	NC	Control	1	4.6
2	15	NC	Control	0	0.0
2	16	Erie	Moderate	0	0.0
2	17	Erie	Control	0	0.0
2	18	Erie	Control	0	20.0
2	19	NC	Low	0	0.0
2	20	NC	Low	1	5.7
2	21	Erie	Moderate	0	0.0
2	22	NC	Moderate	0	10.0
2	23	Erie	Moderate	0	0.0
2	24	Erie	Control	0	5.0
2	25	NC	Low	1	7.7
2	26	NC	Moderate	1	12.8
2	27	NC	Low	0	15.0
2	28	NC	Control	0	0.0
2	29	Erie	Low	0	0.0
2	30	Erie	Low	0	0.0
3	1	NC	Moderate	0	15.0
3	2	NC	Low	0	0.0
3	3	NC	Control	0	10.0
3	4	Erie	Control	0	20.0
3	6	Erie	Low	0	0.0
3	7	Erie	Low	0	10.0
3	8	Erie	Moderate	0	0.0
3	9	NC	Low	1	5.3
3	10	NC	Control	0	20.0

3	11	NC	Control	0	5.0
3	12	Erie	Moderate	0	0.0
3	13	Erie	Control	0	15.0
3	14	Erie	Control	0	10.0
3	16	Erie	Control	0	10.0
3	17	NC	Control	0	5.0
3	18	NC	Control	1	5.3
3	19	Erie	Moderate	1	4.2
3	20	NC	Low	0	0.0
3	21	Erie	Moderate	0	10.0
3	22	Erie	Moderate	1	5.6
3	23	NC	Moderate	0	0.0
3	24	Erie	Low	0	0.0
3	26	NC	Moderate	0	0.0
3	27	NC	Moderate	1	5.2
3	28	NC	Moderate	0	5.0
3	29	NC	Low	0	0.0
3	30	NC	Low	0	0.0
4	1	NC	Low	0	0.0
4	2	NC	Low	0	0.0
4	3	Erie	Low	0	15.0
4	4	Erie	Moderate	0	10.0
4	6	NC	Moderate	0	15.0
4	7	Erie	Low	0	15.0
4	8	NC	Low	1	6.5
4	9	NC	Low	0	0.0
4	11	NC	Moderate	0	0.0
4	12	Erie	Low	1	6.8
4	13	Erie	Low	0	0.0
4	14	Erie	Moderate	0	15.0
4	15	NC	Low	0	5.0
4	17	NC	Control	0	0.0
4	18	NC	Control	1	10.0
4	19	NC	Moderate	0	10.0
4	20	Erie	Control	0	0.0
4	21	Erie	Moderate	0	10.0
4	22	NC	Moderate	0	10.0
4	23	Erie	Moderate	0	10.0
4	24	NC	Control	0	15.0
4	25	NC	Moderate	0	5.0
4	26	NC	Control	0	5.0
4	27	Erie	Control	0	0.0
4	28	NC	Control	1	13.0

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4	29	Erie	Control	1	16.8
4	30	Erie	Control	0	5.0

