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RESEARCH ARTICLE

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Effects of pre-winter cortisol exposure on condition, diet, and morphology of wild juvenile brown trout (*Salmo trutta*)

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Abstract

Winter is an energetically challenging period for many animals in temperate regions because of the relatively harsh environmental conditions and reduction in food availability during this season. Moreover, stressors experienced by individuals in the fall can affect their subsequent foraging strategy and energy stores after exposure has ended, referred to as carryover effects. We used exogenous cortisol manipulation of wild juvenile brown trout (*Salmo trutta*) in the fall to simulate a physiological stress response and then investigated short-term (2 weeks) and long-term (4 months) effects on condition metrics (hepatosomatic index and water muscle content), diet (stomach contents and stable isotopes), and morphology during growth in freshwater. We revealed some short-term impacts, likely due to handling stress, and long-term (seasonal) changes in diet, likely reflecting prey availability. Unfortunately, we had very few recaptures of cortisol-treated fish at long-term sampling, limiting detailed analysis about cortisol effects at that time point. Nonetheless, the fish that were sampled showed elevated stable isotopes, suggestive of a cortisol effect long after exposure. This is one of few studies to investigate whether cortisol influences foraging and morphology during juvenile growth, thus extending the knowledge of proximate mechanisms influencing ecologically-relevant phenotypes.

KEYWORDS
carry-over effects, diet, glucocorticoid, migration, physiology, season, stable isotopes

1 | INTRODUCTION

Stressors are ubiquitous and highly variable in natural systems (Boonstra, 2013; Johnstone et al., 2012; Przeslawski et al., 2015). In temperate and polar regions, winter is a physiologically challenging period for many animals because of the relatively harsh environmental conditions, particularly temperature, and reduction in food availability during this season (Sutton et al., 2021). Some animals (e.g., many fishes) remain active, although often somewhat quiescent compared to other seasons, and continue foraging in winter conditions (Shuter et al., 2012). The winter period itself can be a source of multiple environmental stressors, such as variation in temperature, ice cover, and flow, but this period is often overlooked (Brady et al., 2020). Moreover, stress incurred during other

seasons may impact the probability of surviving the winter period when there are carryover effects from earlier events (e.g., [O'Connor et al., 2010, 2014]). Many juvenile fish are particularly susceptible to overwinter mortality, with fish size and energetic condition influencing survival (Cunjak, 1996; Gosselin et al., 2021; Hurst, 2007; Shuter et al., 2012). However, fewer studies have investigated effects of stressors on those individuals that survive the event (i.e., sublethal impacts; e.g., Louhi et al., 2023; Saboret & Ingram, 2019).

When fish experience stressful events the hypothalamic-pituitary-interrenal (HPI) axis is activated (Birnie-Gauvin et al., 2018; Wendelaar Bonga, 1997). During periods of stress, the circulating levels of glucocorticoid hormones (e.g., cortisol) increases, leading to the mobilization of fatty acids and liver glycogen that provide available energy resources to cope with the stressor (Barton et al., 2002; Mileva et al., 2009). This cascade of events can initiate both physiological and behavioral responses with the ultimate goal of reestablishing homeostasis (Barton et al., 2002). However, prolonged elevation of cortisol can have detrimental effects when resources are diverted from other activities, such as foraging and growth, to focus on survival, which has long-term consequences on behavior and, ultimately, life-history trade-offs (Wingfield et al., 1998). To better understand how organisms in the wild are affected by stressors, we also need to understand how the underlying physiological mechanisms (Baker et al., 2013; Dantzer et al., 2014), including those mediated by hormones (Sapolsky et al., 2000), impact individual performance (Ball & Balthazart, 2008; Denver, 2009).

Exogenous cortisol manipulation is a common technique for assessing the responses of fish to one aspect of the HPI axis (reviewed in MacDougall-Shackleton et al., 2019; Sopinka et al., 2015). Cortisol consistently affects energy allocation, though the extent to which it does so via mobilizing energy reserves versus stimulating foraging behavior (orexigenic activity) is still unclear and has mainly been studied in birds (reviewed in Sopinka et al., 2015; Vera et al., 2017). Additionally, contemporary studies have focused on foraging activity or efficiency, not type of dietary items ingested. The effects of cortisol on body morphology are even less known, with only one fish (Vinterstare et al., 2020) and one amphibian (Middlemis Maher et al., 2013) laboratory study to date, to our knowledge. While diet can affect feeding morphology (Robinson & Parsons, 2002), whether cortisol affects an individual's diet composition and body morphology is not well understood due to a lack of knowledge regarding the proximate mechanisms affecting ecologically-relevant phenotypes. As the diverse role of glucocorticoids continue to be explored, we will start to better understand the ecology, evolution, and mechanisms of action of these important hormones (MacDougall-Shackleton et al., 2019).

