

ORIGINAL ARTICLE

Influence of supraphysiological cortisol manipulation on predator avoidance behaviors and physiological responses to a predation threat in a wild marine teleost fish*

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Abstract

The stress axis in teleost fish attempts to maintain internal homeostasis in the face of allostatic loading. However, stress axis induction has been associated with a higher predation rate in fish. To date, the physiological and behavioral factors associated with this outcome are poorly understood. The purpose of the present study was to investigate the impact of experimental cortisol elevation on anti-predator behavior and physiological responses to predator presence. We hypothesized that semi-chronic cortisol elevation would increase susceptibility to predation by increasing stress-induced risk-taking behaviors. To test this hypothesis, schoolmaster snapper were given cocoa butter implants without cortisol (sham) or with cortisol (50 mg/kg body weight) and tethered to cover. Fish were exposed to either a lemon shark or control conditions for 15-min. Space use and activity were recorded throughout and fish were terminally sampled for blood. Cortisol implantation, relative to shams, resulted in higher blood glucose and plasma cortisol concentrations with a lower plasma lactate concentration. Shark exposure, relative to controls, elicited higher blood glucose and lactate concentrations but had no effect on plasma cortisol concentration. No interactions were detected between shark exposure and cortisol treatment for any physiological trait. Behavioral metrics, including shelter use and activity, were unaffected by either cortisol implantation or shark exposure. Physiological responses to cortisol implantation likely resulted from enhanced gluconeogenic activity, whereas alterations under predator exposure may have been the product of catecholamine mobilization. Further work should address context-specific influences of stress in mediating behavioral responses to predation.

Key words: homeostatic overload, lemon shark, predation, refuging, stress axis, teleost physiology

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*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., L.F.G.G. and S.J.C. All authors contributed to the writing and editing of this work.

INTRODUCTION

With increasing anthropogenic activities and disturbances in the marine environment (e.g. coastal development, fisheries interactions, noise pollution, water quality degradation, environmental change; Gray 1997; Crain

et al. 2009), declines in fish populations and alterations in community and ecosystem structure and function have been observed (Hutchings & Baum 2005; Hutchings & Reynolds 2005; Halpern *et al.* 2007). Anthropogenic disturbances may serve as stressors, perturbing the internal homeostasis of a fish and activating its stress axis (Walker *et al.* 2005; Busch & Hayward 2009; Wright *et al.* 2011; reviewed in Barton & Iwama 1991, Wendelaar Bonga 1997, and Schreck & Tort 2016). The duration and magnitude of these perturbations can have a significant influence on the ability to respond to a future stressor as well as contributing to the animal's allostatic load: the concept incorporating the physiological "costs" of sustained stress axis stimulation (reviewed in Korte *et al.* 2005; Romero *et al.* 2009).

In teleost fish, one arm of the stress axis, the hypothalamic–pituitary–interrenal (HPI) axis, regulates the biosynthesis and secretion of cortisol, the primary corticosteroid (reviewed in Mommsen *et al.* 1999; Schreck & Tort 2016). The re-establishment of internal homeostasis during a stress response is an energetically demanding process (Chan & Woo 1978; Barton & Schreck 1987; Sloman *et al.* 2000; Lankford *et al.* 2005; O'Connor *et al.* 2010; Schreck & Tort 2016) and, as such, the glucocorticoid function of cortisol is important in initiating an upregulation of energy mobilizing processes. Consequently, increases in plasma cortisol levels are often accompanied by an elevation of circulating glucose concentrations, thus meeting the enhanced energetic requirements under a stressor (reviewed in Barton & Iwama 1991; Wendelaar Bonga 1997; Mommsen *et al.* 1999; Schreck & Tort 2016).

While cortisol's actions are generally considered to be beneficial to the organism in surviving a stressor (Wendelaar Bonga 1997; Schreck & Tort 2016), stress axis stimulation can be problematic in other aspects of a fish's life history, including its responses to a predator. Teleosts stressed through air exposure, handling, or exposure to toxicants suffer higher rates of predator-induced mortality relative to unstressed counterparts, with effects occurring over varying timescales and stressor types (Brown *et al.* 1985; Jarvi 1989; Olla & Davis 1989; Olla *et al.* 1992, 1995; Mesa *et al.* 1994, 1998; Danylchuk *et al.* 2007). The specific physiological mechanisms associated with the influence of stress on predator–prey dynamics is currently unknown, but a role for cortisol itself warrants investigation. The metabolic consequences associated with continued HPI axis stimulation could be a contributing factor (Guderley & Portner 2010). Specifically, sustained cortisol el-

evation can result in increases in both routine (Chan & Woo 1978; Morgan & Iwama 1996; De Boeck *et al.* 2001) and resting metabolic rates (i.e. the standard metabolic rate; Sloman *et al.* 2000; O'Connor *et al.* 2010) in a teleost fish, potentially leading to energetic trade-offs that compromise predator avoidance capacity (Fry 1947; Priede 1977; Guderley & Portner 2010; Killen *et al.* 2015).

