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Causes and consequences of voluntary anorexia during the parental care period of wild male smallmouth bass (*Micropterus dolomieu*)

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ABSTRACT

By definition, parental care behaviors increase offspring survival, and individual fitness, at some cost to the parent. In smallmouth bass (*Micropterus dolomieu*), parental males provide sole care for the developing brood that includes an increase in activity during brood defense and decreased foraging resulting in a decline in endogenous energy reserves. No mechanisms have been proposed for cessation of voluntary foraging, though regulation of appetite hormones such as ghrelin have been documented to affect feeding behavior in other fishes. We documented baseline fluctuations in plasma ghrelin concentrations across parental care. Plasma ghrelin concentrations were lowest during the early stages of parental care before increasing as the brood developed to independence. Additionally, we performed an intervention experiment whereby plasma ghrelin levels were artificially increased through an injection of rodent ghrelin at the onset of parental care. Despite measuring a significant increase in plasma ghrelin approximately 1 week after injection, we noted no differences in plasma-borne indicators of recent foraging activity indicating that voluntary anorexia is possibly reinforced by receptor insensitivity to appetite hormones. Finally, we assessed the ultimate consequences of foraging during parental care by feeding fish to satiation and measuring post-prandial changes in swimming performance and aggression. Fish fed to satiation showed significant decreases in burst swimming ability and aggressiveness towards potential brood predators. Voluntary anorexia during smallmouth bass parental care is an adaptive behavior that avoids potentially deleterious declines in swimming performance and aggression apparently through a modulation of production and reception of appetite hormones including ghrelin.

Introduction

Broadly defined, parental care behaviors are any investment into offspring after initial fertilization and serve the function of increasing offspring survival, typically at some cost to the parent (Williams, 1966; Trivers, 1972; Reynolds, 1996). As a behavior, parental care has evolved in numerous species in multiple taxa (Gross and Sargent, 1985; Reynolds, 1996; Møller and Cuervo, 2000; Mas and Kolliker, 2008) and various forms have been documented from simple behaviors such as concealment of fertilized eggs (Gross and Sargent, 1985) to highly complex behaviors such as extended internal gestation followed by feeding of offspring through lactation (Martin, 2007), and teaching of offspring for years (Thornton and Raihani, 2008). Often, concomitant with other parental costs such as decreased opportunity for mating (Magrath and Komdeur, 2003), care givers expend energy during care which can result in a decrease in condition of the parent (Coleman and Fischer, 1991; Smith and Wootton, 1999; Reynolds, 1996; Webb et al., 2002). Though trends in declining condition of parents have been documented, the mechanisms whereby organisms regulate energy utilization during parental care to maximize offspring survival and individual fitness are still largely unknown.

Teleost fish species exhibit a wide range of parental care behaviors (Blumer, 1982; Gross and Sargent, 1985). Of the various forms of parental care, smallmouth bass (*Micropterus dolomieu*) exhibit the most common teleost form, namely uniparental male care (Gross and Sargent, 1985). In spring, when water temperatures reach approximately 15°C, male bass construct nests (small, saucer shaped depressions) in the littoral zone which serves as the site of courtship and fertilization (Coble, 1975; Ridgway, 1988). Shortly after fertilization, the female departs and the male provides care for the developing brood in the form of protection from potential brood predators and maintenance of the nest to prevent silt deposition and to aerate the nest site (Coble, 1975; Ridgway, 1988). During this parental care

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period, which lasts until the brood is independent (typically ~1 month), male bass are highly active while defending the brood (Hinch and Collins, 1991; Cooke et al., 2002). Although the fish restrict their activity to a localized area (e.g., 10 m^2), they can swim as much as 41 km per day while defending the nest (Cooke et al., 2002). In addition, 20% of the time is spent with the male engaged in high intensity activity (i.e., >80% of critical swimming speed; Cooke et al., 2002). Concomitant with this increase in activity, males dramatically decrease foraging (Hinch and Collins, 1991) and suffer drastic declines in energy reserves and nutritional condition as endogenous resources are catabolized to power this activity (Mackereth et al., 1999; Cooke et al., 2006; Hanson and Cooke, 2009). Opportunistic feeding has been shown at very low levels that would not compensate for energy loss associated with parental care activities (Hinch and Collins, 1991; Steinhart et al., 2005), though manipulative experiments have revealed that smallmouth bass can be fed supplemental food while on the nest (Ridgway and Shuter, 1994). While cessation of foraging is a common, and presumably adaptive, feature of bass parental care, to date no research has clarified the proximate causes and ultimate consequences of voluntary anorexia during this time period.