Brown trout (*Salmo trutta*) are a partially migrant species and although there is extensive life-history variation within and among populations (Klemetsen et al., 2003), in most areas juveniles hatch in freshwater tributaries in the spring and spend 1–3 years in their natal stream before either migrating to sea or staying in freshwater and assuming residency (Boel et al., 2014; Cucherousset et al., 2005). A juvenile's "decision" to stay or migrate has been linked to individual condition (Cucherousset et al., 2005; Olsson et al., 2006; Wysujack et al., 2009) though maternal effects and genetic factors also play a role (Van Leeuwen et al., 2017; Páez et al., 2011). Before migration, individuals undergo smoltification, a process which involves physiological, morphological, and behavioral changes (Björnsson et al., 2011). Given the importance of the fall season for smoltification preparation and the fact that it precedes winter, juvenile trout at this time are likely sensitive to perturbations that result in sublethal stressors (Peiman et al., 2017). Such stressors can be caused by natural (e.g., predation attempts, food scarcity) or human (e.g., point source pollution, habitat alterations) sources (Baker et al., 2013; Helmuth, 2009).

Here we tested for the effects of one component of the HPI response by exposing wild juvenile brown trout to an exogenous cortisol manipulation in their natal stream and then assessing their condition, diet, and morphology over short- and long-term time intervals. If cortisol affects energy allocation, we predicted manipulated fish would have decreased assimilation efficiency (poorer condition metrics and enriched nitrogen stable isotopic values reflecting starvation; see below) and/or reduced foraging rates (emptier stomachs). If cortisol affects ecologically-relevant morphology, then manipulated fish should have morphological traits that reflect poor feeding and swimming performance.

2 | METHODS

2.1 | Fish collection

We initially caught juvenile brown trout from October 20 to 25, 2015 along the Gudsø stream in Denmark using single-pass electrofishing gear (see Birnie-Gauvin et al., 2017 for study site details). All trout caught between 12 and 20 cm were anaesthetized in benzocaine (0.03 g L^{-1}), photographed, weighed, measured, and tagged with a 23 mm passive integrated transponder tag (Texas Instruments; RI-TRP-RRHP, 134 kHz, 0.6 g mass in air). At this time a 0.1 mL blood sample was taken from the caudal vein using a 1.5 inch 25-gauge heparinized needle, and approximately 30 scales were removed from the left side above the lateral line and posterior to the dorsal fin. The 834 trout were then randomly assigned one of three treatments: control (no further manipulation), sham, or cortisol.

Cortisol fish received an intracoelomic injection of hydrocortisone 21-hemisuccinate (Sigma-Aldrich) suspended in vegetable shortening (100% vegetable shortening; Crisco) using a dosage of 100 mg kg^{-1} which elevates the levels of circulating cortisol above baseline levels for at least 9 days (see Birnie-Gauvin et al., 2018). While cortisol values of juvenile brown trout using the same treatment protocols administered at similar water temperatures

(6–7°C; Birnie-Gauvin et al., 2018) were elevated above physiological levels at Day 3 posttreatment, by Day 6 values were within the range of stress-induced levels produced naturally in fish (Birnie-Gauvin et al., 2018; Gamperl et al., 1994). Similar cortisol protocols have been successfully employed in other studies using this same brown trout population (Midwood et al., 2014, 2015, 2016). Therefore, we did not specifically measure cortisol levels as part of this study. Sham fish received the same dosage of vegetable shortening without any hydrocortisone.

On November 5 to 7, 2015 (approximately 2 weeks post-treatment; hereafter, the short-term sampling period), we electrofished the same stretch of stream, and retained the first 100 fish from each treatment encountered. We resampled these trout with a photograph, mass, length, and 0.1 mL blood sample using the same methods as during the initial sampling protocol. Scales had not yet regrown and so were not sampled. Twenty trout per treatment ($n = 60$ total) were euthanized by overdose of benzocaine to assess diet by stomach contents and nutritional status (see below).

On February 29 to March 2, 2016 (approximately 16 weeks after treatment; hereafter, the long-term sampling period), the stream was electrofished starting from 750 m downstream and ending 1600 m upstream of our initial sampling locations. Twenty control, 18 sham, and 4 cortisol-treated fish were recaptured, representing all tagged fish encountered. These trout were resampled as before, but additionally had scales removed from the same location as the initial sample. We did not lethally sample these long-term tagged fish for stomach contents as too few fish were recaptured, but we did sacrifice untagged fish at this time point to use for diet and condition metrics. We only analyzed initial isotope samples from the individuals we captured in either the short or long-term to provide repeated samples for comparison.

2.2 | Condition metrics

Daily growth rates (mass and length) and condition factors for fish used in this study have been previously published (Birnie-Gauvin et al., 2017). We assessed two additional condition metrics of individuals that were lethally sampled during each time interval. First, we assessed the hepatosomatic index (HSI) by taking the ratio of wet whole liver weight to body weight. This provides an indication of energy status, because as liver size decreases for a given body size (corresponding to less energy reserves) the HSI values decrease (O'Connor et al., 2013). Second, we assessed white muscle water content which is inversely related to lipid content (Barton et al., 2002; Brett & Groves, 1979; Gravel et al., 2010). We expected individuals under nutritional stress to have higher water content values, reflecting a reduction in lipid levels within the body. To determine water content, we took a sample of muscle from the lateral side of each fish, weighed it, oven dried it for 24 h at 80°C, reweighed each sample, and used the difference in weight expressed as a proportion of the original weight.

