Puff and bite: The relationship between the glucocorticoid stress response and anti-predator performance in checkered puffer (*Sphoeroides testudineus*)

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ABSTRACT

Individual variation in the endocrine stress response has been linked to survival and performance in a variety of species. Here, we evaluate the relationship between the endocrine stress response and anti-predator behaviors in wild checkered puffers (*Sphoeroides testudineus*) captured at Eleuthera Island, Bahamas. The checkered puffer has a unique and easily measurable predator avoidance strategy, which is to inflate or 'puff' to deter potential predators. In this study, we measured baseline and stress-induced circulating glucocorticoid levels, as well as bite force, a performance measure that is relevant to both feeding and predator defence, and 'puff' performance. We found that puff performance and bite force were consistent within individuals, but generally decreased following a standardized stressor. Larger puffers were able to generate a higher bite force, and larger puffers were able to maintain a more robust puff performance following a standardized stressor relative to smaller puffers. In terms of the relationship between the glucocorticoid stress response and performance metrics, we found no relationship between post-stress glucocorticoid levels and either puff performance or bite force. However, we did find that baseline glucocorticoid levels predicted the ability of a puffer to maintain a robust puff response following a repeated stressor, and this relationship was more pronounced in larger individuals. Our work provides a novel example of how baseline glucocorticoids can predict a fitness-related anti-predator behavior.

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1. Introduction

The stress response is a complex physiological response in the face of a real or perceived challenge. Initiated by an increase in glucocorticoid stress hormones (Mommsen et al., 1999; Sapolsky et al., 2000), the suite of physiological and whole-animal changes associated with the stress response heighten performance during a challenge by mobilizing energy resources that facilitate escape from acute stressors, as well as to promote recovery once the challenge has been overcome (Wingfield et al., 1998; Sapolsky et al., 2000; Romero, 2004). However, the stress response may come at a cost to other important components of fitness such as

immunocompetence, growth, and reproduction (Greenberg and Wingfield, 1987; Barton and Iwama, 1991; Barton, 2002; Sapolsky et al., 2000; Moore and Jessop, 2003; Romero et al., 2009; Fuzzen et al., 2011). The stress response therefore represents an important component of fitness, and the optimal stress response will maximize survival through a challenge, while minimizing unnecessary costs to other components of fitness (Wingfield et al., 1998; Ricklefs and Wikelski, 2002).

It is generally thought that low baseline circulating glucocorticoid stress hormones, a robust glucocorticoid response to a challenge, and a rapid return of glucocorticoid to baseline levels, are indicative of an optimal stress response, but empirical evidence is equivocal (see reviews by Breuner et al., 2008; Bonier et al., 2009). In terms of baseline glucocorticoid levels, some studies have found elevated baseline glucocorticoids negatively predict

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reproduction or survival, while other studies have found positive relationship, while other studies find no relationship (see review by Bonier et al., 2009). In terms of stress responsiveness, or the extent to which an individual raises glucocorticoid levels in response to a challenge, evidence is similarly mixed. For example, survival in Galápagos marine iguanas (*Amblyrhynchus cristatus*) during starvation events is negatively related to glucocorticoid responsiveness (Romero and Wikelski, 2001) and positively related to the ability to rapidly terminate a glucocorticoid stress response (Romero and Wikelski, 2010). A robust glucocorticoid response is also negatively associated with survival in European white storks (*Ciconia ciconia*; Blas et al., 2007). In the white-crowned sparrow (*Zonotrichia leucophrys*), Breuner and Hahn (2003) found that greater glucocorticoid reactivity is associated with poorer body conditions. Similarly, lines of zebra finches (*Taeniopygia guttata*) selected for higher glucocorticoid reactivity have smaller adult body size (Roberts et al., 2007). However, glucocorticoid responsiveness is positively correlated with return rates to breeding grounds in a migratory bird, the American redstart (*Setophaga ruticilla*; Angelier et al., 2009). What constitutes an optimal glucocorticoid stress response is therefore likely context-dependent, and may vary based on the environmental conditions or the life-history traits of the individual being measured (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003; Korte et al., 2005).