Cessation of foraging behavior can be induced through modulation of various gut and brain hormones (Badman and Flier, 2005; Abizaid and Horvath, 2008). Amongst these appetite hormones, ghrelin has been previously noted to relate to feeding behavior and lipid deposition in a number of fish species (Unniappan et al., 2004; Unniappan and Peter, 2004; Matsuda et al., 2006; Shepherd et al., 2007; Kaiya et al., 2008). Particularly relevant to this study, ghrelin administration has been noted to stimulate food intake in multiple teleost fishes (Unniappan and Peter, 2004; Kaiya et al., 2008). Ghrelin also stimulates anabolic metabolism, principally the storage of lipids for later use as endogenous energy reserves (Riley et al., 2005; Unniappan and Peter, 2004; Kaiya et al., 2008), and increases in production of growth hormone (Unniappan et al., 2002; Unniappan and Peter, 2004; Kaiya et al., 2008). In the only study of the relationship between gut hormones and energy utilization during the reproductive period in fish, fluctuations in plasma ghrelin immuno-reactive peptide levels have been noted across the spawning period in burbot (*Lota lota*) whereby ghrelin levels were lowest prior to and during spawning (corresponding to decreased foraging) and increased after spawning during resumption of feeding (Mustonen et al., 2002). Since parental care in smallmouth bass is marked by a lack of foraging and catabolism of endogenous energy reserves, decreases in ghrelin production and receptor sensitivity could be the possible mechanism to induce this state.

In accordance with previous work on feeding and nutrition during parental care, we predicted that endogenous plasma ghrelin levels would be lowest during the egg and egg sac fry brood development stages (during times of restricted foraging and increased catabolic demands) and increase during the free swimming fry stage (indicating resumption of foraging by the parental male and increased lipid deposition). If we noted an increase in plasma-borne indicators of recent foraging (measures of dietary minerals, lipids, and protein) in fish treated with exogenous ghrelin, we predicted that parental male bass suppress ghrelin production to maintain a state of voluntary anorexia during parental care. However, if treatment with exogenous ghrelin produced no changes in nutritional status, parental bass would apparently be reducing responsiveness to stimulatory appetite hormones to cease foraging. Functionally, we predicted that cessation of foraging by nesting male bass is necessary to avoid decreases in swimming ability that would occur during digestion of prey items, thereby making a parental male unable to aggressively defend the brood from potential predators. In laboratory studies, researchers have documented that post-prandial blood flow to the gut increases which reduces swimming performance (Thorarensen and Farrell, 2006) and increases heart rate and oxygen consumption (Eliason et al., 2008). As such, we predict that fish which ingest high numbers of prey items should experience decreases in their ability to engage and chase brood predators after feeding.

