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Cortisol does not increase risk of mortality to predation in juvenile bluegill sunfish: A manipulative experimental field study

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Abstract

The hypothalamic-pituitary-interrenal (HPI) or stress axis in teleost fishes produces their primary glucocorticoid, cortisol. Although generally an adaptive response, prolonged HPI axis stimulation can impair organismal performance. Previous work has shown that stressed teleosts have higher mortality to predation than unstressed conspecifics, suggesting a role for HPI axis in modulating predator–prey interactions. Our current study investigated whether elevated cortisol levels altered the predation rate of a wild teleost fish, the bluegill sunfish (*Lepomis macrochirus*). Wild juvenile bluegill were given intraperitoneal implants of cocoa butter (i.e., sham), or cocoa butter containing cortisol or cortisol and the glucocorticoid receptor antagonist RU486. After 24 hr, fish were tethered along the bottom of the lake and their survival under natural predation was recorded following 24 hr. A subset of fish was used to validate the efficacy of cortisol implants in this setting. No treatment effect on survival was observed, suggesting that elevated cortisol has minimal involvement in mediating predator–prey interactions in this context. However, experimental fish may have demonstrated resiliency to physiological perturbations owing to the relatively acute duration of our experimental series, and negative effects might be manifested over a more chronic period.

KEYWORDS
bluegill sunfish, cortisol, predation, stress, teleost, tethering

1 | INTRODUCTION

When teleost fishes are subjected to a stressor, the hypothalamic-pituitary-interrenal (HPI) axis increases production of cortisol, the primary glucocorticoid in this taxon (Barton & Schreck, 1987; Cook, O'Connor, McConnachie, Gilmour, & Cooke, 2012; reviewed in Gorissen & Flik, 2016). Cortisol has multiple functions in teleosts, including enhancing energy substrate synthesis (i.e., gluconeogenesis and glycogenolysis), assisting in re-establishing hydromineral balance, and temporarily diverting energy away from nonessential processes (i.e., growth and reproduction; reviewed in Mommsen, Vijayan, & Moon, 1999; Schreck & Tort, 2016). These effects are believed to be achieved predominately through glucocorticoid receptor (GR) activation (reviewed by Mommsen et al., 1999). Together, the actions of cortisol ensure that individuals have access to the energy needed to offset the deleterious effects of the stressor (Mommsen et al., 1999; Romero, Dickens, & Cyr, 2009; Schreck & Tort, 2016). Thus, the HPI axis acts to coordinate a suite of adaptive mechanisms that help animals cope with challenges in the environment (Romero et al., 2009; Schreck & Tort, 2016).

However, sustained elevations of plasma cortisol can be detrimental to organismal performance. In chronically stressed teleosts,

for example, reductions in somatic growth (Sadoul & Vijayan, 2016), energy reserves (e.g., hepatosomatic index [HSI]; Barton, Schreck, & Barton, 1987; Montero, Izquierdo, Tort, Robaina, & Vergara, 1999), reproductive capacity (Pankhurst, 2016), and immune function (Yada & Tort, 2016) have been observed. Interestingly, stress also appears to influence predator–prey interactions, with stressed teleosts suffering higher rates of predation compared with nonstressed conspecifics (reviewed in Mesa, Poe, Gadomski, & Petersen, 1994; Raby, Packer, Danylchuk, & Cooke, 2014). Although the mechanism (s) underlying this effect remain unclear, stressors have been shown to cause reduced escape distances (Handeland, Järvi, Fernö, & Stefansson, 1996; Killen, Reid, Marras, & Domenici, 2015; McKenzie, Shingles, Claireaux, & Domenici, 2008; Ryer, 2004) and altered shoal cohesion (Pavlidis, Theodoridi, & Tsalafouta, 2015; Piato et al., 2011; Ryer, 2004), suggesting impairment of predator avoidance capacity (reviewed in Godin, 1997).

