Predator burden and past investment affect brood abandonment decisions in a parental care-providing teleost

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Summary

1. Reproductive success of parental care-providing species is contingent upon the level of parental investment into a brood. When the expected fitness contribution of a brood is outweighed by the costs of parental care (e.g. decreasing parent somatic condition), an individual may reduce its level of care, or may altogether abandon a brood in trade-off for potential future reproductive success. Leading parental care hypotheses have established parental stress, oxidative stress, nutrition and androgen condition, as well as reproductive value and the threat of brood depredation, as drivers of investment into parental care.

2. The concomitant effects of parent physiological condition and the threat of brood depredation have yet to be considered in their direct effect on the reproductive success of a parental care-providing individual. Using largemouth bass (*Micropterus salmoides*) as a model parental care-providing species, we investigated the relative influence of these factors, together, on a direct fitness measure: the decision by a parent to abandon its brood.

3. By employing a novel combination of multivariate and information-theoretic modelling, our findings indicate that reproductive success of parental largemouth bass is contingent primarily on the threat of depredation to a brood (i.e. the density of brood predators adjacent to nest locations), past investment into a brood and reproductive value of the brood.

4. Parent physiological condition had limited influence on the decision by largemouth bass to abandon parental care. Modelling indicated that parental circulating androgen concentration and antioxidant capacity may play a minimal role in driving brood abandonment; nutrition and stress condition of the parent did not differ between reproductive outcomes.

5. Findings here suggest that a holistic approach should be implemented when studying parental care and that a definite experimental endpoint, e.g., brood abandonment or reproductive success, be utilized as a direct metric of the cost to fitness of parental care decisions.

Key-words: fitness, largemouth bass, life-history, nutrition, oxidative stress, reproductive success, stress, testosterone

Introduction

Reproductive success of parental care-providing individuals can be influenced by trade-offs between the cost of care, and current and future fitness (Williams 1966; Trivers 1972). Research addressing the cost of parental care has focused primarily on brood size, brood loss, resource availability, predation risk, or endocrine-related physiological condition of a parent (Wingfield *et al.* 1990; Fontaine

& Martin 2006; Gravel & Cooke 2009; Neff & Knapp 2009; Dassow *et al.* 2011), with oxidative stress emerging as an additional potential driver of fitness (Alonso-Alvarez *et al.* 2004; Wiersma *et al.* 2004; Monaghan, Metcalfe and Torres 2009; Wilson *et al.* 2012). Studies that use a holistic approach to test for the relative influence of these factors concurrently are few (Zera & Harshman 2001; Harshman & Zera 2007; Schreck 2010; O'Connor *et al.* 2012); most work to date has quantified these individual factors in isolation. Furthermore, few studies have related these factors to a direct fitness response (e.g. offspring abandonment,

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reproductive success) (Breuner, Patterson & Hahn 2008), with most work extrapolating changes in behaviour to a decrease in fitness (Steinhart *et al.* 2004; Fontaine & Martin 2006; Travers *et al.* 2010).

Physiological responses are inter-correlated and are coupled with the biotic and abiotic environment and life-stage of an individual (Wingfield & Sapolsky 2003; Barton 2002; Ricklefs & Wikelski 2002). Additionally, the threat of predation has a strong influence on parental behaviour and parent physiology. For example, care-providing birds, when threatened by predation, reduce foraging effort and decrease maternal investment into egg mass and clutch size (Fontaine & Martin 2006). This behavioural response is thought to be mediated via a glucocorticoid stress response to the perceived threat of depredation (Cyr & Romero 2007), and an overall decrease in physiological condition in birds nesting in a high predator density environment (Travers *et al.* 2010). In contrast, for the black basses (*Micropterus* spp.), the threat of brood depredation can elicit intensified brood defence behaviours and a subsequent change in circulating androgen concentration (Steinhart *et al.* 2004; Gravel & Cooke 2009; O'Connor *et al.* 2011a). The role of testosterone in mediating parental care, however, is unclear (Hanson *et al.* 2009). Dey *et al.* 2010 demonstrated a reduction in brood defence behaviours following the injection of parental male smallmouth bass (*Micopterus dolomieu*) with an androgen receptor antagonist, yet testosterone concentrations have been observed to increase following aggressive behaviours towards brood predators (O'Connor *et al.* 2011a). No direct link between androgen concentration and reproductive success, however, has been observed.

