

# The role of progeny quality and male size in the nesting success of smallmouth bass: integrating field and laboratory studies

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**Abstract** Smallmouth bass display size-specific variation in reproductive success with larger brood-guarding males in a population more likely to rear offspring to independence than smaller individuals. The exact mechanisms responsible for this size-specific increase in reproductive output have yet to be identified. To assist in this process, we investigated the relationship between the size of brood-guarding male smallmouth bass and offspring quality (in this case, egg physiology, egg morphology, egg size, hatching success and lab survival). Further, we examined how factors such as egg physiology, egg morphology and egg size influenced reproductive success in the wild and hatching success in a controlled laboratory environment. Nesting male smallmouth bass that successfully reared their offspring to independence spawned earliest in the nesting period were the largest individuals, and guarded eggs with greater concentrations of cortisol compared to males that abandoned their offspring prematurely. Offspring survival in the laboratory was not correlated

with offspring survival in the wild, indicating that caution should be used interpreting studies that attempt to relate laboratory-derived survival metrics to the wild. Together, results demonstrate size-specific differences in offspring quality for nesting smallmouth bass, which are correlated with higher concentrations of cortisol in eggs. However, hatching success under laboratory conditions was dissimilar to nesting success in the field relative to cortisol concentrations.

**Keywords** Reproductive success · Egg quality · Cortisol · Year class

## Introduction

For many fish species, mortality during the egg and young embryo stages represent one of the most significant determinants of year class strength. Mortality events during egg and embryo stages can result from a variety of density-dependent and density-independent factors that include temperature change, predation, and timing of reproduction (Cushing 1974; Rice et al. 1993; Cushing and Horwood 1994). For smallmouth bass (*Micropterus dolomieu*), mortality during the larval period has been defined as a bottleneck that can partially influence year class formation (DeVries et al. 2009). Further, year class strength in smallmouth bass is believed to be heavily influenced by the size structure of the adult population, with fitness advantages offered to larger individuals

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(Suski and Ridgway 2007; DeVries et al. 2009). Smallmouth bass appear to mate selectively by size, with larger females preferring to mate with larger males. Paternal nest guarding can last for more than 4 weeks (Ridgway et al. 1991).

Premature abandonment (and thus reproductive failure) by a male prior to brood independence can occur following a number of biotic and abiotic challenges that include storm events, temperature fluctuations, and predation (DeVries et al. 2009). Nesting success occurs in roughly 50% of the males who guard nests each year; however, annual variation can be dramatic ranging from 10 to 90% nesting success (Suski and Ridgway 2007). Offspring from early spawning pairs in a population, (typically the largest parents), are believed to receive an advantage relative to later-spawned (smaller) individuals via an advanced transition to consuming nutrient-rich piscine prey, which offers accelerated growth and nutrient accumulation (Neves 1975; Suski and Ridgway 2007; DeVries et al. 2009). However, work using molecular techniques to identify the genetic origin of fall young-of-the-year smallmouth bass showed that recruitment was driven by a disproportionate contribution of offspring from a small number of spawning pairs within the population, and neither paternal size nor timing of spawning could explain differences in reproductive output within the population (e.g., Gross and Kapuscinski 1997). Thus, mechanisms defining year class formation and fitness for smallmouth bass have not been clearly defined and, in some cases, are incongruous. Such discrepancies and uncertainties suggest that the size of parents may be partially responsible for year class formation, with larger individuals having a fitness advantage, but additional factors (for example, potential influence of non-genetic and/or maternal factors) may play a role in influencing year class formation (Bonduriansky and Day 2009; Wolf and Wade 2009).

Non-genetic (maternal) inheritance involves the vertical transmission of factors other than DNA across generations and can influence phenotypic traits, population dynamics, and fitness for a range of organisms (Bonduriansky and Day 2009). Of particular importance is the degree to which maternal phenotype can influence offspring characteristics and/or quality (Wolf and Wade 2009). The quality of eggs and larvae, in terms of size, nutrient allocation, and hormonal inputs, can vary across females for many

fish species (reviewed in Brooks et al. 1997). Further, this non-genetic variation in quality can have pronounced implications for survival and year class formation (De Jesus et al. 1991; Hwang et al. 1992). In some species, egg characteristics such as lipid concentration, quantity of stress hormones (e.g., cortisol), size, morphology, hatching success, and survivability can impact offspring survival and performance, with many of these characteristics arising from maternal contributions (Sampath-Kumar et al. 1993; Lam 1994; Contreras-Sanchez et al. 1998). Non-genetic (maternal) inheritance through differential allocation to offspring (Sheldon 2000) combined with variation in offspring quality may therefore play a role in observed size-specific differences in larval survival for large male smallmouth bass relative to smaller individuals and may also be a factor driving the disproportionate contribution of offspring to a year class from few individuals within a population (Gross and Kapuscinski 1997; Suski and Ridgway 2007; DeVries et al. 2009).

Based on the number of factors that appear to influence offspring quality and, ultimately brood success in fishes, the objectives of the current study were to (a) quantify the relationship between the size of brood-guarding male smallmouth bass and offspring quality, and (b) relate differences in offspring quality to both reproductive success in the wild and hatching success in a controlled laboratory environment. Due to the elusive nature of wild female smallmouth bass and their abbreviated role in a reproductive bout, we were unable to directly quantify female characteristics other than eggs collected from nests. We predict that larger nesting male smallmouth bass will guard eggs that are larger and contain higher quantities of energy stores due to differential female maternal allocation, and their eggs will exhibit increased hatching success under controlled laboratory conditions.