The metabolic consequences of HPI axis activation also may be problematic on a behavioral level with respect to predation risk. Foraging behavior and general activity are highly dependent on the energetic status of the animal. Energetically compromised individuals are more likely to take on a greater burden of predation risk (e.g. higher activity and foraging duration) to satisfy metabolic demands (reviewed in Lima 1998). For example, parasitized three-spine stickleback (*Gasterosteus aculeatus*) exhibited greater activity levels (Gilles 1987; Godin & Sproul 1988) and quicker behavioral recovery from a predator encounter (e.g. latency to resume feeding; Giles 1983, 1987; Godin & Sproul 1988), and foraged within close proximity to a potential predator (Milinski 1985; Godin & Sproul 1988). Thus, duress (i.e. parasite load) enhanced predation risk in stickleback (Godin & Sproul 1988), with parasitism likely acting to increase both cortisol (Ross *et al.* 2000; Costello 2002) and metabolic load (Fry 1971). In Atlantic salmon (*Salmo salar*), energetic stress corresponded with a reduced latency to resume feeding activities following a predation event (Gotceitas & Godin 1991), as well as foraging at greater distance from cover (Dill & Fraser 1984), suggesting a greater degree of risk-taking behavior in stressed individuals. Furthermore, the duration of time spent in this refuge is highly dependent on a number of factors, including the animal's energetic status and body condition, with poor body conditions and increasing hunger levels corresponding with reduced refuging activity (Sih 1992, 1997; Kraus *et al.* 1998). Given the role of the stress axis in mediating energy metabolism and budgeting, it is reasonable to hypothesize that a stressed teleost fish would accept an elevation of predation risk to optimize energy intake (Sokolova 2013; Schreck & Tort 2016; Lawrence *et al.* 2017).

The objective of the present study was to investigate the impact of HPI axis activity in modulating the behavior and physiology of a teleost fish in response to a predation threat. Because the most visible outcome of HPI axis activation is a rise in circulating cortisol titres, cortisol levels were manipulated and the consequences of elevated cortisol levels on predator–prey interactions

were investigated.

MATERIALS AND METHODS

Experimental animals

Juvenile schoolmaster snapper (*Lutjanus apodus* Walbaum, 1792; 53.6 ± 2.1 g; $N = 57$), selected for their commercial, recreational and ecological importance (Allen 1985), were collected using minnow traps from a mangrove nursery habitat (Page Creek, Eleuthera Island, Bahamas; $24^{\circ}49'04''\text{N}$, $76^{\circ}18'51''\text{W}$) in November and December 2014. Fish were transported to The Cape Eleuthera Institute (Eleuthera Island, Bahamas) and held in a raceway style tank (519 L) containing simulated cover. Juvenile lemon sharks (*Negaprion brevirostris* Poey, 1868; 602 ± 11 mm; $N = 6$) were collected by seine net from a nearby mangrove system (Kemp's Creek, Eleuthera Island, Bahamas; $24^{\circ}48'41.45''\text{N}$, $76^{\circ}18'16.83''\text{W}$). Sharks were held in a large, circular tank (approximately 6420 L) with a sandy substrate. Fish were collected under a scientific collection permit provided by the Bahamian Department of Marine Resources. All tanks were supplied with aerated natural seawater on an overflow system (dissolved oxygen $>85\%$; temperature $24.5 \pm 0.7^{\circ}\text{C}$; pH 8.16 ± 0.04 ; salinity 33.9 ± 0.1 ppt). Both species were maintained on a natural photoperiod (13 D: 11 L) and were fed daily to satiation on chopped sardines. Snapper were fasted overnight (approximately 16-h) in advance of cortisol manipulation and were not fed during the experimental series (approximately 40-h fasting total).

