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An experimental evaluation of the role of the stress axis in mediating predator-prey interactions in wild marine fish

Michael J. Lawrence [a,*], Erika J. Eliason [a,b], Jacob W. Brownscombe [a], Kathleen M. Gilmour [c], John W. Mandelman [d,e], Steven J. Cooke [a]

[a] Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, ON K1S 5B6, Canada
[b] Department of Ecology, Evolution and Marine Biology, University of California at Santa Barbara, Santa Barbara, CA 93106, USA
[c] Department of Biology, University of Ottawa, Ottawa, ON K1N 6N5, Canada
[d] School for the Environment, University of New England, Biddeford, ME 04005, USA
[e] John H. Prescott Marine Laboratory, New England Aquarium, Boston, ME 02110, USA

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ABSTRACT

The hypothalamic-pituitary-interrenal (HPI) axis, through corticosteroid secretion, is an integral mechanism regulating internal homeostasis when vertebrates are faced with a stressor. However, continued HPI-axis stimulation can produce homeostatic overload, where corticosteroids are detrimental to organismal function. This overload condition may play an important role in mediating predator-prey interactions, because chronically/previously stressed animals may have higher rates of predator-induced mortality. However, the mechanism(s) underlying this observation are unknown. Using fish as models, we hypothesized that chronic stress would increase predation susceptibility owing to a poor physiological state (e.g. homeostatic overload) with corresponding suboptimal changes in predator-avoidance behaviour. As cortisol is also required in low quantities to help regulate basic metabolic functions in fish, we expected that a glucocorticoid receptor antagonist (GR; e.g. homeostatic failure) may produce similar effects. Schoolmaster snapper (*Lutjanus apodus*) were given intraperitoneal implants of cocoa butter impregnated with nothing (sham; 5 ml/kg body weight (BW)), cortisol (50 mg/kg BW) or the GR antagonist RU486 (100 mg/kg BW). At 24-h post-implantation, fish were tethered to the seafloor and observed for behavioural metrics associated with predation. Blood samples were collected from a subset of fish to assess the physiological consequences of the implants. Cortisol- and RU486-implanted fish both had significantly higher plasma cortisol concentrations than sham fish, with blood glucose and plasma urea being elevated only in the former. Further, anti-predator behaviours and predation mortality did not differ significantly among treatments. Despite changes in physiological state, predation susceptibility was unaffected, a finding that may reflect the complex relationships linking the physiology and behaviour of an organism as well as potential tethering artefacts.

1. Introduction

In fish, exposure to stressors results in physiological perturbations and can lead to disruptions in homeostasis (reviewed in Barton and Iwama, 1991). Stressors may be physical (e.g. handling, confinement), environmental (e.g. hypoxia, temperature, hypercapnia) and physiological (e.g. exercise, acidosis). During a stress response, circulating levels of the glucocorticoid stress hormone, cortisol, are markedly elevated as a result of activation of the stress axis; the primary mechanism by which physiological disturbances from external and/or internal stressors are mitigated in most vertebrate species (Selye, 1946; reviewed in Hawlena and Schmitz, 2010 & Romero et al., 2009). In teleost fish, the stress axis consists of the hypothalamic-pituitary-interrenal (HPI) axis that is analogous to the mammalian HP-adrenal (HPA) axis (reviewed in Mommsen et al., 1999). The end product of the HPI axis, cortisol, acts via the blood stream as the primary glucocorticoid agent eliciting elevations in gluconeogenic, glycogenic and proteolytic capacities (reviewed in Mommsen et al., 1999 & Wendelaar Bonga, 1997), resulting in a greater degree of energy mobilization, to allow the fish to respond to the stressor.

Elevated circulating levels of cortisol, in both freshwater and marine teleosts, have been associated with increases in circulating glucose (reviewed in Barton and Iwama, 1991, Mommsen et al., 1999, & Wendelaar Bonga, 1997) and free amino acid concentrations (Chan and Woo, 1978; Costas et al., 2011; Wood et al., 1999), as well as elevations in nitrogenous waste excretion (Chan and Woo, 1978; Lawrence et al., 2015; McDonald and Wood, 2004), supporting the significant role of this hormone in mediating energy metabolism. Restoring homeostatic balance in the face of a stressor is an energetically demanding process,

* Corresponding author.
E-mail address: m_lawrence27@live.ca (M.J. Lawrence).

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requiring elevations in various molecular transport activities, protein synthesis, energy mobilization and cardiorespiratory responses, through the regulatory input of cortisol (reviewed in Hawlena and Schmitz, 2010 & Wendelaar Bonga, 1997).

However, elevation of cortisol levels is not entirely beneficial to the physiological operation of a teleost fish. The reactive scope model posits that cortisol can become a detrimental agent to the organism at high circulating concentrations, referred to as a "homeostatic overload"; a condition where cortisol itself damages physiological stability over a more chronic duration (Romero et al., 2009). Under conditions of chronic stress, HPI axis induction in teleost fish can be detrimental to growth and reproduction (reviewed in Wendelaar Bonga, 1997) and the immune system (Espelid et al., 1996; reviewed in Weyts et al., 1999), and can alter behaviour (reviewed in Schreck et al., 1997). This state comes as a result of the diversion of resources away from processes such as reproduction to aid in homeostatic restoration of the individual (Romero et al., 2009). Sensory interpretation and behavioural patterns can be affected (reviewed in Schreck et al., 1997), which in turn can have adverse consequences with regard to ecological processes such as predator-prey dynamics. The idea that stress at the level of the individual may be relevant to ecological processes has generated interest in 'the ecology of stress' (Boonstra, 2013b).

Although empirical studies remain limited, predator-prey interactions seem to be modulated by the stress status of animals (Hawlena and Schmitz, 2010) it has been observed that teleost fish exemplifying a pre-existing stressor (i.e. not predation; handling, toxicants, air exposure, etc), are subject to higher rates of predator-associated mortality among a few fish species, generally reflecting poor predator avoidance capacity (Brown et al., 1985; Järvi, 1989; Mesa et al., 1994, 1998; Olla and Davis, 1989; Olla et al., 1992, 1995). Indeed, the predator-avoidance behaviour of these fish is modulated by being in a pre-stressed state demonstrating reduced escape distances (Allan et al., 2015; Handeland et al., 1996; Järvi, 1989), poor shoaling behaviour (Brown et al., 1985; Handeland et al., 1996; Sullivan et al., 1978), and reduced swimming capacity (Allan et al., 2015; Brown et al., 1985; Danylchuk et al., 2007; Kruzynski et al., 1994), thereby contributing to an overall greater mortality rate. In encountering an acute threat of predation, fish elicit a stress response which includes increases in circulating corticosteroids and cardiovascular activity (Hawlena and Schmitz, 2010); modifications that are of great benefit to the prey in avoiding predation (Hawlena and Schmitz, 2010; Lima and Dill, 1990). However, the beneficial influences of the stress axis under predation might be inhibited in fish already exhibiting a chronic/sub-chronic stimulation of the stress axis through other means (e.g. hypoxia, handling, and acidosis). Chronic cortisol elevation may act to modulate the aerobic scope of the animal (Lankford et al., 2005; Guderley and Pörtner, 2010; Sokolova, 2013) which could affect energetic allocation towards anti-predator responses. Similarly, chronic cortisol elevation could act to de-sensitize the acute stress response (Barton et al., 1987; Barcellos et al., 1999; Cericato et al., 2008; Sloman et al., 2002; McConnachie et al., 2012b) thereby potentially mitigating the beneficial actions of corticosteroids in response to a predation threat. A similar occurrence is likely under conditions where cortisol, produced from a predation stress, is prevented from acting on its regulatory targets. Indeed, blocking GRs through pharmaceuticals (e.g. RU486) diminished cortisol-induced physiological changes including gluconeogenic activities (DiBattista et al., 2006; Vijayan et al., 1994); alterations that may be required to successfully avoid a predator (Hawlena and Schmitz, 2010). However, these ideas have yet to be tested experimentally to any great degree, and data on the linkage between stress-induced changes in teleost physiology and ecosystem dynamics are rather limited, with most studies focused on salmonids facing stressors in a lab setting (Hawlena and Schmitz, 2010; Lima, 1998; Schreck et al., 1997).

The objective of the present study was to experimentally manipulate the stress axis of a teleost fish and relate the resulting physiological changes to behavioural responses to a predator in a natural system. We used juvenile schoolmaster snapper (*Lutjanus apodus*) as a study species because they inhabit nearshore mangrove marine habitats that are rich in predators (Laegdsgaard and Johnson, 2001) and mangroves represent one of the most threatened (Crain et al., 2009) and disturbed ecosystems in the world (Alongi, 2002; Duke et al., 2007; Valiela et al., 2001). Fish were tethered to the seafloor in the coastal zone (Rypel et al., 2007) and observed for predation-related endpoints in the face of a natural predator, the lemon shark (*Negaprion brevirostris* [Poey, 1868]). We predicted that elevated cortisol would result in a homeostatic overload causing altered behaviour and impaired physiological condition, in turn elevating susceptibility to predator-induced mortality compared to sham-treated fish. Furthermore, blocking cortisol receptors (through RU486) was predicted to also elevate predator-induced mortality by mitigating the beneficial physiological effects of the acute cortisol response to the predator (stressor), thereby preventing the animal from responding appropriately, relative to a baseline state, to a predation threat.