In addition to mediating behavioural changes, physiological responses are competitive such that investment into one physiological function comes at the cost of resources to other endocrine responses, growth or allocation to parental care (Ricklefs & Wikelski 2002). Both testosterone and the glucocorticoid stress response compromise immune function (Barton 2002; Hau 2007), and testosterone-mediated male aggression has been implicated as being detrimental to parental care behaviours and longevity of the parental individual (Wingfield *et al.* 1990). Despite the cost of aggression, recent work suggests that androgens may play an integral role in paternal care and brood defence (Magee, Neff & Knapp 2006; Dey *et al.* 2010; O'Connor *et al.* 2011a). Similarly, the ability of a parental care provider to neutralize cell-damage by oxidative reactive oxygen species (ROS) is reduced at a potential cost to reproduction, self-preservation and subsequent future reproductive capacity (Alonso-Alvarez *et al.* 2004; Wiersma *et al.* 2004; Monaghan, Metcalfe & Torres 2009). The energetic demand of brood defence and nest maintenance, together exacerbated by allocation of energy to a sustained physiological challenge, results in decreased body condition of a care provider (Steinhart *et al.* 2004; Hanson & Cooke 2009), and a subsequent decrease in offspring condition (Eriksrad *et al.* 1997). Limited evidence suggests

that nest site and male experience, when compared with parental male physiological condition, may contribute to reproductive success in some species (O'Connor *et al.* 2012), yet it remains largely unclear whether varying physiological and environmental conditions have a hierarchal effect on parental behaviours. Specifically, it is unknown how this suite of physiological factors influences the decision by a parent to abandon its offspring in relation to nest predation risk, thereby incurring a potential fitness cost to parental care-providing individuals.

Using largemouth bass (*Micropterus salmoides*; Lacépède 1802) as a model species, we employed the use of both a multivariate and an information-theoretic approach to test whether a suite of physiological and environmental factors, when examined concurrently, influence the parental decision to abandon a brood. Specifically, the objectives of the study were to quantify how parent nutritional condition, stress, androgen concentration, and oxidative stress, as well as naturally occurring brood predator density, compete in driving brood abandonment decisions in largemouth bass, with all of these factors being compared in the same experimental setting. The approach used here offers a novel design for studying parental decisions in that: (i) the relative influences of physiological and environmental (predator) variables on parental care decisions were compared concurrently; (ii) the population examined was wild and free-swimming without environmental variable manipulation; and (iii) a direct measure of reproductive success (brood abandonment) was the end point in the study.

Materials and methods

STUDY AREA AND SPECIES

A closed population of largemouth bass was studied in Mills Lac (45 °47′46″ N, 74 °46′54″ W), a mesotrophic lake near Kenauk, Quebec, Canada. Largemouth bass begin spawning as water temperatures approach 15 °C, when males construct a saucer-shaped nest in the littoral substrate and court females, which subsequently deposit eggs (Philipp *et al.* 1997). Following fertilization, the female departs the nest, leaving the male to provide sole parental care for the following 3–5 weeks, until offspring develop to the stage at which they become free-swimming and independent of parental care (Philipp *et al.* 1997).

During the parental care period, paternal bass refrain from actively foraging, yet demonstrate a marked increase in energetic investment into care of their brood compared to energetic demands outside of the reproductive season (Cooke, Philipp & Weatherhead 2002; Hanson, Abizaid & Cooke 2009). Parental care activities include fanning eggs to prevent accumulation of sediment and maintain sufficient oxygenation, and engagement in agonistic behaviours in defence of the brood from nest predators, here bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) (Gravel & Cooke 2009). At the northern extent of largemouth bass range, consistent with the latitude at which Mills Lac is located, males rarely attempt more than a single spawning bout per reproductive season, thus abandonment of a brood premature to offspring independence almost certainly results in complete forfeiture of reproductive value for that year (Steinhart *et al.* 2008).

FIELD TECHNIQUES

From May to June 2010, snorkel surveys were performed parallel to the entire length of the shoreline of Mills Lac to locate nesting largemouth bass. A uniquely numbered polyvinyl chloride (PVC) tag was placed approximately 1 m from each nest for identification purposes, and the location of each nest was recorded on a waterproof map by the snorkeler. Developmental stage of each brood (brood age) was estimated from newly fertilized eggs (≤ 1 day old, translucent with an oil droplet) to egg sac fry (c. 7-day old, larvae with yolk sac still present) (O'Connor *et al.* 2009), the final larval stage before offspring are capable of swimming. Relative spawn date (i.e. the consecutive order of spawning, starting with the first observed date of spawn, May 13, as 1) was determined based on estimated brood age. The number of offspring in each nest (brood size, BS) was visually estimated using a proven technique and assigned a categorical ranking from 1 (few) to 5 (many) (Suski *et al.* 2003). Brood predator density at each nest site was quantified via instantaneous count of the number of bluegill and pumpkinseed within a 2 m radius of the nest upon approach and departure from the nest (adapted from Gravel & Cooke 2009). The mean of the two predator counts was termed 'pre-capture predator burden' and constituted an estimate of the threat of brood depredation as perceived by the care-providing male (Gravel & Cooke 2009).