Tissue	Tank	Populatio n	Treatmen t	<i>hif-1α</i>	<i>igf-1β</i>	<i>cyp19a1</i> <i>a</i>	<i>socs3</i>	<i>hsp70</i>
Brain	3	Erie	Control	1.01	0.92	1.09	1.65	1.22
Brain	4	Erie	Control	0.72	0.57	0.41	0.78	0.63
Brain	5	Erie	Control	0.95	1.34	1.06	1.42	0.96
Brain	6	Erie	Control	1.09	1.48	1.03	1.05	1.00
Brain	11	Erie	Control	1.25	1.84	1.18	1.37	1.32
Brain	14	Erie	Control	1.26	1.93	1.44	1.31	1.34
Brain	7	Erie	Exposure	1.26	1.32	3.10	1.98	1.51
Brain	15	Erie	Exposure	0.97	0.99	1.02	1.55	0.79
Brain	18	Erie	Exposure	1.27	1.85	2.46	3.00	1.56
Brain	19	Erie	Exposure	1.45	2.25	3.58	2.93	2.38
Brain	20	Erie	Exposure	0.88	0.71	0.33	0.98	0.69
Brain	22	Erie	Exposure	1.47	2.19	1.58	1.80	1.13
Brain	1	NC	Control	1.00	1.00	1.00	1.00	1.00
Brain	2	NC	Control	1.00	0.85	0.78	1.31	0.80
Brain	10	NC	Control	0.96	0.89	0.95	1.49	1.02
Brain	13	NC	Control	1.23	1.38	3.46	2.34	1.48
Brain	16	NC	Control	0.56	0.22	0.54	0.71	0.43
Brain	17	NC	Control	1.03	0.68	0.70	0.48	0.61
Brain	8	NC	Exposure	1.10	1.10	1.24	1.04	0.92
Brain	9	NC	Exposure	0.86	0.84	0.98	1.37	0.93
Brain	12	NC	Exposure	1.86	2.60	2.01	2.27	1.61
Brain	21	NC	Exposure	1.20	1.25	2.03	3.02	1.50
Brain	23	NC	Exposure	0.93	0.75	2.51	1.38	1.18
Brain	24	NC	Exposure	0.65	0.40	0.45	1.31	0.90
Gill	3	Erie	Control	1.29	1.44	0.22	0.38	0.87
Gill	4	Erie	Control	1.14	0.59	1.29	0.54	0.76
Gill	5	Erie	Control	1.17	0.27	0.31	0.03	0.06
Gill	6	Erie	Control	1.05	1.21	0.86	0.31	0.37
Gill	11	Erie	Control	3.01	10.42	0.63	0.60	1.11
Gill	14	Erie	Control	1.26	3.94	0.94	0.52	1.08
Gill	7	Erie	Exposure	1.71	0.65	0.70	0.06	0.08
Gill	15	Erie	Exposure	2.59	10.67	1.61	0.99	1.08
Gill	18	Erie	Exposure	1.57	2.25	0.23	0.17	0.29
Gill	19	Erie	Exposure	1.16	3.31	1.03	0.74	0.78
Gill	20	Erie	Exposure	1.54	4.48	0.54	0.26	0.35
Gill	22	Erie	Exposure	1.88	4.44	1.42	0.69	0.75
Gill	1	NC	Control	1.00	1.00	1.00	1.00	1.00
Gill	2	NC	Control	1.75	5.31	1.14	0.59	0.83
Gill	10	NC	Control	0.90	0.51	0.76	0.58	0.49
Gill	13	NC	Control	1.36	3.73	1.64	0.74	0.68