In the current study, we examined baseline and stress-induced circulating glucocorticoid levels in relation to two performance metrics in the checkered puffer (*Sphoeroides testudineus*). First, we looked at a unique puffer predator avoidance strategy, which is to inflate or 'puff' when threatened. Puffing increases the body size of the fish, and makes them difficult to subdue and consume (Randall, 1967; Recher and Recher, 1968; Myer, 1989). Second, we looked at a more common performance metric, bite force. Checkered puffers are durophagous, feeding on hard-shelled prey. In all durophagous vertebrates, bite force is important for feeding (Wainwright, 1988; Hernandez and Motta, 1997; Grubich, 2005; Berumen and Pratchett, 2008) and may influence dietary range (see Mara et al., 2010 for overview). Increased bite force allows exploitation of prey unavailable to conspecifics and other species (Hernandez and Motta, 1997; Berumen and Pratchett, 2008), thereby reducing inter- and intra-specific competition (Wainwright, 1988; Grubich, 2005). In another durophagous species, the northern map turtle (*Graptemys geographica*) bite force is strongly correlated to increased dietary range, body condition and reproductive output (Bulté et al., 2008). Bite force may also be relevant as a measure of competitive ability in resource contests with conspecifics (e.g., Vanhooydonck et al., 2005; Lailvaux and Irschick, 2007), and as a measure of the effectiveness of biting as an anti-predator behavior (e.g., Greene, 1988; Hertz et al., 1982). Given the importance of glucocorticoids in mobilizing energy resources during a challenge, and given that both 'puff' response and bite-force are energetically costly behaviors associated entirely or in part with anti-predator behavior in puffers, we predicted that in this context, glucocorticoid responsiveness would be positively associated with our performance metrics. We predicted that puffers with lower baseline glucocorticoid levels and higher post-stress glucocorticoid levels would have increased puff performance as well as increased bite force relative to puffers with higher baseline glucocorticoid levels and lower post-stress glucocorticoid levels.

2. Methods

2.1. Study site and study animals

Between February 22–25 and June 1–12, 2012, checkered puffers (*n* = 110) were collected from Plum and Page Creeks on Eleuthera Island, Bahamas (Plum: N 24°45′45″, W 76°15′6″; Page: N 24°49′04″, W 76°18′51″). Puffers were corralled into a seine net set at the mouth of the creeks on an outgoing tide and transported in aerated coolers to the Cape Eleuthera Institute (CEI: N 24°50′05″, W 76°20′32″). At CEI, puffers were held in 1250 L aerated flow-through saltwater tanks at ambient temperature (29.2 ± 2.7 °C), and were allowed to acclimate to laboratory conditions between 2 and 7 days before experimentation. During acclimation, puffers were fed an assortment of fresh fish every 2 days. The holding tank was cleaned every 4 days. Fish were fasted and tanks were not cleaned for 48 h prior to experimentation to avoid disturbing the fish close to experimentation. Following experiments, all puffers were weighed (g) using a portable electronic balance and then placed in a foam-lined trough to obtain a total length (TL) measurement (mm). All techniques were performed without anesthesia (see Cooke et al., 2005 for rationale), and all samples were collected in accordance with the guidelines of the Canadian Council on Animal Care as administered by Carleton University (B12-01). All fish were released back into the ocean upon recovery at the conclusion of the experiment.
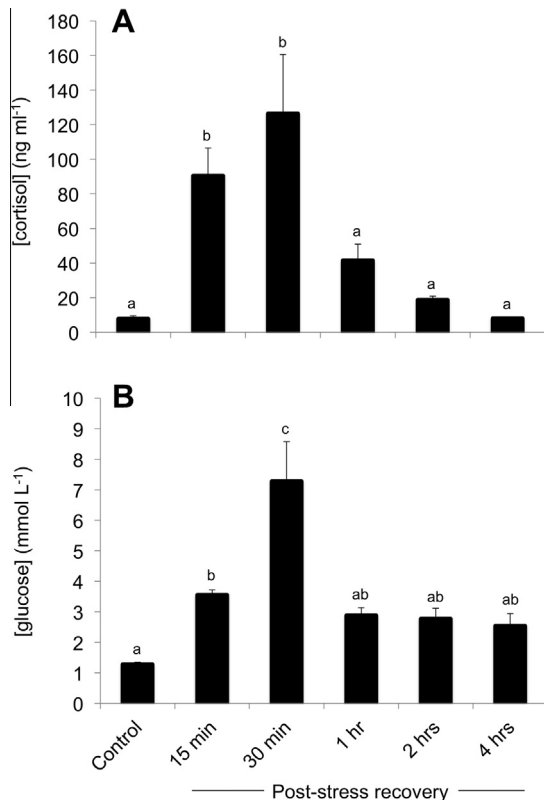
2.2. Cortisol recovery profiles