Fasted snapper were given an intraperitoneal injection of hydrocortisone 21-hemisuccinate (50 mg/kg body mass; $N = 30$; Sigma-Aldrich, Oakville, ON, Canada) suspended in cocoa butter (5 mL/kg body mass) warmed to be in liquid form; sham-treated animals ($N = 27$) received the cocoa butter vehicle alone. The cortisol dose was based on that in Cull *et al.* (2015) for use in a tropical teleost as well as being a common dosage used in the teleost literature (reviewed in Gamperl *et al.* 1994; Mommsen *et al.* 1999). Fish were fasted to standardize hunger status in the animals given that hunger is an important trait regulating risk assessment (Milinski 1993). Because the work occurred at a remote field site, an a priori validation study could not be conducted so we relied on the doses in the literature. At the same time, an anchoring point for a tether was made by creating a small hole on the lower jaw with a fine suturing needle (1/2 circle, cutting edge, size 14; Integra Miltex, Plainsboro, NJ, USA) according to the proce-

dures of Rypel *et al.* (2007). Anesthesia was not used on these animals in an attempt to minimize handling stress as well as to avoid physiological perturbations resulting from anesthesia usage (Wagner & Cooke 2005). Animals were allowed to recover for 24 h in a small mesh chamber that was maintained under ambient seawater conditions (as above). This 24-h period also allowed for cortisol to reach a homeostatic overload state to mimic a semi-chronic stressor. All procedures were in accordance within the standards of the Canadian Council on Animal Care (CCAC) under authorization from Carleton University's Animal Care Council (AUP-100612).

Behavior trials

Behavioral assessment trials were conducted in a large, outdoor circular tank (approximately 6420 L) that was shielded from the elements by a roof. A sandy substrate was placed on the bottom of the tank, with a trio of conch shells (10-cm spacing) being located 84-cm from the center of the tank, where a stand pipe (8.9-cm outer diameter) was located. The tank was divided in half with a fine mesh seine net. Prior to behavior trials, the tank was maintained with water on a flow-through arrangement using ambient filtered seawater (see above). Water was allowed to flow through the system overnight, and water flow was stopped before any experimental procedures began.

In preparation for behavior trials, a snapper was fitted with a 1.5-m long tether as described in Lawrence *et al.* (2017). Use of the tether was necessary to complement previous stress-predation work that had been carried out in this species. The fish was moved to the behavioral arena (see above) with the tether being secured to the outer conch shell. The fish was allowed to acclimate in the arena for 5-min prior to the experiment. The snapper was then exposed, for 15-min, to 1 of 2 possible scenarios: control conditions or the presence of a lemon shark. A single lemon shark randomly selected from the pool of animals was added to the behavioral arena on the opposite side of the net from the tethered snapper. Sharks were never in a fasted state during trials to avoid active hunting by the animals. The behavioral responses of the snapper were monitored during this time using a Go-Pro Hero camera (Go-Pro, San Mateo, CA, USA; Struthers *et al.* 2015) mounted directly above the tank.

After the behavior trial, snapper were killed by cerebral percussion and a blood sample (approximately 200 μL) was withdrawn by caudal venipuncture into a heparinized (Na^+ heparin, 10 000 USP units/mL; Sandoz Canada, Boucherville, QC, Canada) 1-mL syringe us-

ing a 23-G needle taking no more than 3 minutes (Lawrence *et al.* 2018). Glucose and lactate concentrations were measured immediately, and the remaining blood was centrifuged (2000 g; Mandel Scientific, Guelph, ON, Canada) for 1-min. Plasma was decanted, frozen and stored at -20°C for later analysis of plasma cortisol and ion concentrations.

Behavioral and blood analyses

Concentrations of blood glucose (Accu-Chek Compact Plus, Hoffman-La Roche, Mississauga, ON, Canada) and lactate (Lactate Plus, Nova Biomedical Canada, Mississauga, ON, Canada) were measured using medical-grade, hand-held analyzers previously validated for use in teleost fish (Wells & Pankhurst 1999; Serra-Llinares *et al.* 2012; reviewed in Stoot *et al.* 2014). Plasma cortisol concentrations were measured using a previously validated (Gamperl *et al.* 1994), commercially available radioimmunoassay kit (ImmunoChem Cortisol Coated Tube RIA Kit, MP Biomedicals, Solon, OH, USA). Intra-assay and inter-assay variation was 3.1% and 1.8%, respectively. Plasma Cl^{-} and Na^{+} concentrations were determined using, respectively, a colorimetric assay (Zall *et al.* 1956) and flame spectrophotometry (Varian Spectra AA 220FS, Varian, Palo Alto, CA, USA). The chloride assay was carried out in triplicate at room temperature (approximately 22°C) using a 96-well microplate reader (SpectraMax, Molecular Devices, Sunnyvale, CA, USA).