2. Methods

All experimental procedures were conducted in accordance with the guidelines provided by the Carleton University Animal Care Committee (AUP-100612) under the supervision of the Canadian Council of Animal Care (CCAC). All animals were obtained under a scientific collection permit from the Bahamian Department of Marine Resources.

2.1. Animal collection and preparation

Juvenile schoolmaster snapper [*Lutjanus apodus* (Walbaum, 1792); 27.8 ± 1.8 g; $N = 96$] were collected from a nearby mangrove creek (Page Creek, Eleuthera Island, Bahamas; 24°49′04″N: 76°18′51″W) which consisted of a mixed sea grass/sandy bottom habitat bordered by mangrove prop roots. Fish obtained using a baited minnow trap from November 15th–December 13th, 2014 were then transported to the Cape Eleuthera Institute (Eleuthera Island, Bahamas; 24°50′06.70″ N, 76°19′31.69″W) and held in a well-aerated raceway-style tank (519 l) under ambient flow-through seawater conditions (dissolved oxygen >85%; temperature 25.5 ± 0.5 °C; pH 8.29 ± 0.1; salinity 34.1 ± 0.1 ppt) under a natural photoperiod (13 h D:11 h L). Animals were fed chopped sardines to satiation on a daily basis and were allowed to acclimate to the holding conditions for at least 24-h prior to any experimental manipulation.

Fasted fish (~16 h) were weighed and a small hole was made in the ventral surface of the lower jaw with a fine surgical needle (1/2 circle, cutting edge, size 14, Integra Miltex, Plainsboro, NJ, USA) to serve as a tether anchoring point (Rypel et al., 2007). Cocoa butter implants (5 ml/kg BW; $N = 25$; Cull et al., 2015) were administered by intraperitoneal injection (Gamperl et al., 1994). Implants contained either hydrocortisone 21-hemisuccinate (50 mg/kg BW; $N = 31$; Cull et al., 2015; Sigma-Aldrich, Oakville, ON, Canada) or RU486 (100 mg/kg BW; $N = 25$; Doyon et al., 2006; Sigma-Aldrich, Oakville, ON, Canada); sham injections of cocoa butter alone served as an injection control group ($N = 27$). Animals recovered for 24-h, in segregated black crates submerged in the holding tanks maintained under ambient seawater conditions as described above, to allow the implanted drugs to reach biologically active concentrations in circulation (Bernier et al., 1999; McConnachie et al., 2012a, 2012b).

2.2. Experimental procedure and study site

A subset of fish ($N = 11$–15 per treatment) was not included in the field experiments but instead was retained to determine the physiological effects associated with each implant group at 24 h post-implantation, the time at which field experiments were carried out. Animals were euthanized through cerebral concussion and a blood sample (~0.5 ml) was withdrawn via caudal venipuncture using a heparinized

(Na+ heparin, 10, 000 USP units/ml; Sandoz Canada Inc., Boucherville, QC, Canada) 1 ml syringe and 23 G needle. Whole blood was immediately assessed for glucose and lactate concentrations as well as haematocrit. Blood samples were then centrifuged (2000 *g*; Mandel Scientific, Guelph, ON, Canada) for 1 min and decanted plasma was promptly frozen and stored at −20 °C for subsequent analyses of cortisol, Na+, Cl−, and urea concentrations.

To assess the validity of cortisol implants in reflecting a "naturally" high cortisol level in schoolmaster snapper, an additional group of control (no implant) fish ($N = 13$) was chased to exhaustion in a manner similar to McDonald et al. (1989), using a 5 min chase time. Exercise has been shown to be a stressor that elicits a significant elevation in circulating cortisol in teleosts (reviewed in Milligan, 1996 & Davison, 1997). Fish were euthanized and a blood sample was collected (as described above) 30 min after exercise. This sampling time was selected because plasma [cortisol] reaches its peak value at 30 min post-stressor in a number of teleost fish (Herbert and Steffensen, 2005; Milligan and Wood, 1987).

For individuals used in field experimentation, a 1.5 m long tether (15-lb. test monofilament fishing line) was attached to the prepared hole described above using a triple surgical box knot, similar to Rypel et al. (2007). The procedure took <1 min and animals were then allowed to recover for 20–30 min in individual, 19-L buckets with ample aeration.

The study site consisted of 1.2 km of coastline adjacent to the Cape Eleuthera Institute. It consisted of a similar habitat type to that found within the collection site (i.e. seagrass and macroalgae with a sandy/rocky substrate), thereby representing natural cover that the animals would have access to in their home mangrove creek (Murchie et al., 2015). The shoreline also had sparse patches of mangroves but tethering experiments occurred away from mangroves because of the potential for tangling of tethered fish. The site was subdivided into four 150 m-long blocks each separated by 200 m. Each block contained three steel anchors that were separated by 50 m from one another. Water depth at these locations ranged from 0.3–1.0 m, varying with the daily tidal cycles.

Each experimental trial consisted of tethering one fish from each implant group (sham $N = 16$; cortisol, $N = 16$; RU486 $N = 13$) to an anchor within a block site. To control for day-to-day weather and tidal patterns, fish were assigned to each block- and anchor-site using a randomized block design. Once anchored to the seafloor, a fish was able to move freely within a 1.5 m radius for 15 min, and video was recorded via a Go-Pro Hero camera (Go-Pro, San Mateo, CA, USA) that was placed approximately 1.6 m behind the animal on the beach-facing side. Cameras used a wide angle field of view and were shot at 30 frames per second in 1080p high definition to maximize video capture conditions (Struthers et al., 2015). Water clarity was exceptional with visibility in excess of 10 m. Parameters measured included mortality, time to predator arrival and time to predation event (see below).

2.3. Behavioural and plasma analyses

Video observations were made over a 15 min experimental timeframe. Time to predator arrival constituted the time required for a predator to arrive at the tether site once the experimental trial had begun and reflects the conspicuousness of the prey to the predator, which can include visual, olfactory and mechanical cues, thereby serving as a proxy for predation risk (see Lima and Dill, 1990). Time to predation event was the time at which the prey item was consumed by the predator from the onset of the experimental trial. This metric reflected any capacity of the animal to avoid the predator during the interaction and, consequently, acted as a proxy of anti-predator defensive behaviour. Similarly, mortality within the 15 min viewing frame also served as a proxy of an anti-predator response. When multiple predators were observed at the tether, it was impossible to identify when a specific individual arrived, and consequently, $t = 0$ was standardized to be from the occurrence of the first predator to visit the site. Juvenile lemon sharks, *Negaprion brevirostris*, were the only predators observed during this experiment. It is worth highlighting that juvenile lemon sharks, the sole predator observed here, exhibit habitat co-localization with a collective of snapper species (Newman et al., 2007, 2010). Furthermore, gut content assessments of lemon sharks have indicated that teleosts comprise >70% of a lemon shark's diet (reviewed in Wetherbee et al., 1990) with snappers being documented as a prey species in juvenile sharks (Cortés and Gruber, 1990; Newman et al., 2010). Additionally, schoolmaster snapper are often found moving independently of a school during their juvenile life stage (Springer and McErlean, 1962; Rooker and Dennis, 1991; reviewed in Mateo et al., 2010). Taken together, this evidence supports the notion that our experimental design provided an accurate portrayal of a natural trophic system representing a common predator-prey interaction.

Blood glucose and lactate concentrations were assessed using portable glucose (Accu-Chek Compact Plus, Hoffman-La Roche Limited, Mississauga, ON, Canada) and lactate (Lactate Plus, Nova Biomedical Corporation Canada Ltd., Mississauga, ON, Canada) meters, respectively. These meters have been validated for use in teleost fish (reviewed in Stoot et al., 2014). Haematocrit was determined through the use of heparinized microcapillary tubes (ammonium heparin; Drumond Scientific Co., Broomall, PA, USA) that were centrifuged for 5 min at 4400 *g* (Zipocrit Centrifuge, LW Scientific, Lawrenceville, GA, USA). Total plasma cortisol was assessed using a commercially-available radioimunnoassay kit (ImmunoChem Cortisol Coated Tube RIA Kit, MP Biomedicals, Solon, OH, USA) that has been validated for use in teleost fish (Gamperl et al., 1994). Inter-assay and intra-assay variabilities were 5.54% and <14%, respectively. Concentrations of plasma urea and Cl− were determined using colourimetric assays employing the methods of Rahmatullah and Boyde (1980) and Zall et al. (1956), respectively. Colourimetric assays were read at room temperature (~22 °C) on a microplate reader (SpectraMax, Molecular Devices, Sunnyvale, CA, USA). Plasma Na+ concentrations were determined through flame spectrophotometry (Varian Spectra AA 220FS, Varian Inc., Palo Alto, CA, USA). Plasma urea and Cl− samples were measured in triplicate whereas plasma Na+ was measured in duplicate.