Gill	16	NC	Control	2.46	6.37	1.08	0.67	1.00
Gill	17	NC	Control	1.76	5.10	0.35	0.23	0.80
Gill	8	NC	Exposure	2.36	7.86	2.12	0.80	1.11
Gill	9	NC	Exposure	2.11	10.27	1.24	0.67	1.06
Gill	12	NC	Exposure	2.10	7.17	0.28	0.36	0.87
Gill	21	NC	Exposure	1.07	3.35	1.06	0.07	0.13
Gill	23	NC	Exposure	0.68	0.95	0.08	0.13	0.16
Gill	24	NC	Exposure	2.71	10.32	2.20	1.18	1.07
Liver	3	Erie	Control	1.77	0.57	0.11	0.17	0.29
Liver	4	Erie	Control	1.54	3.48	1.66	1.67	1.98
Liver	5	Erie	Control	2.08	3.94	2.26	2.44	2.18
Liver	6	Erie	Control	0.83	2.01	0.25	0.75	0.98
Liver	11	Erie	Control	1.33	1.75	1.28	0.82	0.90
Liver	14	Erie	Control	1.43	1.15	0.28	0.53	0.49
Liver	7	Erie	Exposure	2.08	6.54	2.43	1.53	1.75
Liver	15	Erie	Exposure	1.51	2.50	1.76	1.25	1.33
Liver	18	Erie	Exposure	0.95	1.49	0.79	0.43	0.35
Liver	19	Erie	Exposure	1.31	1.63	3.48	2.14	1.01
Liver	20	Erie	Exposure	0.97	0.71	0.18	0.21	0.17
Liver	22	Erie	Exposure	4.38	1.34	2.06	1.24	1.33
Liver	1	NC	Control	1.00	1.00	1.00	1.00	1.00
Liver	2	NC	Control	1.45	2.02	1.70	1.45	1.49
Liver	10	NC	Control	9.78	1.41	0.62	0.28	0.79
Liver	13	NC	Control	1.86	6.23	2.48	2.77	2.74
Liver	16	NC	Control	2.20	2.94	7.91	1.81	3.10
Liver	17	NC	Control	0.95	1.14	0.73	0.57	0.54
Liver	8	NC	Exposure	1.07	2.49	1.92	0.59	0.96
Liver	9	NC	Exposure	1.42	1.72	1.00	0.65	0.93
Liver	12	NC	Exposure	1.23	1.53	0.53	0.46	0.59
Liver	21	NC	Exposure	1.54	1.09	1.60	0.60	0.53
Liver	23	NC	Exposure	0.97	2.76	1.29	0.86	0.85
Liver	24	NC	Exposure	1.55	1.65	6.93	3.27	4.77
Muscle	3	Erie	Control	1.43	2.08	3.14	1.85	1.47
Muscle	4	Erie	Control	1.24	0.79	1.45	0.87	0.93
Muscle	5	Erie	Control	1.01	1.36	1.54	1.03	1.22
Muscle	6	Erie	Control	0.96	1.88	1.24	1.10	0.83
Muscle	11	Erie	Control	1.12	1.96	1.92	1.88	1.10
Muscle	14	Erie	Control	1.22	1.52	1.58	0.61	1.24
Muscle	7	Erie	Exposure	1.20	1.71	2.07	1.23	1.23
Muscle	15	Erie	Exposure	1.28	1.38	0.60	0.80	1.14
Muscle	18	Erie	Exposure	1.77	1.01	1.91	1.47	1.35
Muscle	19	Erie	Exposure	1.21	1.01	2.05	1.31	0.90
Muscle	20	Erie	Exposure	1.01	2.55	0.79	0.66	1.22

Muscle	22	Erie	Exposure	1.21	1.62	2.67	1.64	1.42
Muscle	1	NC	Control	1.00	1.00	1.00	1.00	1.00
Muscle	2	NC	Control	1.32	1.28	1.25	1.20	1.68
Muscle	10	NC	Control	1.39	1.83	1.25	1.43	0.75
Muscle	13	NC	Control	1.12	0.61	1.34	1.46	0.80
Muscle	16	NC	Control	0.99	0.81	1.38	0.85	1.25
Muscle	17	NC	Control	1.16	1.15	1.81	0.86	0.95
Muscle	8	NC	Exposure	1.56	2.25	1.55	1.09	1.28
Muscle	9	NC	Exposure	1.16	1.15	1.01	0.94	1.07
Muscle	12	NC	Exposure	1.62	2.19	1.83	1.22	1.15
Muscle	21	NC	Exposure	1.17	0.61	1.22	0.95	0.94
Muscle	23	NC	Exposure	1.13	1.17	0.38	0.38	1.11
Muscle	24	NC	Exposure	1.31	1.08	2.06	1.68	1.11

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Table B3. Experiment 3 (Chronic Exposure) growth, consumption, and [Hb] data