First, we subjected a subset of fish to a standardized stress challenge and sampled them during the recovery period to identify the maximum cortisol concentration (cortisol being the primary glucocorticoid in fish; Mommsen et al., 1999) for puffers and the time at which the maximum cortisol peak occurs. Puffers (*n* = 42; mass = 123 ± 3 g; TL = 178 ± 3 mm; mean ± standard error of the mean [SEM]) were placed in individual opaque aerated chambers (12.5 L) with constant flow-through saltwater for 24 h. Fish were then randomly assigned to one of six treatment and sampling groups: (1) control (*n* = 8), (2) stress treatment, with sampling 15 min post-stressor (*n* = 7), (3) stress treatment, with sampling 30 min post-stressor (*n* = 8), (4) stress treatment, with sampling 1 h post-stressor (*n* = 7), (5) stress treatment, with sampling 2 h post-stressor (*n* = 7), and (6) stress treatment, with sampling 4 h post-stressor (*n* = 5). With the exception of the control group, puffers in each of the treatment groups were subjected to an acute standardized stressor by holding them at the air–water interface for 5 min in a rubber-mesh dip net, and then returning them to their individual chambers for recovery for the designated duration until sampling. Fish in all groups were then non-lethally sampled for 0.5 mL of blood by caudal venipuncture using a heparinized 1 mL syringe and 21 gauge, 2.5 cm needle. To avoid sampling-induced stress, each blood sample was withdrawn in under 3 min after opening the individual chamber (Romero and Reed, 2005). Control fish were sampled after being held in an individual chamber for 24 h, with no exposure to the standardized stress treatment.

2.3. Cortisol levels relative to puff performance and bite force

Based on data from the cortisol recovery profiles, we determined that the maximum values of stress-induced cortisol concentrations occur 30 min post-stressor in puffers (Fig. 1). All sampling for maximal cortisol concentrations during successive trials therefore occurred 30 min post-stressor.

To explore the relationship between baseline and stress-induced cortisol levels, puff performance, and bite force, puffers (*n* = 48) were collected, acclimated, and held in individual opaque experimental chambers as described above for 24 h prior to experimentation. After 24 h in the experimental chambers, fish were air-exposed for 3 min. During this time, their baseline bite force was measured with a custom-built force transducer system (modeled after Lailvaux and Irschick, 2007; Bulté et al., 2008).

Fig. 1. (A) Plasma cortisol and (B) plasma glucose recovery profile in the checkered puffer (*Sphoeroides testudineus*) following a 5 min standardized stressor. Error bars represent standard error of the mean (SEM). Different letters indicate statistically significant differences among sampling time points (Tukey's HSD post hoc test following a significant ANOVA; $\alpha = 0.05$). Sample sizes are as follows: (1) control, $n = 8$; (2) sampling 15 min post-stressor, $n = 7$; (3) sampling 30 min post-stressor, $n = 8$; (4) sampling 1 h post-stressor, $n = 7$; (5) sampling 2 h post-stressor, $n = 7$; (6) sampling 4 h post-stressor, $n = 5$.

The force transducer was composed of a load cell and a custom built DC amplifier. The load cell was constructed from an aluminum block ($75 \times 12 \times 12$ mm) with material removed from the center portion to create a thin-walled (1 mm), 15 mm long channel. Loads applied to one end of the aluminum block therefore caused deformation of the thinned regions in the center, that were detected by thin-foil type resistive strain gauges bonded to adjacent surfaces of the block at the thinned regions. The paired strain gauges were connected in a Wheatstone bridge configuration. The amplifier unit supplied an excitation voltage to the bridge and changes in resistance of the strain gauges produced a change in voltage proportional to the load applied to the cell. A multimeter (Agilent True RMS Multimeter, Model U1233A) was then used to display voltage changes from the load cell. The bite force meter was calibrated using a series of loads of known weights, and the calibrated output of the unit was linear, with little drift due to thermal instability (less than 0.05% of full scale). All puffers were also sampled for 0.5 mL of blood within this 3 min period, using the methods described above (Section 2.2), which served as a baseline sample. The intensity of the 'puff' over the course of the 3 min initial sampling period was also monitored to generate a baseline puff score. Puffs were assigned a score from 0 to 3. A score of 0 was assigned if there was no puff; a score of 1 indicated that the fish was producing equal to or less than half a puff (i.e., there was a low level of inflation); a score of 2 indicated that the fish was producing between half a puff and a full puff (i.e., the fish was highly inflated, but could still inflate further); and 3 being a full puff (i.e., the fish's skin was tight to the touch and subsequent inflation

attempts resulted in no further expansion). Each puff score was assigned a percentage of time used over the 3 min, and then weighted according to its score. As a result, each puff score is presented as a value between 0 and 3 (i.e., 0 being no puff at all for the entire sampling period, and 3 being a consistent full puff over the course of the 3 min sampling period).

Following the sampling, all fish were held in a rubber mesh net at the air–water interface for 2 min (i.e., for a 5 min total air exposure stressor), and subsequently returned to their individual chambers. Once released into the chamber, the time the fish required to deflate was recorded. Thirty minutes after the standardized stressor (the time identified as the maximal cortisol response), all puffers were again collected to record their post-stress bite force using the methods described above, and sampled for 0.5 mL of blood while monitoring their post-stress puff score. Puffers were then returned to their individual chambers where the time to deflate was again recorded. Out of the 48 fish, blood samples could not be obtained for one or both sampling periods for 10 fish, resulting in a final sample size of 38 fish (mass = 153 ± 6 g; TL = 200 ± 3 mm; mean ± SEM).