Behavioral metrics were collected for the first 10-min of exposure to the predator or control conditions, and included activity, time spent in cover, time in proximity to the net and time spent in the open. Activity scores were determined using a line crossing analysis employing a 2×2 body length (BL) grid overlaid on the video recording. A line crossing was defined as a fish's body completely crossing a line in the horizontal axis. The animal was considered to be in cover when it was within 1 BL of either the outer rim of the conch shell trio or the standpipe in the center of the tank. Proximity to the net was defined to occur when the fish was within 1 BL of the net but not including the 1 BL radius around the standpipe. Fish not occupying these regions were considered to be in the open.

Statistical analysis

Unless otherwise noted, statistical analysis was carried out using SigmaPlot v11.0 (Systat Software, San Jose, CA, USA). The statistical limit of significance was $\alpha = 0.05$. Values are reported as the mean ± 1 SE

(N). Blood and plasma parameters as well as activity scores were assessed using 2-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests when $P < 0.05$. Because of the supraphysiological levels of plasma cortisol in cortisol-treated fish, Student's t -tests were used to compare cortisol concentrations between cortisol-treated and sham-treated fish within an exposure series as well as to compare sham-treated fish between predator exposure groups.

Analysis of the percentage of time spent in cover was performed within the R statistical environment (R Core Development Team 2016). Given the nature of the data, we used a negative binomial generalized linear model (MASS package, Venables & Ripley 2002) where time spent in cover (number of seconds + 1) was the response variable. Factors included cortisol treatment (cortisol *vs* sham), predator treatment (shark present *vs* control), and the interaction between cortisol treatment and predator treatment. The model was validated after checking the spread of the residuals against each covariate, and checking the residuals for overdispersion (i.e. the occurrence of more variance in the data than predicted by a statistical model, Bolker *et al.* 2009).

RESULTS

Blood analyses

Cortisol-treated fish exhibited significantly higher plasma cortisol levels than sham fish (Student's t -tests, $P < 0.001$ and $P < 0.001$; Fig. 1a). Plasma cortisol concentrations were not affected by shark exposure in sham-treated snapper (Student's t -test, $P = 0.143$; Fig. 1a). Shark-exposed snapper had higher blood glucose concentrations than fish exposed to control conditions (2-way ANOVA, $P = 0.041$; Fig. 1b). Similarly, cortisol-treated fish exhibited significantly higher blood glucose concentrations than sham-treated fish ($P = 0.032$; Fig. 1b); there was no interaction between shark exposure and cortisol implantation ($P = 0.636$). Blood lactate levels increased in response to shark exposure (2-way ANOVA, $P = 0.041$; Fig. 1c) and were higher in sham-treated fish relative to cortisol-treated fish ($P = 0.004$; Fig. 1c). There was no interaction between shark exposure and cortisol implantation on blood lactate levels ($P = 0.350$). Hematocrit and plasma Na^{+} and Cl^{-} concentrations were generally unaffected by either shark exposure or cortisol treatment (Table 1), although cortisol-treated snapper exhibited significantly higher plasma Cl^{-} concentrations relative to shams (Table 1).