Following completion of each snorkel survey, newly discovered largemouth bass with broods aged ≤ 7-day old were located using the geographical location of the nest as recorded on a map by the snorkeler, captured via conventional hook-and-line angling from a boat and placed directly into a foam-lined trough containing fresh lake water for blood sampling. Blood was sampled via the caudal vessel using a heparin-rinsed 21-ga needle and 1 mL syringe and then transferred to a 1·5 mL vial for centrifugation at $2000 \times g$ for 3 min. Plasma was separated from erythrocytes and placed into a dry shipper charged with liquid nitrogen for subsequent laboratory analysis. Following blood drawing, total length (TL) of each male was measured to the nearest mm, a small section of the lower caudal fin was removed to mark the individual as having been captured, and the fish was placed into a 75 L cooler containing fresh lake water for a standardized 10-min holding period, the timing of which commenced upon successful hooking of the male during angling. The 10-min holding period allowed for the standardization of handling techniques and recovery of the male largemouth bass following plasma sampling procedures, and acted as a common stressor for each male (O'Connor *et al.* 2011b). The duration of time between hooking a fish and completion of blood sampling was <2 min, a handling time sufficient to avoid detection of any angling-induced physiological response (Romero & Reed 2005; Hanson *et al.* 2009). While the male was away from the brood, an observer in a boat situated 2 m from the nest quantified the occurrence of depredation by bluegill and pumpkinseed. No measurable brood depredation occurred while the male was away, as verified by a follow-up snorkel survey.

Following the 10-min holding period, each male was released approximately 2 m from its respective nest and resumed paternal care behaviours within 30 s of release. Upon the males' return to his brood, an observer in the bow of the boat and positioned 2 m from the nest provided an instantaneous count of the number of brood predators within a 2 m radius of the nest (Gravel & Cooke 2013). This final predator count ('post-capture predator burden') represents the number of brood predators the male encountered upon return to the nest and was indicative of potential brood depredation that may have occurred while the nest was unguarded, as perceived by the returning male (Gravel & Cooke 2009).

To quantify the rate of brood abandonment, we employed a commonly used proxy for reproductive success in *Micropterus* spp. following a common stressor (Philipp *et al.* 1997; Suski *et al.*

2003). Snorkel surveys of each nesting male were conducted 24 h following angling and blood sampling procedures; males present on their nests during this survey, as verified by the lower caudal fin clip applied during capture, were considered to have maintained parental care. Males not present at a nest, or if the nest was devoid of egg or fry (i.e. fully depredated), were deemed to have forfeited care of the current brood. All males that maintained paternal care in the current study beyond the 24 h observation were successful in raising broods to the free-swimming fry stage, as observed during subsequent snorkel transects, and were assumed to have reared broods to independence. Thus, parental males that did not abandon their brood following capture are considered to have been successful in reproduction. In addition to active field sampling, water temperatures (± 1 °C) were recorded every hour at a depth of 1 m throughout the duration of the experiment using a commercially available thermal logger (iButton Thermocron® DS1921H; Maxim Integrated Products, Inc., San Jose, California, USA). Water temperatures from iButtons within proximity to the sampling area were used to generate mean daily water temperature of Mills Lac for each day of the study.

PHYSIOLOGICAL ANALYSES

Plasma cortisol was quantified via a colorimetric competitive enzyme-linked immunoassay, (ELISA; Enzo Life Sciences Cortisol ELISA Kit ADI-900-071; Farmingdale, New York, USA), a technique previously validated for quantification of cortisol concentrations in largemouth bass plasma (Sink, Lochman & Fecteau 2008). Quantification of plasma potassium (K^+) and sodium (Na^+) occurred by flame atomic mass spectrometry (Cole-Palmer Instruments Model 2655-00, Vernon Hills, Illinois, USA), while chloride (Cl^-) concentration was quantified by coulometric titration (Labconco Co. Digital Chloridometer Model 4425000, Kansas City, Missouri, USA). Plasma glucose was quantified via a colorimetric enzymatic assay adapted from Lowry & Passonneau (1972). Plasma protein concentration was determined by total solid refractometry (Reichert VET 360 model 137536L0; Depew, New York, USA) (Wells & Pankhurst 1999), and plasma cholesterol was quantified with a commercially available colorimetric assay (BioAssay Systems EnzyChrom™ AF Cholesterol Assay Kit E2CH-100; Hayward, California, USA). Concentrations of 11-ketotestosterone (11-KT), the primary androgen in teleost fishes, were determined by colorometric ELISA (Cayman Chemical 11-KT Testosterone EIA Kit 582751; Ann Arbor, Michigan, USA).

Oxidative stress of each paternal largemouth bass was assed via two variables describing oxidative damage and antioxidant capacity. Malondialdehyde (MDA), the byproduct of peroxidation of polyunsaturated fatty acids and an indicator of oxidative damage to cell lipids, was quantified by colorimetric determination of thiobarbituric acid reactive substances (TBARS) following the reaction between MDA, and thiobarbituric acid (Cayman Chemical TBARS Assay Kit 10009055). The capacity for each male to prevent oxidative damage (total antioxidant capacity, TAC) was determined via plasma analysis as the collective free-radical-scavenging capacity of endogenously and exogenously derived enzymes and macromolecules (Cayman Chemical Antioxidant Kit 709001). Commercially available assays were performed according to manufacturer specification, and all colorimetric analyses were quantified by microplate spectrometry (Molecular Devices Spectramax Plus model 384, Sunnyvale, California, USA).