2.3 | Diet

We examined the diet of a random subsample of fish upon initial capture, a random subsample of tagged fish per treatment during short-term recapture (see above), and a random subsample of non-tagged fish upon long-term capture (see Supporting Information S1: Table 1 for sample sizes). Stomach contents (including intestines) were kept frozen until examined under a dissecting microscope. Prey were identified to Order or Family and further classified as aquatic (zoobenthos, snails, dipteran pupae, worms) or terrestrial (terrestrial insects), as terrestrial insects would involve a different foraging strategy (i.e., feeding at the water's surface). Prey were recorded as wet mass which we used to calculate stomach fullness (mass of all prey divided by mass of full stomach), proportion of terrestrial food (terrestrial prey mass divided by total mass of all prey), and a categorical metric of specialization (where individuals were terrestrial specialists if terrestrial food mass was >50% total prey mass). As proportion of aquatic food and aquatic specialization are complementary numbers to proportion of terrestrial food and terrestrial specialization, respectively (proportions sum to 1), we do not analyze terrestrial and aquatic metrics separately.

Stable isotopes can be used to infer an individual's habitat and resource use (Kelly, 2000). Terrestrial insects have a depleted $\delta^{13}C$ signature compared to aquatic insects (Akamatsu et al., 2004), and nitrogen stable isotopes ($\delta^{15}N$) are more enriched with each trophic level (Cabana & Rasmussen, 1996). Thus, carbon stable isotope values can be used as a proxy for habitat origin of prey and nitrogen stable isotopes as an indicator of trophic position (e.g., Colborne & Robinson, 2013). However, $\delta^{15}N$ also becomes enriched during starvation when endogenous proteins are used for maintenance (Hatch, 2012).

Blood plasma has a fast isotopic turnover (2–3 days; Vander Zanden et al., 2015) and so reflects recent diet. Blood samples were prepared for stable isotope analysis by spinning whole blood in the field at 6000 rpm for 2 min, then separating the plasma and red blood cell components. Plasma samples were stored on ice for <8 h before transferring it to a −20°C freezer until analysis. Plasma samples were oven-dried for 24 h at 60°C, ground into fine pieces, and then weighed into tin capsules.

Fish scales grow both outwardly and by underplating, and thus whole-scale isotope values are biased to the last few months of feeding (Hutchinson & Trueman, 2006). Natal scales of individuals initially collected in late October are biased towards their summer diet from approximately June onwards. Regrown scales collected from individuals resampled at the long-term interval would only reflect diet since treatment, assuming exogenously-derived nutrients are used for new scale formation. Regrown scales are easily distinguished from natal scales by their large central plate, as well as irregular and few circuli due to rapid growth during the replacement phase. Scales from recaptured trout were examined under a microscope and only regrown scales were used for long-term isotope data. Scales were cleaned using distilled water, oven-dried for 24 h, cut into fine pieces, and weighed into tin capsules. All plasma

and scale isotope samples were analyzed at the Trophic Ecology Lab at the Great Lakes Institute for Environmental Research (GLIER; University of Windsor).

2.4 | Body morphology

We took a picture of the right side of each anesthetized fish using a Nikon camera mounted on a tripod in the field. We used tpsDig2 (ver. 2.25) to place landmarks on each fish and we extracted linear measurements using CoordGen8 (Integrated Morphometrics Package Suite; H. David Sheets; Canisius College). We chose measurements related to ecologically relevant morphology, with two related to feeding (upper maxilla length, snout length), and three related to swimming (pectoral fin length, distance between insertion points for pectoral and anal fins, caudal fin depth) (Figure 1). We regressed each body metric against standard length as measured in the field to analyze size-corrected differences in morphological traits.

2.5 | Data analysis

Condition (HSI index, water content) and diet (stomach fullness, proportion of terrestrial food) were assessed using two analysis of variance (ANOVA) models with treatment or time as the covariate, while terrestrial specialist was analyzed using two logistic regression models: one that analyzed treatment (control, sham, cortisol) at the short-term time point; and one that analyzed time (initial, short-term, long-term) with control fish. We examined the change in diet (stable isotope values) and morphological traits using a general linear mixed effects model to compare time, treatment, and their interaction, with individual as a random effect to account for repeated measures over time. In all cases, when appropriate, Tukey's post hoc comparisons were used to compare groups.

We used base R for ANOVA models, with GLM models run using the lmer function from the lme4 package (Bates et al., 2014), and Tukey's comparisons were made using the emmeans package (Lenth, 2019).

3 | RESULTS

During the initial sampling period 834 fish were collected and assigned to either control ($n = 276$), sham ($n = 282$), or cortisol ($n = 276$) treatments. During the short-term sampling period (approximately 2 weeks after initial capture), we sampled 100 tagged individuals from each treatment. At the long-term sampling point (approximately 16 weeks after initial capture), we captured 20 control fish (7.2% of tagged sample), 18 sham fish (6.4%), and 4 (1.4%) cortisol treated fish, some of which had also been captured at the short-term (Supporting Information S1: Table 1). Thus, although we present results testing for long term effects of cortisol, the data available for this category were limited and should be interpreted with caution. Presumably the cortisol treatment contributed to mortality based on reductions in recapture relative to controls and shams.