The observation of higher predation rates among stressed teleosts suggests that the HPI axis has a role in modulating predator–prey interactions. However, at this time, studies addressing the specific role of cortisol in mediating predator–prey interactions are quite scant, representing an interesting avenue for future research, especially given cortisol's potential role in modifying life history attributes in fishes (Crossin, Love, Cooke, & Williams, 2015; Sopinka et al., 2016). More specifically, in previous work chronic cortisol administration failed to alter mortality (Lawrence et al., 2017) and antipredator behaviors (Lawrence, Eliason et al., 2017, 2018) in tethered schoolmaster snapper (*Lutjanusapodus*). Similarly, cortisol treatment in checkered pufferfish (*Sphoeroides testudineus*) failed to impede defense capabilities (i.e., puff scores), which suggests that antipredator responses may be unaffected (Cull et al., 2015; Pleizier, Wilson, Shultz, & Cooke, 2015). Thus, the purpose of the current experiment was to test experimentally whether chronic cortisol elevation affects predator-induced mortality in a wild, freshwater teleost fish, the bluegill sunfish (*Lepomis macrochirus*). We hypothesized that experimental elevation of cortisol levels would result in higher mortality in cortisol-treated fish compared with sham controls and RU486-treated fish. We tested this hypothesis with bluegill sunfish that were implanted with cocoa butter (i.e., a sham), or with cocoa butter containing cortisol, or cortisol and the GR antagonist, RU486. Twenty four hours after implant, fish were tethered in the shallow nearshore waters of a lake and left undisturbed and exposed to natural predators for 24 hr, following which the surviving fish were recovered and mortalities noted.

2 | MATERIALS AND METHODS

2.1 | Fish collection, housing, and implantation procedures

Juvenile bluegill sunfish (mean ± standard error (*SE*), wet mass = 5.4 ± 0.1 g) were seized from shallow nearshore habitats in Lake Opinicon (44.5590°N, 76.3280°W; Ontario, Canada) during the months of May and June of 2018 (under Ontario Ministry of Natural Resources and Forestry permit #1089028). Lake Opinicon is a shallow and weedy euphotic lake with a diverse predator community including teleost, avian, and mammalian piscivores (Keast, 1978; Keast, Harker, & Turnbull, 1978). Seining was the preferred capture method to avoid possible selection of specific behavioral phenotypes (Gutowsky, Sullivan, Wilson, & Cooke, 2017; Wilson, Binder, McGrath, Cooke, & Godin, 2011), thus ensuring a random collection of fish in our samples. Following capture, fish were sorted by hand to select individuals of 5–8 cm total length, because previous work on sunfish predation used similarly-sized individuals with success (Gotceitas & Colgan, 1989). Fish were promptly transported to the nearby Queen's University Biological Station (Elgin, ON, Canada) where they were held in large, outdoor flow-through tanks (~435 L) supplied with fresh lake water (19.7 ± 0.3°C; >90% O_2 saturation) and exposed to natural sunlight and darkness. Animals were held under these conditions for 24 hr before implantation. All experimental series were conducted in accordance with the guidelines set by the Canadian Council on Animal Care under administration of the Carleton University Animal Care Committee (AUP# 104262 and 106523).