STATISTICAL ANALYSES

Due to high dimensionality and the potential for biological interrelatedness among the 10 physiological variables quantified (Table 1) (Zera & Harshman 2001; Travers *et al.* 2010), a multivariate principal component analysis (PCA) was used to reduce blood

plasma variables into newly defined terms. Principal components (PCs) with eigenvalues >1·0 were retained and rotated orthogonally, and parameters were considered to contribute maximally to each PC if rotated eigenscores were >0·40 or <−0·40 (Green 1991; Peres-Neto, Jackson & Somers 2003; Gingerich & Suski 2011).

To define the factors influencing abandonment decisions by paternal largemouth bass, biologically significant generalized linear models (GLMs) containing the newly derived PC terms based on physiological parameters, male TL, BS, brood stage, brood predator burden and mean daily water temperature as model terms, were developed, *a priori*, as predictors of the binomial response: abandon a brood, or not abandon a brood. To avoid potential pitfalls of stepwise regression modelling (Whittingham *et al.* 2006), a maximum likelihood information-theoretic approach was implemented to test model fit (Akaike 1973). The generated GLMs were ranked by best-fit using Akaike's Information Criterion (AIC) score, corrected for small sample size (AIC_c) (Burnham & Anderson 2002). The model with lowest AIC_c score was considered to be the most parsimonious; models with a difference in AIC_c score from the best-fit model (ΔAIC_c) <2·0 were considered best competing models, and models with a ΔAIC_c < 6·0 were considered to have substantially less support (Burnham & Anderson 2002). Variables from best-fit models were graphically represented by box plot, where appropriate. All analyses were performed using JMP version 10.0 (SAS Institute, Inc., Cary, North Carolina, USA), and all means are reported ± standard error (SE).

Results

A total of 55 brood-guarding largemouth bass were sampled during the study, of which 70% were successfully in raising a brood to independence, and 30% abandoned their brood within 24 h of the standardized stressor. Blood-based physiological characteristics were sorted into four PCs with eigenvalues >1·0, and together explain 69·7% of the observed variation in male physiological con-

dition (Table 2). PC1 describes male nutritional condition and was characterized by high plasma protein and cholesterol, and also by high MDA concentration (Table 2). PC2 describes male androgen condition, as characterized by positive loading by 11-KT (Table 2). PC3 is most substantially represented by negatively loaded TAC, with positive glucose and Na^+ loads. Cortisol loaded negatively into PC4, and Cl^- loaded positively, suggesting that PC4 represents male stress condition.

The best-fit GLM for predicting brood abandonment by parental largemouth bass contained brood stage and mean predator burden (AIC_c = 62·42, Table 3). This model represents an increased likelihood of brood abandonment for male largemouth bass nesting in areas with higher brood predator density (Fig. 1a), and for male largemouth bass with older broods (Fig. 1b). The addition of BS to the GLM resulted in a highly competitive AIC_c (ΔAIC_c = 1·86, Table 3; Fig. 1c), thus as next best-fit model indicated that, in addition to increasing brood age and high predator burden, the likelihood for brood abandonment by largemouth bass increases with lower reproductive value. Models including blood-based physiological PC terms did not produce highly competitive models, nor did models with male TL and mean daily water temperature. Models containing PC2 and PC3 together with mean predator burden did, however, provide minimal support (i.e. ΔAIC_c < 6·0) for predicting brood abandonment, indicating that male 11-KT concentrations and male antioxidant capacity may play a limited role in mediating parental care decisions in largemouth bass (Table 3).

Table 1. Mean, minimum, maximum and standard error (SE) for brood-related, predator density and physiological metrics collected from brood-guarding largemouth bass. Sample size is $N = 55$ for all variables

	Mean	Min	Max	SE
TL (mm)	310·5	213	428	7·24
Relative spawn date (day)	8·0	1	31	0·71
Mean daily temperature (°C)	18·0	12·3	22·9	0·27
Brood size	2·8	1	5	0·71
Brood age (day)	3·5	1	7	0·31
Pre-capture pred. burden	3·9	0	17·5	0·61
Post-capture pred. burden	3·0	0	18	0·6
Mean pred. burden	3·5	0	16·5	0·46
Plasma Cl^- (Meq/L)	103·9	64	120	1·54
Plasma Na^+ (Meq/L)	190·5	112·8	234·8	3·13
Plasma K^+ (Meq/L)	4·7	2·4	8·1	0·16
Plasma cortisol (pg/mL)	8·7	0·3	57·3	1·4
Plasma glucose (mM)	3·8	2·1	8·1	0·17
Plasma protein (g/mL)	0·1	0·04	0·09	0·02
Plasma cholesterol (mg/dL)	503·2	327·7	667	11·42
Plasma 11-KT (ng/mL)	1021·2	27·3	2854·6	100
Plasma TAC (mM)	0·3	0·07	0·45	0·02
Plasma MDA (μM)	340·3	115·25	882	21·7

TL, male total length; 11-KT, 11-ketotestosterone; TAC, total antioxidant capacity; MDA, malondialdehyde.