3.1 | Condition metrics

HSI differed among time points ($F_{2,41} = 10.66$, $p < 0.001$) with initial values decreasing in the short term ($p = 0.01$) and then increasing in the long term ($p = 0.0002$), so that initial and long-term values did not differ ($p = 0.19$, Figure 2a). Water content also differed among time points ($F_{2,41} = 9.03$, $p = 0.0006$) with similar initial and short-term values ($p = 0.70$) that decreased at the long-term time point (initial-long: $p = 0.004$; short-long: $p = 0.0006$; Figure 2b).

At the short-term time point, the HSI index was affected by treatment ($F_{2,51} = 6.64$, $p = 0.003$; Figure 3a), with control values lower than cortisol ($p = 0.002$) but not different from sham ($p = 0.15$), nor was sham different from cortisol ($p = 0.19$). Water content was not affected by treatment ($F_{2,51} = 1.15$, $p = 0.33$; Figure 3b).

3.2 | Diet

Stomach fullness did not differ among time points ($F_{2,40} = 0.19$, $p = 0.88$). The proportion of prey type changed over time

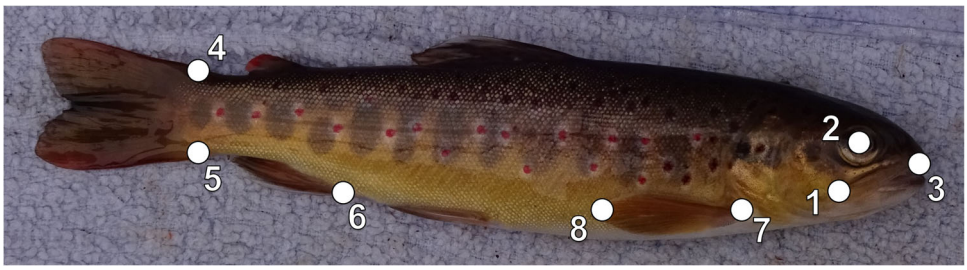

FIGURE 1 Placement of landmarks on juvenile brown trout used for linear measurements, generating two feeding traits (upper maxilla length: point 1–2, snout length: point 2–3) and three swimming traits (pectoral fin length: point 7–8, distance between insertion points for pectoral and anal fins: point 7–6, caudal fin depth: point 4–5).

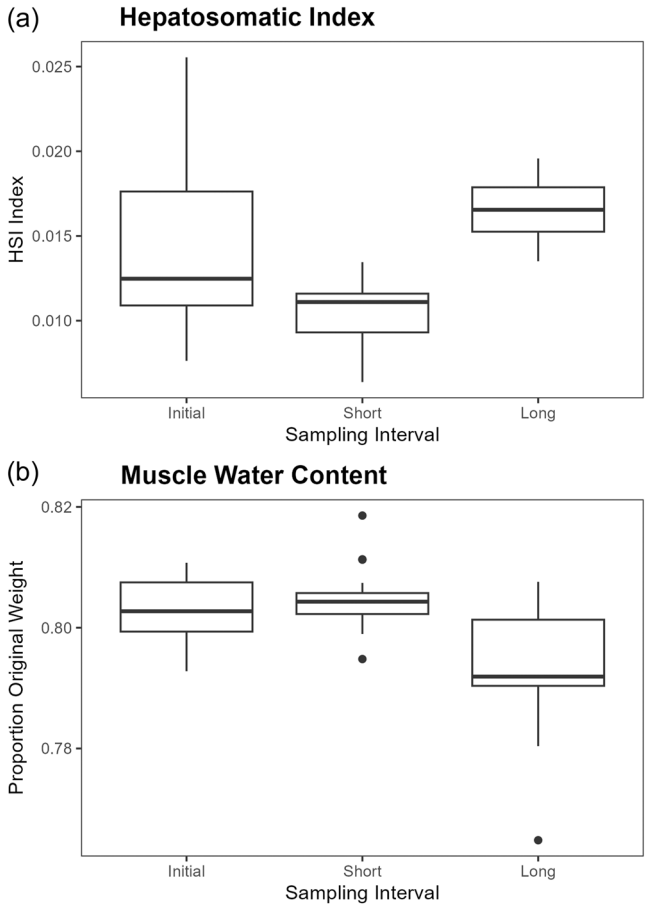

FIGURE 2 Condition measurements of (a) liver hepatosomatic index (HSI) and (b) muscle water content for juvenile brown trout across three treatments (control, sham, and cortisol implants) at three time periods (initial collection, short-term: 2 weeks, and long-term: 4 months). The boxplot outlines represent the lower and upper quartiles, the line in the middle of the box represents the median values, and values outside the whiskers represent potential outliers.