Tank	Treatment	Population	Growth (mm)	Consumption (g · g ⁻¹ · d ⁻¹)	[Hb]
1	Control	NC	0.33	0.00	47.6
2	Control	NC	0.67	$3.62 \cdot 10^{-4}$	45.4
3	Low	Erie	-0.33	$5.89 \cdot 10^{-5}$	37.1
4	Low	NC	0.00	$2.79 \cdot 10^{-5}$	42.3
5	Control	NC	0.33	$4.40 \cdot 10^{-4}$	46.3
6	Low	NC	-0.33	$4.38 \cdot 10^{-4}$	34.8
7	Moderate	Erie	0.33	$6.81 \cdot 10^{-4}$	50.7
8	Moderate	Erie	0.00	$9.40 \cdot 10^{-5}$	49.6
9	Moderate	NC	1.00	$3.53 \cdot 10^{-4}$	37.8
10	Control	Erie	0.33	$7.49 \cdot 10^{-5}$	52.9
11	Moderate	NC	0.33	$1.513 \cdot 10^{-3}$	43.0
12	Moderate	Erie	0.00	$8.18 \cdot 10^{-5}$	46.1
13	Low	Erie	-0.33	$6.75 \cdot 10^{-4}$	46.2
14	Low	NC	0.33	$2.60 \cdot 10^{-4}$	53.2
15	Moderate	NC	-0.33	0.00	45.1
16	Control	Erie	0.00	0.00	34.9
17	Moderate	NC	0.00	0.00	44.1
18	Control	NC	-0.33	$9.01 \cdot 10^{-4}$	44.3
19	Control	Erie	0.00	$5.41 \cdot 10^{-4}$	36.9
20	Low	Erie	0.67	$1.83 \cdot 10^{-3}$	45.9
21	Control	Erie	0.33	$1.21 \cdot 10^{-4}$	47.5
22	Low	Erie	0.33	0.00	50.4
23	Moderate	Erie	0.00	0.00	49.8
24	Low	NC	-0.67	$1.00 \cdot 10^{-3}$	35.3

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Table B4. Experiment 3 (Chronic Exposure) fold data

Tissue	Tank	Populatio n	Treatmen t	<i>hif-1α</i>	<i>igf-1β</i>	<i>cyp19a1</i> <i>a</i>	<i>socs3</i>	<i>hsp70</i>
Brain	10	Erie	Control	1.03	1.65	1.22	0.91	1.22
Brain	16	Erie	Control	1.12	1.44	0.77	1.46	1.10
Brain	19	Erie	Control	0.99	1.61	1.20	1.41	1.38
Brain	21	Erie	Control	1.00	1.60	1.23	1.27	1.13
Brain	7	Erie	Moderate	0.98	1.18	0.67	0.74	0.73
Brain	8	Erie	Moderate	0.91	1.51	0.77	1.06	0.85
Brain	12	Erie	Moderate	0.99	1.34	1.33	1.70	1.04
Brain	23	Erie	Moderate	1.02	0.80	0.75	1.29	1.02
Brain	3	Erie	Low	0.97	0.65	0.65	1.31	0.74
Brain	13	Erie	Low	0.92	0.96	1.36	1.24	1.19
Brain	20	Erie	Low	1.06	1.81	1.07	1.31	1.43
Brain	22	Erie	Low	0.96	1.23	1.23	1.92	1.10
Brain	1	NC	Control	0.85	1.11	0.83	0.95	0.90
Brain	2	NC	Control	1.00	1.42	1.16	0.72	0.82
Brain	5	NC	Control	0.99	1.29	1.30	1.09	1.34
Brain	18	NC	Control	1.00	1.00	1.00	1.00	1.00
Brain	9	NC	Moderate	1.00	1.04	0.95	0.89	1.01
Brain	11	NC	Moderate	1.05	0.84	1.23	1.31	1.02
Brain	15	NC	Moderate	0.99	0.79	0.80	0.91	0.74
Brain	17	NC	Moderate	0.89	0.57	0.91	0.92	0.99
Brain	4	NC	Low	1.10	0.95	1.41	1.72	1.02
Brain	6	NC	Low	0.90	0.36	1.13	1.17	1.13
Brain	14	NC	Low	1.03	1.40	1.31	1.85	1.32
Brain	24	NC	Low	0.81	0.41	0.55	0.86	0.88
Gill	10	Erie	Control	0.71	1.00	0.71	0.52	0.60
Gill	16	Erie	Control	1.21	1.43	0.50	0.61	0.43
Gill	19	Erie	Control	1.18	1.24	0.88	1.00	1.44
Gill	21	Erie	Control	0.52	0.17	0.10	0.40	0.76
Gill	7	Erie	Moderate	0.77	0.93	0.91	0.48	0.84
Gill	8	Erie	Moderate	0.75	0.68	0.83	0.55	0.63
Gill	12	Erie	Moderate	0.71	0.27	0.81	0.85	0.60
Gill	23	Erie	Moderate	0.71	0.11	0.49	0.27	0.77
Gill	3	Erie	Low	0.84	1.09	0.83	0.68	0.87
Gill	13	Erie	Low	0.70	0.46	1.37	0.44	0.52
Gill	20	Erie	Low	0.83	0.69	0.85	0.44	0.48
Gill	22	Erie	Low	0.70	0.31	0.41	0.68	0.67
Gill	1	NC	Control	0.64	0.22	0.57	0.52	0.64
Gill	2	NC	Control	0.73	0.43	0.43	0.46	0.71
Gill	5	NC	Control	0.68	0.51	0.87	0.59	1.02
Gill	18	NC	Control	1.00	1.00	1.00	1.00	1.00