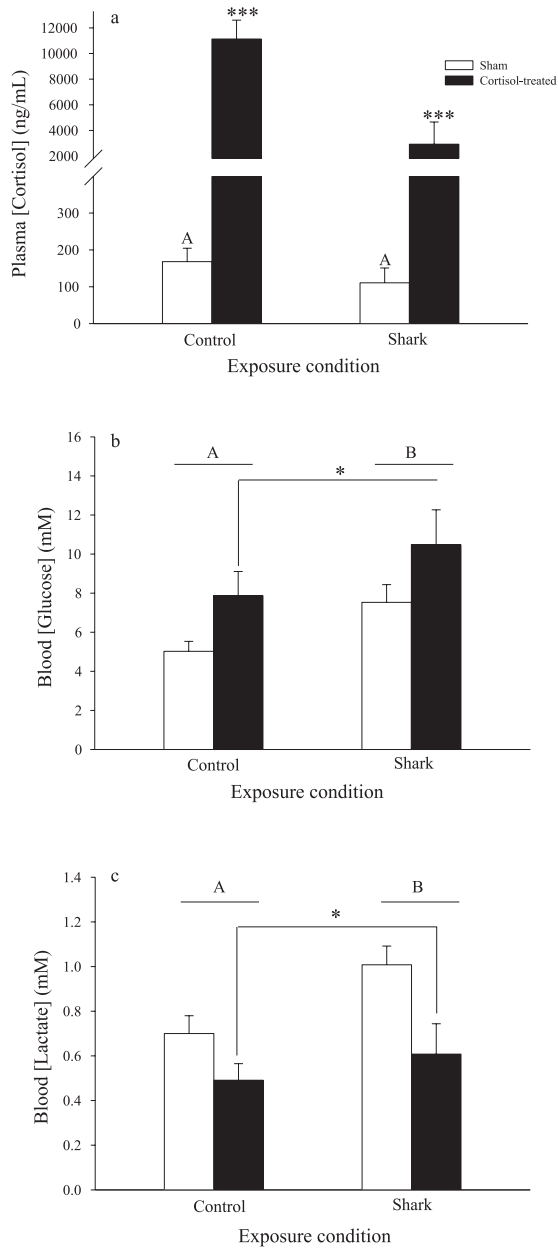


Figure 1 Concentrations of plasma cortisol (a), blood glucose (b) and blood lactate (c) in schoolmaster snapper, 24-h after receiving a cocoa butter implant (sham; 5 mL/kg body mass; white bars; $N \leq 13$) or a cocoa butter implant containing cortisol (50 mg/kg body mass; black bars; $N \leq 12$) and in response to exposure to a lemon shark ($N = 13$) or control conditions ($N \leq 11$). Samples, cortisol notwithstanding (see Methods), were analyzed using a 2-way ANOVA and a Tukey post-hoc test where a significant interaction was detected. Unique letters represent statistically significant ($P < 0.05$) differences between shark and control exposure groups, whereas asterisks (*; $P < 0.05$) denote statistically significant differences between implant groups. Values are reported as the mean \pm SE (N).

Activity patterns

Schoolmaster snapper activity was similar between cortisol-treated and sham animals (2-way ANOVA, $P = 0.784$) and between control and shark exposure ($P = 0.571$), with mean activity ranging between 27.5 and 43.5 line crossings during the 10-min observation period (Fig. 2). No significant interaction was detected between cortisol treatment and predator exposure ($P = 0.450$)

Cover use

While variable, snapper were generally found to associate with cover in most instances. There were no statistically significant effects of either shark exposure or cortisol treatment on the percentage of time snapper spent in cover ($P > 0.05$ in all cases). However, sham-treated snapper exposed to control conditions exhibited the lowest median percent use of cover. By contrast, use of cover in cortisol-treated animals exposed to control conditions was comparable to that of predator-exposed animals (Fig. 3).

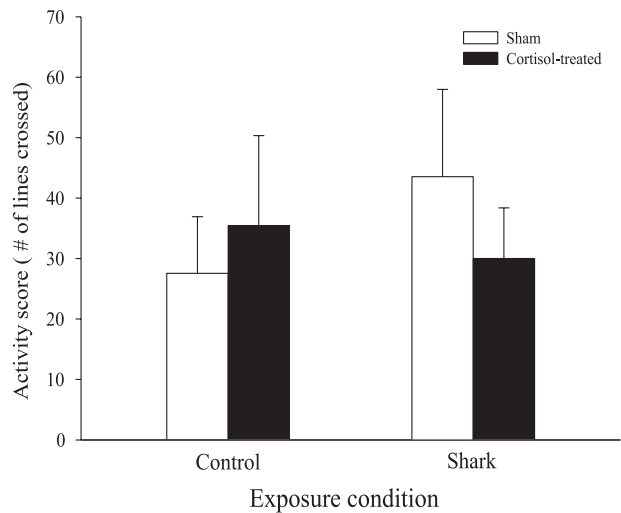


Figure 2 Activity score of schoolmaster snapper during a 10-min exposure to either a lemon shark ($N = 13$) or control conditions (i.e. no predator; $N \leq 11$), 24-h after receiving a cocoa butter implant (sham; 5 ml/kg body mass; white bars; $N \leq 13$) or a cocoa butter implant containing cortisol (50 mg/kg body mass; black bars; $N \leq 13$). Samples were analyzed using a 2-way ANOVA. No significant effects of either shark exposure ($P = 0.571$) or implant treatment ($P = 0.784$) were found. Values are reported as the mean \pm SE (N).