## Materials and methods

### Field data collection

Thirty-seven nesting male smallmouth bass guarding newly spawned eggs (<2 days old) were located by snorkelers in Lake Opinicon (South Eastern Ontario, Canada, 44°33′55.34″N; 76°19′26.48″W) between

May 9th and May 19th, 2008. Because we were particularly interested in testing hypotheses concerning offspring quality related to the size of the parental male, we intentionally sought nesting males that spanned a range of sizes (Table 1; sizes were normally distributed, as confirmed by Shapiro–Wilk  $W$  Test goodness of fit test). When a male was discovered by the snorkeler, they were marked with a uniquely numbered white polyvinyl chloride (PVC) tag, and spawn date was recorded. To generate a relative spawning order, the first day of egg deposition within our population was assigned a spawn date of one, and the relative spawn date of all subsequent spawning events in the study was numbered in relation to this spawning date.

Each brood-guarding male selected for the study was angled by the snorkeler using conventional angling gear and taken to a nearby boat where it was weighed to the nearest gram and measured for total length (nearest mm). The number of eggs in each nest was then determined by following Raffetto et al. (1990), where the snorkeler estimated the number of eggs in each nest by extrapolating from counts in 3, randomly selected 2 cm  $\times$  2 cm grid cells. The snorkeler then collected approximately 100 eggs from the nest with a turkey baster and transferred the eggs to a glass jar filled with lake water collected at the nest site. The glass was placed in a closed cooler until returned to the aquatic research facility at the Queen's University Biology Station (QUBS) for processing

(within approximately 3 h). During these procedures, the snorkeler defended the brood, until the males returned and resumed nest defense. A weather monitoring station at QUBS, which recorded lake temperature every 60 min (Campbell Scientific thermocouple probes, Model 105T; [www.campbellsci.com](http://www.campbellsci.com)) indicated that, on the days of egg collections at the time of day when eggs were collected, mean surface water temperatures were  $17.3 \pm 0.07^\circ\text{C}$  (standard error, S.E.).

Snorkelers monitored the development of broods in Lake Opinicon by visiting each tagged nest every 3–4 days for 4–5 weeks. During these nest checks, snorkelers recorded whether the male was present or if he had prematurely abandoned his brood. These regular visits continued for approximately 4 weeks, at which time broods had developed to the brown fry stage (advanced fry that were approximately 4–5 weeks old that had begun to swim near the nest area). The male was considered to have successfully reared his brood to independence once offspring developed into the brown fry stage (Ridgway and Friesen 1992; Suski et al. 2003).

#### Sample processing and hatching success

At QUBS, three aliquots of 20 eggs were blot dried on a paper towel, placed in 1.5-ml microcentrifuge tubes and immediately frozen in liquid nitrogen for laboratory analysis of physiological parameters (described

**Table 1** Minimum, maximum, mean and standard error values for different characteristics of spawning smallmouth bass, and smallmouth bass eggs, sampled from  $N = 37$  nest in Lake Opinicon, Ontario

Parameter	$N$	Minimum	Maximum	Mean	Standard error
Relative spawn date	37	1	11	3	0.5
Number of eggs in nest	37	851	9,744	3,295	293
Male mass (g)	37	155	1,412	726	53
Male total length (mm)	37	234	464	369	9
Egg mass (mg)	31	4.94	9.20	6.93	0.16
# Eggs that hatched in lab (out of 20)	37	0	20	12	1
Volume of egg ( $\mu\text{l}$ )	37	5.05	9.30	6.95	0.16
Volume of oil droplet ( $\mu\text{l}$ )	37	0.40	1.27	0.75	0.03
% Volume of egg oil droplet	37	7.4	16.7	10.8	0.4
Triglycerides ( $\text{mmol l}^{-1} \text{egg}^{-1}$ )	31	0.28	0.73	0.42	0.02
Cortisol ( $\text{pg ml}^{-1} \text{egg}^{-1}$ )	30	2.15	17.64	7.44	0.56

Approximately 7 eggs per nest (ranged from 5 to 20 eggs depending on parameter) were sampled to generate values. These variables were used to generate the principle components uses in the PCA model shown in Table 2

below). An additional 10 eggs were blot dried and weighed on an analytical balance to the nearest 1.0 mg to generate a mean egg mass for each nest. In addition, a subset of eggs was used to quantify hatching success for each nest. For this, 20 eggs were first transferred to a glass jar filled with 800 ml of ambient Lake Opinicon water. This glass jar with eggs was placed in a water bath filled to cover the lower 75% of each jar, and the water bath was outfitted with a temperature controlled, submersible heater (Clecco Cleveland Process Corporation 0716-18 IS-H) set to 18°C ( $\pm 1^\circ\text{C}$ ). This water temperature was chosen as an approximate mean water temperature that offspring would be exposed to during their development in the lake, verified by the automated temperature logging system described above. A low-pressure blower provided aeration through an air stone placed approximately 1 cm below the water's surface of each jar, and approximately 50% of the water in each jar was exchanged daily with fresh Lake Opinicon water to prevent build-up of waste. Each jar was examined twice daily to quantify the number of eggs that had hatched (defined by the presence of a tail and/or locomotion of hatched larvae) and also to remove any dead eggs (identified by an opaque coloration and/or fungal growth). The hatching success experiment was terminated once all eggs had hatched or died.

#### Egg morphology

To quantify the size of eggs and oil droplets, Image Pro 4.0 software (Media Cybernetics, Inc., Bethesda MD) was used to determine the radius of five eggs per nest under a digital dissection microscope (10 $\times$  power), and two radii (horizontal and vertical) measured for each egg. Radii values were averaged to calculate a mean radius, which was subsequently used to generate a volume for each egg. The same technique was used to obtain a measure of mean oil droplet volume for each egg.