2.4. Statistical analyses

SigmaPlot v11.0 (Systat Software Inc., San Jose, CA, USA) was employed for all statistical evaluation. Statistically-significant effects were accepted at $\alpha = 0.05$ with values, when appropriate, being reported as the mean ± 1 s.e.m. (N). A one-way analysis of variance (ANOVA) was used to compare blood/plasma parameters among treatments. Where significant differences were found, a Tukey's HSD post-hoc test was employed. A multiple logistical regression model was used to test for differences in snapper mortality among treatments. A log-rank survival analysis was employed to assess the effects of treatment group on predator arrival time and time to predation.

3. Results

3.1. Baseline blood and plasma parameters

Plasma cortisol concentrations reached their highest levels in cortisol-treated fish, being 111- and 13.5-fold greater than values for sham and RU486-treated fish, respectively (Fig. 1A). Plasma [cortisol] in RU486-treated fish was higher (8.2×) than plasma [cortisol] in sham-implanted fish (Fig. 1A). Treatment with cortisol (50 mg/kg BW) led to a nearly 2.4-fold higher blood [glucose] relative to sham-implanted fish (Fig. 1B). Blood glucose levels did not differ between RU486 and sham treated fish (Fig. 1B). Plasma urea levels were 1.7× higher in cortisol-treated fish relative to the sham group. However, RU486 treated fish had comparable plasma urea concentrations to both sham and

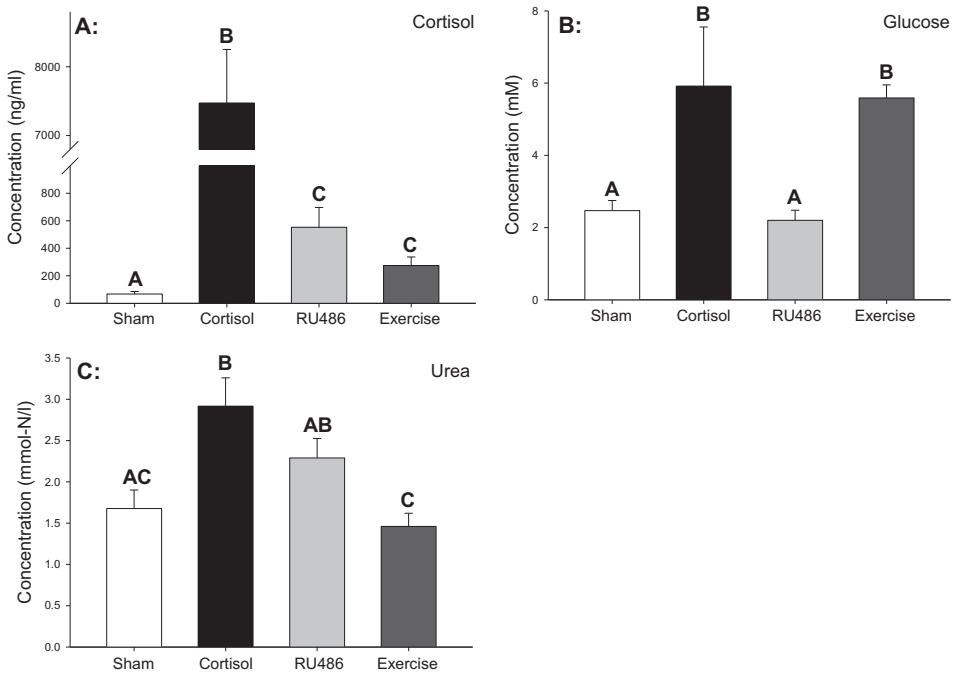

Fig. 1. Concentrations of plasma cortisol (A), blood glucose (B) and plasma urea (C) in schoolmaster snapper, 24 h after receiving a cocoa butter implant on its own (sham; 5 ml/kg BW; white bars; $N \leq 8$), containing cortisol (50 mg/kg BW; black bars; $N \leq 6$), or RU486 (100 mg/kg BW; grey bars; $N \leq 11$) and post 30 min exhaustive exercise (dark grey bars; $N \leq 13$). Values are reported as means ±1 s.e.m. Samples were analyzed using a one-way ANOVA (Cortisol, $P < 0.001$; glucose, $P < 0.001$; urea, $P < 0.001$) coupled with a Tukey post-hoc test. Unique letters represent statistically significant ($P < 0.05$) differences among treatment groups.

cortisol fish (Fig. 1C). Plasma concentrations of Na+, Cl− and lactate, and haematocrit did not differ among implant groups (Table 1).

In fish allowed to recover for 30-min from exhaustive exercise, circulating levels of glucose (2.3×), lactate (7.8×) and cortisol (4.1×) were all appreciably higher when compared to values for sham-treated fish (Fig. 1). Cortisol-treated fish had blood glucose levels that were comparable to those of exercised fish despite having higher plasma cortisol (27×) and urea (2×) concentrations (Fig. 1). RU486 treated fish had lower blood glucose (0.4×), higher plasma urea (1.6×) and comparable plasma cortisol concentrations relative to exercised fish (Fig. 1). Haematocrit was also significantly higher in exercised fish, by 32%, 28% and 29% relative to sham, cortisol, and RU486-treated fish, respectively (Table 1). Plasma concentrations of Na+ and Cl− were unaffected by exercise (Table 1).

3.2. Field behavioural assessments

Juvenile lemon sharks (*Negaprion brevirostris*) were the only predator observed to frequent the tethering stations. Overall mortality rates for sham, cortisol-treated and RU486-treated fish were 76.9%, 58.3% and 63.6%, respectively. Snapper mortality was unaffected by implantation group ($P = 0.471$) when modelled by the function $Logit\ P = 1.002 - (0.315 * implant\ group)$. Both the "time to predator arrival" (Fig. 2) and the "time to predation event" (Fig. 3) were equal among treatment groups with sharks finding and consuming snapper in a non-discriminate fashion ($P > 0.05$).

4. Discussion

4.1. Overview

Schoolmaster snapper were implanted with either cortisol or the GR antagonist RU486 in an attempt to understand the physiological implications of the stress axis in mediating predator-prey interactions. Under cortisol treatment, it was expected that homeostatic overload from cortisol implantation would have energetically compromised the snapper causing a decrease in predator avoidance capacity. Similarly, the blockage of cortisol-mediated effects via treatment with the GR-antagonist RU486 would have likely compromised the animal's ability to energetically respond to cortisol produced in response to the stressor of predation. In both instances, HPI axis modification did not alter snapper predation, with predator-induced mortality and predation avoidance capacity being uniform among all treatment groups. The absence of predation differences may reflect the complexity of relationships between

Table 1
Blood and plasma parameters for a schoolmaster snapper with sham, cortisol and RU486 implants and after a 30 min recovery from exhaustive exercise.

Parameter	Treatment Group			
	Sham	Cortisol	RU486	Exercise
Plasma [Na+]	180.8 ± 6.5 ($N = 4$)	176.3 ± 4.8 ($N = 6$)	180.4 ± 5.3 ($N = 11$)	182.7 ± 2.5 ($N = 12$)
Plasma [Cl−]	148.0 ± 10.6 ($N = 4$)	156.2 ± 6.2 ($N = 6$)	145.4 ± 5.3 ($N = 11$)	158.98 ± 2.3 ($N = 12$)
Haematocrit (%)	23.7 ± 2.0a ($N = 7$)	24.5 ± 2.3ab ($N = 6$)	24.3 ± 1.4a ($N = 9$)	31.2 ± 1.9b ($N = 13$)
Blood [lactate]	0.5 ± 0.1a ($N = 8$)	0.1 ± 0.1a ($N = 6$)	0.2 ± 0.1a ($N = 12$)	3.5 ± 0.5b ($N = 13$)

Values are presented as mean ± 1 s.e.m. and, unless otherwise noted, are presented as mM. A one-way ANOVA was used to determine statistical differences among treatment groups (Na+, $P = 0.82$; Cl−, $P = 0.15$; Haematocrit, $P = 0.01$; lactate, $P < 0.001$) and was coupled with a Tukey post-hoc test. Unique letters represent statistically significant ($P < 0.05$) results among treatment groups.