2.4. Sample analyses

Whole blood samples were held in water–ice slurries for no more than 1 h before whole blood glucose concentrations were quantified on site using an Accu-Chek® Compact Plus glucose meter (Roche Diagnostics, Basel, Switzerland; see Cooke et al., 2008 for validation). Remaining whole blood was then centrifuged at 2000g for 5 min to separate erythrocytes from plasma (Capsule HF-120, Tomy Seiko Co., LTD, Tokyo, Japan). Plasma samples were stored at -20 °C and then transferred to a -80 °C freezer until cortisol immunoassay analysis. Plasma cortisol was quantified using colorimetric competitive enzyme-linked immunoassay (ELISA; Enzo Life Sciences Cortisol ELISA Kit ADI-900-071; Farmingdale, New York, USA), a technique previously validated for measuring cortisol concentrations in a variety of fish species (Sink et al., 2008). Samples were read by a SpectraMax Plus 384 absorbance microplate reader following manufacturer recommendations.

2.5. Statistical analyses

Statistical analyses were conducted using R version 3.0.2 (R Core Team, 2013). Residuals were examined for normal distributions using Shapiro–Wilks tests, and homogeneity of variance was assessed using Bartlett's tests. Three outliers (1 baseline cortisol value, and 2 post-stress time to deflate values) were identified, and these values were truncated to the 99th percentile (Osborne, 2008). All variables were scaled by their standard deviation and centered by their means to make estimates comparable for all model terms (Schielzeth, 2010). Unmanipulated data are presented in figures, and unless otherwise noted, values are presented as mean ± standard error of the mean (SEM). For all models and model terms, $\alpha = 0.05$.

2.5.1. Cortisol recovery profiles

To determine the recovery profile, analysis of variance (ANOVA) models were performed with cortisol concentration and glucose concentration as the dependent variables, and post-stress sampling time (i.e., control, 15, 30 min, 1, 2 and 4 h post-stressor) as the independent variable. Following a significant ANOVA ($\alpha = 0.05$), Tukey's HSD post hoc tests were used to quantify differences among groups.

2.5.2. Are performance metrics consistent within individuals?

Pearson's correlations were used to determine whether individuals' performance metrics (puff score, time to deflate, and

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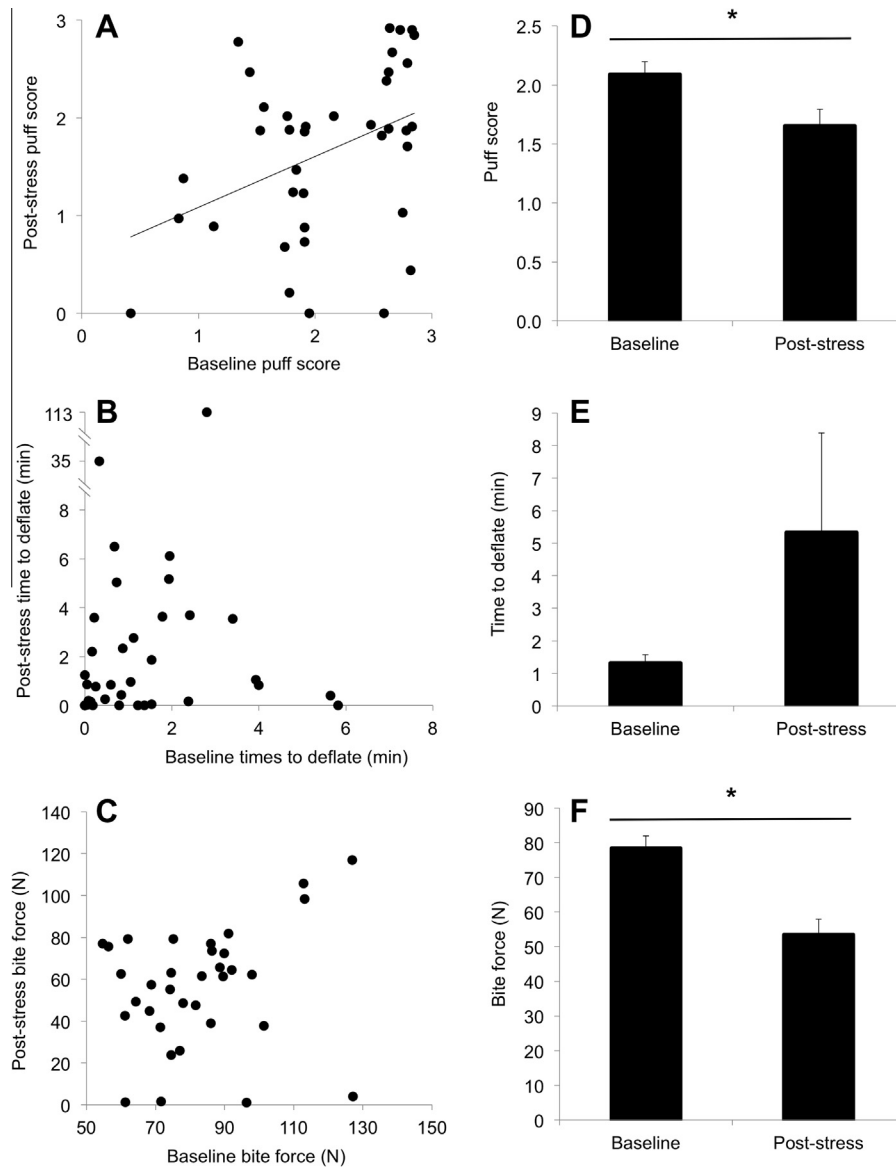
Fig. 2. The (A) significant correlations ($p = 0.02$) between baseline and post-stress puff score, (B) non-significant relationship ($p = 0.79$) between baseline and post-stress time to deflate, and (C) the marginally non-significant correlation ($p = 0.05$) between baseline and post-stress bite force in individual checkered puffer (*Sphoeroides testudineus*). Both (D) puff score and (F) bite force generally decreased post-stress relative to baseline measurements, with no difference in (E) time to deflate relative to sampling period. For all tests, $\alpha = 0.05$. Note that outliers (2 post-stress time to deflate values) were truncated to the 99th percentile (Osborne, 2008) for the statistical analyses, but unmanipulated data is shown. Sample size is $n = 38$. See Section 2 for full statistical details.