Bluegill were implanted with cocoa butter containing either cortisol (hydrocortisone 21-hemisuccinate; 25 mg kg^{-1} body weight [BW]), or cortisol (25 mg kg^{-1} BW) with RU486 (Mifepristone; 50 mg kg^{-1} BW), or were implanted with cocoa butter alone (5 ml kg^{-1} BW) as a sham control. Implants were deposited into the peritoneal cavity through the ventral surface of the fish using a 1 ml syringe and an 18 G needle. The use of cortisol-treated cocoa butter implants has been validated for use in teleost fishes (Gamperl, Vijayan, & Boutilier, 1994) and has been widely used in centrarchid fishes (e.g., Algera et al., 2017; McConnachie, O'Connor, Gilmour, Iwama, & Cooke, 2012; O'Connor et al., 2011; Zolderdo et al., 2016). The cortisol dosage used here was based on prior work (Algera et al., 2017; Brown, MacLatchy, Hara, & Eales, 1991). Cocoa butter solutions containing cortisol were prepared following the methods of Hoogenboom et al. (2011). The RU486 dose (50 mg kg^{-1} BW) was based on prior work, where RU486 has been used extensively as an antagonist of GRs (Bernier, Lin, & Peter, 1999; Lawrence et al., 2017; Vijayan, Reddy, Leatherland, & Moon, 1994). We did not include a no-treatment (i.e., no implant) control group in our experimental design because we were primarily interested in the relative effects of exogenous cortisol rather than how stressors associated with fish handling may have affected predator-induced mortality. Furthermore, prior work indicated that plasma [cortisol] is comparable between sham-treated and non-treated bluegill sunfish (McConnachie et al., 2012). Following implantation, fish were held in indoor, flow-through tanks (~211 L; T = 20.9 ± 0.4 °C; O_2 saturation > 90%) for 24 hr to ensure that the pharmaceutical agents reached biologically active concentrations in the blood before exposure to predation risk, as in Lawrence et al. (2017). Fish were not fed at any time while in captivity.

2.2 | Assessment of predatory mortality in the wild

Tethering has been used effectively to assess the risk of predation on teleost fish in natural aquatic environments (see Elvidge & Brown,

2014; Lawrence et al., 2017; Rypel, Layman, & Arrington, 2007). As in previous work (Lawrence et al., 2017; Rypel et al., 2007), tethers consisted of a single piece of monofilament fishing line (1.5-m in length; 2.72 kg test monofilament) attached to the lower jaw of the fish. A small sewing needle was used to pierce the flesh in the jaw so that the tether could be knotted directly to the fish (Lawrence et al., 2017). Tethers were secured to the animal just before deployment in the field.

Tethering was conducted at one of the four sites in Lake Opinicon, namely, Keast Beach (44°33′53.1″N 76°19′33.9″W), Cow Island (44°34′02.8″N 76°19′11.4″W), Birch Bay (44°33′56.0″N 76° 19′40.1″W), and Eight Acre Island (44°33′34.3″N 76°19′20.4″W). These sites were selected because they have naturally occurring populations of *Lepomis spp.* and a diverse predator community. The sites were similar in depth (~1.5–1.8 m) and substratum composition, having a sandy/muddy bottom with sparse woody debris, small rocks, and natural vegetation. At each site, a 60-m length of lead-core line with 5-m interval markers was anchored into the substrate. The interval markers ensured consistent spacing among individual tether lines. A random number generator was used to select the particular tethering site. On a given day of experimentation, upwards of 12 fish were used, consisting of four individuals from each of the three treatment groups (i.e., $N = 4$/treatment/site). Sample sizes for each of the tethering treatments can be found in Table 1. Unbalanced sample sizes amongst our treatment groups for tethered fish are the result of uncontrollable occurrences in the field (e.g., overnight holding tank mortalities, fish establishing a nest overnight near a tethering site, missing tether stake, etc.). Animals were transported in coolers to their predetermined tether site and were fitted with tethers (see above). Tether lines were attached to a large metal stake (22.9 cm long) that was driven into the substrate at the predetermined location by hand by a snorkeler. The order in which animals were placed along the line, at 5-m separations, was such that no two adjacent fish were of the same treatment group (i.e., sham, cortisol, RU486 + cortisol) and was changed daily using systematic randomization. Tethered fish were left for 24 hr and survivors were collected the following day. Fish that were not present on their tether line at the time of collection were assumed to have been consumed by a predator. Fish that survived the overnight tethering period were euthanized as per our approved animal care protocol (AUP# 106523).