#### Survivability

To determine laboratory survival, approximately 20 egg sac fry (ESF) were removed from 28 of the original 37 nests (nine nests experienced premature abandonment by the male prior to rearing broods to the ESF stage). These 20 ESF from each nest were placed in glass jars filled with ambient Lake Opinicon water

and were also transferred to the aquatic facility at QUBS where they were held in a manner identical to egg holding described above. Egg sac fry were checked twice daily to quantify mortality (defined as immobility, loss of coloration, and the inability to maintain equilibrium), and the time (hours) for each jar (nest) to reach 50% mortality was determined.

#### Brown fry energetics

Twenty brown fry were collected from those nests where males were successful at raising offspring to this stage ( $N = 12$ ). These fry were collected in the same fashion as the egg collection (see above), with the only modification being dip nets were used to collect specimens. Collected brown fry were transported to QUBS where they were euthanized by cervical dislocation (followed by pithing) and immediately frozen in liquid nitrogen for subsequent lipid analysis at the University of Illinois.

#### Physiological parameters of eggs and brown fry

Following the homogenization of 3–5 eggs in 245  $\mu\text{l}$  of 5% triton-X with a hand-held homogenizer, the concentration of triglycerides in eggs was determined enzymatically using a commercially available kit (BioAssay Systems, Hayward, CA, Cat# ETGA-200) and a microplate spectrophotometer (Molecular Devices, Spectra Max Plus 384, Model # 05362, Union City, CA). The concentration of cortisol in eggs was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from Assay Designs (Enzo Life Sciences) (Plymouth Meeting PA; Kit ADI-900-071) following the homogenization of 15–20 eggs in 200  $\mu\text{l}$  of assay buffer provided in the kit, and passing the resulting supernatant through filtered microcentrifuge tubes (pore size 0.65  $\mu\text{m}$ ). This product has low cross-reactivity with other hormones produced by fishes that could interfere with binding (Assay Designs Kit #900-071 Insert). In addition, because a number of eggs per nest were pooled for determination of cortisol concentrations, all data generated fell within the acceptable limits of the standard curve for the assay once preliminary dilution curves were generated. Cortisol concentration was determined using a logistic curve fitting program provided by the manufacturer after the dilution factors and number of eggs per sample had

been factored into calculations ( $\text{pg ml}^{-1} \text{egg}^{-1}$ ). The concentration of whole-body lipid in brown fry was determined following methods described in Bligh and Dyer (1959) after first grinding whole fry under liquid nitrogen and drying the resulting powder at  $80^\circ\text{C}$ .

### Statistical analyses

A *t* test was used to compare the size of males that successfully reared their broods to the brown fry stage with males that prematurely abandoned their broods prior to the brown fry stage. Two regressions were used to assess the relationships between male size (independent), and relative spawn date and the number of eggs in each male's nest (dependent variables) (Zar 1999). These three characteristics were left out of the PCA analyses since they have been previously found to be correlated with male size.

A multivariate principal component analysis (PCA) was used to relate characteristics of brood-guarding males and their eggs. This statistical test was used because of potential biases and shortcomings with stepwise multiple regressions, with particular emphasis on complex data, including data sets with many continuous response variables (Hurvich and Tsai 1990; Stephens et al. 2005; Whittingham et al. 2006). For the PCA, relative spawn date, the estimated number of eggs in a nest, egg mass, proportional laboratory egg hatching success, egg volume, oil droplet volume, percent volume of oil droplet, concentration of triglycerides in eggs, and egg cortisol concentrations were used in this model. The time required for egg sac fry to reach 50% mortality in the laboratory, as well as the concentration of lipids in brown fry, had low sample sizes due to premature nest abandonment by brood-guarding males and were excluded from PCA models to facilitate analyses. Principal factors with eigenvalues greater than 1 were used to determine the relationship between the two independent variables: male size and abandonment (probability of rearing offspring to brown fry stage) and subjected to varimax factor rotation, which maximizes the amount of variation explained by each factor (Kaiser 1960; Hanson et al. 2007). Factors with eigenvectors greater than 0.4 or less than  $-0.4$  were used to characterize each principal component (Kaiser 1960). These rotated principle components were regressed (simple linear regression) against male size to determine the relationship between egg/nest

characteristics and male size. Further, principle component scores were compared between males that successfully reared their broods to the brown fry stage and males that abandoned their broods prior to reaching the brown fry stage.