292 bite force) were correlated between the baseline and post-stress
293 measurements. Paired *t*-tests were then used to determine
294 whether the performance metrics generally increased, decreased,
295 or remained constant between the two measurement periods.

296 *2.5.3. Do baseline circulating cortisol concentrations predict*
297 *performance?*
298   To determine whether baseline circulating cortisol concentra-
299 tions predict initial performance metrics, post-stress performance
300 metrics, or both, we ran general linear models with the perfor-
301 mance measures (baseline puff score, time to deflate, and bite
302 force; post-stress puff score, time to deflate, and bite force) as the
303 dependent variables, and baseline cortisol concentration and
304 mass as independent variables. We included the interaction effect
305 between baseline cortisol concentration and mass in all initial
306 models, and dropped it from the final model if it was not sig-
307 nificant ($\alpha = 0.05$).

308 *2.5.4. Does cortisol responsiveness predict performance?*
309   To determine whether cortisol responsiveness is related to ini-
310 tial performance metrics, post-stress performance metrics, or both,
311 we ran general linear models with the performance measures
312 (baseline puff score, time to deflate, and bite force; post-stress puff
313 score, time to deflate, and bite force) as the dependent variables,
314 and post-stress cortisol concentration and mass as the indepen-
315 dent variables. As above (Section 2.5.3), we included the interac-
316 tion effect between baseline cortisol concentration and mass in
317 all initial models, and dropped it from the final model if it was
318 not significant ($\alpha = 0.05$).

319 **3. Results**

320 *3.1. Cortisol recovery profiles*

321   Following the standardized stressor, checkered puffers dis-
322 played a maximum cortisol response of $145.9 \pm 31.0 \, \text{ng mL}^{-1}$

Table 1

Results of general linear models exploring the effect of baseline cortisol concentrations, body mass, and the interaction effect on baseline and post-stress performance metrics in checkered puffers (*Sphoeroides testudineus*). Significant effects are indicated by bold italics. Non-significant interaction effects were dropped from final models. For all models and effects, $\alpha = 0.05$. See Section 2 for full statistical details.

Dependent variable		Independent variable	t-Value	p-Value
Baseline	Puff score	Baseline cortisol	1.50	0.14
		Mass	1.59	0.12
	Time to deflate	Baseline cortisol	−0.34	0.74
		Mass	0.49	0.63
	Bite force	Baseline cortisol	0.14	0.89
		Mass	***5.22***	***<0.001***
Post-stress	Puff score	Baseline cortisol	1.57	0.13
		Mass	***4.17***	***<0.001***
		Baseline cortisol × mass	***2.54***	***0.02***
	Time to deflate	***Baseline cortisol***	***3.04***	***0.005***
		Mass	1.11	0.28
		Baseline cortisol × mass	***3.04***	***0.004***
	Bite force	Baseline cortisol	1.42	0.16
		Mass	***3.48***	***0.001***