2.3 | Validation of cortisol implants and physiological indices

The second group of bluegill (mass = 10.3 ± 2.1 g; $N = 65$; see Table 1 for specific sample sizes) were implanted as described above and held for 24 or 48 hr after implant in individual, blacked-out compartments (as described by McConnachie et al., 2012) supplied with flowing water. A larger size class (size range: 8–10 cm TL) was used here to ensure sufficient volumes of blood for analytical procedures. Blood (~100 μl) was collected via caudal venipuncture (23 G needle on a 1 ml syringe; 10,000 USP units/ml of sodium heparin; Sandoz, Boucherville, Canada) with the procedure taking less than 3 min to ensure that samples were representative of baseline conditions (Lawrence, Jain-Schlaepfer et al., 2018). Whole blood was immediately assessed for blood [glucose] using a portable glucose meter (Accu-Chek Compact Plus; Hoffman-La Roche Limited, Mississauga, ON, Canada) that has been validated for use in teleost fishes (Serra-Llinares, & Tveiten, 2012; Wells & Pankhurst, 1999; reviewed in Stoot et al., 2014). The remaining blood was centrifuged (2 min at 2,000*g*; Mandel Scientific, Guelph, ON, Canada) and the plasma fraction was flash frozen and stored at −80°C for later analysis of plasma [cortisol]. Plasma [cortisol] was determined using a commercially available radioimmunoassay kit (ImmuChem Cortisol Coated Tube RIA Kit; MP Biomedicals, Solon, OH) that has been validated for use in teleosts (Gamperl et al., 1994). Intra-assay variation was 5.8% and all samples were measured in a single assay. Following blood sample collection, fish were euthanized via cerebral percussion and weighed. The liver was dissected out and weighed to determine HSI. HSI was calculated using the method of Busacker, Adelman, and Goolish (1990), where liver mass (m_L) is divided by total mass of the fish (m_F) and multiplied by 100%, $HSI = (m_L/m_F) * 100\%$. HSI is a relevant index here because previous work has shown it to be under the regulatory control of cortisol (Mommsen et al., 1999) and it is commonly used as a stress index (Sopinka, Donaldson, O'Connor, Suski, & Cooke, 2016).

TABLE 1 Sample sizes for the physiological indices measured at 24 and 48 hr after implant. The total number of fish that were tethered in the experiment is also included. Treatment groups consisted of fish implanted with cocoa butter as a sham control, cocoa butter containing cortisol (25 mg kg^{-1} body weight [BW]), or cocoa butter containing cortisol + RU486 (cortisol = 25 mg kg^{-1} BW and RU486 = 50 mg kg^{-1} BW)

	Treatment group					
	Sham		Cortisol		Cortisol + RU486	
Parameters	*24 hr*	*48 hr*	*24 hr*	*48 hr*	*24 hr*	*48 hr*
Plasma [cortisol]	10	5	6	7	8	4
Blood [glucose]	12	8	12	9	10	12
Hepatosomatic index	12	9	12	9	11	12
Tethered fish	57		54		47	

2.4 | Data analyses

Statistical analyses of blood and tissue data from the cortisol implant validation portion of the study were conducted using SigmaPlot v11.0 (Systat Software Inc., San Jose, CA). Each physiological metric was analyzed separately using an independent two-way analysis of variance (ANOVA) model with the main effects of treatment (sham, cortisol, cortisol + RU486) and time (24 and 48 hr after implant) as the fixed independent factors. An interaction term between treatment and time was also included in this model. Nonnormal data were log transformed. Data are presented as means ± *SE* unless otherwise noted, and statistical significance is reported at $\alpha = 0.05$.

We analyzed survival data using a generalized linear model fitted to a binomial distribution in the R statistical environment (version 1.1.423; RStudio Team, 2015). Here, treatment group (i.e., sham, cortisol, cortisol + RU486) was the main fixed factor in the model, with the tethering line site location in the lake (i.e., the four sites listed above) included as a covariate. Model fitting, using a likelihood ratio test (Zuur, Ieno, Walker, Saveliev, & Smith, 2009), was used to determine the overall effect of each model term (i.e., treatment group and site location) on fish survival. Using an effect size derived from a similar study on schoolmaster snapper (Lawrence et al., 2017) and G*Power (v 3.1.9.2; Heinrich Heine University of Dusseldorf, Düsseldorf, Northrhine-Westphalia, Germany) (Faul et al. 2007, 2009), we carried out a post-hoc power analysis that revealed a power value of 0.865, which suggests that we had a high probability of observing an effect in this study if one indeed existed (Cohen, 1992).