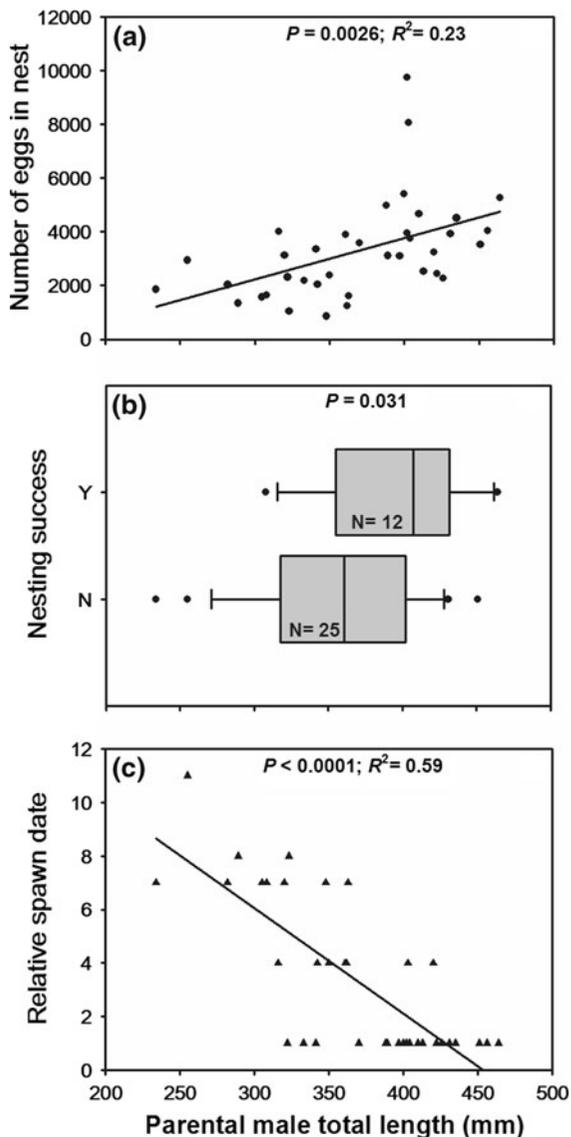
All statistical analyses were performed using JMP version 7.0 (SAS Institute, Cary, NC). The level of significance ( $\alpha$ ) for all tests was 0.05 (Zar 1999). All values are reported as means  $\pm$  S.E. where appropriate.

### Results

Summary statistics for variables associated with spawning characteristics of nesting male smallmouth bass, as well as with egg characteristics, are outlined in Table 1. Egg mass varied almost twofold across nests; oil droplet volume and triglycerides varied approximately threefold, while cortisol concentration per egg ranged almost eightfold across all nests, highlighting the variability in egg characteristics across smallmouth bass nests and males.

The total length of nesting smallmouth bass used in this study ranged from 234 to 464 mm (mean =  $369 \pm 9$  mm). The estimated number of eggs in each nest ranged from 851 to 9,744 (mean =  $3,295 \pm 293$ ) and was positively related to male size (linear regression,  $P = 0.0026$ ,  $R^2 = 0.23$ , Fig. 1a). The 25 male smallmouth bass that abandoned their broods prematurely (mean total length = 355 mm) were on average 11% smaller than the 12 males that successfully reared their broods to independence (mean total length = 398 mm) ( $t_{35} = -2.26$ ,  $P = 0.0305$ , Fig. 1b). Larger males also spawned earlier in the season than did smaller males (linear regression,  $P < 0.0001$ ,  $R^2 = 0.59$ , Fig. 1c).

Principal component analysis produced four factors with eigenvalues greater than 0.4 or less than  $-0.4$ , which described 81.3% of the variance in the egg variables examined (Table 2). Principal Component 1 (PC1) accounted for 37.4% of the model variance and was characterized by high positive factor loadings for the number of eggs in a nest, total volume of oil droplet, and percent volume of oil droplet, and negatively for spawn date (Table 2). Therefore, PC1 represented characteristics of maternal fecundity and inputs of nutrients. Egg mass, egg volume, and oil droplet volume had high positive factor loadings for



**Fig. 1** Relationship between size of parental male smallmouth bass and **a** number of eggs received by the male in a mating, **b** whether or not the parental male successfully reared his brood to independence during the spawning season (where Y = yes and N = no not successful), and **c** relative order of spawning within an individual spawning season. Results of statistical analyses are reported on figures, and data were analyzed using **a** regression, **b** *t* test, and **c** regression. Thirty-seven nests were used to generate each *panel*

principal component two (PC2) (Table 2). This factor accounted for 19.1% of the model variance and mainly described egg morphology and size. Principal component three (PC3) accounted for 12.8% of the variance and was negatively related to spawn date,

and hatching success and positively correlated with cortisol concentration in eggs (Table 2). Principal component 4 (PC4) was characterized by a positive weighting for egg triglyceride concentration and accounted for 12.0% of variance (Table 2).

Of the principal components generated, only PC1 and PC3 were related to male size, with both scores increasing with the size of the brood-guarding male (Table 3). The other two principal components (PC2 and PC4) did not correlate with male size or male nesting success in the wild ( $P > 0.05$ , Table 3). In addition, only PC3 differed significantly between males that successfully reared broods to independence and those that abandoned nests prematurely, with males that successfully reared their broods to independence having significantly higher PC3 scores than males that abandoned prematurely (Table 3; Fig. 2).

Twenty-eight of the original 37 males reared their offspring to the egg sac fry stage. However, only 12 males successfully guarded broods that reached the brown fry stage. Due to these combined small sample sizes, and because of potential problems with blank data in PCA (McGarigal et al. 2000), brown fry lipid concentration and egg sac fry time to 50% mortality were left out of the PCA and regressed separately. Neither of these two variables were significantly correlated with male size ( $N = 28$ ,  $P = 0.17$  and  $N = 12$ ,  $P = 0.013$ , respectively).

The time (in hours) required for egg sac fry to reach 50% mortality in the laboratory was not correlated with nest success in the wild ( $P = 0.48$ ). In addition, the duration of time (in hours) for egg sac fry to reach 50% mortality in the laboratory varied by approximately 13.5% between nests (mean  $499 \pm 3.4$  h or almost 21 days, range 462–534 h).