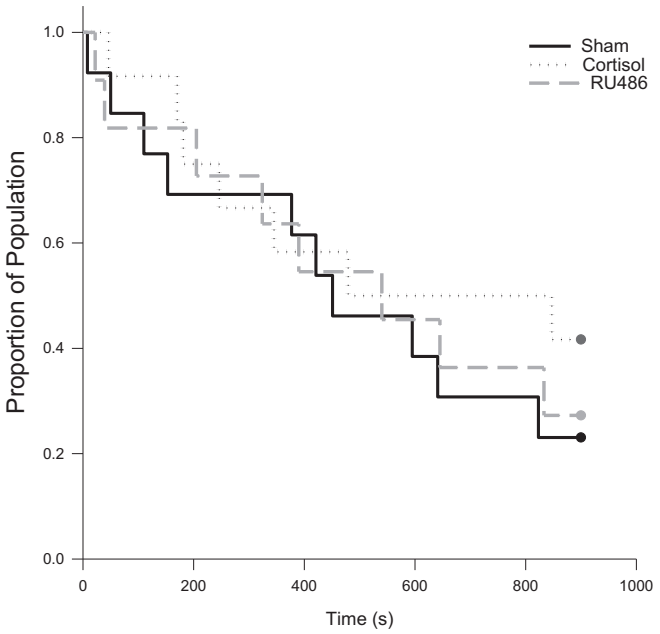

Fig. 2. Survival analysis of time to predator arrival among three implantation groups of schoolmaster snapper with implants containing either a sham (5 ml/kg BW; solid line; $N = 13$), cortisol (50 mg/kg BW; dotted line; $N = 12$), or RU486 (100 mg/kg BW; dashed line; $N = 11$). No significant effects of implantation group were found ($P > 0.05$). Individuals that did not have a predator arrive were censored from the analysis (filled circles).

physiological function and behaviour. Additionally, artefacts associated with tethering may have masked treatment differences in our behavioural metrics.

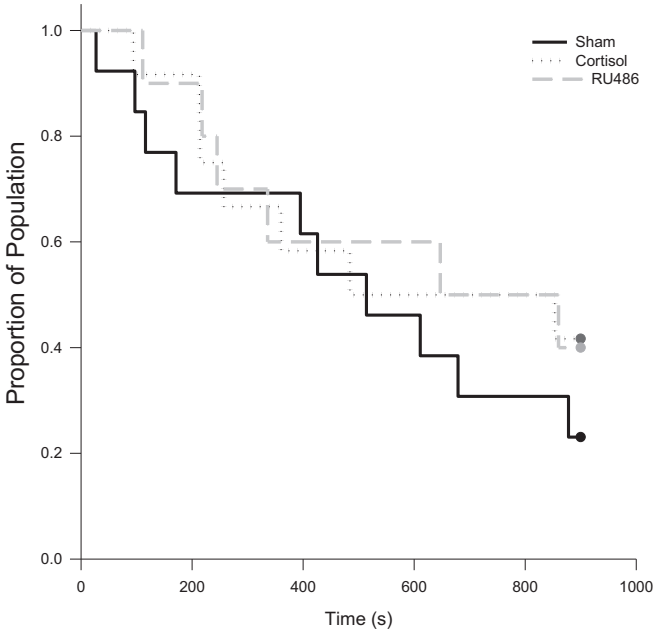

Fig. 3. Survival analysis of time to predation event among the three treatment groups of schoolmaster snapper with implants containing either a sham (5 ml/kg BW cocoa butter alone; solid line; $N = 13$), cortisol (50 mg/kg BW; dotted line; $N = 12$), or RU486 (100 mg/kg BW; dashed line; $N = 10$). No significant effects of implantation group were found ($P > 0.05$). Individuals that did not have a predation event were censored from the analysis (filled circles).

4.2. Validation of pharmacological implants

In schoolmaster snapper, plasma cortisol concentrations were far higher than those observed in chased fish, reaching supra-physiological levels, and corresponded with an elevation in blood [glucose] (Fig. 1A) relative to sham fish. Cortisol elevation under stressful conditions stimulates the mobilization of energy reserves and up-regulates gluconeogenic pathways, at the enzymatic and molecular levels, via glucocorticoid receptor (GR) stimulation (reviewed in Aluru and Vijayan, 2009 & Mommsen et al., 1999). As such, elevated cortisol levels in the blood of teleosts are often accompanied by increases in blood [glucose] (De Boeck et al., 2001; Jentoft et al., 2005; McConnachie et al., 2012b; reviewed in Mommsen et al., 1999), which are used as an indicator of stress axis induction (Barton and Iwama, 1991). The supra-physiological levels of plasma cortisol in our fish may have been a concern given cortisol's autoregulation of GR expression (Aluru and Vijayan, 2007; Maule and Schreck, 1991; Pottinger et al., 1994; Sathiyaa and Vijayan, 2003; Shrimpton and Mccormick, 1999; Terova et al., 2005; Vijayan et al., 2003), which may generate an insensitivity to the hormone (Maule and Schreck, 1991; Sathiyaa and Vijayan, 2003). However, previous work has demonstrated that teleostean tissue is still responsive, at the gluconeogenic level, to cortisol even under sustained cortisol elevations (Sathiyaa and Vijayan, 2003; Vijayan et al., 2003). Given that blood glucose was still elevated under cortisol administration in schoolmaster snapper and that the biochemical pathways mediating gluconeogenesis are still active under similar conditions in other teleosts (McConnachie et al., 2012b; Sathiyaa and Vijayan, 2003; Vijayan et al., 2003), the HPI axis is still likely to be responsive in our fish. Under these conditions, high levels of cortisol could stimulate an increased rate of GR ubiquitination/degradation (Boone and Vijayan, 2002; Sathiyaa and Vijayan, 2003; Vijayan et al., 2003). However, it is believed that cortisol can still interact with the receptor prior to this event and likely explains the discrepancy between the continued responsiveness of the stress axis under cortisol levels that would instigate autoregulation (Sathiyaa and Vijayan, 2003).

RU486 treatment also functioned as expected with snapper demonstrating an increase in plasma cortisol concentrations while blood glucose concentrations remained at control levels. RU486 is a well-established antagonist of GRs in teleost fish with its effect characterized across a number of species (reviewed in Mommsen et al., 1999). The rise in plasma cortisol concentrations under RU486 treatment represents a failure in the negative feedback actions of cortisol as RU486 blocks GRs on the upstream regulators of cortisol biosynthesis, resulting in a sustained release of ACTH and cortisol production by extension (Bradford et al., 1992; Bernier et al., 1999; Reddy et al., 1995; Veillette et al., 1995, 2007); an effect similar to what has been documented in mammals (Gaillard et al., 1984; Healy et al., 1985; Heindorff et al., 1994). Loss of negative feedback likely explains the increase in plasma cortisol observed here and lends support to the effectiveness of RU486 in blocking glucocorticoid responses in these animals. The lack of change in blood [glucose] despite the significant increase in plasma [cortisol] similarly supports the effectiveness of RU486 treatment.

Urea metabolism was elevated by cortisol treatment, relative to sham fish (Fig. 1C). Amino acid catabolism has been found to be tightly associated with cortisol (Chan and Woo, 1978; Milligan, 1997; Mommsen et al., 1992; Vijayan et al., 1996). However, this process produces toxic ammonia as a by-product (Randall and Tsui, 2002); ammonia must be either excreted or detoxified by the fish internally (Arillo et al., 1981; Fromm and Gillette, 1968; Lawrence et al., 2015; Mommsen and Walsh, 1992; Olson and Fromm, 1971). Some teleosts are capable of converting ammonia into a less toxic form, urea, and therefore the increase in plasma [urea] in cortisol-treated snapper may reflect a detoxification pathway (Arillo et al., 1981; Fromm and Gillette, 1968; Mommsen and Walsh, 1992; Olson and Fromm, 1971). This observation supports the notion that energy reserves are mobilized during elevations in cortisol in schoolmaster snapper.

As expected, chasing caused significant increases in haematocrit and blood lactate (Table 1) levels consistent with the mobilization of catecholamine stress hormones and use of anaerobic metabolism, respectively (Milligan, 1996; Milligan and Wood, 1987; Pearson and Stevens, 1991; Wood, 1991). Thus, chasing served as a stressor, and cortisol levels in chased fish could be used as an index of the endogenous cortisol response to a stressor in schoolmaster snapper. Under exogenous cortisol loading (e.g. cortisol implants), plasma cortisol levels often peak within the first 24 h of application before stabilizing above resting levels for an extended duration (e.g. days to weeks; Gamperl et al., 1994). Peak cortisol values, during exogenous loading, often reflect those observed during periods of elevated endogenous production in teleost fish (e.g. acute stressors; reviewed in Barton and Iwama, 1991) thereby representing comparable responses. It is important to note that the physiological changes reflected here may not be representative of those experienced by an animal being actively hunted by its predator. It would be expected that acute predation exposures would yield increases in haematocrit and blood glucose in response to a predation threat as part of the catecholamine mediated 'fight or flight' response (reviewed in Randall and Perry, 1992 & Cannon, 1929).