Materials and methods

Baseline sampling of endogenous ghrelin across parental care

All procedures used in this study were developed with approvals and guidance from the Canadian Council on Animal Care administered by Carleton University and Queen's University. To determine natural appetite hormone fluctuation, sampling occurred from May 27th to June 12th, 2008 in a lake in eastern Ontario, Canada (44° 32′ N, 76° 00′ W). Snorkel surveys of the littoral zone were conducted to locate smallmouth bass that were actively guarding nests with newly deposited eggs (>1 day old) at the commencement of the study. Upon location of an active bass nest, the snorkeler placed a numbered polyvinyl chloride (PVC) tile near the nest and recorded nest location and number of eggs within the nest (visual, categorical assessment rating the size of the brood from low of 1 to high of 5; Suski and Philipp, 2004). At this point, individual fish were captured via heavy-action recreational fishing equipment from either the boat or underwater (by the diver) and landed at the boat in under 20 s to minimize physiological disturbance related to anaerobic exercise. Fish were then placed in a foam lined sampling trough filled with fresh lake water and non-lethally blood sampled by the caudal puncture method using a 1.5″, 21 gauge vacutainer syringe (Houston, 1990). Approximately 1.5 mL (representing approximately 3.7% of total blood volume) of blood was collected in a 3 mL, flat-bottomed vacutainer treated with lithium heparin to prevent blood coagulation and was then placed into a water-ice slurry. Blood samples were centrifuged immediately at 10,000 × *g* for 5 min (Clay Adams Compact II Centrifuge). Two separate blood samples were stored in liquid nitrogen for later analysis: an unmodified sample to be used for nutritional analysis as described in Hanson and Cooke (2009) and a sample for ghrelin analysis preserved with 10 μL *p*-hydroxymercuribenzoic acid (PHMB) and 10 μL HCl per 1 mL of plasma to reduce protease activity. Finally, total length was measured prior to release of the individual within 5 m of the nest (sampling time mean ± SD; 128 ± 49 s). During the time that the fish were removed from the nest, the diver protected the nest from potential brood predators using a blunt pole. The preceding sampling procedure was repeated at three stages of brood development representing the entirety of the parental care period in smallmouth bass. Briefly, the brood development stages at which the male was sampled were fresh eggs (sampled within 1 day of spawning; $n = 49$), egg sac fry (newly hatched embryos, approximately 1.5 weeks after spawning; $n = 14$), and free swimming fry (larvae swim <1 m above and around the nest, prior to independence, approximately 3 weeks after spawning; $n = 12$).

Samples were analyzed for concentrations of plasma-borne ghrelin which has previously been identified as a primary appetite hormone in a number of fish species (Unniappan and Peter, 2004; Kaiya et al., 2008). Plasma samples were assayed in duplicate to determine the content of active (acylated) ghrelin using a commercially available radioimmunoassay (RIA) kit (Millipore, Billerica, MA) that targets the biologically active region of the ghrelin molecule (which is evolutionarily conserved across multiple taxa including fish [Kaiya et al., 2008]), using a similar method as described by Picha et al. (2009). All samples were assayed together and had an intra-assay variability of 9.5%. Plasma ghrelin levels measured in this method were within the physiological range previously reported for teleost fish (Kaiya et al., 2008).

To determine the differences in plasma appetite hormone concentrations between stages of brood development during parental care, the mean plasma ghrelin concentrations of each brood

development group were compared by one-way analysis of variance (ANOVA) (Zar, 1999). All analyses were performed in the statistical package JMP v 7.0 and the level of significance for all tests (α) was assessed at 0.05. All values presented represent means ± SD unless otherwise noted.

Experimental manipulation with exogenous ghrelin

To determine the role of ghrelin in regulating voluntary anorexia during parental care, a manipulation experiment was conducted concurrently with endogenous ghrelin sampling. Location of individual bass nests, capture of animals, blood sampling for nutritional and ghrelin analyses, and sample storage followed the methods described above. After capture at the egg stage, but prior to release, individual bass were randomly placed in one of two treatment groups. Fish in the control group ($n=11$; TL [mean ± SD] = 390 ± 59 mm) were released without further intervention. Fish in the exogenous ghrelin group ($n=12$; TL = 393 ± 43 mm) were intraperitoneally injected with rodent ghrelin (π-Proteomics, Huntsville, Alabama) via 1″, 21 gauge hypodermic needle at a dosage of 100 μg of ghrelin (dissolved in physiological saline) per kg of fish. Previous studies have noted that injections of acylated rat ghrelin at similar dosages induce feeding behavior in teleost fishes (Shepherd et al., 2007). After experimental intervention, fish were released at the site of the nest. All experimental fish were recaptured when the brood developed to the egg sac fry stage and blood was sampled for nutritional and ghrelin analyses. There is a closed season for bass fishing during the reproductive period so it was illegal for members of the public to target or harvest fish from the study site.