## Discussion

For smallmouth bass in the current study, brood-guarding males that successfully reared offspring to the brown fry stage were shown to be the largest individuals, and those that spawned earlier in the nesting period. Previous work with smallmouth bass has demonstrated that larger males in a population spawn earlier in a year, likely due to elevated lipid stores relative to smaller conspecifics upon emergence from winter (Mackereth et al. 1999), or because both large males and large females are reproductively

**Table 2** Principle components summarizing egg and spawning characteristics for nesting male smallmouth bass

	PC1	PC2	PC3	PC4
<i>Parameters</i>				
Spawn date	<b>-0.63</b>	-0.10	<b>-0.68</b>	0.096
Number of eggs in nest	<b>0.78</b>	-0.11	0.14	-0.22
Egg mass (g)	0.0070	<b>0.83</b>	0.36	-0.22
Egg hatching success	0.29	-0.36	<b>-0.53</b>	-0.30
Egg volume ( $\mu$ l)	0.24	<b>0.87</b>	0.057	0.015
Volume of oil droplet ( $\mu$ l)	<b>0.79</b>	<b>0.57</b>	-0.038	0.14
Volume of oil droplet (%)	<b>0.86</b>	0.23	-0.049	0.13
Egg triglycerides (mmol/l/egg)	0.0046	-0.10	0.040	<b>0.93</b>
Egg cortisol (pg/ml/egg)	0.081	0.12	<b>0.91</b>	0.0035
% Variance explained	37	19	13	12
Eigenvalue	3.4	1.7	1.2	1.1

Variables were loaded into four principle components (PC1, PC2, PC3, and PC4). Characteristics contributing maximally to each principle component are in bold and are greater than 0.4 or less than -0.4

**Table 3** Results from regression analysis and *t* tests relating either size of male, or nesting success, with principle component scores (PC1–PC4) for components identified in Table 2

Independent variable	Response variable	$R^2$	<i>N</i>	<i>F</i> or <i>T</i> stat.	<i>P</i> value
Male total length (mm)	PC1	0.18	25	5.1	0.034*
	PC2	<0.001	25	0.006	0.94
	PC3	0.41	25	15	0.001*
	PC4	<0.001	25	0.20	0.66
Nest success in the wild	PC1	N/A	25	-0.86	0.64
	PC2	N/A	25	-3.1	0.27
	PC3	N/A	25	-1.1	0.005*
	PC4	N/A	25	0.48	0.40

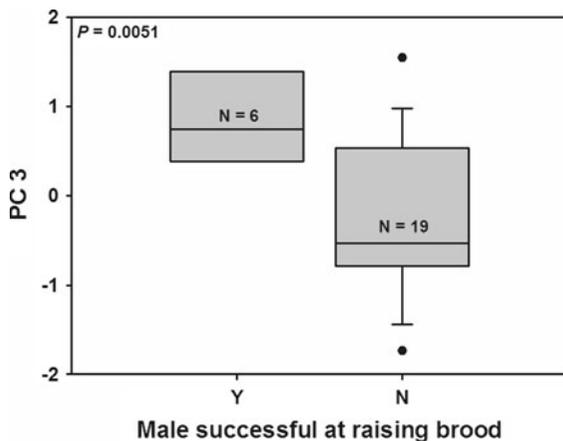
A nest was said to be successful if the attending male raised his brood to the brown fry stage (approximately 4–6 weeks after egg deposition)

\* Indicates a *P* value less than 0.05

active earlier in the spring relative to smaller conspecifics (Wiegmann et al. 1992). A positive relationship between male size and quantity of eggs in a nest has also been reported previously for smallmouth bass (Suski and Ridgway 2007; Suski and Philipp 2004). Next to climate influence, male size has been identified as the second most important factor in predicting male nesting success (Suski and Ridgway 2007). It has been hypothesized that the positive relationship between male size and egg quantity results from larger, more fecund, females choosing to mate with larger nesting males, which is partially driven by elevated energy stores in larger males relative to

smaller individuals following winter (Ridgway and Friesen 1992). Increased energy stores following winter may also provide larger males with additional fuel to sustain parental care activities relative to smaller males that are more likely to prematurely abandon their broods prior to independence (Mackereth et al. 1999; Suski and Ridgway 2007; Hanson and Cooke 2009).

In the current study, reproductive success of nesting smallmouth bass was also correlated with elevated concentrations of cortisol in the eggs of larger nesting males, representing another factor that might contribute to nesting success of larger individuals relative to



**Fig. 2** Principle Component 3 (PC3) score for nesting male smallmouth bass that were successful at rearing their broods to independence compared to males that abandoned broods prematurely. PC3 scores were compared using a *t* test and the *P* value is shown on the panel. Sample sizes are shown on individual bars

smaller conspecifics. Cortisol has been found in the eggs of many fish species and is added during maternal oogenesis (De Jesus et al. 1991; Hwang et al. 1992; Contreras-Sanchez et al. 1998). Cortisol is the primary stress hormone in fishes and is released to help maintain homeostasis and mineral balance and to regulate growth and energy stores during environmental challenges (Wendelaar Bonga 1997; Mommsen et al. 1999). The presence of cortisol in eggs has previously been shown to exert both positive and negative impacts on the quality and survival of fish offspring, highlighting that the specific role of cortisol in developing fish embryos has yet to be clearly defined (De Jesus et al. 1991). For example, cortisol can enhance the stimulatory action of thyroid hormones in larval fish (De Jesus et al. 1990), increase larval survival in Asian sea bass (*Lates calcarifer*) (Sampath-Kumar et al. 1993) and, in Mozambique tilapia (*Tilapia mossambica*), stimulate larval growth (Lam 1994), all suggesting a beneficial impact of cortisol for developing fishes. In contrast, McCormick (2006) found that female damselfish (*Pomacentrus amboinensis*) breeding in higher densities had higher egg cortisol concentrations and subsequently reduced larvae size, suggesting a negative impact of egg cortisol concentration on fitness. For smallmouth bass in the current study, large males that spawn early in the nesting cycle and guard broods higher concentrations of cortisol in their eggs are more likely to successfully

rear broods to independence. Therefore, hormonal benefits, manifested in increased concentrations of egg cortisol, might have provided larger males superior eggs (those with greater cortisol) and contributed to improved nesting success of larger male smallmouth bass relative to smaller conspecifics.