4.3. Physiological impacts of stress and the potential to alter predation susceptibility

We predicted that fish exposed to increased cortisol concentrations would be more susceptible to predation as a result of a homeostatic overload condition; however, predation related parameters were unaffected by cortisol treatment in schoolmaster snapper. This finding contrasts with the work of others where predator-induced mortality increased in stressed individuals (Danylchuk et al., 2007; Järvi, 1989; Mesa, 1994; Olla et al., 1992, 1995; reviewed in Beitinger, 1990 and Mesa et al., 1994). With stress axis activation influencing energy metabolism in fish (e.g. increasing standard metabolic rate), aerobic scope is likely to be reduced with cortisol administration (Barton and Schreck, 1987; Farrell et al., 2008; Guderley and Pörtner, 2010), reducing the potential to inhibit predator avoidance capacity through changes in swimming performance (Guderley and Pörtner, 2010; Priede, 1977); a contributor to mounting a successful escape response (reviewed in Godin, 1997). Although signs that energy metabolism was affected in cortisol-treated fish were observed in the present study, the metabolic consequences of stress axis induction were not investigated. Stress-associated changes in the metabolism of teleosts should be investigated as a potential mediator of alterations in predation susceptibility.

To date, the influence of GR blocking in the face of predation has not been ascertained in fish (Crossin et al., 2015). In response to an acute predation exposure, teleosts upregulate cortisol secretion as a preparative metabolic mechanism to support anti-predator defenses (Woodley and Peterson, 2003; Barcellos et al., 2007). Thus, we predicted that under GR blocking via RU486, fish would experience a greater risk of predation as a result of a failure of physiological mechanisms supporting anti-predator defenses including alterations in energetic supplies. We observed no difference in glucose supply with GR blockage (Fig. 1A) suggesting that these animals were not energetically compromised under resting, unstressed conditions. Under exposure to a predator, the HPI axis is likely to be induced to provide the necessary physiological changes for successful predator evasion (Clinchy et al., 2013; Hawlena and Schmitz, 2010). However, with GRs blocked by RU486, the beneficial influences of cortisol (see Section 1), would not be conferred in the tethered RU486 treated fish making them potentially more susceptible to a predation event. As well, it should be noted that RU486 does not antagonise catecholamine-mediated responses in fish. Catecholamine secretion is critical for an animal's survival of a predation event because it increases evasion potential primarily through alterations in energy mobilization (Cannon, 1929; Galhardo and Oliveira, 2009; reviewed in & Fabbri et al., 1998 & Wendelaar Bonga, 1997). Because the predator-prey interactions observed here were acute (e.g. minutes), the actions of catecholamines may have been more important than those of cortisol.

4.4. Physiological changes may not regulate behaviour

Alterations in stress hormones including glucocorticoids are thought to be an important regulator of behaviour in predator-prey systems (reviewed in Hawlena and Schmitz, 2010). However, in numerous studies with teleost fish, cortisol implantation has resulted in clear signs of physiological stress but failed to influence the behaviour of the animal (Cull et al., 2015; Dey et al., 2010; Nagrodski et al., 2013; O'Connor et al., 2009; Pleizier et al., 2015). For example, in checkered pufferfish (*Sphoeroides testudineus*) implanted with cortisol to elevate plasma [cortisol], behavioural metrics, including both swimming performance and anti-predator responses (e.g. puffing), were unaffected (Cull et al., 2015; Pleizier et al., 2015). If swimming performance in snapper of the present study was similarly unaffected by cortisol implantation, then the observed homogeneity in behavioural parameters is not surprising. This apparent disconnect between behaviour and physiology suggests that simple physiological effects may not correspond directly with a change in the behaviour of an animal (Crossin et al., 2015; Pleizier et al., 2015; Sopinka et al., 2015). Indeed, the notion of complex relationships between levels of scale (e.g. physiological function and behaviour) holds true across a number of vertebrate taxa, where the strength of the association depends on a number of internal and external factors (Crossin et al., 2015; Sopinka et al., 2015). As such, the act of raising cortisol alone in snapper may not be sufficient to cause behaviour changes and predatory avoidance impairment. Perhaps elevated cortisol in tandem with metabolic and neurophysiological changes associated with stress axis induction (reviewed in Barton and Iwama, 1991, Sapolsky et al., 2000 & Wendelaar Bonga, 1997) are necessary to elicit behavioural scale impacts on the animal's survival in the face of a predator. It is also worth noting that the relatively short duration of elevated cortisol exposure (24 h) may not have caused the fish to suffer any impacts of energetic misallocation/diversion; there were likely ample energy stores at this time to draw on (Romero et al., 2009). Over more extended durations of cortisol elevation, heightened energetic consumption may elicit a change in behavioural patterns, particularly with respect to foraging dynamics, that could increase conspicuousness to a predator (reviewed in Milinski, 1993 & Godin, 1997). Thus, it could be speculated that the effects of cortisol elevation may be more pronounced over longer exposure durations. While cortisol alone may not be a strong influence in mediating predator-prey interactions, the accompanying traits that coincide with physiological stress (not investigated here) may perhaps yield a mechanism for the alterations in predation avoidance capacities observed in the literature.

4.5. Potential tethering artefacts

Tethering experiments, historically, have been debated as a reliable tool in assessing predation (Aronson and Heck, 1995; Aronson et al., 2001; Kneib and Scheele, 2000; Peterson and Black, 1994). In close encounters with predators, many teleosts rely upon burst swimming and highly mobile escape responses to avoid predation (Godin, 1997; Lima and Dill, 1990). As tethers restrict movement, they pose a significant issue for organisms that rely upon mobile escape responses, in contrast to species that employ crypsis. Indeed, tethered (mobile) prey items have been found to be limited in their normal escape response behaviour, thereby suffering a greater rate of predation when compared to their free counterparts (Barbeau and Scheibling, 1994; Curran and Able, 1998; Haywood et al., 2003; Kneib and Scheele, 2000; reviewed by Peterson and Black, 1994). Furthermore, any negative impacts of cortisol or RU486 treatment on swimming performance (see Section 4.3) are likely to have been muted under tethering owing to the limited range of motion available (Laegdsgaard and Johnson, 2001), resulting in a high capture probability among all of our treatment groups (Lima

and Dill, 1990). It is worth noting that the experimental fish did have access to natural structure (macroalgae and coral rubble) while tethered which could have provided of a means of predator evasion unlike tethering studies conducted in the lab.

Predator density also can influence the effectiveness of tether-based systems. Previous work has found that increasing predator density corresponds with a greater proportion of predator-induced mortality (Kneib and Scheele, 2000). Although the shark population density has not yet been characterized in the waters surrounding the Cape Eleuthera Institute, a number of individual sharks frequented the study area (>5/km^2). Furthermore, the tethering of fish may enhance predator localization of prey through the release of olfactory cues resulting from the tether itself damaging the skin and tissues (Curran and Able, 1998). In a similar case, shark predation on angled bonefish upon release was believed to be enhanced by the emittance of stress-related olfactory cues from the prey in question (Danylchuk et al., 2007). Sharks, with acute sensory abilities, were likely also detecting the movements and sounds associated with the setup of the tether thus enhancing prey localization. Additionally, tethered animals are more likely to display erratic/altered behaviour that may be beneficial to predator detection of the prey item (Barshaw and Able, 1990; Peterson and Black, 1994). Together, these complications with tethering could potentially skew our data as the snapper experienced a greater predation rate and were more "conspicuous" to predators (Peterson and Black, 1994), making it difficult to detect differences in mortality and predator evasion capacity linked to the experimental treatments.

4.6. Conclusions

We predicted that cortisol and RU486 treatments should generate a higher susceptibility to predation due to physiological impairment from each of the respective treatments. However, despite physiological changes associated with hormonal manipulation, predation related parameters were not affected. Although prior evidence found that chronically/previously stressed fish experience higher rates of predator-induced mortality (reviewed in Beitinger, 1990 and Mesa et al., 1994), our findings suggest that cortisol elevations alone may not be enough to alter these interactions. Additionally, artefacts associated with tethering procedures may have confounded our experimental design. Understanding how stress at the level of the individual, particularly in the face of extensive anthropogenic disturbances and environmental change in aquatic systems (Helmuth, 2009), influences individual fitness and ecological processes such as predator-prey relationships represents a timely research area in the realm of the "ecology of stress" (Boonstra, 2013a, 2013b).

Contributions

M.J.L., E.J.E., J.W.B., and S.J.C. designed the project. The experiments, data collection and analyses were done by M.J.L., E.J.E., K.M.G., and S.J.C. All authors contributed to the writing and editing of this work.