In the laboratory, samples were analyzed for concentrations of plasma-borne biochemical indicators of individual nutritional status (total protein, triglycerides, and cholesterol) as well as dietary minerals (phosphorus, magnesium, and calcium) and enzymatic indicators of recent feeding (alkaline phosphatase [ALP; enzyme number 3.1.3.1]) (Wagner and Congleton, 2004; Congleton and Wagner, 2006; Hanson and Cooke, 2009). All biochemical analyses were conducted on a Roche Hitachi 917 analyzer (Basal, Switzerland) based upon the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standard reference model. All nutritional assays followed procedural guidelines for standardization and quality assurance established by the Veterinary Laboratory Association Quality Assurance Program, College of American Pathologists, and the Canadian Food Inspection Agency External Proficiency Panel. Plasma ghrelin levels were analyzed concurrently with samples from the previous portion of the study.

To determine the effect of exogenous ghrelin on feeding activity, the mean plasma concentrations of ghrelin and each nutritional factor mentioned above were compared between brood development stages by repeated measures analysis of variance (repeated measures MANOVA) (Zar, 1999).

Swimming performance experiment

To determine the effect of feeding and digestion on swimming performance, a separate study was conducted between May 15th and 17th, 2008. In total, 36 parental male smallmouth bass guarding broods at the egg stage were included in the study. After locating a nest, fish were randomly assigned to the following treatment groups: (1) a group that was not fed ($n=12$; TL = 384.7 ± 43 mm), (2) a group that was fed local crayfish (*Orconectes virilis*) until satiation and then sampled 3 h after feeding ($n=12$; TL = 417 ± 33 mm), and (3) a group that was fed crayfish until satiation and then sampled 24 h after feeding ($n=12$; TL = 467 ± 50 mm). Sampling times were based upon previous work documenting the duration of increased gut blood flow during digestion (Thorarensen and Farrell, 2006). A snorkeler fed individual bass by dropping crayfish into the nest and satiation was determined when the bass stopped ingesting food items. When appropriate, fish were captured via heavy-action recreational angling gear in less than 10 s, placed in an annular swim flume filled with fresh lake water (Portz, 2007), and chased to exhaustion by application of tactile stimulus to the caudal region of the fish to induce burst swimming (Kieffer, 2000). Swim trials were digitally recorded with a video camera placed above the flume and analyzed to determine the number of burst swimming events (a measure of maximum swimming performance combining both burst and sustained swimming) and time elapsed prior to exhaustion (a measure of aerobic swimming performance) (Beamish, 1978; Drucker, 1996; Portz, 2007). Fish were then released within 5 m of their nest. This protocol for swimming trials allowed fish to be returned to the nest without long term removal which would be required if we were using other swimming protocols such as critical swimming speed tests. Moreover, the swim flume was of sufficient size that it could be safely mounted on our 24 foot research vessel. Differences between burst swimming performance between the treatment groups was assessed by one-way ANOVA and Tukey's post-hoc test (Zar, 1999). The same statistical method was applied to determine differences in time to exhaustion between treatment groups (Zar, 1999).

Aggression experiment

To determine the effect of foraging and exogenous ghrelin on parental aggression towards a potential brood predator, 21 male smallmouth bass guarding eggs were located on May 28th, 2008. Upon location of individual nests, a snorkeler subjected each male to an aggression test wherein a glass jar (volume = 3.78 L) containing a small nest predator (bluegill, *Lepomis macrochirus*, TL = 172 ± 29 mm) was placed on the rim of the nest and the number of aggressive acts ('hits' when a male made physical contact with the jar) performed by the parental male in a 1-min time period was enumerated. Fish were then randomly assigned to the following two treatment groups: a control group ($n=7$) that was not captured or treated and the ghrelin injection group ($n=7$; TL = 416 ± 47 mm) described above. Fish from the ghrelin treatment group were captured via standard recreational angling gear in under 10 s and placed in a foam lined surgery trough filled with fresh lake water and were intraperitoneally injected following study protocols. Twenty-four hours later, a snorkeler relocated each nest and fed parental males to satiation following the methods described above. Three hours after feeding, each individual was subjected to the aggression test. The ghrelin treated fish from this experiment were recaptured following the second aggression experiment and blood was sampled following above described methods to determine the concentration of plasma ghrelin 24 h after injection with exogenous ghrelin.