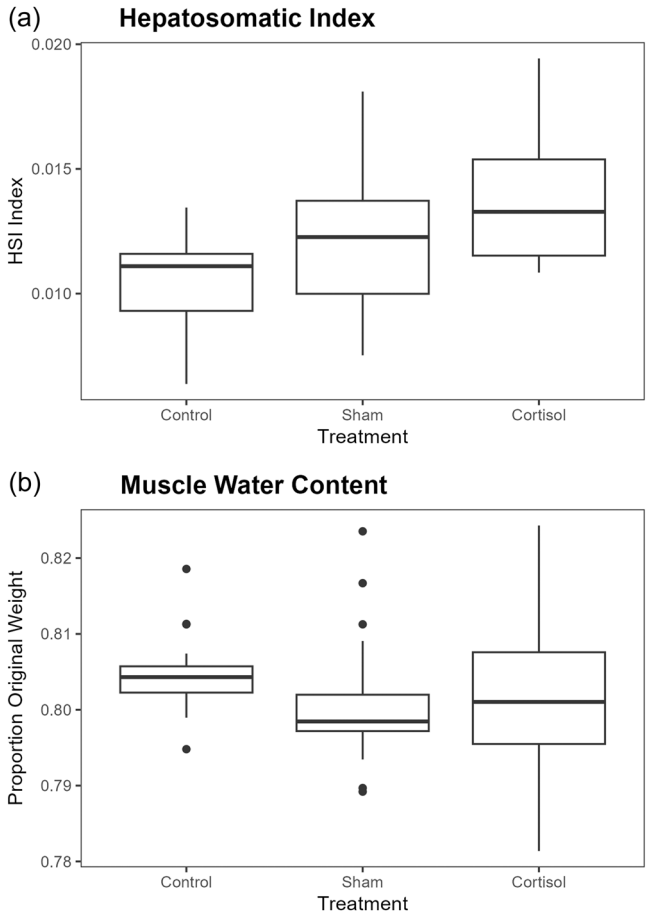

FIGURE 3 Measures of (a) liver hepatosomatic index (HSI) and (b) muscle water content for juvenile brown trout collected during the short-term sampling period (i.e., 2 weeks after initial collection) across three treatment groups (control, sham, and cortisol implants). Outlines of the boxplots show the lower and upper quartiles, the middle line in the box represents the median, and any values outside the whiskers represented potential outliers.

($F_{2,40} = 4.30$, $p = 0.02$), as fish were initially eating both terrestrial and aquatic prey but switched to eating mainly aquatic prey at the long-term point ($p = 0.03$), with no difference between initial and short ($p = 0.07$) or short and long ($p = 0.84$) time points. Though there was a signal of change in specialization across time points (deviance = 6.59, $df = 2.40$, $p = 0.04$), no post hoc tests were significant (initial-long: $p = 0.12$; initial-short: $p = 0.17$; short-long: $p = 0.77$).

Stomach fullness did not differ among treatments at the short-term time point ($F_{2,48} = 0.13$, $p = 0.88$). Neither proportion of prey type ($F_{2,48} = 0.14$, $p = 0.87$) nor specialization (deviance = 0.12, $df = 2.48$, $p = 0.94$) was affected by treatment.

Time had a strong effect on stable isotope values in plasma and scales, while treatment did not (Table 1; Figure 4; Supporting Information S1: Table 1). Blood plasma $\delta^{13}C$ was similar between the short and initial time points but became enriched at the long-term point (Table 1). Blood plasma $\delta^{15}N$ became enriched between the initial and short-term time point and stayed similarly enriched in the long term for all treatments (time × treatment, Table 1). Both $\delta^{13}C$ and $\delta^{15}N$ scale values became enriched between the initial and long-term time points (Table 1).

3.3 | Morphology

3.3.1 | Feeding morphology

Both snout length ($F_{2,302.72} = 73.12$, $p < 0.001$) and upper maxilla length ($F_{2,192.33} = 20.65$, $p < 0.001$) changed over time (all other model effects $p \geq 0.06$). Snout length decreased from initial samples (0.99 ± 0.01 cm, $n = 619$) to the short-term sampling period (0.89 ± 0.01 cm, $n = 157$; $p < 0.001$), then increased in the long-term samples to values higher than initial (1.08 ± 0.02 cm, $n = 37$; $p < 0.001$). Upper maxilla length did not change from initial (1.41 ± 0.01 cm, $n = 617$) to short-term samples (1.39 ± 0.02, $n = 157$, $p = 0.29$), but then increased in the long-term samples (1.58 ± 0.03, $n = 37$; $p < 0.001$).