Table 2. Principal component (PCA) of parental male largemouth bass physiological correlates measured in blood plasma. Principal components (PCs) with eigenvalues >1·0 were retained following orthogonal rotation. Principal components are described by factors that loaded maximally (values ≥ 0.4 or ≤ -0.4) and are presented in bold. PCs together describe 72·4% of the variation in male largemouth bass physiological condition

Description	PC1 Nutritional condition	PC2 Androgen concentration	PC3 Antioxidant capacity	PC4 Stress
Cl^-	0·12	−0·16	−0·26	**0·76**
Na^+	0·13	0·17	**0·60**	0·30
K^+	−0·07	**−0·78**	0·12	0·34
Glucose	0·12	−0·25	**0·66**	0·03
Cortisol	0·07	−0·07	−0·16	**−0·72**
Protein	**0·91**	−0·22	0·11	0·05
Cholesterol	**0·90**	0·17	0·03	−0·11
11-KT	−0·12	**0·85**	−0·07	0·21
TAC	0·00	0·11	**−0·76**	0·28
MDA	**0·87**	−0·04	0·10	0·10
Eigenvalue	2·6	1·7	1·5	1·2
% var. explained	27·4	15·4	15·1	14·5

11-KT, 11-ketotestosterone; TAC, total antioxidant capacity; MDA, malondialdehyde.

Table 3. Model results for the most likely predictors of brood abandonment by parental male largemouth bass, ranked by increasing AIC_c score. The model with the lowest AIC_c score is the most parsimonious; models with $\Delta AIC_c < 2.0$ are considered to be highly competitive, and models with $\Delta AIC_c < 6.0$ are considered to have substantially less support as predictors for brood abandonment by male largemouth bass. Additional models with AIC_c weights <0.00 are not included

Model	AIC_c	ΔAIC_c	AIC_c weight	Model likelihood	No. parameters	Deviance
$Pred_{mean}$+Stage	62·42	0·00	0·33	1·00	3	55·94
$Pred_{mean}$+Stage+BS	64·27	1·86	0·13	0·40	4	55·48
$Pred_{mean}$+PC2	65·46	3·04	0·07	0·22	3	59·00
Stage	65·65	3·24	0·07	0·20	2	61·42
$Pred_{mean}$	65·72	3·31	0·06	0·19	2	61·50
BS+Stage+BS*Stage	65·79	3·38	0·06	0·18	4	60·00
$Pred_{pre-capture}$	66·58	4·16	0·04	0·12	2	62·35
$Pred_{mean}$+PC2+$Pred_{mean}$*PC2	66·73	4·32	0·04	0·12	4	57·93
PC2	67·14	4·73	0·03	0·09	2	62·91
Stage+PC3+Stage*PC3	67·57	5·15	0·03	0·08	4	58·77
TL+$Pred_{mean}$	67·86	5·45	0·02	0·07	3	61·21
Stage*PC3	68·15	5·74	0·02	0·06	3	63·91
$Pred_{post-capture}$	68·77	6·36	0·01	0·04	2	64·54
Temp	69·61	7·20	0·01	0·03	2	55·94
Stage+PC2+Stage*PC2	69·40	6·99	0·01	0·03	4	60·84
PC1	70·11	7·69	0·01	0·02	2	65·88
PC3	70·17	7·75	0·01	0·02	2	65·94
PC4	70·37	7·96	0·01	0·02	2	66·14
Temp+PC2+Temp*PC2	70·40	7·99	0·01	0·02	4	61·60
Date	70·43	8·02	0·01	0·02	2	66·20
BS	70·44	8·02	0·01	0·02	2	66·20
TL	70·56	8·14	0·01	0·02	2	66·33
PC1+PC3+PC1*PC3	70·92	8·51	0·00	0·01	4	62·12
Stage*PC4	71·40	8·98	0·00	0·01	3	65·33
Stage*PC1	71·84	9·42	0·00	0·01	3	66·27

$Pred_{mean}$, mean predator burden; $Pred_{pre-capture}$, pre-capture predator burden; $Pred_{post-capture}$, post-capture predator burden; Stage, brood developmental stage; PC, principal component; Temp, mean daily temperature; BS, brood size; TL, male largemouth bass total length.