Differences between the weight of crayfish (g) consumed by individuals in each treatment group were assessed by Students t-test (Zar, 1999). Additionally, simple linear regression was used to determine the relationship between the weight of crayfish consumed and percent change in aggression between the two days (Zar, 1999).

Results

Baseline sampling of endogenous ghrelin across parental care

Baseline plasma ghrelin levels fluctuated across the parental care period in relation to the stage of brood development (one-way ANOVA, d.f. = 2, 72, $F=16.56$, $P<0.001$; Table 1; Fig. 1). Specifically, plasma ghrelin levels were lowest during the egg and egg sac fry stages, and highest during the free swimming fry stage of brood development (Table 1 and Fig. 1).

Table 1
Parental male smallmouth bass plasma ghrelin concentrations at three stages of brood development during parental care.

	Egg	Egg sac fry	Free swimming fry
Non-injected control fish (pg/mL)	55.71 ± 25.33 (15.10–164.90) $n=49$	82.83 ± 29.59 (31.19–133.90) $n=14$	207.61 ± 200.31 (64.67–798.40) $n=12$
Exogenous ghrelin injected fish (pg/mL)	59.59 ± 37.12 (23.57–164.9) $n=12$	101.15 ± 51.27 (38.14–226.70) $n=12$	N/A

Values are presented as mean ± SD with minimum and maximum in parentheses.

Experimental manipulation with exogenous ghrelin

Twenty-four hours after injection, plasma ghrelin levels (as measured in a subset of wild fish) were increased to 382.92 ± 182.49 pg/mL, almost seven times greater than the mean value of 55.71 ± 25.32 pg/mL found for fish at the egg stage that were not subjected to intervention. Prior to exogenous ghrelin manipulation, there were no significant differences between groups in plasma ghrelin levels at the egg stage of brood development (repeated measures MANOVA; Tables 1 and 2). Additionally, indicators of nutritional status and recent foraging activity did not differ significantly between groups at the egg stage (Tables 2 and 3). Following injection, plasma ghrelin levels in injected fish increased ~170% from the egg stage to the egg sac fry stage (repeated measures MANOVA; Tables 1 and 2 and Fig. 2). However, no blood-borne indicators of nutritional status or recent foraging activity differed between groups (Tables 2 and 3).

Swimming performance experiment

Fish that were not fed showed a higher number of burst swimming events than either group of fed fish indicating a loss of swimming performance as a result of digestion (one-way ANOVA, d.f. = 2, 24, $F=3.45$, $P=0.048$, Fig. 3A). Additionally, swimming performance was impaired by digestion starting as early as 3 h after feeding and lasting for up to 24 h after feeding (one-way ANOVA, d.f. = 2, 24, $F=3.45$, $P=0.048$, Fig. 3A). However, there was no difference in time elapsed until exhaustion between the treatment groups (one-way ANOVA, d.f. = 2, 23, $F=1.96$, $P=0.16$, Fig. 3B).

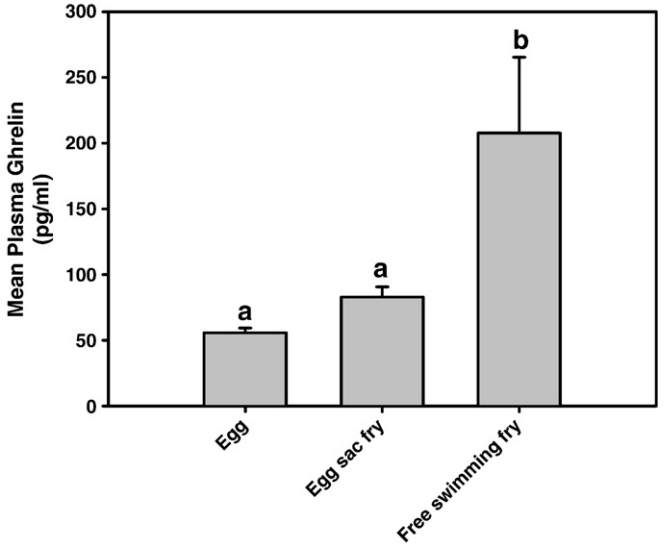

Fig. 1. Changes in baseline plasma ghrelin levels in nest guarding male smallmouth bass across three stages of brood development (egg, egg sac fry, and free swimming fry) during the parental care period. Letter assignments of "a" and "b" denote significant ($P<0.05$) differences among brood development stages. Error bars show mean ± SE.