Independent factor	Result	Post-hoc test	*p*-value
Plasma $\delta^{13}C$			
Time	$F_{2,\ 205.59} = 32.73$, $p < 0.01$	Initial - Short	0.29
		Initial - Long	0.01
		Short - Long	0.01
Treatment	$F_{2,\ 208.31} = 2.00$, $p = 0.14$		
Interaction effect	$F_{4,\ 203.75} = 0.79$, $p = 0.54$		
Plasma $\delta^{15}N$			
Time	$F_{2,\ 177.78} = 65.39$, $p < 0.01$	Initial - Short	0.01
		Initial - Long	0.23
		Short - Long	0.89
Treatment	$F_{2,\ 202.17} = 1.11$, $p = 0.33$		
Interaction effect	$F_{4,\ 177.62} = 3.49$, $p = 0.01$		
Scales $\delta^{13}C$			
Treatment	$F_{2,\ 65.73} = 0.04$, $p = 0.97$		
Time	$F_{2,\ 58.16} = 17.55$, $p < 0.01$		
Interaction effect	$F_{4,\ 53.37} = 1.92$, $p = 0.16$		
Scales $\delta^{15}N$			
Treatment	$F_{2,\ 62.64} = 0.36$, $p = 0.70$		
Time	$F_{2,\ 46.00} = 33.16$, $p < 0.01$		
Interaction effect	$F_{4,\ 43.88} = 0.98$, $p = 0.38$		

TABLE 1 Linear mixed effects model results for brown trout stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of blood plasma and scale tissues measured over three time periods for fish assigned to one of three treatments (control, sham, cortisol implant). Scale samples were taken at only two intervals (initial and long-term time periods) and, therefore, post-hoc comparisons of time periods were only necessary for blood plasma samples. For interpretation of the plasma $\delta^{15}N$ interaction effect, see text.

3.3.2 | Swimming morphology

Pectoral fin length decreased from initial to short-term sampling in the sham treatment ($p = 0.0125$) but did not change in control or cortisol treatments ($p \geq 0.26$), then increased in control ($p < 0.0001$) and sham ($p < 0.0001$) but not in cortisol ($p = 0.99$) at the long-term sampling point. Caudal tail depth and distance between pectoral and anal fin insertion points was not affected by treatment or time (all $p \geq 0.08$) (Table 2).

4 | DISCUSSION

In the short term, several metrics indicated that our fish experienced adverse handling effects. HSI, a measure of liver size, was lowest at the short-term sampling time, indicating all fish lost energy stores. Surprisingly, HSI was also lower in control than cortisol-treated fish and sham fish were intermediate in HSI, possibly because vegetable shortening, which both cortisol and sham fish received, could have been absorbed internally, offsetting some of the energy losses after handling in those two groups of fish (Birnie-Gauvin et al., 2018). Blood plasma $\delta^{15}N$ also became enriched at the short-term sampling time, which could indicate protein catabolism (Hatch, 2012) from increased use of endogenous energy stores due to handling effects. Other physiological metrics measured in these same fish also unexpectedly decreased in the short term: fish had lower oxidative stress levels and low molecular weight antioxidants (Birnie-Gauvin et al., 2017), attributed to either the onset of winter conditions, or handling stress. Brown trout can take 2 weeks to recover just from netting into a small tank for 2 min (Pickering et al., 1982), which is far less invasive than our handling procedures that involved electrofishing, anesthesia, an incision and tagging, and blood/scale removal for all individuals. Additionally, changes in morphology over the short term were in an unexpected direction, with snout length becoming smaller for a given body size and pectoral fin only shrinking in sham treated fish. Though changes in body morphology have not been linked to handling stress before, this remains a possibility; alternatively, photographs taken from fish in the field could have contained some unintended sampling time differences, biasing measurements for snout length (though this would not account for treatment effects seen in pectoral fin length, as photographs from different treatments were interspersed on any given day).

We found no evidence that cortisol treatment affected foraging success or foraging strategy, as neither stomach fullness, proportion of terrestrial/aquatic prey, nor specialization on either prey type was affected by treatment. Consistent with previous studies, cortisol fish had lower specific growth rates than sham or control treatments (Birnie-Gauvin et al., 2017) which supports our conclusion that the