Discussion

The level of investment into parental care by reproducing organisms can vary with changes in the environment, predation threat and endocrine-mediated physiological condition of the care-providing individual (Zera & Harshman 2001; Ghalambor & Martin 2002; Ricklefs & Wikelski 2002; Breuner, Patterson & Hahn 2008); rarely have changes in parental care behaviour been related to a fitness cost (O'Connor *et al.* 2012). Scrutiny of the best-fit models here indicate that the perceived threat of brood depredation, together with past parental investment and parent androgen condition, plays a primary role in driving premature brood abandonment by paternal largemouth bass.

Male largemouth bass that abandoned parental care following a standardized stressor were guarding broods in areas of high predator burden relative to males that successfully raised a brood, indicating that predator densities can incur a direct fitness cost on care-providing individuals. The indirect effect of predation pressure on parental care behaviours has been previously documented, with the occurrence of nest predators inducing a reduction in foraging rates and mate feeding in several passerine birds (Ghalambor & Martin 2002; Fontaine & Martin 2006), and decreased investment into clutch size and egg mass by female song sparrows (*Melospiza melodia*) (Travers *et al.* 2010). In contrast, male smallmouth bass nesting in areas of high predator density engage in more frequent aggressive behaviours relative to conspecifics nesting in lakes of low brood predator density (Steinhart *et al.* 2004; Gravel & Cooke 2009). Predator burden, however, has not yet been linked to a direct reduction in reproductive success in centrarchid fishes. Although limited brood loss due to depredation may occur throughout the parental care period (Steinhart *et al.* 2008), it is unlikely that significant brood depredation occurs while a male bass is actively defending his brood, and no measurable brood loss occurred in the current study while males were away from their nest during plasma collection. Thus, mean predator burden represents the threat of predation as perceived by the brood-guarding male, independent of actual loss in reproductive value (Gravel & Cooke 2013). Male largemouth bass may have been less willing to continue guarding their brood following capture, though, resulting in heightened rates of brood depredation following capture of the male (Suski *et al.* 2003). Despite uncertainty in what occurred within the 24 h post-capture period, the current study provides evidence that the threat of brood depredation, when used as a basis for brood abandonment decisions, can have a direct negative effect on the fitness of an individual.
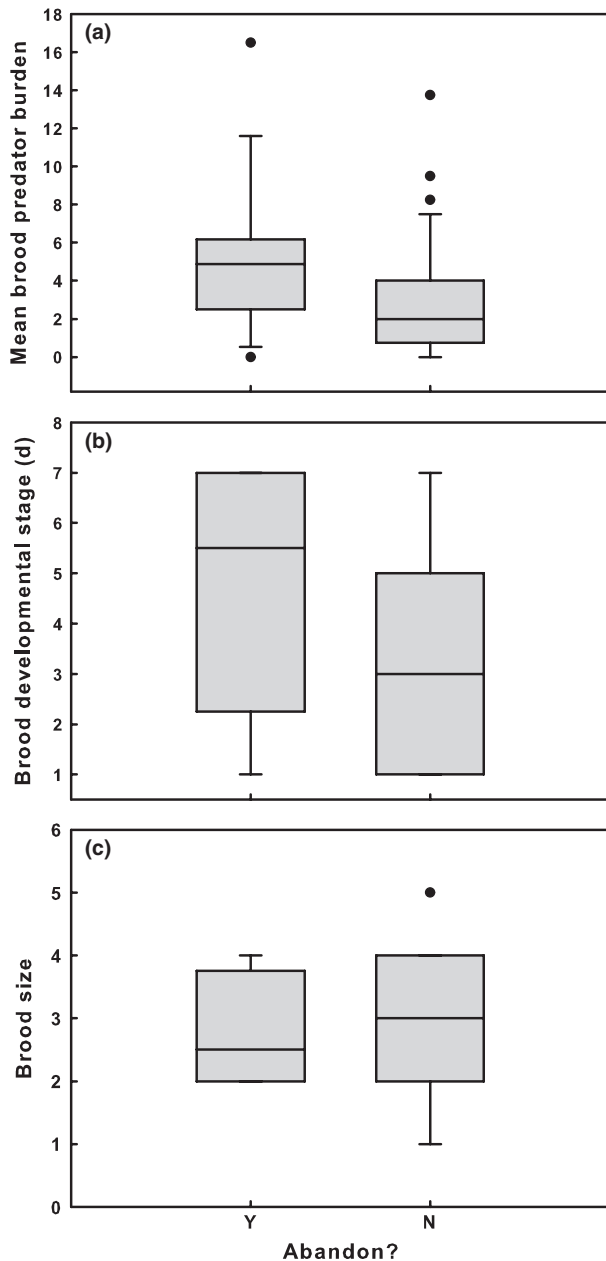
Fig. 1. Box plots comparing: (a) mean brood predator burden, (b) brood developmental stage and (c) brood size between males that abandoned their brood (Abandon? = Y) and those that were successful in raising a brood (Abandon? = N). Mean predator burden, brood stage and brood size constituted the best-fit generalized linear models (GLMs) as ranked by AIC$_c$ score.

