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Do physical habitat complexity and predator cues influence the baseline and stress-induced glucocorticoid levels of a mangrove-associated fish? In prep for: Comparative biochemistry and physiology - Part A

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ABSTRACT

As human populations continue to expand, increases in coastal development have led to the alteration of much of the world's mangrove habitat, creating problems for the multitude of species that inhabit these unique ecosystems. Habitat alteration often leads to changes in habitat complexity and predation risk, which may serve as additional stressors for those species that rely on mangroves for protection from predators. However, few studies have been conducted to date to assess the effects of these specific stressors on glucocorticoid (GC) stress hormone levels in wild fish populations. Using the checkered puffer as a model, our study sought to examine the effects of physical habitat complexity and predator environment on baseline and acute stress-induced GC levels. This was accomplished by examining changes in glucose and cortisol concentrations of fish placed in artificial environments for short periods (several hours) where substrate type and the presence of mangrove roots and predator cues were manipulated. Our results suggest that baseline and stress-induced GC levels are not significantly influenced by changes in physical habitat complexity or the predator environment using the experimental protocol that we applied. Although more research is required, the current study suggests that checkered puffers may be capable of withstanding changes in habitat complexity and increases in predation risk without experiencing adverse GC-mediated physiological effects, possibly as a result of the puffers' unique morphological and chemical defenses that help them to avoid predation in the wild.

1. Introduction

Human urbanisation has led to dramatic alterations in biodiversity and ecosystem structure and function (Hooper et al., 2012; Vitousek et al., 1997). Although all ecosystems have been altered, coastal marine systems have been particularly impacted (Crain et al., 2009; Gray, 1997). Coastal ecosystems have been subjected to shoreline modification (shoreline hardening, removal of vegetation), dredging, and input of pollutants (including chemicals, silt and nutrients; Alongi, 2002; Buchan, 2000), causing negative impacts on biota that span multiple levels of biological organization (i.e. from the ecosystem to the cell; Helmuth, 2009).

Mangrove habitats in particular are being threatened by coastal development, having undergone global declines of about one third since the early 1950s (Alongi, 2002). The destruction of mangrove habitats is devastating for natural systems, as these environments support thousands of species of flora and fauna (Katherisan and Bingham, 2001; Nagelkerken et al., 2008). This is particularly salient for those species of marine fishes and invertebrates whose early life-stages use the complex mangrove prop-root systems as habitat during early ontogeny. Mangroves therefore contribute directly to biodiversity and can also generate massive economic benefits (Rönnbäck, 1999). For example, it is estimated that 80% of global commercial fish catches rely directly or indirectly on mangrove systems (Sandilyan and Katherisan, 2012). Given that many aquatic prey species rely on the physical complexity of the mangrove ecosystem for protection from predators (Buchan, 2000; MacDonald et al., 2009), destruction of mangrove communities may influence the stress physiology of these prey species.

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When fish are exposed to a stressor, activation of the hypothalamic-pituitary-interrenal (HPI) axis results in the release of glucocorticoid (GC) stress hormones such as cortisol into the blood, leading to a mobilization of energy stores such as glucose that help fish restore homeostasis and thereby cope with the stressor (Barton, 2002; Wendelaar Bonga, 1997). Although this process, hereafter referred to as the stress response, plays a fundamental role in helping fish respond to changes in their environment (Wingfield, 2008; Wingfield et al., 2011), chronically elevated levels of stress hormones can have negative fitness implications (Breuner et al., 2008; Romero et al., 2009). While there is a general understanding that baseline and post-stress GC levels increase in response to environmental change (Bonier et al., 2009; Breuner et al., 2008), few studies have been conducted on wild animals to determine the effects of specific changes on GC secretion (Boonstra, 2013).

Given the importance of mangroves to fish populations around the globe, the negative fitness implications of elevated stress, and the ecosystem services generated by healthy fish populations (Holmlund and Hammer, 1999), there is a growing desire to understand how stressors associated with coastal development affect GC secretion in wild fish populations. Development in tropical regions often involves the removal of mangrove trees (Alongi, 2002), resulting in a loss of physical habitat complexity and, in theory, an associated increase in the risk of predation for prey fish species. While a number of studies have been conducted on terrestrial animals examining the effects of predation risk on prey physiology and demography (e.g. Clinchy et al., 2013; Creel et al., 2009; Sheriff et al., 2011; Zanette et al., 2011), comparatively few studies have been conducted on aquatic species examining how factors such as habitat alteration and the predator environment influence baseline GC levels and the ability of fish to respond to other stressors.