Table 1 Blood and plasma parameters for schoolmaster snapper implanted with either cortisol (50 mg/kg BW) or vehicle alone (sham) and exposed to either a lemon shark or control conditions

Parameter	Exposure			
	Control		Shark	
	Sham	Cortisol-treated	Sham	Cortisol-treated
Hematocrit (%)	27.6 ± 2.1 (8)	23.9 ± 1.8 (10)	26.0 ± 1.5 (12)	26.3 ± 0.6 (10)
Plasma [Na ⁺] (mM)	166.2 ± 11.5 (8)	164.8 ± 7.8 (9)	152.4 ± 6.4 (7)	177.6 ± 4.6 (10)
Plasma [Cl ⁻] (mM)	166.0 ± 11.0 (8)	174.1 ± 8.4* (9)	155.6 ± 5.6 (7)	183.8 ± 5.7* (10)

Values are presented as mean ± 1 SE (*N*), with numbers (*N*) presented in parentheses. Two-way ANOVA was used to determine statistical differences among treatment groups. No statistically significant effects were detected for hematocrit or plasma [Na⁺]. For plasma [Cl⁻], implant group *P* = 0.031, predator *P* = 0.970, implant x predator *P* = 0.220 and asterisks (*) represent the statistically significant main effect of the implant group.

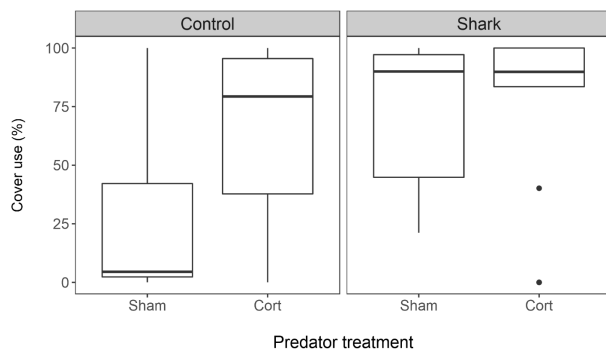


Figure 3 Cover use (% of observation period) for cortisol-treated and sham-treated schoolmaster snapper when exposed to a shark predator or to control conditions. Dark horizontal lines are the median values. Boxes denote the interquartile range (1st to 3rd quartile) with sample sizes. Whiskers are 1.5× the upper or lower interquartile range to the highest or lowest value within the interquartile range. Outliers are shown as black points extending beyond the whiskers.

DISCUSSION

Overview

It was hypothesized that, as a result of an expected increase in metabolic energy expenditure with semi-chronic elevation of cortisol concentrations, cortisol-treated fish would demonstrate greater risk-taking behavior. In contrast to this hypothesis, cortisol treatment did not influence snapper behavior patterns, with all animals tending to associate with cover and exhibiting consistent activity throughout the experiment. A number of factors may have contributed to this result,

including a high predation risk coefficient (i.e. lack of food for which to forage), the relatively short duration of cortisol elevation, the assessment of behavior during daylight hours, and a possible disconnect between physiological function and behavior. Animals appeared to respond to the threat of predation through a rise in blood glucose levels, likely mediated through the actions of catecholamines as part of the fight-or-flight response (Cannon 1929; Godin 1997). Future work should assess how risk-taking behaviors are influenced by cortisol manipulation in a more ecologically-relevant setting that includes access to food.

Physiological validation of implants

Snapper given cortisol implants had higher concentrations of blood glucose and plasma cortisol than sham fish. The relationship between blood glucose and plasma cortisol concentrations is consistent with cortisol's regulation of gluconeogenic pathways (Vijayan *et al.* 2003; Aluru & Vijayan 2007; Choi *et al.* 2007; Wiseman *et al.* 2007; reviewed in Mommsen *et al.* 1999, Aluru & Vijayan 2009; Schreck & Tort 2016). However, plasma cortisol concentrations were far in excess of what has been observed in this species in response to a stressor (30-min post-exhaustive exercise), where values reached approximately 270 ng/mL (Lawrence *et al.* 2017). Despite using a standardized implantation procedure (Gamperl *et al.* 1994), cortisol concentrations in the plasma of implanted fish were supraphysiological relative to other species at comparable dosages and time points, which may be a product of the fish's environment and/or metabolic tendencies (reviewed in Mommsen *et al.* 1999). It should be noted that sham-treated fish also had higher plasma [cortisol]; a baseline cortisol

titer for this species has been reported at approximately 67 ng/mL (Lawrence *et al.* 2017). This elevation likely reflects handling, tethering and the implantation of the animals (Wendelaar Bonga 1997; Schreck & Tort 2016). This cortisol elevation may also explain why there were no detectable effects between shark-exposed and control-exposed sham fish.