Many variables that have previously been shown to be important factors defining egg and offspring quality for fishes did not appear to influence reproductive success of male smallmouth bass. Although PC1 and PC2 showed high factor loadings for characteristics such as egg size, morphology, and oil droplet volume, only PC1 correlated with male size, and neither of these components varied significantly across males that reared their broods to independence compared to males that prematurely abandoned their nests. Offspring from larger male smallmouth bass also had similar concentrations of lipids in brown fry and time required to reach 50% mortality when compared to smaller nesting males. Furthermore, PC4 loaded heavily for the concentration of triglycerides in eggs; however, this principle component was not correlated with male size or nesting success. Together, these egg morphology and presumably, physiological differences did not appear to influence reproductive success in male smallmouth bass.

Interestingly, older, and larger, female rockfish (*Sebastes melanops*) produce offspring that grow three times as fast and survive starvation longer than the offspring of younger females as the eggs from older females have larger energy-rich oil droplets (Berkeley et al. 2004). Faster larval growth, like that seen in offspring produced by older female rockfish, can provide a locomotory advantage that enhances larval predator avoidance and prey capture (Blaxter 1986; Bailey and Houde 1989) and is often attributed to higher maternal quality (Leggett and Deblois 1994). Another egg characteristic that can positively influence offspring survival is egg size (Hendry et al. 2001). In Chinook salmon (*Oncorhynchus tshawytscha*), for example, egg size can vary up to 300% across different females, with the largest eggs demonstrating increased hatching success (Rombough 1985). For the eggs of smallmouth bass, despite 2–3-fold differences in factors such as size, oil droplet, and triglyceride concentrations across individuals, we found no evidence that these factor influenced either hatching success in the lab, or nest success in the field.

Variation in the relative importance of egg quality characteristics between salmonids and smallmouth bass might be related to the parental care provided by smallmouth bass. Semelparous fish such as salmon from the genus *Oncorhynchus* do not invest in offspring following nest site selection and egg deposition, potentially necessitating elevated pre-hatching investment to ensure survival. Smallmouth bass, however, guard their broods from predators following egg deposition (Hinch and Collins 1991; Ridgway et al. 1991), and this provisioning by the attending male may compensate for a lack of maternal resources such as triglycerides compared to salmonids. This brood-guarding behavior has also been shown to define reproductive success for this species (Suski and Ridgway 2007). Together, traditional egg quality characteristics such as egg size, triglycerides, and the volume of oil droplet vary across different nesting male smallmouth bass but are not correlated with the size of a brood-guarding male and further do not appear to influence the likelihood of a male rearing his brood to independence.

While the concentration of cortisol in smallmouth bass eggs was positively correlated with the probability of a male guarding his brood to independence, cortisol concentration (and hence male size) did not influence hatching success in the laboratory portion of the study. In addition, survival of offspring in the laboratory was not related to nest success in the wild, further demonstrating discrepancies between lab-based metrics and those in the field. Knotek and Orth (1998) suggested that high egg mortality in smallmouth bass nests, particularly in nests of large males that have high numbers of eggs, may result from density-dependent factors resulting from high numbers of eggs (e.g., accumulation of fungus). The conditions used as part of our laboratory study may have maintained eggs in sufficiently low numbers common across all nests to prevent density-dependent impacts from occurring, thereby precluding the observation of high mortality rates for larger individuals. Alternatively, benefits associated with cortisol concentration in eggs might only become apparent under fluctuating or adverse biotic/abiotic conditions in the field, such as temperature change and/or the presence of fungal infections, conferring survival advantages in the wild relative to stable laboratory conditions. Additionally, Marshall et al. (2010) defined potential problems associated with studies that rear offspring

from different mothers in common environments, which may remove environmental variability that can allow survival of different phenotypes in the wild. However, despite the observed increases in daily egg mortality in nests from large males, Knotek and Orth (1998) reported that large parental males with higher egg numbers still maintained larger broods throughout parental care and contributed a higher proportion of juveniles than males with smaller egg numbers, indicating a proficiency for large males to successfully contribute offspring relative to smaller individuals.

Physiological constraints, including low cortisol concentrations in eggs, might also be a plausible explanation for observed egg mortalities in smaller parental smallmouth bass, although this possibility was not investigated by earlier studies. Laboratory and field results in the current study become increasingly important when the number of previous investigations that have documented elevated hatching success under controlled laboratory or aquaculture conditions without complimentary field observations are considered (e.g., Hwang et al. 1992; Sampath-Kumar et al. 1993; Schultz et al. 1996; Contreras-Sanchez et al. 1998). Discrepancies between physiological parameters observed in the lab and field have recently been reported by Sloman et al. (2008) who concluded that physiological correlates of dominance exist among wild salmonid populations, but factors that define dominance in the wild may differ from factors controlling dominance in the laboratory. Regardless of the mechanism, the current study showed that hatching success for smallmouth bass eggs in the laboratory was negatively correlated with cortisol concentration, despite the fact that these eggs came from nests that were more likely to be reared to independence in the field.