3 | RESULTS

3.1 | Implant validation and physiological responses

Treatment significantly affected plasma [cortisol] in bluegill ($F = 60.75$; $df = 2,39$; $p < 0.001$; Figure 1a). Plasma [cortisol] was 5- and 15-times higher in cortisol- and cortisol + RU486-treated fish, respectively, than in sham-treated fish, at 24-hr after implant. By 48 hr, cortisol- and RU486 + cortisol-treated fish exhibited plasma [cortisol] that was, respectively, 4.5- and 23-times higher than that of shams (Figure 1a). Although a significant effect of time on plasma [cortisol] was observed ($F = 5.69$; $df = 1,39$; $p = 0.023$), the interaction of treatment group and time was not significant ($F = 0.67$; $df = 2,39$; $p = 0.518$). Despite a trend for blood [glucose] to be elevated in cortisol-treated fish, treatment effects on blood [glucose] were not statistically significant ($F = 3.15$; $df = 2,57$; $p = 0.051$; Figure 1b). Time did not have significant effects on blood [glucose] ($F = 0.81$; $df = 1,57$; $p = 0.371$), nor was there an interaction between the main effects ($F = 0.39$; $df = 2, 57$; $p = 0.676$). Similarly, none of treatment ($F = 1.69$; $df = 2,59$; $p = 0.193$), time ($F = 1.94$; $df = 1,59$; $p = 0.169$), or the interaction of these factors ($F = 0.40$; $df = 2,59$; $p = 0.672$) had significant effects on HSI (Figure 2).