30 min post-stressor. After 1 h, circulating cortisol concentrations were not significantly higher than control values ($F_{5,28} = 10.10$, $p < 0.001$; Fig. 1A). Puffers also exhibited peak glucose levels of 6.3 ± 1.0 mmol L^{-1} 30 min post-stressor, and circulating glucose concentrations were not significantly higher than control values by the 1 h time point ($F_{5,28} = 13.86$, $p < 0.001$, Fig. 1B). Therefore,
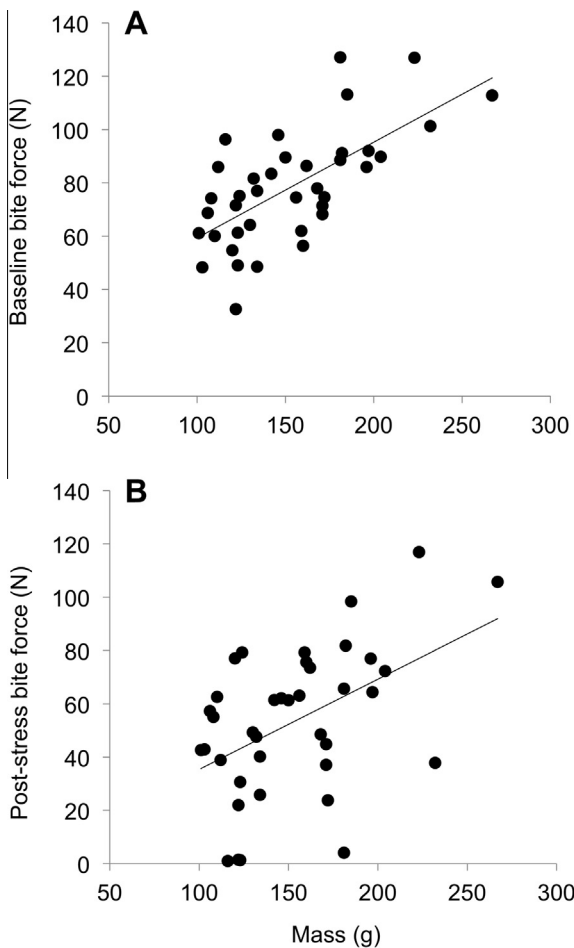
Fig. 3. The significant relationships between body size (mass) and (A) baseline and (B) post-stress bite force in checkered puffer (*Sphoeroides testudineus*). For both tests, $\alpha = 0.05$. Sample size is $n = 38$. See Section 2 for full statistical details.

we used a 30 min time point to assess maximum post-stress physiological measures for all subsequent aspects of the study.

3.2. Are performance metrics consistent within individuals?

Baseline and post-stress puffing performances were significantly correlated ($r_{37} = 0.39$, $p = 0.02$, Fig. 2A). There was a similar trend for bite force, although this relationship was marginally non-significant ($r_{37} = 0.32$, $p = 0.05$, Fig. 2C). There was no relationship between baseline and post-stress time to deflate ($r_{37} = 0.04$, $p = 0.79$, Fig. 2B). Both puff score ($t_{37} = 3.13$, $p = 0.003$, Fig. 2D) and bite force ($t_{37} = 5.26$, $p < 0.001$, Fig. 2F) decreased post-stress relative to baseline measurements, with no consistent pattern in time to deflate relative to sampling period ($t_{37} = 1.49$, $p = 0.14$, Fig. 2E). Together, results suggest that performance metrics are consistent within individuals, but overall performance for all individuals tends to decrease following a stressor.

3.3. Do baseline circulating cortisol concentrations predict performance?

We found no effect of either baseline circulating cortisol concentration or body mass on baseline puff performance (measured as baseline puff score, and baseline time to deflate; Table 1). Neither baseline nor post-stress bite force was related to baseline circulating cortisol concentration, but both measures of bite force were significantly positively influenced by body mass (Table 1 and Fig. 3A and B).

Both post-stress puff score and post-stress time to deflate were significantly influenced by an interaction between body mass and baseline circulating cortisol concentrations (Table 1 and Fig. 4A and B). Fish with higher baseline circulating cortisol were better able to maintain a robust post-stress puff score (Fig. 4A) and took longer to deflate (Fig. 4B), and this pattern was more pronounced in fish with larger body sizes relative to fish with smaller body sizes (Fig. 4A and B).

3.4. Does cortisol responsiveness predict performance?

As with baseline cortisol concentrations, bite force was unaffected by post-stress circulating cortisol values, but significantly positive influenced by body mass (Table 2 and Fig. 3A and B).

Neither baseline nor post-stress puff performance were related to post-stress circulating cortisol concentrations (Table 2). Baseline puff score, baseline time to deflate, and post-stress time to deflate were also unrelated to body mass (Table 2), but post-stress puff score was positively correlated with body mass (Table 2).