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References

Allan, B.J., Domenici, P., Munday, P.L., McCormick, M.I., 2015. Feeling the heat: the effect of acute temperature changes on predator–prey interactions in coral reef fish. Conserv. Physiol. 3 (1), cov011.
Alongi, D.M., 2002. Present state and future of the world's mangrove forests. Environ. Conserv. 29 (03), 331–349.
Aluru, N., Vijayan, M.M., 2007. Hepatic transcriptome response to glucocorticoid receptor activation in rainbow trout. Physiol. Genomics 31 (3), 483–491.
Aluru, N., Vijayan, M.M., 2009. Stress transcriptomics in fish: a role for genomic cortisol signaling. Gen. Comp. Endocrinol. 164 (2), 142–150.
Arillo, A., Margiocco, C., Melodia, F., Mensi, P., Schenone, G., 1981. Ammonia toxicity mechanism in fish: studies on rainbow trout (*Salmo gairdneri* Rich.). Ecotoxicol. Environ. Saf. 5 (3), 316–328.
Aronson, R.B., Heck, K.L., 1995. Tethering experiments and hypothesis testing in ecology. Mar. Ecol. Prog. Ser. 121 (1), 307–309.
Aronson, R.B., Heck, K.L., Valentine, J.F., 2001. Measuring predation with tethering experiments. Mar. Ecol. Ser. 214, 311–312.
Barbeau, M.A., Scheibling, R.E., 1994. Procedural effects of prey tethering experiments: predation of juvenile scallops by crabs and sea stars. Mar. Ecol. Prog. Ser. 111, 305–310.
Barcellos, L.J.G., Nicolaiewsky, S., De Souza, S.M.G., Lulhier, F., 1999. Plasmatic levels of cortisol in the response to acute stress in Nile tilapia, *Oreochromis niloticus* (L.), previously exposed to chronic stress. Aquac. Res. 30 (6), 437–444.
Barcellos, L.J.G., Ritter, F., Kreutz, L.C., Quevedo, R.M., da Silva, L.B., Bedin, A.C., Finco, J., Cericato, L., 2007. Whole-body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*. Aquaculture 272 (1), 774–778.
Barshaw, D.E., Able, K.W., 1990. Tethering as a technique for assessing predation rates in different habitats: an evaluation using juvenile lobsters *Homarus americanus*. Fish. Bull. 88 (2), 415–417.
Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1, 3–26.
Barton, B.A., Schreck, C.B., 1987. Metabolic cost of acute physical stress in juvenile steelhead. Trans. Am. Fish. Soc. 116 (2), 257–263.
Barton, B.A., Schreck, C.B., Barton, L.D., 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis. Aquat. Org. 2 (3), 173–185.
Beitinger, T.L., 1990. Behavioral reactions for the assessment of stress in fishes. J. Great Lakes Res. 16 (4), 495–528.
Bernier, N.J., Lin, X., Peter, R.E., 1999. Differential expression of corticotropin-releasing factor (CRF) and urotensin I precursor genes, and evidence of CRF gene expression regulated by cortisol in goldfish brain. Gen. Comp. Endocrinol. 116 (3), 461–477.
Boone, A.N., Vijayan, M.M., 2002. Glucocorticoid-mediated attenuation of hsp70 response in trout hepatocytes involves the proteasome. Am. J. Regul. Integr. Comp. Physiol. 283, 680–687.
Boonstra, R., 2013a. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. Funct. Ecol. 27 (1), 11–23.
Boonstra, R., 2013b. The ecology of stress: a marriage of disciplines. Funct. Ecol. 27 (1), 7–10.
Bradford, C.S., Fitzpatrick, M.S., Schreck, C.B., 1992. Evidence for ultra-short-loop feedback in ACTH-induced interrenal steroidogenesis in coho salmon: acute self-suppression of cortisol secretion in vitro. Gen. Comp. Endocrinol. 87 (2), 292–299.
Brown, J.A., Johansen, P.H., Colgan, P.W., Mathers, R.A., 1985. Changes in the predator-avoidance behaviour of juvenile guppies (*Poecilia reticulata*) exposed to pentachlorophenol. Can. J. Zool. 63 (9), 2001–2005.
Cannon, W.B., 1929. Bodily Changes in Pain, Hunger, Fear and Rage. Appleton, New York.
Cericato, L., Neto, J.G.M., Fagundes, M., Kreutz, L.C., Quevedo, R.M., Finco, J., Santos da Rosa, J.G., Koakoski, G., Centenaro, L., Pottker, E., Anziliero, D., Barcellos, L.J.G., 2008. Cortisol response to acute stress in jundiá *Rhamdia quelen* acutely exposed to sub-lethal concentrations of agrichemicals. Comp. Biochem. Physiol. C 148 (3), 281–286.
Chan, D.K., Woo, N.Y., 1978. Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. Gen. Comp. Endocrinol. 35 (3), 205–215.
Clinchy, M., Sheriff, M.J., Zanette, L.Y., 2013. Predator-induced stress and the ecology of fear. Funct. Ecol. 27 (1), 56–65.
Cortés, E., Gruber, S.H., 1990. Diet, feeding habits and estimates of daily ration of young lemon sharks, *Negaprion brevirostris* (Poey). Copeia 204–218.
Costas, B., Aragão, C., Ruiz-Jarabo, I., Vargas-Chacoff, L., Arjona, F.J., Dinis, M.T., Mancera, J.M., Conceição, L.E., 2011. Feed deprivation in Senegalese sole (*Solea senegalensis* Kaup, 1858) juveniles: effects on blood plasma metabolites and free amino acid levels. Fish Physiol. Biochem. 37 (3), 495–504.
Crain, C.M., Halpern, B.S., Beck, M.W., Kappel, C.V., 2009. Understanding and managing human threats to the coastal marine environment. Ann. N. Y. Acad. Sci. 1162 (1), 39–62.
Crossin, G., Love, O.P., Cooke, S., Williams, T., 2015. Glucocorticoid manipulations in free-living animals: considerations of dose delivery, life-history context, and reproductive state. Funct. Ecol. http://dx.doi.org/10.1111/1365-2435.12482.
Cull, F., Suski, C.D., Shultz, A., Danylchuk, A.J., O'Connor, C.M., Murchie, K.J., Cooke, S.J., 2015. Consequences of experimental cortisol manipulations on the thermal biology of the checkered puffer (*Sphoeroides testudineus*) in laboratory and field environments. J. Therm. Biol. 47, 63–74.

Curran, M.C., Able, K.W., 1998. The value of tethering fishes (winter flounder and tautog) as a tool for assessing predation rates. Mar. Ecol. Prog. Ser. 163, 45–51.

Danylchuk, S.E., Danylchuk, A.J., Cooke, S.J., Goldberg, T.L., Koppelman, J., Philipp, D.P., 2007. Effects of recreational angling on the post-release behavior and predation of bonefish (*Albula vulpes*): the role of equilibrium status at the time of release. J. Exp. Mar. Biol. Ecol. 346 (1), 127–133.

Davison, W., 1997. The effects of exercise training on teleost fish, a review of recent literature. Comp. Biochem. Physiol. A 117 (1), 67–75.

De Boeck, G., Alsop, D., Wood, C., 2001. Cortisol effects on aerobic and anaerobic metabolism, nitrogen excretion, and whole-body composition in juvenile rainbow trout. Physiol. Biochem. Zool. 74 (6), 858–868.

Dey, C.J., O'Connor, C.M., Gilmour, K.M., Van Der Kraak, G., Cooke, S.J., 2010. Behavioral and physiological responses of a wild teleost fish to cortisol and androgen manipulation during parental care. Horm. Behav. 58 (4), 599–605.

DiBattista, J.D., Levesque, H.M., Moon, T.W., Gilmour, K.M., 2006. Growth depression in socially subordinate rainbow trout *Oncorhynchus mykiss*: more than a fasting effect. Physiol. Biochem. Zool. 79 (4), 675–687.

Doyon, C., Leclair, J., Trudeau, V.L., Moon, T.W., 2006. Corticotropin-releasing factor and neuropeptide Y mRNA levels are modified by glucocorticoids in rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 146 (2), 126–135.

Duke, N.C., Meynecke, J.O., Dittmann, S., Ellison, A.M., Anger, K., Berger, U., Cannicci, S., Diele, K., Ewel, K.C., Field, C.D., Koedam, N., Lee, S.Y., Marchand, C., Nordhaus, I., Dahdouh-Guebas, F., 2007. A world without mangroves? Science 317 (5834), 41–42.

Espelid, S., Løkken, G.B., Steiro, K., Bøgwald, J., 1996. Effects of cortisol and stress on the immune system in Atlantic salmon (*Salmo salar* L.). Fish Shellfish Imuunol. 6 (2), 95–110.

Fabbri, E., Capuzzo, A., Moon, T.W., 1998. The role of circulating catecholamines in the regulation of fish metabolism: an overview. Comp. Biochem. Physiol. C 120 (2), 177–192.

Farrell, A.P., Hinch, S.G., Cooke, S.J., Patterson, D.A., Crossin, G.T., Lapointe, M., Mathes, M.T., 2008. Pacific salmon in hot water: applying aerobic scope models and biotelemetry to predict the success of spawning migrations. Physiol. Biochem. Zool. 81 (6), 697–709.

Fromm, P.O., Gillette, J.R., 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 26 (3), 887–896.

Gaillard, R.C., Riondel, A., Muller, A.F., Herrmann, W., Baulieu, E.E., 1984. RU 486: a steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day. Proc. Natl. Acad. Sci. 81 (12), 3879–3882.