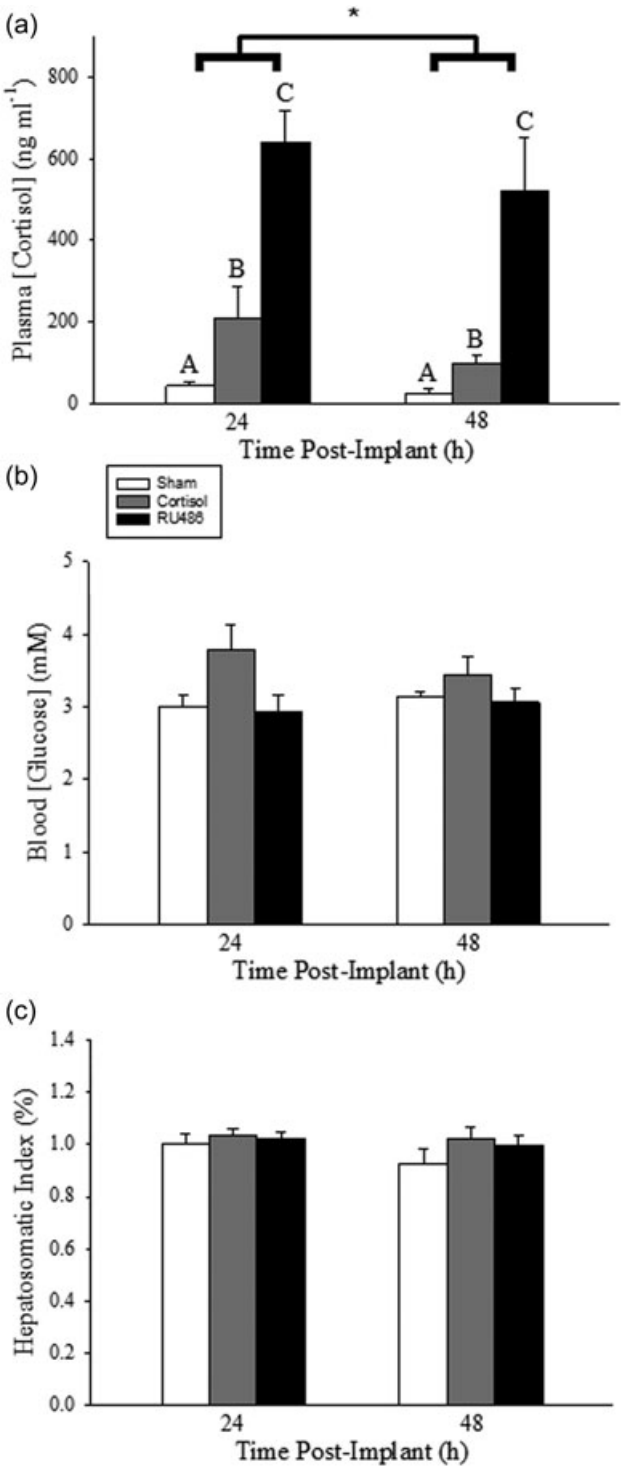

FIGURE 1 Plasma [cortisol] (a), blood [glucose] (b), and hepatosomatic index (HSI; c) of sham-treated (white bars; 5 ml kg^{-1} body weight [BW] cocoa butter; $N = 5$–12), cortisol-treated (black bars; 25 mg kg^{-1} BW cortisol in cocoa butter; $N = 6$–12), and cortisol + RU486-treated (grey bars; 25 mg kg^{-1} BW cortisol and 50 mg kg^{-1} in cocoa butter; $N = 4$–12) bluegill sunfish sampled 24 or 48 hr after implant. Differences in the results between the two after implant sampling times are denoted by an asterisk (*$p < 0.05$), whereas treatment level effects within a sampling time are denoted by unique letters ($p < 0.05$). Statistical effects of time and treatment group on response variables were ascertained with a 2-way ANOVA model (see Results section). ANOVA: analysis of variance

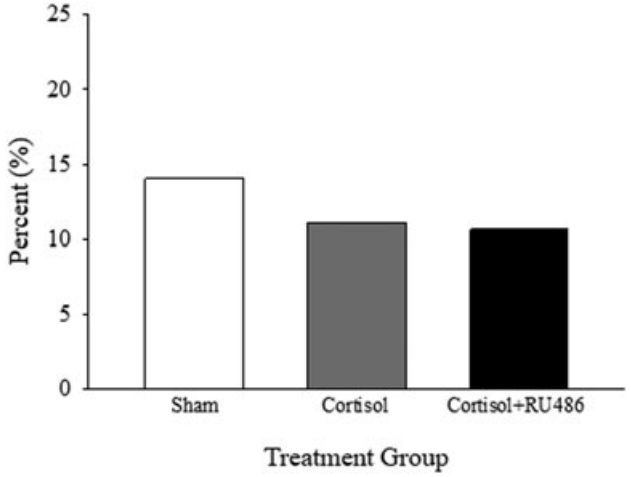

FIGURE 2 Percent survival of sham-treated (white bars; 5 ml kg^{-1} body weight [BW] cocoa butter; $N = 57$), cortisol-treated (black bars; 25 mg kg^{-1} BW cortisol in cocoa butter; $N = 54$), and cortisol + RU486-treated (grey bars; 25 mg kg^{-1} BW cortisol and 50 mg kg^{-1} in cocoa butter; $N = 47$) bluegill sunfish after 24 hr of being tethered in a nearshore lentic environment

3.2 | Tethering survival

Survival rates of sham-, cortisol- and cortisol + RU486-treated sunfish were comparable at 14.0%, 11.1%, and 10.6%, respectively (residual deviance = 115.77; $df = 2$; $p = 0.844$). Furthermore, there was no effect of tethering site location on the survival of bluegill (residual deviance = 112.81; $df = 3$; $p = 0.398$). Opportunistic use of video as well as visual observations suggested that the primary predators included largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), northern pike (*Esox lucius*), rock bass (*Ambloplitesrupestris*), brown bullhead (*Ameiurusnebulosus*), and common loons (*Gaviaimmer*), as these animals were observed feeding on tethered fish and/or were in the vicinity of tethering lines. Common snapping turtles (*Chelydra serpentina*) and western osprey (*Pandionhaliaetus*) also inhabit the lake and may represent additional sources of predation. Survivors were often found within nearby vegetation upon collection following the 24-hr predator exposure period.