The objective of our study was therefore to investigate whether exposure to varying levels of physical habitat complexity and predation risk influences the stress responsiveness of fish from a mangrove community. This was accomplished by examining the baseline and stress-induced changes in glucose and cortisol concentrations of fish placed in artificial environments in which substrate type and the presence of mangrove roots and predator cues were manipulated. The checkered puffer (*Sphoeroides testudineus*) was chosen as a model species because of its wide distribution, ease of capture, and abundance in mangrove communities (MacDonald et al., 2009; Shipp, 1974). Checkered puffers are thought to rely heavily on mangrove habitats for protection from predators (MacDonald et al., 2009), sheltering in the roots and blending in with the heterogeneous substrate using their cryptic dorsal colouration (Austin and Austin, 1971; Targett, 1978). Therefore, we hypothesized that homogeneous substrate, the absence of mangrove roots, and the presence of predator cues would result in higher baseline and post-stress glucose and cortisol concentrations in the puffers as well as a magnified physiological stress response.

2. Materials and methods

2.1. Fish capture and acclimation

This study was conducted in July 2014 at the Cape Eleuthera Institute (CEI) located on Eleuthera, The Bahamas. All research was conducted in accordance with an approved Canadian Council for Animal Care protocol (B12-08) and with a Scientific Collection permit furnished by the Bahamas Department of Marine Resources. A total of 110 checkered puffers (TL = 178 ± 20 mm, mass = 130 ± 37 g; mean ± standard error of the mean [SEM]) were captured from Page Creek (N 24°49′ 04.7″, W 076°18′51.6″), an undisturbed, mangrove-lined tidal creek at the southern end of Eleuthera. Capture was achieved by directing fish down the creek into a seine net during low tide. All fish were then transported to CEI in aerated coolers. At CEI, fish were held in a 1831-L tank that was constantly aerated and supplied with filtered, UV-sterilized flow-through seawater (28.8 ± 0.4 °C). This tank was devoid of any substrate or other habitat characteristics, and fish were held in the tank for a minimum of 72 h prior to experimentation to allow sufficient time for acclimation to laboratory conditions (see Fig. 1 for schematic of the experimental protocol). The puffers were maintained on a diet of chopped sardines (*Sardinella aurita*) throughout the study period, with feeding and cleaning of the holding tank occurring every other day at least 12 h prior to the beginning of the subsequent day's trials. All fish were returned to Page Creek at the conclusion of the study.

2.2. Experimental design

2.2.1. Habitat complexity experiment

In this experiment, we examined the effect of the presence of mangrove roots and substrate type on the baseline and stress-induced GC levels of the puffers. The substrate in mangrove communities is often heterogeneous, with components such as leaf litter, grasses, and small stones that help the checkered puffer to camouflage itself against the bottom (Austin and Austin, 1971). However, the dredging that often accompanies the removal of mangrove trees may result in a homogeneous substrate, making it more difficult for checkered puffers to use the substrate to avoid predators. To examine the effects of mangrove removal and substrate homogenization on the puffers' baseline and stress-induced GC levels, fish ($n = 44$) were assigned to one of four treatment groups: roots present/natural substrate (control group), roots present/homogeneous substrate, roots absent/natural substrate, and roots absent/homogeneous substrate.

Trials were conducted using four opaque experimental aquaria, each filled with approximately 40 L of seawater. Aquaria were constantly aerated using airstones, and the bottom of each aquarium was covered with a 2.0–2.5 cm-deep layer of beach sand. Sand in the 'natural substrate' treatments was scattered with small leaves, rocks, and twigs gathered from a nearby mangrove habitat, while the 'homogeneous substrate' treatments contained plain sand. Mangrove cover was provided by one of two sets of live red mangrove (*Rhizophora mangle*) roots that had 5 branches extending under the water, providing shelter for approximately 25–30% of the aquarium.

2.2.2. Predator environment experiment

In this experiment, we examined the effect of the presence of mangrove roots and chemical predator cues on the baseline and stress-induced GC levels of the puffers. Fish are capable of detecting or inferring the presence of predators using a variety of chemical olfactory cues, including predator odours, disturbance pheromones from conspecifics, and injury-released alarm cues from conspecifics (Wisenden, 2000). This experiment used a conspecific alarm cue in the form of a whole-body extract to simulate the presence of a predator. To examine the effects of mangrove removal and increased predation risk on the puffers' baseline and stress-induced GC levels, fish ($n = 45$; different from fish used in the habitat complexity experiment) were assigned to one of four treatment groups: roots present/predator cue absent (control group), roots present/predator cue present, roots absent/predator cue absent, and roots absent/predator cue present.

The conspecific alarm cue used in this experiment was taken from one of the puffers captured at Page Creek. Preparation of the cue was carried out similar to the methods of Brown and Smith (1997). However, because the presence of a damage-released alarm cue has not previously been tested in checkered puffers, the entire fish was used to account for the possibility of alarm cues located outside of the skin and visceral tissue (Meuthen et al., 2014). The puffer (mass = 102 g) was euthanized via cerebral percussion and blended with 350 mL of tap water. The skin of the puffer had to be removed because it was too tough to blend. Once removed, fifty 1-inch cuts were made in the skin with a scalpel; the skin was then rinsed with 50 mL of water, which was added to the blender, and the mixture was homogenized for an additional 1 min. After homogenization, the solution was poured through a cotton filter and more water was added to bring the filtrate to a final