Galhardo, L., Oliveira, R.F., 2009. Psychological stress and welfare in fish. Annu. Rev. Biomed. Sci. 1, 1–20.

Gamperl, A.K., Vijayan, M.M., Boutilier, R.G., 1994. Experimental control of stress hormone levels in fishes: techniques and applications. Rev. Fish Biol. Fish. 4 (2), 215–255.

Godin, J.G.J., 1997. Evading predators. In: Godin, J.G.J. (Ed.), Behavioural Ecology of Teleost Fishes. Oxford University Press Inc., New York, pp. 191–236.

Guderley, H., Pörtner, H.O., 2010. Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. Can. J. Zool. 88 (8), 753–763.

Handeland, S.O., Järvi, T., Fernö, A., Stefansson, S.O., 1996. Osmotic stress, antipredatory behaviour, and mortality of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. 53 (12), 2673–2680.

Hawlena, D., Schmitz, O.J., 2010. Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. Am. Nat. 176 (5), 537–556.

Haywood, M.D.E., Manson, F.J., Loneragan, N.R., Toscas, P.J., 2003. Investigation of artifacts from chronographic tethering experiments—interactions between tethers and predators. J. Exp. Mar. Biol. Ecol. 290 (2), 271–292.

Healy, D.L., Chrousos, G.P., Schulte, H.M., Gold, P.W., Hodgen, G.D., 1985. Increased adrenocorticotropin, cortisol, and arginine vasopressin secretion in primates after the antiglucocorticoid steroid RU 486: dose response relationships. J. Clin. Endocrinol. Metab. 60 (1), 1–4.

Heindorff, H., Almdal, T., Vilstrup, H., 1994. The in vivo effect of interleukin-1β on urea synthesis is mediated by glucocorticoids in rats. Eur. J. Clin. Investig. 24 (6), 388–392.

Helmuth, B., 2009. From cells to coastlines: how can we use physiology to forecast the impacts of climate change? J. Exp. Biol. 212 (6), 753–760.

Herbert, N.A., Steffensen, J.F., 2005. The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. Mar. Biol. 147 (6), 1403–1412.

Järvi, T., 1989. The effect of osmotic stress on the anti-predatory behaviour of Atlantic salmon smolts: a test of the 'Maladaptive Anti-predator Behaviour' hypothesis. Nord. J. Freshw. Res. 68, 63–71.

Jentoft, S., Aastveit, A.H., Torjesen, P.A., Andersen, Ø., 2005. Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. A 141 (3), 353–358.

Kneib, R.T., Scheele, C.E.H., 2000. Does tethering of mobile prey measure relative predation potential? An empirical test using mummichogs and grass shrimp. Mar. Ecol. Prog. Ser. 198, 181–190.

Kruzynski, G.M., Birtwell, I.K., Chew, G.L., 1994. Behavioural approaches to demonstrate the ecological significance of exposure of juvenile Pacific salmon (genus *Oncorhynchus*) to the antisapstain fungicide TCMTB. J. Aquat. Ecosyst. Health 3 (2), 113–127.

Laegdsgaard, P., Johnson, C., 2001. Why do juvenile fish utilise mangrove habitats? J. Exp. Mar. Biol. Ecol. 257, 229–253.

Lankford, S.E., Adams, T.E., Miller, R.A., Cech, J.J., 2005. The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. Physiol. Biochem. Zool. 78 (4), 599–609.

Lawrence, M.J., Wright, P.A., Wood, C.M., 2015. Physiological and molecular responses of the goldfish kidney (*Carassius auratus*) to metabolic acidosis, and potential mechanisms of renal ammonia transport. J. Exp. Biol. (jeb-117689).

Lima, S.L., 1998. Stress and decision-making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Adv. Study Behav. 27, 215–290.

Lima, S.L., Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68 (4), 619–640.

Mateo, I., Durbin, E.G., Appeldoorn, R.S., Adams, A.J., Juanes, F., Kingsley, R., Swart, P., Durant, D., 2010. Role of mangroves as nurseries for French grunt *Haemulon flavolineatum* and schoolmaster *Lutjanus apodus* assessed by otolith elemental fingerprints. Mar. Ecol. Prog. Ser. 402, 197–212.

Maule, A.G., Schreck, C.B., 1991. Stress and cortisol treatment changed affinity and number of glucocorticoid receptors in leukocytes and gill of coho salmon. Gen. Comp. Endocrinol. 84 (1), 83–93.

McConnachie, S.H., Cook, K.V., Patterson, D.A., Gilmour, K.M., Hinch, S.G., Farrell, A.P., Cooke, S.J., 2012a. Consequences of acute stress and cortisol manipulation on the physiology, behavior, and reproductive outcome of female Pacific salmon on spawning grounds. Horm. Behav. 62 (1), 67–76.

McConnachie, S.H., O'Connor, C.M., Gilmour, K.M., Iwama, G.K., Cooke, S.J., 2012b. Supraphysiological cortisol elevation alters the response of wild bluegill sunfish to subsequent stressors. J. Exp. Zool. A 317 (5), 321–332.

McDonald, M.D., Wood, C.M., 2004. The effect of chronic cortisol elevation on urea metabolism and excretion in the rainbow trout (*Oncorhynchus mykiss*). J. Comp. Physiol. B 174 (1), 71–81.

McDonald, D.G., Tang, Y., Boutilier, R.G., 1989. The role of β-adrenoreceptors in the recovery from exhaustive exercise of freshwater-adapted rainbow trout. J. Exp. Biol. 147 (1), 471–491.

Mesa, M.G., 1994. Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile Chinook salmon. Trans. Am. Fish. Soc. 123 (5), 786–793.

Mesa, M.G., Poe, T.P., Gadomski, D.M., Petersen, J., 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. J. Fish Biol. 45 (sA), 81–96.

Mesa, M.G., Poe, T.P., Maule, A.G., Schreck, C.B., 1998. Vulnerability to predation and physiological stress responses in juvenile chinook salmon (*Oncorhynchus tshawytscha*) experimentally infected with *Renibacterium salmoninarum*. Can. J. Fish. Aquat. Sci. 55 (7), 1599–1606.

Milinski, M., 1993. Predation risk and feeding behaviour. Behaviour of Teleost Fishes, pp. 285–305.

Milligan, C.L., 1996. Metabolic recovery from exhaustive exercise in rainbow trout. Comp. Biochem. Physiol. A 113 (1), 51–60.

Milligan, C.L., 1997. The role of cortisol in amino acid mobilization and metabolism following exhaustive exercise in rainbow trout (*Oncorhynchus mykiss* Walbaum). Fish Physiol. Biochem. 16 (2), 119–128.

Milligan, C.L., Wood, C.M., 1987. Regulation of blood oxygen transport and red cell pHi after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). J. Exp. Biol. 133 (1), 263–282.

Mommsen, T.P., Walsh, P.J., 1992. Biochemical and environmental perspectives on nitrogen metabolism in fishes. Experientia 48 (6), 583–593.

Mommsen, T.P., Danulat, E., Walsh, P.J., 1992. Metabolic actions of glucagon and dexamethasone in liver of the ureogenic teleost *Opsanus beta*. Gen. Comp. Endocrinol. 85 (2), 316–326.

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9 (3), 211–268.

Murchie, K.J., Clark Danylchuk, S., Danylchuk, A.J., Cooke, S.J., 2015. Fish community and habitat assessments of three adjacent tidal creeks on Cape Eleuthera, The Bahamas. In: Murchie, K.J., Daneshgar, P.P. (Eds.), Proceedings of the Second International Symposium on Mangroves as Fish Habitat. Mazatlán, Mexico. American Fisheries Society, Bethesda, Maryland, pp. 67–80.

Nagrodski, A., Murchie, K.J., Stamplecoskie, K.M., Suski, C.D., Cooke, S.J., 2013. Effects of an experimental short-term cortisol challenge on the behaviour of wild creek chub *Semotilus atromaculatus* in mesocosm and stream environments. J. Fish Biol. 82 (4), 1138–1158.

Newman, S.P., Handy, R.D., Gruber, S.H., 2007. Spatial and temporal variations in mangrove and seagrass faunal communities at Bimini, Bahamas. Bull. Mar. Sci. 80 (3), 529–553.

Newman, S.P., Handy, R.D., Gruber, S.H., 2010. Diet and prey preference of juvenile lemon sharks *Negaprion brevirostris*. Mar. Ecol. Prog. Ser. 398, 221–234.

O'Connor, C.M., Gilmour, K.M., Arlinghaus, R., Van Der Kraak, G., Cooke, S.J., 2009. Stress and parental care in a wild teleost fish: insights from exogenous supraphysiological cortisol implants. Physiol. Biochem. Zool. 82 (6), 709–719.

Olla, B.L., Davis, M.W., 1989. The role of learning and stress in predator avoidance of hatchery-reared coho salmon (*Oncorhynchus kisutch*) juveniles. Aquaculture 76 (3), 209–214.