Behavioral responses to a predation threat

In contrast to the hypothesis, risk-taking behaviors were not influenced by cortisol treatment but some evidence of assessment of predation risk was present. Shark-exposed, sham-treated fish exhibited increased refuge use relative to their control-exposed counterparts, although the difference was not statistically significant. The apparent lack of cortisol treatment effect on snapper behavior may have been a consequence of the duration of the implant. The relatively short duration of elevated cortisol (24 h) may not have had substantial consequences for the energetic status of the animal. Indication of depleted energy stores with cortisol implantation has been reported over more chronic durations (e.g. hepatosomatic index; Davis *et al.* 1985; Barton *et al.* 1987; Davis *et al.* 2003). As such, the snapper here likely had sufficient reserves to draw upon and may not be expected to assume additional predation risk (reviewed in Lima 1998). However, fasting over a 24-h period has been shown to elicit significant changes in teleost risk-taking behaviors when exposed to a predation threat (Godin & Sproul 1988; Gotceitas & Godin 1991), demonstrating that predator–prey interactions, in the context of energetic budgeting, is a complex system involving the interaction of a number of physiological processes (Lima & Dill 1990). Furthermore, glucose was mobilized in fish when exposed to experimental cortisol elevation, which suggests either increased synthesis (i.e. gluconeogenesis) or elevated turnover of glycogen stores (glycogenolysis), which are both metabolic consequences of elevated cortisol exposure (reviewed in Mommsen *et al.* 1999). As risk represents an interaction of a number of endogenous (i.e. physiological state) and exogenous (e.g. predation threat, food availability and cover) factors (Lima & Dill 1990), outcomes in risk taking and the associated behaviors become difficult to predict. As such, snapper could be behaving in a manner that minimizes risk while maximizing fitness under its current set of conditions.

The activity patterns of snapper may also have played a significant role in determining its behavioral responses in the present study. The prop roots of mangrove trees

are the primary habitat of juvenile schoolmaster snapper during daylight hours, offering shelter from predators (Nagelkerken *et al.* 2000a,b; Nagelkerken & van der Velde 2004; MacDonald *et al.* 2009). Prop root sheltering constitutes 60–70% of their daily spatial use patterns, with the animals also spending a significant proportion of their time in areas where overhead cover is lacking (MacDonald *et al.* 2009). In addition, foraging during daylight hours constitutes only 2% of their total activity budget; this species is predominately a nocturnal feeder (Nagelkerken *et al.* 2000a; MacDonald *et al.* 2009). Thus, the conditions of the present study may not have been optimal for detecting differences in predator-avoidance behavior in the context of foraging-risk management.

Memory may play an important role in mediating predator–prey interactions. Stress and cortisol can have a significant influence over cognitive functions in teleost fishes, including memory and associated processes (Ellis *et al.* 2012; Sorensen *et al.* 2013; Noakes & Jones 2016). Indeed, memory, from both a predator's hunting performance and from a prey's predator-avoidance capacity, is deeply rooted in experience from previous encounters and can modulate the interaction between the two organisms (Mitchell & Lima 2002; Weisel *et al.* 2015). Although not investigated here, cortisol may have modulated the cognitive function of the snapper, affecting memory-related anti-predator responses and resulting in altered behavioral dynamics. This possibility remains speculative at this time but presents an interesting avenue for future research.

Physiology and behavior: A complex relationship

The lack of behavioral responses to cortisol administration in the present study adds to a growing body of literature that has failed to detect direct effects of cortisol on a range of behaviors (Crossin *et al.* 2015; Sopinka *et al.* 2015). For example, cortisol treatment failed to alter the locomotory activity of largemouth bass (*Micropterus salmoides*; O'Connor *et al.* 2010) and creek chub (*Semotilus atromaculatus*; Nagrodiski *et al.* 2012). Similarly, anti-predator behaviors in checkered pufferfish (*Sphoeroides testudineus*) were not influenced by cortisol treatment, despite significant physiological effects (Cull *et al.* 2015; Pleizier *et al.* 2015). These observations suggest that the interaction between physiology and behavior is inherently complex and likely requires a number of physiological inputs other than just plasma cortisol concentrations to induce a change (Crossin *et al.* 2015; Sopinka *et al.* 2015). It is also possible that

cortisol may not play a direct role in mediating predator–prey interactions in wild fish, although the current body of literature does suggest a role for the stress axis at large in mediating these responses (reviewed in Mesa *et al.* 1994).