Table 2
Results of repeated measure multiple analysis of variance (MANOVA) comparing indicators of nutrition and recent foraging activity among two groups of parental smallmouth bass (one uninjected control group and one group injected with rodent ghrelin) across the first two stages of brood development (egg and egg sac fry).

	Source	d.f.	*F*-ratio	*P*-value
Ghrelin (pg/mL)	Brood stage	1, 20	4.29	0.05
	Treatment	1, 20	3.09	0.09
	Brood Stage*treatment	***1, 20***	***6.19***	***0.02***
Alkaline phosphatase (ALP) (U/L)	***Brood stage***	***1, 21***	***13.16***	***0.002***
	Treatment	1, 21	0.02	0.90
	Brood Stage*treatment	1, 21	0.04	0.84
Calcium (mM)	***Brood stage***	***1, 21***	***10.83***	***<0.001***
	Treatment	1, 21	0.07	0.80
	Brood Stage*treatment	1, 21	0.88	0.36
Cholesterol (mM)	Brood stage	1, 21	0.81	0.38
	Treatment	1, 21	0.29	0.60
	Brood Stage*treatment	1, 21	0.05	0.83
Magnesium (mM)	***Brood stage***	***1, 21***	***41.20***	***<0.001***
	Treatment	1, 21	0.13	0.73
	Brood Stage*treatment	1, 21	2.80	0.11
Phosphorous (mM)	***Brood stage***	***1, 21***	***8.65***	***<0.001***
	Treatment	1, 21	0.51	0.48
	Brood Stage*treatment	1, 21	0.13	0.73
Triglycerides (mM)	Brood stage	1, 21	0.73	0.40
	Treatment	1, 21	0.05	0.83
	Brood Stage*treatment	1, 21	2.27	0.15
Total protein (g/L)	***Brood stage***	***1, 21***	***6.07***	***0.03***
	Treatment	1, 21	0.41	0.53
	Brood Stage*treatment	1, 21	0.06	0.81

Significant differences at $\alpha=0.05$ are indicated by bold and italicized font.

Feeding experiment

There were no differences in crayfish consumption (g) between the control (20.30 ± 15.67 g) and ghrelin injected (8.79 ± 13.86 g) treatment groups (Student's t-test, d.f. = 8, $t=1.31$, $P=0.22$). Amongst fish that ingested food items, there was a negative relationship between the weight of crayfish consumed and percent

Table 3
Concentrations of plasma-borne indicators associated with nutritional status and recent foraging activity for two groups of experimentally manipulated nest guarding male smallmouth bass (non-manipulated controls and fish injected with rodent ghrelin) at two stages of brood development during parental care.