4 | DISCUSSION

4.1 | Physiological effects of implants

Cortisol concentrations in cortisol-treated fish appeared to reach a peak of ~209 ng ml^{-1} at 24 hr after implant, declining thereafter, which is consistent with previous work on centrarchids (McConnachie et al., 2012). These plasma cortisol levels were well within the physiologically-relevant range because peak values were similar to those measured in bluegill experiencing acute stressors (Cook et al., 2012; Wilson et al., 2011). Cortisol was elevated for substantially longer than would be the case for an acute stressor (Sopinka et al., 2015), but the physiological consequences of prolonged cortisol elevation would be expected to become more debilitating with time. Plasma cortisol titers in fish treated with cortisol + RU486 were substantially higher than those in either cortisol- or sham-treated animals. This difference could possibly stem from blockade of GR in the HPI axis itself by RU486, abolishing cortisol-induced negative feedback resulting in a rise in plasma cortisol levels (Bernier et al., 1999; Bradford, Fitzpatrick, & Schreck, 1992; Lawrence et al., 2017; Reddy, Vijayan, Leatherland, & Moon, 1995; Veillette, Sundell, & Specker, 1995). However, we are unsure of the exact reason driving this effect. Thus, effective blockade of GR was likely achieved in the bluegill studied here, and RU486-treated animals were probably not affected adversely by chronic cortisol elevation.

Both blood [glucose] and HSI were unaffected by the treatment. Cortisol treatment in teleosts (reviewed in Mommsen et al., 1999) and specifically in centrarchids (Dey, O'Connor, Gilmour, Van Der Kraak, & Cooke, 2010; McConnachie et al., 2012; Zolderdo et al., 2016) typically results in elevated blood [glucose], because cortisol upregulates gluconeogenic enzymes (Chan & Woo, 1978; Foster & Moon, 1986; Vijayan, Mommsen, Glémet, & Moon, 1996; reviewed in Mommsen et al., 1999). However, this effect is not ubiquitous and cortisol treatment does not always lead to hyperglycemia (Andersen, Reid, Moon, & Perry, 1991; Davis, Torrance, Parker, & Suttle, 1985; Vijayan et al., 1994). Although marginally nonsignificant statistically ($p = 0.051$), the clear trend in our results for elevated blood [glucose] in cortisol-treated fish is consistent with the impact of cortisol treatment on gluconeogenesis. In agreement with literature demonstrating that RU486 treatment blocks the effect of cortisol on gluconeogenic activity (Reddy et al., 1995; Vijayan & Leatherland, 1992), blood [glucose] in cortisol + RU486-treated bluegill was similar to that observed in sham-treated fish, adding to evidence that the desired blockade was effective. As observed previously in teleosts (Leatherland, 1987; Mommsen, Danulat, & Walsh, 1992; Storer, 1967; Vijayan et al., 1994), HSI was unaffected by cortisol treatment in the present study, suggesting that hepatic glycogen reserves, for which HSI can serve as a proxy (Chellappa, Huntingford, Strang, & Thomson, 1995; Lambert & Dutil, 1997), likely were not affected by cortisol treatment.

4.2 | Cortisol and predation mortality

Contrary to prediction, cortisol treatment had no impact on the vulnerability of tethered bluegill sunfish to predation; all implant groups experienced similar survivorship. Because of cortisol's potential role in modulating antipredator behavior (e.g., Hawlena & Schmitz, 2010; Pavlidis et al., 2015; Raby et al., 2014; Ryer, 2004), a greater risk of mortality to predation was predicted for cortisol-treated fish compared with fish in the other two treatment groups. Previous empirical studies are consistent with our negative findings. For example, cortisol treatment failed to alter survivorship and antipredator behavior in the schoolmaster snapper (Lawrence, Eliason et al., 2017, 2018). Similarly, cortisol treatment failed to alter antipredator behavior in checkered pufferfish (Cull et al., 2015; Pleizier et al., 2015) and pumpkinseed sunfish (*Lepomis gibbosus*; Lawrence, Godin, & Cooke, 2018).