Olla, B.L., Davis, M.W., Schreck, C.B., 1992. Notes: comparison of predator avoidance capabilities with corticosteroid levels induced by stress in juvenile coho salmon. Trans. Am. Fish. Soc. 121 (4), 544–547.

Olla, B.L., Davis, M.W., Schreck, C.B., 1995. Stress-induced impairment of predator evasion and non-predator mortality in Pacific salmon. Aquac. Res. 26 (6), 393–398.

Olson, K.R., Fromm, P.D., 1971. Excretion of urea by two teleosts exposed to different concentrations of ambient ammonia. Comp. Biochem. Physiol. A 40 (4), 999–1007.

Pearson, M.P., Stevens, E.D., 1991. Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 9 (1), 39–50.

Peterson, C.H., Black, R., 1994. An experimentalist's challenge: when artifacts of intervention interact with treatments. Mar. Ecol. Prog. Ser. 111 (3), 289–297.

Pleizier, N., Wilson, A.D., Shultz, A.D., Cooke, S.J., 2015. Puffed and bothered: personality, performance, and the effects of stress on checkered pufferfish. Physiol. Behav. 152, 68–78.

Pottinger, T.G., Knudsen, F.R., Wilson, J., 1994. Stress-induced changes in the affinity and abundance of cytosolic cortisol-binding sites in the liver of rainbow trout, *Oncorhynchus mykiss* (Walbaum), are not accompanied by changes in measurable nuclear binding. Fish Physiol. Biochem. 12 (6), 499–511.
Priede, I.G., 1977. Natural selection for energetic efficiency and the relationship between activity level and mortality. Nature 267, 610–611.
Rahmatullah, M., Boyde, T.R.C., 1980. Improvements in the determination of urea using diacetyl monoxime; methods with and without deproteinisation. Clin. Chim. Acta 107, 3–9.
Randall, D.J., Perry, S.F., 1992. 4 catecholamines. Fish Physiol. 12, 255–300.
Randall, D.J., Tsui, T.K.N., 2002. Ammonia toxicity in fish. Mar. Pollut. Bull. 45 (1), 17–23.
Reddy, P.K., Vijayan, M.M., Leatherland, J.F., Moon, T.W., 1995. Does RU486 modify hormonal responses to handling stressor and cortisol treatment in fed and fasted rainbow trout? J. Fish Biol. 46 (2), 341–359.
Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. Horm. Behav. 55 (3), 375–389.
Rooker, J.R., Dennis, G.D., 1991. Diel, lunar and seasonal changes in a mangrove fish assemblage off southwestern Puerto Rico. Bull. Mar. Sci. 49 (3), 684–698.
Rypel, A.L., Layman, C.A., Arrington, D.A., 2007. Water depth modifies relative predation risk for a motile fish taxon in Bahamian tidal creeks. Estuar. Coasts 30 (3), 518–525.
Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21 (1), 55–89.
Sathiyaa, R., Vijayan, M.M., 2003. Autoregulation of glucocorticoid receptor by cortisol in rainbow trout hepatocytes. Am. J. Phys. Cell Physiol. 284 (6), C1508–C1515.
Schreck, C.B., Olla, B.L., Davis, M.W., 1997. Behavioral responses to stress. Fish Stress Health Aquac. 62, 145–170.
Selye, H., 1946. The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol. Metab. 6 (2), 117–230.
Shrimpton, J.M., Mccormick, S.D., 1999. Responsiveness of gill Na+/K+-ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. J. Exp. Biol. 202 (8), 987–995.
Sloman, K.A., Montpetit, C.J., Gilmour, K.M., 2002. Modulation of catecholamine release and cortisol secretion by social interactions in the rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 127 (2), 136–146.
Sokolova, I.M., 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integr. Comp. Biol. ict028.
Sopinka, N.M., Patterson, L.D., Redfern, J.C., Pleizier, N.K., Belanger, C.B., Midwood, J.D., Crossin, G.T., Cooke, S.J., 2015. Manipulating glucocorticoids in wild animals: basic and applied perspectives. Conserv. Physiol. 3 (1), cov031.
Springer, V.G., McErlean, A.J., 1962. A study of the behavior of some tagged south Florida coral reef fishes. Am. Midl. Nat. 386–397.
Stoot, L.J., Cairns, N.A., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J.W., Clark, T.D., Cooke, S.J., 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. Conserv. Physiol. 2 (1), cou011.
Struthers, D.P., Danylchuk, A.J., Wilson, A.D., Cooke, S.J., 2015. Action cameras: bringing aquatic and fisheries research into view. Fisheries 40 (10), 502–512.
Sullivan, J.F., Atchison, G.J., Kolar, D.J., McIntosh, A.W., 1978. Changes in the predator-prey behavior of fathead minnows (*Pimephales promelas*) and largemouth bass (*Micropterus salmoides*) caused by cadmium. J. Fish. Res. Board Can. 35 (4), 446–451.
Terova, G., Gornati, R., Rimoldi, S., Bernardini, G., Saroglia, M., 2005. Quantification of a glucocorticoid receptor in sea bass (*Dicentrarchus labrax*, L.) reared at high stocking density. Gene 357 (2), 144–151.
Valiela, I., Bowen, J.L., York, J.K., 2001. Mangrove Forests: One of the World's Threatened Major Tropical Environments at least 35% of the area of mangrove forests has been lost in the past two decades, losses that exceed those for tropical rain forests and coral reefs, two other well-known threatened environments. Bioscience 51 (10), 807–815.
Veillette, P.A., Sundell, K., Specker, J.L., 1995. Cortisol mediates the increase in intestinal fluid absorption in Atlantic salmon during parr-smolt transformation. Gen. Comp. Endocrinol. 97 (2), 250–258.
Veillette, P.A., Serrano, X., Garcia, M.M., Specker, J.L., 2007. Evidence for the onset of feedback regulation of cortisol in larval summer flounder. Gen. Comp. Endocrinol. 154 (1), 105–110.
Vijayan, M.M., Reddy, P.K., Leatherland, J.F., Moon, T.W., 1994. The effects of cortisol on hepatocyte metabolism in rainbow trout: a study using the steroid analogue RU486. Gen. Comp. Endocrinol. 96 (1), 75–84.
Vijayan, M.M., Mommsen, T.P., Glemet, H.C., Moon, T.W., 1996. Metabolic effects of cortisol treatment in a marine teleost, the sea raven. J. Exp. Biol. 199 (7), 1509–1514.
Vijayan, M.M., Raptis, S., Sathiyaa, R., 2003. Cortisol treatment affects glucocorticoid receptor and glucocorticoid-responsive genes in the liver of rainbow trout. Gen. Comp. Endocrinol. 132 (2), 256–263.
Wendelaar Bonga, S.W., 1997. The stress response in fish. Physiol. Rev. 77 (3), 591–625.
Wetherbee, B.M., Gruber, S., Cortes, E., 1990. Diet feeding habits, digestion and consumption in sharks, with special reference to the lemon shark. *Negrapion brevirostris*. NOAA Technical Report, NM FS 90(1), pp. 29–47.
Weyts, F.A.A., Cohen, N., Flik, G., Verburg-van Kemenade, B.M.L., 1999. Interactions between the immune system and the hypothalamo-pituitary-interrenal axis in fish. Fish Shellfish Imuunol. 9 (1), 1–20.
Wood, C.M., 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. J. Exp. Biol. 160 (1), 285–308.
Wood, C.M., Milligan, C.L., Walsh, P.J., 1999. Renal responses of trout to chronic respiratory and metabolic acidoses and metabolic alkalosis. Am. J. Phys. Regul. Integr. Comp. Phys. 277 (2), R482–R492.
Woodley, C.M., Peterson, M.S., 2003. Measuring responses to simulated predation threat using behavioral and physiological metrics: the role of aquatic vegetation. Oecologia 136 (1), 155–160.
Zall, D.M., Fisher, D., Garner, M.Q., 1956. Photometric determination of chlorides in water. Anal. Chem. 28 (11), 1665–1668.

Glossary

Glucocorticoid receptor (GR): A steroid hormone receptor that, in teleosts, acts as one of the primary target receptors for cortisol and allows the hypothalamic-pituitary-interrenal axis neuroendocrine signal to be transduced to an individual cell.
Hypothalamic-pituitary-interrenal (HPI) axis: The neuroendocrine system responsible for mediating the stress response and cortisol biosynthesis/secretion in teleost fish.
Homeostatic overload: A condition where a physiological mediator exceeds its normal maximal threshold for a particular response resulting in damage to the organism rather than providing the beneficial effects it normally confers.
RU486: A pharmacological antagonist of the glucocorticoid receptor in teleost fish.
Standard metabolic rate (SMR): The sum of the metabolic processes allocated towards basic physiological processes needed to sustain an ectothermic organism. It is measured in individuals that are fasted and completely at rest.
ACTH: Adrenocorticotropic hormone is an important regulatory hormone in mediating cortisol biosynthesis as part of the hypothalamic-pituitary-interrenal axis.