	Egg—control	Egg—ghrelin injected	Egg sac fry—control	Egg sac fry—ghrelin injected
Alkaline phosphatase (ALP) (U/L)	21.64 ± 9.61 (13–40) $n=11$	21.75 ± 13.83 (9–48) $n=12$	12.92 ± 5.42 (7–27) $n=11$	12.42 ± 4.34 (6–22) $n=12$
Calcium (mM)	2.48 ± 0.11 (2.29–2.70) $n=11$	2.52 ± 0.15 (2.22–2.81) $n=12$	2.59 ± 0.11 (2.43–2.75) $n=11$	2.59 ± 0.18 (2.41–3.04) $n=12$
Cholesterol (mM)	11.95 ± 2.84 (7.3–17.3) $n=11$	11.18 ± 3.79 (5.3–16.5) $n=12$	11.93 ± 2.71 (8.3–17.0) $n=11$	11.38 ± 3.63 (5.7–17.5) $n=12$
Magnesium (mM)	1.19 ± 0.12 (1.04–1.41) $n=11$	1.25 ± 0.16 (1.01–1.51) $n=12$	1.08 ± 0.06 (1.00–1.19) $n=11$	1.06 ± 0.07 (0.95–1.12) $n=12$
Phosphorous (mM)	1.76 ± 0.35 (1.3–2.5) $n=11$	1.81 ± 0.30 (1.4–2.4) $n=12$	1.58 ± 0.13 (1.4–1.8) $n=11$	1.66 ± 0.20 (1.4–2.0) $n=12$
Triglycerides (mM)	2.72 ± 0.50 (1.91–3.76) $n=11$	2.45 ± 0.46 (1.51–3.28) $n=12$	2.39 ± 0.63 (1.78–3.68) $n=11$	2.54 ± 1.05 (1.18–4.80) $n=12$
Total protein (g/L)	41.00 ± 2.57 (37–44) $n=11$	40.25 ± 3.86 (34–46) $n=12$	40.83 ± 2.31 (37–43) $n=11$	39.25 ± 3.89 (33–47) $n=12$

Values are presented as mean ± SD with minimum and maximum in parentheses.

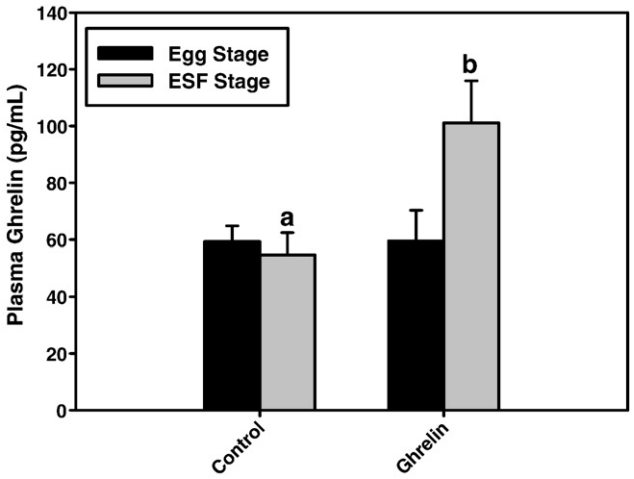

Fig. 2. Changes in plasma ghrelin levels across two stages of brood development (egg [dark bars] and ~1.5 weeks later at the egg sac fry ["ESF," light bars]) in nest guarding male smallmouth bass that were non-injected controls or subjected to exogenous ghrelin injection at the egg stage during the parental care period. Letter assignments of "a" and "b" denote significant ($P<0.05$) differences among brood development stages. Error bars show mean ± SE.