Physiological responses to a predation threat

Acute predation stress in teleosts has been associated with increases in circulating glucocorticoids (Rehnberg *et al.* 1987; Woodley & Peterson 2003; Remage-Healey *et al.* 2006; Barcellos *et al.* 2007; Schreck & Tort 2016), blood [glucose] (Rehnberg and Schreck 1987; Jarvi 1990) and tissue-specific heat shock proteins (Kagawa *et al.* 1999), in addition to an elevation in cardiorespiratory variables (e.g. heart rate, ventilation, cardiac output; Holopainen *et al.* 1997; Cooke *et al.* 2003; Sundstrom *et al.* 2005; Sunardi *et al.* 2007). These physiological responses support the animal's energetic and locomotory needs as it flees from a predator (Wendelaar Bonga 1997; Wingfield 2003; Hawlena & Schmitz 2010; Schreck & Tort 2016). In schoolmaster snapper, blood [glucose] was significantly increased in response to shark exposure. Because sham-treated fish had no change in plasma cortisol in response to shark exposure, the glucose response likely was mediated by the actions of catecholamine hormones rather than cortisol. In most vertebrates, catecholamines act as the primary hormone in mediating acute anti-predator responses (Cannon 1929; Hawlena & Schmitz 2010; Perry & Capaldo 2011).

Blood lactate levels increased in response to a predator. However, the change in blood lactate was quite small and may have been associated with transient hypoxia generated through a freeze response: a behavioral adaptation that induces bradycardia and reduced ventilation to lessen the prey's conspicuousness to a predator (Cooke *et al.* 2003; Shingles *et al.* 2005).

CONCLUSIONS

Cortisol implantation in schoolmaster snapper was sufficient to elevate plasma [cortisol] to supraphysiological levels. This effect corresponded with higher blood [glucose] relative to sham fish, likely as a result of cortisol's actions on energy metabolism. Shark exposure caused an increase in blood [glucose], which was likely mediated by catecholamines because plasma [cortisol] did not change with shark exposure. Behavioral indices were not significantly affected by cortisol treatment or shark exposure. The lack of effect may be explained by

a high-risk situation deterring movement outside the refuge (i.e. no food present coupled with the animal having sufficient energy reserves) in addition to the fact that, during daylight hours, this species usually remains under cover. Despite the absence of significant effects in the present study, stress is believed to be an important and highly relevant factor in mediating behavioral, population and ecological level effects in wild fish (Hawlena & Schmitz 2010; Boonstra 2013). Indeed, the ecology of stress is becoming ever more relevant in today's world where anthropogenic activities may enhance both the frequency and magnitude of stressful events in aquatic systems (Boonstra 2013; Crespi *et al.* 2013; Wingfield 2013). Given the potential importance of stress in mediating predator–prey interactions, further work on the relationship between stress and predator–prey interactions is warranted (Schreck *et al.* 1997; Guderley & Portner 2010; Hawlena & Schmitz 2010; Lawrence *et al.* 2017).

ACKNOWLEDGMENTS

M.J. Lawrence is supported by an NSERC PGS-D. S.J. Cooke is supported by NSERC and the Canada Research Chairs Program. E.J. Eliason was supported by an NSERC PDF. J.W. Brownscombe is supported by NSERC and The Berkeley Marine Conservation Fellowship from The American Fisheries Society. K.M. Gilmour is supported by NSERC. J.W. Mandelman is supported by the New England Aquarium. The authors would like to thank the Cape Eleuthera Institute, Edd Brooks and Zach Zuckerman for the support and resources to make this project possible. The authors would also like to thank Jean-Guy J. Godin for input on behavioral metrics and analyses used in this study. In addition, the authors would like to thank Petra Szekeres for assistance in collecting the fish used in this project.

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Cite this article as:

Lawrence MJ, Eliason EJ, Brownscombe JW *et al.* (2018). Influence of supraphysiological cortisol manipulation on predator avoidance behaviors and physiological responses to a predation threat in a wild marine teleost fish. *Integrative Zoology* **13**, 206–18.