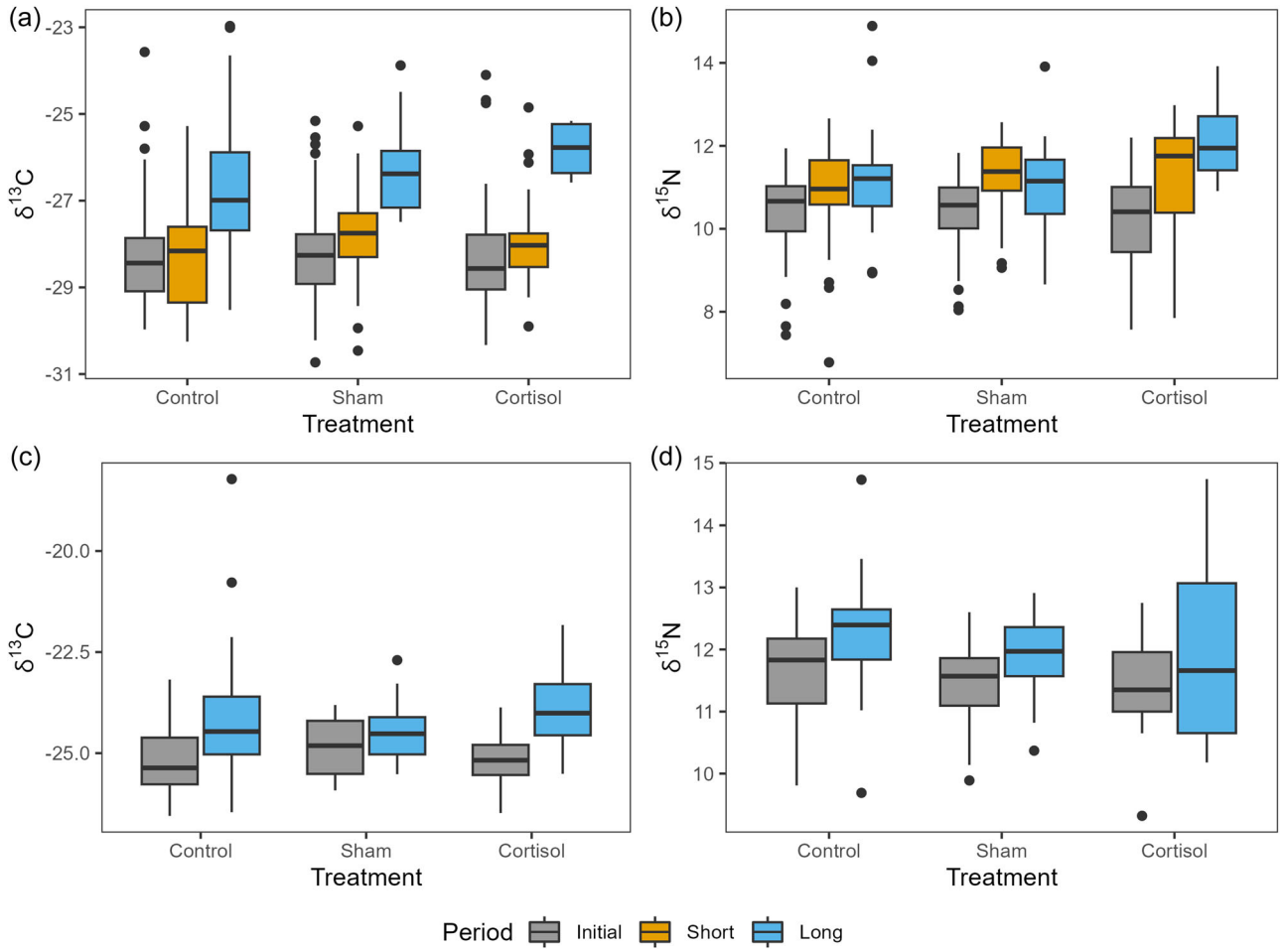

FIGURE 4 Stable isotope values of juvenile brown trout blood plasma (a, b) and scales (c, d) for fish assigned to one of three treatments (control, sham, or cortisol implants) measured over three periods (initial collection, short-term: 2 weeks, long-term: 4 months). Scale samples were collected only during the initial and long-term periods. The boxplot outlines represent the lower and upper quartiles, the line in the box represents the median value, and values outside the whiskers represent potential outliers.

TABLE 2 Summary of juvenile brown trout linear measures (mean ± 1 standard error) related to swimming morphology (total pectoral fin length, caudal depth, and distance between pectoral and anal fins) across three sampling periods (initial sampling, short-term: 2 weeks after initial, and long-term: 4 months after initial) and three treatment groups (control, sham, and cortisol treated).

Measure	Sampling interval	Control	Sham	Cortisol
Pectoral fin length	Initial	2.46 ± 0.03 (*n* = 198)	2.49 ± 0.03 (*n* = 210)	2.47 ± 0.03 (*n* = 199)
	Short-term	2.43 ± 0.06 (*n* = 42)	2.41 ± 0.03 (*n* = 53)	2.39 ± 0.04 (*n* = 59)
	Long-term	2.50 ± 0.07 (*n* = 17)	2.47 ± 0.04 (n = 16)	2.03 ± 0.04 (*n* = 2)
Caudal depth	Initial	1.25 ± 0.01 (*n* = 201)	1.28 ± 0.01 (*n* = 212)	1.25 ± 0.01 (*n* = 203)
	Short-term	1.22 ± 0.03 (*n* = 42)	1.21 ± 0.02 (*n* = 54)	1.19 ± 0.02 (*n* = 60)
	Long-term	1.33 ± 0.05 (*n* = 18)	1.29 ± 0.02 (*n* = 16)	1.11 ± 0.03 (*n* = 3)
Pectoral-anal fin distance	Initial	6.90 ± 0.08 (*n* = 205)	7.04 ± 0.08 (*n* = 212)	6.92 ± 0.08 (*n* = 204)
	Short-term	6.67 ± 0.19 (*n* = 42)	6.66 ± 0.11 (*n* = 54)	6.60 ± 0.11 (*n* = 61)
	Long-term	7.51 ± 0.31 (*n* = 18)	7.09 ± 0.12 (*n* = 16)	5.95 ± 0.17 (*n* = 3)

effects of cortisol occurred via mobilizing energy reserves instead of affecting foraging behavior (Colborne & Robinson, 2013; Doi et al., 2017), similar to other studies that found that cortisol decreases food conversion efficiency but not food intake (Bernier et al., 2004; Pfalzgraff et al., 2021).