Further analysis of the best-fit model indicates that, when included with mean brood predator burden, past parental investment may also drive abandonment decisions. Modelling indicated that male largemouth bass with offspring at a later stage of brood development are more likely to abandon following a stressor. Parental care theory suggests that the relative value of a brood increases as offspring approach independence (Trivers 1972; Östlund-Nilsson 2002). In an attempt to control for the influence of past parental investment (i.e. brood age) on parental decisions, largemouth bass were

included in the current study only if their offspring were non-mobile, endogenously feeding (i.e. eggs or egg sac fry ≤ 7 day old) and fully dependent upon paternal care for protection from predators. Brood abandonment at this stage is likely to result in complete forfeiture of reproductive value for the paternal largemouth bass (Philipp *et al.* 1997), thus the relative fitness cost of brood abandonment on male fitness was the same across these early brood stages. Cooke, Philipp & Weatherhead (2002) confirmed an increase in energetic investment by paternal largemouth bass as offspring developed toward the free-swimming fry stage, and a decrease in nutritional and body condition has been observed in the black basses as the care period progresses (Gillooly & Baylis 1999; Steinhart *et al.* 2004). Inclusion of nutritional condition (PC1) as a covariate with brood stage did not yield any competitive model, thus parental investment was not likely to have been limited by a decrease in male nutritional condition over the duration of care. Alternatively, this finding offers some evidence that, for largemouth bass, the value of a brood does not increase with prior investment.

The hormone 11-ketotestosterone (11-KT) is considered to be the primary androgen in teleost fishes (Borg 1994) and has been shown to correlate with circulating levels of testosterone (T) in paternal bluegill (Magee, Neff & Knapp 2006). PC2, comprised of male plasma 11-KT and K$^+$ concentration, was not included as a significant term in any best-fitting model. PC2 was included, however, in several models lending minimal support (i.e. ΔAIC$_c$ < 6·0) for 11-KT as a potential driver of parental investment. The exact function of male androgens in parental care behaviour across taxa has not been clearly defined, and likely varies across species and brood developmental stage (Dufty 1989; Wingfield *et al.* 1990; Hau 2007). Androgens are believed to play a role in spermatogenesis, sexually dimorphic trait development, and nest construction in teleost fishes (Borg 1994). Dey *et al.* (2010) showed that aggression in brood-guarding smallmouth bass was significantly attenuated following experimental injection of the androgen receptor antagonist cyproterone acetate, and circulating androgen concentrations increased following antagonistic behaviours by smallmouth bass against brood predators (O'Connor *et al.* 2011a), suggesting androgens to be important for brood defence behaviours. In contrast, Neff & Knapp (2009) demonstrated that neither 11-KT nor testosterone (T) influenced nest-tending behaviours in paternal bluegill and also that 11-KT concentration was negatively correlated with aggressive response to nest intrusion by a simulated brood predator. Hanson *et al.* (2009) observed no correlation between aggression in male smallmouth bass and circulating concentrations of T. Together, these studies indicate that androgen concentrations are dynamic in their effect on nest defence behaviours by a care-providing fish, yet no influence of androgens has been observed to result in a direct decrease in reproductive success (O'Connor *et al.* 2012). Modelling

from the current study offers limited evidence that androgen concentration may influence fitness as a mediator of brood abandonment decisions and may play a role in the decision by an individual to maintain continued parental investment. However, the role of androgens in mediating parental care is complex, and further work is needed to deduce the mechanistic role of androgen's effect on reproductive success and care behaviours.

Interestingly, several variables previously shown to influence parental care behaviours and decisions in fishes were not significant drivers of brood abandonment decisions in largemouth bass. Principal components describing nutritional condition (PC1) and stress condition (PC4) were not indicated by modelling as viable predictors of brood abandonment in largemouth bass. Resource limitation is often cited as a primary constraint on parental investment (Williams 1966; Trivers 1972; Smith & Wootton 1995), and parental body condition generally decreases as a proximate cost of prolonged care (Gillooly & Baylis 1999; Dearborn 2001; Cooke, Philipp & Weatherhead 2002). Poor body condition can hinder parental behaviours and compromise future reproductive effort by an individual (Williams 1966; Trivers 1972; Gillooly & Baylis 1999) and has also been observed to increase the likelihood for offspring abandonment (Erikstad et al. 1997). In centrarchid fishes, the allocation of resources to a brood is high because parental males cease foraging, resulting in a decline in body condition as parental care progresses (Gillooly & Baylis 1999; Cooke, Philipp & Weatherhead 2002; Hanson & Cooke 2009). PC1, as a metric for nutritional condition, did not contribute to any best-fit model. This may be attributed to the limited time of parental care under scrutiny (i.e. all broods were ≤ 7 day old at the time of plasma sampling), or potentially because male largemouth bass, if emerging from overwinter in poor body condition, may opt out of attempting a reproductive bout altogether (Fullerton et al. 2000). As the parental care period progresses, voluntary foraging is induced in parental bass by the production of ghrelin, so it is unlikely that nutritional condition becomes limiting to energy expenditure beyond the eggsac-fry stage (Hanson, Abizaid & Cooke 2009). In agreement with Travers et al. (2010), results from this study demonstrate that nutritional condition is not likely to influence parental care decisions in paternal largemouth bass when considered in conjunction with environmental and other physiological variables.