For smallmouth bass, reproductive output within a spawning event might be influenced through large males receiving many high quality eggs in their nest due to differential female allocation of resources to eggs, particularly the deposition of cortisol. Larger male smallmouth bass also have increased energy stores to help fuel nest guarding behaviors, and thus, have an increased ability to provide parental care for extended periods (Suski and Ridgway 2007). This combination of factors may partially explain the size-selective advantage in reproductive output of larger smallmouth bass relative to smaller individuals. Future studies should attempt to define mechanisms

responsible for the relationship between size and cortisol in this species, the results of which can help define reproductive success and year class strength for this species.

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## References

- Bailey KM, Houde ED (1989) Predation on eggs and larvae of marine fishes and the recruitment problem. *Adv Mar Biol* 25:1–83. doi:10.1016/S0065-2881(08)60187-X
- Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in marine fish, *Sebastes melanops*. *Ecology* 85:1258–1264. doi:10.1890/03-0706
- Blaxter JHS (1986) Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. *Trans Am Fish Soc* 115:98–114. doi:10.1577/1548-8659(1986)115<98:NLFCDO>2.0.CO;2
- Bligh EG, Dyer WJ (1959) A rapid method for total lipid extraction and purification. *Can J Biochem Physiol* 37:911–917
- Bonduriansky R, Day T (2009) Nongentic inheritance and its evolutionary implications. *Annu Rev Ecol Evol Syst* 40:103–125. doi:10.1146/annurev.ecolsys.39.110707.173441
- Brooks S, Tyler CR, Sumpter JP (1997) Egg quality in fish: what makes a good egg? *Rev Fish Biol Fish* 7:387–416. doi:10.1023/A:1018400130692
- Contreras-Sanchez WM, Schreck CB, Fitzpatrick MS, Pereira CB (1998) Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). *Biol Reprod* 58:439–447. doi:10.1095/biolreprod58.2.439
- Cushing DH (1974) The natural regulation of fish populations. In: Harden Jones FR (ed) *Sea fisheries research*. Elek Science, London, pp 399–412
- Cushing DH, Horwood JW (1994) The growth and death of fish larvae. *J Plankton Res* 16:291–300
- De Jesus EG, Inui Y, Hirano T (1990) Cortisol enhances the stimulating action of thyroid hormones on dorsal fin-ray resorption of flounder larvae in vitro. *Gen Comp Endocrinol* 79:167–173. doi:10.1016/0016-6480(90)90101-Q
- De Jesus EG, Hirano T, Inui Y (1991) Changes in cortisol and thyroid hormone concentrations during early development and metamorphosis in the Japanese flounder, *Paralichthys olivaceus*. *Gen Comp Endocrinol* 82:369–376. doi:10.1016/0016-6480(91)90312-T
- DeVries DR, Garvey JE, Wright RA (2009) Early life history and recruitment. In: Cooke SJ, Philipp DP (eds) *Centrarchid fishes, diversity, biology and conservation*. Blackwell Publishing, West Sussex, UK, pp 105–133
- Gross ML, Kapuscinski AR (1997) Reproductive success of smallmouth bass estimated and evaluated from family-specific DNA fingerprints. *Ecology* 78:1424–1430. doi:10.1890/0012-9658(1997)078[1424:RSOSBE]2.0.CO;2
- Hanson KC, Cooke SJ (2009) Why does size matter? A test of the benefits of female mate choice in a teleost fish. *Physiol Biochem Zool* 82:617–624. doi:10.1086/605934
- Hanson KC, Hasler CT, Suski CD, Cooke SJ (2007) Morphological correlates of swimming activity in wild largemouth bass (*Micropterus salmoides*) in their natural environment. *Comp Biochem Physiol A Mol Integr Physiol* 148:913–920. doi:10.1016/j.cbpa.2007.09.013
- Hendry AO, Day T, Cooper AB (2001) Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *Am Nat* 157:387–407. doi:10.1086/319316
- Hinch SG, Collins NC (1991) Importance of diurnal and nocturnal nest defense in the energy budget of male smallmouth bass: insights from direct video observations. *Trans Am Fish Soc* 120:657–663. doi:10.1577/1548-8659(1991)120<0657:IODANN>2.3.CO;2
- Hurvich CM, Tsai CL (1990) The impact of model selection on inference in linear regression. *Am Stat* 44(3):214–217
- Hwang PP, Wu SM, Lin JH, Wu LS (1992) Cortisol content of eggs and larvae of teleosts. *Gen Comp Endocrinol* 86:189–196. doi:10.1016/0016-6480(92)90101-O
- Kaiser HF (1960) The application of electronic computers to factor analysis. *Educ Psychol Meas* 20:141–151. doi:10.1177/001316446002000116
- Knotek WL, Orth DJ (1998) Survival for specific life intervals of smallmouth bass, *Micropterus dolomieu*, during parental care. *Environ Biol Fish* 51:285–296. doi:10.1023/A:1007497401717
- Lam TJ (1994) Hormones and egg/larval quality in fish. *J World Aquac Soc* 25:2–12. doi:10.1111/j.1749-7345.1994.tb00798.x
- Leggett WC, Deblois E (1994) Recruitment in marine fishes—is it regulated by starvation and predation in the egg and larval stages. *Neth J Sea Res* 32:119–134. doi:10.1016/0077-7579(94)90036-1
- Mackereth RW, Noakes DLG, Ridgway MS (1999) Size-based variation in somatic energy reserves and parental expenditure by male smallmouth bass, *Micropterus dolomieu*. *Environ Biol Fish* 56:263–275. doi:10.1023/A:1007580323816
- Marshall DJ, Heppell SS, Munch SB, Warner RR (2010) The relationship between maternal phenotype and offspring