Collectively, these findings suggest that cortisol may not be an important mediator of predator–prey interactions in teleost fishes. However, we are cautious in this interpretation because cortisol implants in vertebrates often produce variable behavioral responses that are context dependent (Crossin et al., 2016; Sopinka et al., 2015). In these instances, physiological disturbances do not necessarily result in behavioral-level effects, suggesting a disconnect between these two levels of scale. For example, cortisol-treated creek chub (*Semotilus atromaculatus*) displayed no change in fine-scale spatial use patterns despite the chronic elevation of plasma [cortisol]; (Nagrodski, Murchie, Stamplecoskie, Suski, & Cooke, 2013). Similarly, negative findings were noted for some (Algera, Gutowsky, Zolderdo, & Cooke, 2017; Dey et al., 2010; Lawrence, Godin et al., 2018; O'Connor, Gilmour, Arlinghaus, Van Der Kraak, & Cooke, 2009; Zolderdo et al. 2016), but not all (Algera, Brownscombe et al., 2017; O'Connor et al., 2010) studies of cortisol-treated centrarchids. Such conflicting effects highlight the inherent complexity and context-dependent nature of physiology-behavior interactions (Crossin et al., 2016; Killen, Marras, Metcalfe, McKenzie, & Domenici, 2013; Sopinka et al., 2015). Thus, in the particular context of the present study, it may be that changes in plasma cortisol alone are insufficient to alter bluegill behavior, which may require additional input associated with the stress response or an additional environmental challenge to yield cortisol/stress-related effects on predator-induced mortality (Killen et al., 2013; Spagnoli, Lawrence, & Kent, 2016).

Alternatively, the time frame selected for this study may have been too short for the deleterious effects of cortisol to manifest. For example, in chronically-stressed zebrafish (*Danio rerio*), behavioral impairment became apparent at 14 days of chronic stress with the animal being able to cope over more acute durations (~7 days; Piato et al., 2011). Likewise, behavioral performance can be maintained under cortisol treatment, but the fish suffer consequences at a later time (i.e., mortality, loss of fecundity, etc.; Algera, Gutowsky et al., 2017; Nagrodski et al., 2013; O'Connor et al., 2009; Zolderdo et al., 2016). However, we are limited in the interpretation of our findings in the current study because behavioral traits were not measured. It would, therefore, be of interest to repeat our current experiment over a longer period and with a behavioral assessment to ascertain whether the duration of elevated cortisol affects antipredator capacity.

5 | CONCLUSIONS

We tested the hypothesis that cortisol treatment increases predator-induced mortality in juvenile bluegill sunfish. Despite elevated plasma cortisol levels, no effects of treatment were observed on the survival of tethered sunfish. Our results suggest that chronic cortisol elevation may not increase susceptibility to predation in this species. However, context-dependent effects, disconnects between physiological and behavioral processes, and/or the relatively acute experimental duration may also explain the lack of effect. We acknowledge in addition that tethering, as an experimental methodology, can limit natural predator-avoidance tactics as well as making organisms more conspicuous to predation, which may act to mask treatment-level effects (reviewed in Lawrence et al., 2017). Nevertheless, tethering serves as an important tool in estimating relative rates of predation in wild fish in ecologically-relevant settings (Elvidge & Brown, 2014; Lawrence et al., 2017; Rypel et al., 2007). Further study is needed to address how cortisol influences fine-scale risk-taking and antipredator behaviors.

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AUTHORS CONTRIBUTION

All authors contributed to the design of the experiment. The experimental trials were conducted by M. J. L and A. J. Z. Data analyses were performed by M. J. L. The manuscript was written by M. J. L., with all authors contributing to revisions.

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