Gill	9	NC	Moderate	0.71	0.38	0.35	0.42	0.42
Gill	11	NC	Moderate	0.94	1.39	1.26	0.67	1.00
Gill	15	NC	Moderate	0.86	0.66	0.84	0.56	1.40
Gill	17	NC	Moderate	0.93	0.50	1.53	1.17	1.42
Gill	4	NC	Low	1.35	2.33	1.20	1.01	0.92
Gill	6	NC	Low	0.77	1.08	0.79	0.97	1.07
Gill	14	NC	Low	0.78	1.06	0.88	0.71	0.76
Gill	24	NC	Low	0.53	0.53	0.14	0.30	0.39
Liver	10	Erie	Control	0.72	1.11	0.66	1.73	0.94
Liver	16	Erie	Control	0.86	1.06	0.65	1.48	1.41
Liver	19	Erie	Control	0.86	1.16	0.82	1.31	1.09
Liver	21	Erie	Control	0.94	0.97	0.48	0.82	0.83
Liver	7	Erie	Moderate	0.87	1.23	0.44	0.64	0.93
Liver	8	Erie	Moderate	1.02	1.07	0.50	1.10	1.43
Liver	12	Erie	Moderate	1.63	1.01	1.41	1.45	1.20
Liver	23	Erie	Moderate	1.15	1.51	1.33	0.89	1.24
Liver	3	Erie	Low	0.85	1.22	0.42	0.89	1.07
Liver	13	Erie	Low	1.09	1.59	0.79	1.00	1.69
Liver	20	Erie	Low	1.06	1.03	0.35	1.62	1.49
Liver	22	Erie	Low	0.92	1.51	0.50	1.12	1.46
Liver	1	NC	Control	0.99	1.19	0.77	2.93	1.41
Liver	2	NC	Control	0.51	0.12	0.36	0.67	0.26
Liver	5	NC	Control	1.00	1.16	1.00	2.10	1.80
Liver	18	NC	Control	0.46	1.00	1.99	1.00	1.00
Liver	9	NC	Moderate	0.73	1.27	0.83	1.25	1.09
Liver	11	NC	Moderate	0.98	1.32	0.77	0.77	1.19
Liver	15	NC	Moderate	0.92	1.30	1.32	1.57	1.11
Liver	17	NC	Moderate	0.84	1.04	1.81	0.66	0.86
Liver	4	NC	Low	0.90	1.20	1.79	1.22	1.22
Liver	6	NC	Low	0.96	1.13	0.56	0.80	1.43
Liver	14	NC	Low	0.87	1.29	1.13	2.72	1.43
Liver	24	NC	Low	0.90	1.03	0.89	1.01	1.36
Muscle	10	Erie	Control	1.38	3.52	0.53	0.38	0.53
Muscle	16	Erie	Control	0.90	2.77	0.75	0.48	0.28
Muscle	19	Erie	Control	1.41	0.83	1.52	0.56	0.71
Muscle	21	Erie	Control	1.17	0.72	2.22	0.88	0.62
Muscle	7	Erie	Moderate	1.46	1.94	1.87	0.80	0.61
Muscle	8	Erie	Moderate	1.37	1.33	1.74	0.67	0.57
Muscle	12	Erie	Moderate	1.18	2.46	3.27	1.19	0.52
Muscle	23	Erie	Moderate	1.03	1.27	0.88	0.41	0.31
Muscle	3	Erie	Low	1.33	0.78	2.06	0.74	0.49
Muscle	13	Erie	Low	1.67	3.51	2.06	0.91	0.56
Muscle	20	Erie	Low	1.02	0.95	0.43	0.47	0.63