4. Discussion

In the current study, we found that puff performance and bite force were consistent within individuals across time, but generally decreased following a standardized stressor. Larger puffers were consistently able to generate a higher bite force, which was unsurprising given that bite force is positively associated with the size of an animal in a number of species (e.g., Wainwright et al., 2004; Grubich et al., 2008). However, we also found that larger puffers were able to maintain a more robust puff score following a standardized stressor than smaller puffers. In terms of the relationship between performance metrics and the glucocorticoid stress response, we found that baseline glucocorticoid levels predicted the ability of a puffer to maintain a robust puff response and deflate quickly following a repeated stressor, particularly in larger individuals.
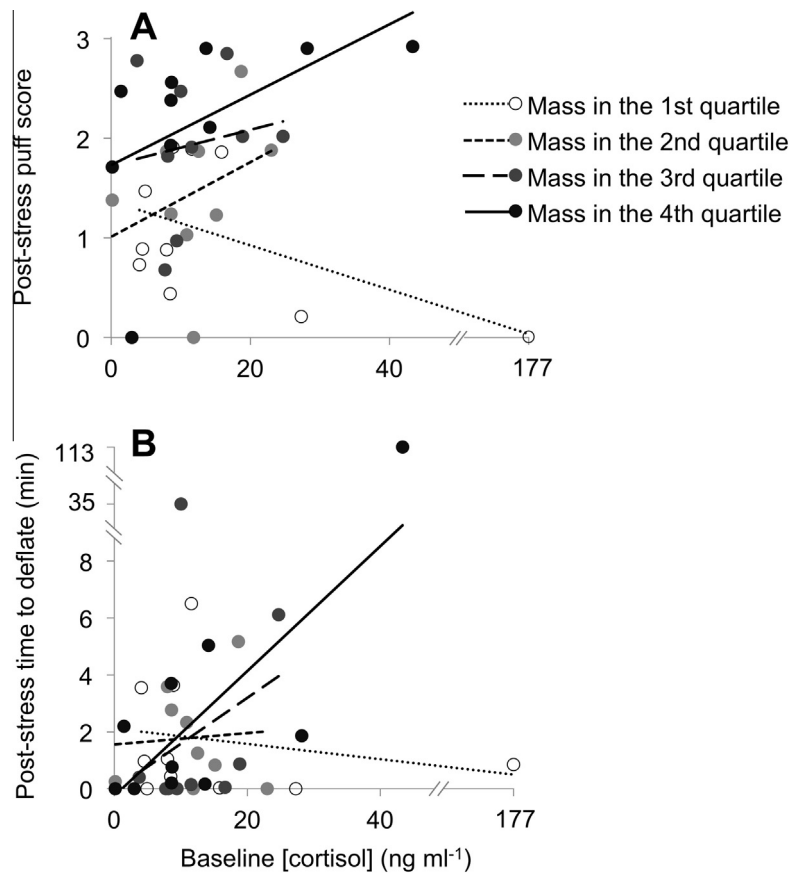
Fig. 4. The interaction between body size (mass) and baseline circulating cortisol concentrations has a significant effect on (A) post-stress puff score and (B) post-stress time to deflate in checkered puffer (*Sphoeroides testudineus*). Note that in the linear model used to explore these relationships, both baseline circulating cortisol concentrations and mass are continuous variable. To visualize the interaction between two continuous variables, the relationship between baseline circulating cortisol and performance metrics is graphed for checkered puffers of varying body size. The first quartile for body size included puffer with masses up to 122 g; the second quartile had masses between 123 and 148 g; the third quartile had masses between 149 and 178 g; and the fourth quartile included puffers with masses 179 g or greater. Outliers (1 baseline cortisol value, and 2 post-stress time to deflate values) were truncated to the 99th percentile (Osborne, 2008) for the statistical analyses, but unmanipulated data is shown. For all effects, $\alpha = 0.05$. Sample size is $n = 38$. See Section 2 for full statistical details, and Table 1 for full statistical results.

Table 2
Results of general linear models exploring the effect of post-stress cortisol concentrations, body mass, and the interaction effect on baseline and post-stress performance metrics in checkered puffers (*Sphoeroides testudineus*). Significant effects are indicated by bold italics. Non-significant interaction effects were dropped from final models. For all models and effects, $\alpha = 0.05$. See Section 2 for full statistical details.

Dependent variable		Independent variable	*t*-Value	*p*-Value
Baseline	Puff score	Post-stress cortisol	0.57	0.57
		Mass	1.49	0.14
	Time to deflate	Post-stress cortisol	−0.43	0.66
		Mass	0.34	0.73
	Bite force	Post-stress cortisol	−0.76	0.45
		Mass	***4.63***	***<0.001***
Post-stress	Puff score	Post-stress cortisol	−0.88	0.39
		Mass	***3.25***	***0.002***
	Time to deflate	Post-stress cortisol	−1.27	0.21
		Mass	0.18	0.86
	Bite force	Post-stress cortisol	1.88	0.07
		Mass	***3.82***	***<0.001***