Fish were initially eating both terrestrial and aquatic prey but switched to eating mainly aquatic prey at the long-term point. This explains the enriched $\delta^{13}C$ in the long term, as aquatic insects have enriched $\delta^{13}C$ over terrestrial insects (Akamatsu et al., 2004). As well, aquatic insects have enriched $\delta^{15}N$ compared to terrestrial insects (Akamatsu et al., 2004), so this also matches our results showing enriched $\delta^{15}N$ in both tissue types. Fish had more energy at the long-term sampling period compared to initial and short-term, as water content was lowest at the long-term point and this metric is inversely related to lipid content. We do not know how the abundance of prey types changed seasonally, and so cannot assess whether differences were due to changes in foraging preference or simply reflecting seasonal changes in prey availability. The latter is more likely as there are fewer terrestrial insects available in the winter months. Regardless, this switch to aquatic prey types seemed beneficial for the fish as they had greater lipid content although that could also simply be a function of more time to accumulate or consolidate energy resources.

In the long term, we had low recapture rates of cortisol treated fish, similar to other studies in this system (e.g., Midwood et al., 2015). Cortisol treatment may have resulted in mortality from suppression of the immune response (Sapolsky et al., 2000) or increased the probability of juveniles migrating out of the system before long term resampling (Birnie-Gauvin et al., 2019; Peiman et al., 2017); studies suggest cortisol manipulation does not affect behavioral responses to predation (Lawrence, Godin, et al., 2019; Lawrence, Zolderdo, et al., 2019). Low recaptures affected our ability to make robust conclusions about treatment effects in the long term. For instance, plasma isotope values for both $\delta^{13}C$ and $\delta^{15}N$ seemed to be most enriched in cortisol treated fish, but we had insufficient power to detect any statistical differences. Thus, this measure of long-term effects of cortisol remains only a tantalizing possibility.

In fish, body form and functional morphology is routinely linked to performance (Wainwright, 1994). For example, fish with relatively short pectoral fins are better swimmers (Jones et al., 2020; Rouleau et al., 2010) and thicker caudal peduncles generate higher critical swimming speeds (Hawkins & Quinn, 1996). Brown trout that are terrestrial feeders have body forms that are streamlined, with shorter fins and longer snouts (terminal mouths) (Stelkens et al., 2012; Závorka et al., 2020). While we found the relative length of both the maxilla and snout increased in the long term, this does not match the switch to more aquatic prey, most of which cling to the benthic substrate in our system (chironomid larvae, snails, worms, amphipods). Actively benthic-feeding trout should have a subterminal mouth which would result in more compact head shapes. Thus, feeding morphology changes were independent of prey type consumed. We found no compelling evidence of changes in body morphology due to cortisol treatment. The only studies to investigate this causal relationship found that cortisol implants resulted in shallower bodies in crucian carp (*Carassius carassius*) (Vinterstare et al., 2020) and larger tails in tadpoles (*Rana sylvatica*) (Middlemis Maher et al., 2013). Both were investigating whether cortisol treatments mimicked morphological changes induced by predators; Vinterstare et al. (2020) found changes were in the opposite direction, while Middlemis Maher et al. (2013) found they matched. Whether cortisol could also affect performance-related morphology for feeding and sustained swimming is still unknown, as due to our low cortisol treatment sample size in the long term, we cannot make conclusions about morphology changes over 4 months. Whether cortisol would first induce changes in feeding or swimming morphology, and so changes in diet or habitat use would follow as an indirect effect, or vice versa, and whether there are trade-offs in cortisol-induced changes in foraging behavior, activity, and type of prey available (Meo et al., 2021), are some of the many remaining interesting avenues for investigation.

5 | CONCLUSIONS

Stressful conditions that challenge homeostasis are ubiquitous across ecosystems and all individuals are likely to be exposed to various stressors during development. However, in this era of global change the level and duration of stress exposure for animals is likely increasing across many systems, ranging from invasive species to siltation and changes in flow. Understanding the potential long-term mechanistic impacts of these conditions to wild fishes not only informs about their ecology and physiology but can also inform restoration and conservation initiatives (Cooke et al., 2023). However, we observed that juvenile brown trout likely experienced negative effects of handling over at least 2 weeks, which may have masked any effect of cortisol treatment on foraging behavior. This is an important consideration in future studies investigating any responses in fish less than 2 weeks after treatment. Understanding the physiological impacts of stress responses on the development of organisms is necessary to fully appreciate how stress impacts wild organisms during critical life stages (Baker et al., 2013; Crossin et al., 2016; Dantzer et al., 2014) and studies can manipulate more than one component of the HPI axis. Our ability to make conclusions about long term impacts of cortisol are lacking due to only 1.4% of treated fish recaptured, reflecting the inherent risks of using wild fish in natural environments, and so we encourage further investigation with larger initial sample sizes into the long-term impacts of cortisol and other glucocorticoid hormones on the ecology and behavior of wild fishes.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be made available upon reasonable request to the corresponding author (Scott Colborne; colborne@msu.edu).

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SUPPORTING INFORMATION

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