Muscle	22	Erie	Low	1.21	1.20	2.08	0.54	0.47
Muscle	1	NC	Control	1.13	0.80	0.83	0.45	0.87
Muscle	2	NC	Control	1.49	1.67	1.74	0.74	0.61
Muscle	5	NC	Control	1.00	0.90	1.00	0.47	0.39
Muscle	18	NC	Control	1.33	1.00	2.73	1.00	1.00
Muscle	9	NC	Moderate	0.96	0.47	1.97	0.83	0.61
Muscle	11	NC	Moderate	1.01	2.45	1.05	0.48	0.67
Muscle	15	NC	Moderate	1.25	0.96	1.10	0.45	0.72
Muscle	17	NC	Moderate	1.17	2.69	1.68	0.49	0.46
Muscle	4	NC	Low	1.08	0.35	0.92	0.57	0.30
Muscle	6	NC	Low	1.31	2.12	1.50	0.81	0.77
Muscle	14	NC	Low	1.03	0.78	2.93	0.80	0.95
Muscle	24	NC	Low	0.99	1.57	1.61	0.57	0.36

Supplemental file 3. Bayesian analysis parameter estimates

Table C1. Parameter estimates from Bayesian analysis of Experiment 1: Foraging. β indicates the effect of the respective factor, ‘f’ is the proportion of the posterior distribution with the same sign as β (i.e., the confidence that β has a positive or negative effect), and ‘95% CI overlap’ indicates if the credible interval around β overlaps with zero.

Response	Value	Population		Treatment		Random Effect	
		Erie	NC	Control	Moderate	Low	Trial
Movement	β	-1.49	0.13	-0.02	-0.42	0.37	-0.56
	f	0.50	0.51	0.50	0.51	0.50	0.52
	95% CI overlap	Yes	Yes	Yes	Yes	Yes	Yes
Time	β	1.23	3.22	1.77	0.53	1.74	4.05
	f	0.58	0.70	0.63	0.54	0.62	0.73
	95% CI overlap	Yes	Yes	Yes	Yes	Yes	Yes

Table C2. Parameter estimates from Bayesian analysis of Experiment 2: Acute Exposure. β indicates the effect of the respective factor, ‘f’ is the proportion of the posterior distribution with the same sign as β (i.e., the confidence that β has a positive or negative effect), and ‘95% CI overlap’ indicates if the credible interval around β overlaps with zero.

Tissue	Response	Value	Population		Treatment		Random Effect	
			Erie	NC	Control	Exposure	Day	Tank
Brain	[Hb]	β	17.35	22.96	23.17	19.70	0.60	1.50
		f	0.63	0.68	0.68	0.65	0.52	0.56
		95% CI overlap	True	True	True	True	True	True
	hif	β	-0.56	-0.61	0.19	0.24	0.18	
		f	0.51	0.51	0.50	0.50	0.52	
		95% CI overlap	True	True	True	True	True	
	igf	β	0.16	-0.04	-1.20	-1.10	0.65	
		f	0.50	0.50	0.51	0.51	0.53	
		95% CI overlap	True	True	True	True	True	
	cyp	β	-0.48	-0.50	0.24	0.41	-0.02	
		f	0.50	0.50	0.50	0.50	0.50	
		95% CI overlap	True	True	True	True	True	
	socs3	β	0.69	0.63	0.40	0.58	-0.82	
		f	0.50	0.50	0.50	0.51	0.53	
		95% CI overlap	True	True	True	True	True	
	hsp70	β	-0.37	-0.44	-0.09	0.02	0.14	
		f	0.49	0.49	0.50	0.50	0.51	
		95% CI overlap	True	True	True	True	True	
Gill	hif	β	-1.19	-1.18	1.28	1.35	-0.03	