- quality: do older mothers really produce the best offspring? Ecology 91:2862–2873. doi:[10.1890/09-0156.1](https://doi.org/10.1890/09-0156.1)
- McCormick MI (2006) Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish. Ecology 87:1104–1109. doi:[10.1890/00129658\(2006\)87\[1104:MMCLTS\]2.0.CO;2](https://doi.org/10.1890/00129658(2006)87[1104:MMCLTS]2.0.CO;2)
- McGarigal K, Cushman S, Stafford SG (2000) Multivariate statistics for wildlife and ecology research. Springer, New York
- Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanism of action, and metabolic regulation. Rev Fish Biol Fish 9:211–268. doi:[10.1023/A:1008924418720](https://doi.org/10.1023/A:1008924418720)
- Neves RJ (1975) Factors affecting fry production of smallmouth bass (*Micropterus dolomieu*) in South Branch Lake, Maine. Trans Am Fish Soc 104:83–87. doi:[10.1577/15488659\(1975\)104<83:FAFPOS>2.0.CO;2](https://doi.org/10.1577/15488659(1975)104<83:FAFPOS>2.0.CO;2)
- Raffetto NS, Baylis JR, Serns SL (1990) Complete estimates of reproductive success in a closed population of smallmouth bass (*Micropterus dolomieu*). Ecology 71:1523–1535. doi:[10.2307/1938289](https://doi.org/10.2307/1938289)
- Rice JA, Miller TJ, Rose KA, Crowder LE, Marschall EA, Trebitz AS, DeAngelis DL (1993) Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Can J Fish Aquat Sci 50:133–142
- Ridgway MS, Friesen TG (1992) Annual variation in parental care in smallmouth bass (*Micropterus dolomieu*). Environ Biol Fishes 35:243–255. doi:[10.1007/BF00001890](https://doi.org/10.1007/BF00001890)
- Ridgway MS, Shuter BJ, Post EE (1991) The relative influence of body size and territorial behaviour on nesting asynchrony in male smallmouth bass *Micropterus dolomieu* (Pisces: Centrarchidae). J Anim Ecol 60:665–681
- Rombough PJ (1985) Initial egg weight, time to maximum alevin wet weight, and optimal ponding times for Chinook salmon (*Oncorhynchus tshawytscha*). Can J Fish Aquat Sci 42:287–291. doi:[10.1139/f85-036](https://doi.org/10.1139/f85-036)
- Sampath-Kumar R, Munro AD, Lee J, Lam TJ (1993) Exogenous cortisol promotes survival of Asian seabass (*Lates calcarifer*) hatchlings exposed to hypersalinity but not hyposalinity shock. Aquaculture 116:247–255. doi:[10.1016/0044-8486\(93\)90012-N](https://doi.org/10.1016/0044-8486(93)90012-N)
- Schultz ET, Reynolds KE, Conover DO (1996) Countergradient variation in growth among newly hatched *Fundulus heteroclitus*: geographic differences revealed by common-environment experiments. Funct Ecol 10:366–374
- Sheldon BC (2000) Differential allocation: tests, mechanisms and implications. Trends Ecol Evol 15:397–402
- Sloman KA, Baker D, Winberg S, Wilson RW (2008) Are there physiological correlates of dominance in natural trout populations? Anim Behav 76:1279–1287. doi:[10.1016/j.anbehav.2008.06.012](https://doi.org/10.1016/j.anbehav.2008.06.012)
- Stephens PA, Buskirk SW, Hayward GD, Martinez del Rio C (2005) Information theory and hypothesis testing: a call for pluralism. J App Ecol 42:4–12. doi:[10.1111/j.1365-2664.2005.01002.x](https://doi.org/10.1111/j.1365-2664.2005.01002.x)
- Suski CD, Philipp DP (2004) Factors affecting the vulnerability to angling of nesting male largemouth bass and smallmouth bass. Trans Am Fish Soc 133:1100–1106. doi:[10.1577/T03-079.1](https://doi.org/10.1577/T03-079.1)
- Suski CD, Ridgway MS (2007) Climate and body size influence nest survival in a fish with parental care. J Anim Ecol 76:730–739. doi:[10.1111/j.1365-2656.2007.01242.x](https://doi.org/10.1111/j.1365-2656.2007.01242.x)
- Suski CD, Svec JH, Ludden JB, Phelan FJS, Philipp DP (2003) The effect of catch-and-release angling on the parental care behavior of male smallmouth bass. Trans Am Fish Soc 132:210–218. doi:[10.1577/1548-8659\(2003\)132<0210:TEOCAR>2.0.CO;2](https://doi.org/10.1577/1548-8659(2003)132<0210:TEOCAR>2.0.CO;2)
- Wendelaar Bonga SB (1997) The stress response in fish. Physiol Rev 77:591–625
- Whittingham MJ, Stephens PA, Bradbury RB, Freckleton RP (2006) Why do we still use stepwise modelling in ecology and behaviour? J Anim Ecol 75:1182–1189. doi:[10.1111/j.1365-2656.2006.01141.x](https://doi.org/10.1111/j.1365-2656.2006.01141.x)
- Wiegmann DD, Baylis JR (1995) Male body size and paternal behavior in smallmouth bass, *Micropterus dolomieu* (Pisces: Centrarchidae). Evolution 46:1740–1753
- Wiegmann DD, Baylis JR, Hoff MH (1992) Sexual selection and fitness variation in a population of smallmouth bass, *Micropterus dolomieu* (Pisces: Centrarchidae). Evolution 46:1740–1753
- Wolf JB, Wade MJ (2009) What are maternal effects (and what are they not)? Phil Trans R Soc B 364:1107–1115. doi:[10.1098/rstb.2008.0238](https://doi.org/10.1098/rstb.2008.0238)
- Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Englewood Cliffs, NJ