Male stress (i.e. cortisol and subsequent plasma ion concentration; PC4) was not identified as a likely driver of brood abandonment in any competitive model. The stress response is important in ensuring the survival of an individual. During reproduction, though, the stress response re-allocates resources to maintain homeostasis and can negatively impact reproductive success by attenuating parental investment (Ricklefs & Wikelski 2002; Cyr & Romero 2007; Breuner, Patterson & Hahn 2008; Schreck 2010). In largemouth bass, however, the implantation of parental males with exogenous cortisol did not induce

changes in parental behaviour, thus the stress response has not previously been linked to a reduction of parental care activities (O'Connor et al. 2009; Dey et al. 2010). As reviewed by Wingfield & Sapolsky (2003), individuals may temporarily attenuate or mask the stress response to minimize behavioural changes associated with maintaining homeostasis, thereby avoiding a cost to current reproductive effort. The attenuation of a stress response in brood-guarding bass is further substantiated by the current study in that PC4, characterized by cortisol and Cl⁻, did not have a pertinent predictive role in any model explaining reproductive success. The evidence against stress-mediated behavioural changes in largemouth bass, together with the lack of stress-mediated brood abandonment in the current study, indicates the stress response likely does not play a primary role in driving parental decisions in largemouth bass.

Total antioxidant capacity, when included in models with brood stage, offered little support as a predictor for brood abandonment in largemouth bass. TAC, a measure of free-radical-scavenging capacity, and MDA, a product of lipid peroxidation, did not load into the same PC. MDA concentration did, however, exhibit a positive correlation with circulating cholesterol and protein concentration in PC1, suggesting a potential oxidative cost of mobilization of energy reserves to maintain high energetic demand of parental care activities. Oxidative stress can negatively impact growth and post-breeding survival of an individual due to investment into anti-oxidant production, repair of damaged cells, or faster accumulation of oxidative damage (Monaghan, Metcalfe & Torres 2009). Exercise, such as increased muscle activity by paternal bass into brood fanning and predator chasing, increases the production of damaging ROS as a byproduct of aerobic respiration (Leeuwenburgh & Heinecke 2001; Cooke, Philipp & Weatherhead 2002), yet oxidative stress was not observed to occur in brood-guarding bass (Wilson et al. 2012). While the occurrence of ROS alone does not constitute oxidative stress, TAC did diminish with brood age in the current study; given the minimal model support for antioxidant capacity as a driver of abandonment in largemouth bass, the role of oxidative stress cannot be completely discounted. For instance, reproductively active individuals have diminished capacity for scavenging ROS, as demonstrated by increased susceptibility by zebra finch *Taeniopygia guttata* to oxidative damage with increased breeding effort (Alonso-Alvarez et al. 2004; Monaghan, Metcalfe & Torres 2009). Antioxidant capacity, and oxidative stress as a whole, may play a limiting factor in later stages of brood abandonment. Based on MDA concentration being uncorrelated with TAC in the current study, and in accordance with previous findings on the effect of oxidative stress on parental care in black bass (Wilson et al. 2012), reproduction in largemouth bass is unlikely to incur an oxidative cost and is not likely to induce premature brood abandonment by parental males when compared with other variables.

The influence of stress, predation risk, resource limitation and androgen condition on reproductive success of care-providing organisms has been well-established in the literature (Zera & Harshman 2001; Ricklefs & Wikelski 2002), with recent findings suggesting that oxidative stress can also incur a fitness cost to an individual (Alonso-Alvarez *et al.* 2004; Monaghan, Metcalfe & Torres 2009). The current study employed a novel, holistic approach to determine how predator burden, stress, oxidative stress, nutritional condition and androgen concentration of a care provider compare in their influence on potential fitness decisions, using brood abandonment as a direct fitness affect. Findings here indicate that, when considered along with leading hypotheses for limits to parental care, predator burden, past investment and reproductive value are most important in driving the decision by paternal largemouth bass to forfeit a brood. Compiled, these variables have strong implications for the resiliency of care-providing organisms in the face of ecosystem change; increasing predator densities associated with urban development and invasive species are cited to have non-lethal ecological consequences on the behaviours of prey and affect reproductive success through nest predation (Lima 1998; Ludwig *et al.* 2012). The findings in this study suggest that increased brood predator density could have an additive, direct fitness cost as brood abandonment may occur independent of a nest predation event. The framework put forth here addresses the lack of empirical evidence as to how different factors, when examined concurrently, affect fitness (Zera & Harshman 2001; Schreck 2010), and provides further implications for the use of direct fitness measures for future studies into the cost of parental care.

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