Liver		f	0.51	0.51	0.51	0.51	0.49
		95% CI overlap	True	True	True	True	True
		β	0.97	1.19	-0.20	0.11	-0.35
	igf	f	0.51	0.51	0.51	0.50	0.51
		95% CI overlap	True	True	True	True	True
		β	0.09	0.17	-0.06	-0.05	-0.14
	cyp	f	0.50	0.50	0.49	0.49	0.51
		95% CI overlap	True	True	True	True	True
		β	-0.19	-0.02	-0.90	-0.97	-0.18
	socs3	f	0.51	0.51	0.51	0.50	0.50
		95% CI overlap	True	True	True	True	True
		β	-0.59	-0.45	0.54	0.42	0.11
	hsp70	f	0.51	0.51	0.51	0.51	0.49
		95% CI overlap	True	True	True	True	True
		β	-0.67	-0.66	0.38	0.31	0.51
	hif	f	0.50	0.50	0.50	0.49	0.53
		95% CI overlap	True	True	True	True	True
		β	0.61	0.63	0.23	0.21	0.04
	igf	f	0.51	0.51	0.50	0.50	0.48
		95% CI overlap	True	True	True	True	True
		β	-0.41	-0.16	-0.30	-0.12	0.29
	cyp	f	0.49	0.49	0.51	0.51	0.52
		95% CI overlap	True	True	True	True	True
		β	-0.41	-0.16	-0.30	-0.12	0.29

Muscle	socs3	β	-0.66	-0.62	0.16	0.13	0.26
		f	0.51	0.51	0.50	0.50	0.50
		95% CI overlap	True	True	True	True	True
	hsp70	β	-2.51	-2.36	2.14	2.05	0.55
		f	0.51	0.51	0.51	0.51	0.54
		95% CI overlap	True	True	True	True	True
	hif	β	0.03	0.04	-0.24	-0.19	0.02
		f	0.50	0.50	0.51	0.51	0.50
		95% CI overlap	True	True	True	True	True
	igf	β	-1.49	-1.60	1.66	1.69	0.05
		f	0.50	0.50	0.51	0.51	0.50
		95% CI overlap	True	True	True	True	True
	cyp	β	2.37	2.26	-1.51	-1.56	-0.36
		f	0.51	0.51	0.51	0.51	0.52
		95% CI overlap	True	True	True	True	True
	socs3	β	0.10	0.06	-0.15	-0.18	0.09
		f	0.50	0.50	0.50	0.50	0.50
		95% CI overlap	True	True	True	True	True
	hsp70	β	-0.40	-0.43	-0.07	-0.03	0.56
		f	0.50	0.50	0.50	0.50	0.50
		95% CI overlap	True	True	True	True	True

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Table C3. Parameter estimates from Bayesian analysis of Experiment 3: Chronic Exposure. β indicates the effect of the respective factor, ‘f’ is the proportion of the posterior distribution with the same sign as β (i.e., the confidence that β has a positive or negative effect), and ‘95% CI overlap’ indicates if the credible interval around β overlaps with zero.

Tissue	Response	Value	Population		Treatment			Random Effect
			Erie	NC	Control	Moderate	Low	Tank
Brain	Consumption	β	-0.47	-0.39	0.98	0.97	1.21	
		f	0.51	0.51	0.50	0.50	0.51	
		95% CI overlap	True	True	True	True	True	
	Growth	β	0.85	0.86	-0.41	-0.44	-0.65	
		f	0.50	0.50	0.51	0.51	0.51	
		95% CI overlap	True	True	True	True	True	
	[Hb]	β	29.19	26.78	16.73	18.07	15.45	-0.24
		f	0.74	0.72	0.64	0.65	0.63	0.49
		95% CI overlap	True	True	True	True	True	True
	hif	β	1.67	1.65	-1.52	-1.53	-1.54	
		f	0.51	0.51	0.51	0.51	0.51	
		95% CI overlap	True	True	True	True	True	
	igf	β	0.09	-0.08	0.17	0.02	-0.04	
		f	0.49	0.51	0.51	0.51	0.49	
		95% CI overlap	True	True	True	True	True	
	cyp	β	-0.05	-0.04	0.05	-0.03	0.03	
		f	0.50	0.50	0.50	0.50	0.50	
		95% CI overlap	True	True	True	True	True	