Stress responsiveness (i.e., the extent to which an individual responds to a given challenge) is both repeatable (Cockrem, 2007; Cook et al., 2011; Rensel and Schoech, 2011) and heritable (Pottinger and Pickering, 1997; Pottinger and Carrick, 1999; Almasi et al., 2010) in many species. High stress responsiveness tends to be associated with a suite of other metabolic and behavioral traits, including decreased growth, reduced aggression, and increased anti-predator behavior relative to traits measured in individuals with low stress responsiveness (Breuner and Hahn, 2003; Øverli et al., 2007; Roberts et al., 2007). Thus, in the current study, we had predicted that energetically costly anti-predator behaviors would be positively correlated with high stress responsiveness, measured as elevated post-stress circulating cortisol concentrations. Puffing is a mechanical defense against piscine and avian predators (Winterbottom, 1974; Brainerd, 1994) that require a complex set of muscles to achieve a full and effective puff (Wainwright et al., 1995). Generating high bite force is similarly energetically costly (Huber et al., 2005). We found that larger puffers were consistently able to generate a higher bite force, and larger puffers were better able to produce a robust puff response following our standardized stress protocol, which speaks to the cost of these anti-predator behaviors. However, we found no relationship between post-stress circulating cortisol concentrations and our performance measures. In this case, it appears that stress responsiveness is not strongly related to bite force or puff performance.

Baseline glucocorticoid levels tend be less repeatable within individuals (Romero and Reed, 2008; Rensel and Schoech, 2011), and are often examined as physiological indices of the relative condition or the typical levels of baseline stress experienced by an individual. Low glucocorticoid levels are typically thought to indicate relatively good condition, or relatively low levels of baseline stress, while higher baseline glucocorticoid levels are through to

suggest poor condition or elevated environmental challenges (Bonier et al., 2009). Thus, in the current study, we had predicted that energetically costly anti-predator behaviors would be negatively correlated with baseline cortisol levels, indicating that fish in better condition or facing lower levels of background stress would be able to launch a more effective anti-predator response. Instead, we found a positive correlation between baseline circulating cortisol levels, and puff performance following a standardized stressor. Sapolsky et al. (2000) suggest that animals with higher baseline circulating glucocorticoid levels may be better prepared to face challenges, such as predator attacks, and our results are consistent with this idea.

Interestingly, we found no relationship between baseline cortisol levels and baseline puff performance, which suggests that fish with higher baseline cortisol levels do not necessarily produce a more robust initial puff, but are better able to maintain their puff performance when faced with repeated stressors. Given that the initial bite force and puff performance occurred before stored energy reserves could be mobilized, these initial performances were likely fueled by readily available energy sources, as glycogen or creatine phosphate. However, following a stressor, stored energy reserves are quickly mobilized and then decline (e.g., Vijayan and Moon, 1992), and so the relationship between baseline cortisol values and post-stress puff performance may indicate that fish with higher baseline cortisol values have more total stored energy available to handle multiple consecutive stressors. The relationship between baseline circulating cortisol levels and post-stress puff performance is also complicated by an interaction effect with body mass, and larger fish have a stronger relationship between baseline circulating cortisol levels and post-stress puff performance than smaller fish. Somatic energy reserves are positively correlated with body size in fish (Brett, 1995; Mackereth et al., 1999; Crossin et al., 2004), and the interaction between body size and baseline cortisol values may arise because smaller fish with fewer energy reserves are less able to produce a robust puff following a standardized stressor, which reduces the potential variation in puff performance in smaller fish, and therefore reduces the potential variation that can be attributed to differences in baseline cortisol levels.

Overall, our results suggest overall that baseline glucocorticoid levels predicted the ability of a puffer to maintain a robust puff response and deflate quickly following a repeated stressor, particularly in larger individuals. While these results are contrary to our predictions, they are consistent with some previous research showing a positive relationship between baseline glucocorticoids and performance measures. For example, survival in translocated European rabbits (*Oryctolagus cuniculus*) is positively related to baseline glucocorticoids (Cabezas et al., 2007). Both the previous study and our current study found a relationship between baseline glucocorticoid levels and performance measures in wild animals that were captured and held in captivity, which is a process that in itself is likely to influence stress responses. The baseline glucocorticoid values that we found in the checkered puffers in the current study were similar to those obtained when sampling checkered puffers in the field immediately after capture (Jennifer Magel, Carleton University, unpublished data). However, there is opportunity for more work to identify how capture-and-holding might influence the relationship between glucocorticoid levels and performance measures in wild animals.

In summary, we found no relationship in the checkered puffer between glucocorticoids responsiveness and any of our performance measures, but we did find that increased baseline circulating glucocorticoids positively predicted the ability of fish to maintain a robust puff performance when faced with repeated stressors. Our results contribute to the emerging and complex picture of performance in relation to circulating glucocorticoids, and provide a novel example of a positive correlation between baseline circulating glucocorticoids and an anti-predator behavior in a wild animal.

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