Gill	socs3	β	2.12	2.05	-2.06	-2.06	-1.95
		f	0.53	0.53	0.53	0.53	0.53
		95% CI overlap	True	True	True	True	True
	hsp70	β	-0.74	-0.76	0.86	0.78	0.85
		f	0.51	0.51	0.51	0.51	0.51
		95% CI overlap	True	True	True	True	True
	hif	β	0.19	0.20	-0.07	-0.08	-0.08
		f	0.51	0.51	0.50	0.50	0.50
		95% CI overlap	True	True	True	True	True
	igf	β	-0.03	0.08	-0.22	-0.30	-0.10
		f	0.50	0.50	0.50	0.50	0.50
		95% CI overlap	True	True	True	True	True
	cyp	β	1.53	1.58	-1.86	-1.68	-1.75
		f	0.51	0.51	0.52	0.52	0.52
		95% CI overlap	True	True	True	True	True
	socs3	β	-1.32	-1.24	1.34	1.32	1.35
		f	0.52	0.52	0.51	0.51	0.51
		95% CI overlap	True	True	True	True	True
	hsp70	β	-0.66	-0.57	0.67	0.69	0.61
		f	0.51	0.51	0.51	0.51	0.51
		95% CI overlap	True	True	True	True	True
Liver	hif	β	1.69	1.61	-1.84	-1.73	-1.75
		f	0.51	0.51	0.52	0.51	0.51

		95% CI overlap	True	True	True	True	True
	igf	β	-0.16	-0.25	-0.27	-0.10	-0.09
		f	0.50	0.50	0.51	0.51	0.50
		95% CI overlap	True	True	True	True	True
	cyp	β	-1.91	-1.71	1.54	1.64	1.51
		f	0.52	0.51	0.52	0.52	0.52
		95% CI overlap	True	True	True	True	True
	socs3	β	-1.49	-1.46	1.39	1.25	1.34
		f	0.51	0.51	0.51	0.51	0.51
		95% CI overlap	True	True	True	True	True
	hsp70	β	0.47	0.43	-0.23	-0.16	-0.07
		f	0.50	0.50	0.51	0.51	0.51
		95% CI overlap	True	True	True	True	True
Muscle	hif	β	-1.08	-1.12	1.30	1.30	1.29
		f	0.51	0.51	0.51	0.51	0.51
		95% CI overlap	True	True	True	True	True
	igf	β	-1.16	-1.29	1.26	1.21	1.33
		f	0.52	0.52	0.52	0.52	0.52
		95% CI overlap	True	True	True	True	True
	cyp	β	0.76	0.79	-0.66	-0.57	-0.55
		f	0.50	0.50	0.50	0.50	0.50
		95% CI overlap	True	True	True	True	True
	socs3	β	-0.81	-0.83	0.37	0.42	0.39

	f	0.51	0.5	0.50	0.50	0.50
			:>}} 1			
	95% CI overlap	True	True	True	True	True
hsp70	β	-0.16	-0.09	-0.14	-0.18	-0.17
	f	0.49	0.49	0.51	0.51	0.51
	95% CI overlap	True	True	True	True	True

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Figure D1. Mean fold change $\pm$ 80% CI for all genes examined in Experiment 2: Acute exposure, including brain (a), gill (b), liver (c), and muscle (d) tissues.

Figure D2. Mean fold change $\pm$ 80% CI for all genes examined in Experiment 3: Chronic exposure, including brain (a), gill (b), liver (c), and muscle (d) tissues.