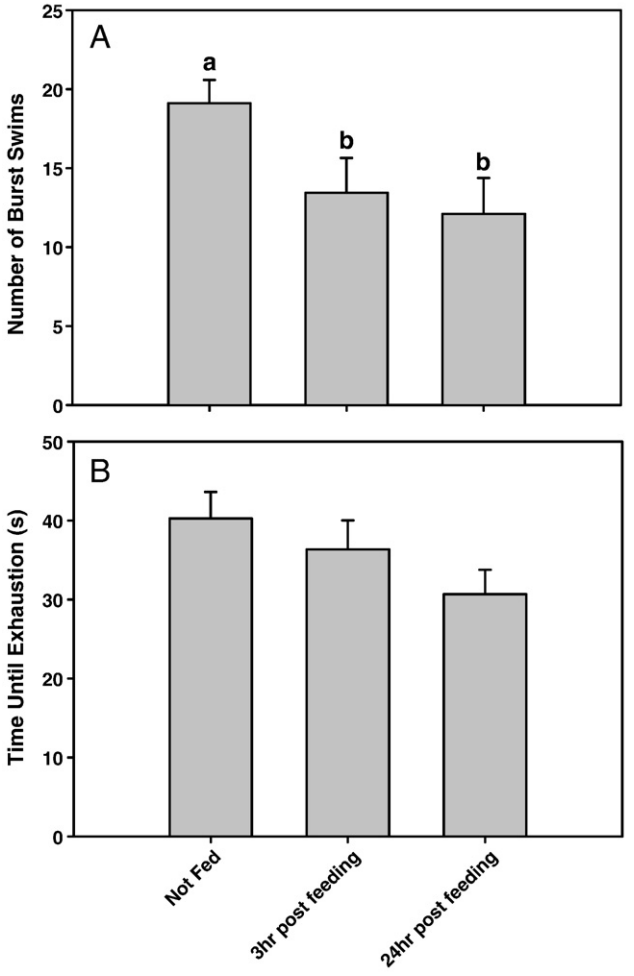

Fig. 3. Changes in swimming performance (number of burst swims (A) and time until exhaustion(s) (B)) among nest guarding male smallmouth bass subjected to swimming trials at three time periods (non-fed controls, 3 h after feeding to satiation, and 24 h after satiation). Letter assignments of "a" and "b" denote significant ($P<0.05$) differences among brood development stages. Error bars show mean ± SE.

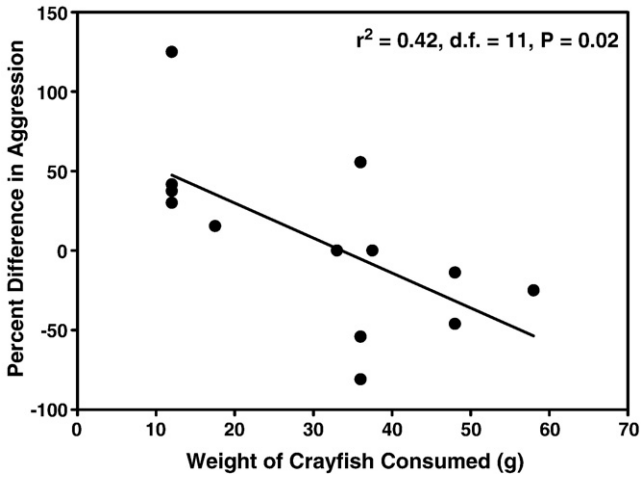

Fig. 4. The relationship between percent change in nest guarding male smallmouth bass aggression towards a simulated brood predator and the weight of forage consumed 24 h after feeding to satiation.

change in aggression between the two days, with fish that consumed greater a greater amount of crayfish showing the greatest reductions in aggression towards a potential brood predator (simple linear regression, d.f. = 12, $F = 8.09$, $P = 0.016$, Fig. 4).

Discussion

In the current study, plasma ghrelin levels were lowest during the egg (55.71 ± 25.32 pg/mL) and egg sac fry (82.83 ± 29.59 pg/mL) stages of brood development before increasing to 207.61 ± 200.30 pg/mL during the free swimming fry stage (Fig. 1). During the early stages of parental care when the male defends the brood in a localized area around the nest, foraging behavior dramatically decreases (Ridgway, 1988; Hinch and Collins, 1991; Cooke et al., 2002). Previous research has speculated that decreased foraging behavior is a result of decreased opportunity of finding suitable forage in the area of the nest (Ridgway, 1988; Hinch and Collins, 1991), though individual bass have been noted to remove potential food items (small bodied fishes, invertebrates) from the vicinity of the nest without consuming them (Steven Cooke, unpublished). As ghrelin has been noted to be a hormonal cue initiating voluntary foraging in teleost fishes (Unniappan and Peter, 2004; Volkoff et al., 2005), decreases in plasma ghrelin may be necessary to reduce hunger and induce voluntary anorexia during parental care.

Concomitant with this decline in foraging, parental male bass are extremely active (Cooke et al., 2002) with localized movements powered through mobilization of endogenous energy reserves in the form of muscle and liver lipid stores (Mackereth et al., 1999). This energetic dilemma featuring a massive increase in activity with a massive decrease in energy uptake through foraging results in a loss of endogenous energy reserves (Mackereth et al., 1999; Cooke et al., 2006), decreases in indicators of nutritional physiology (Hanson and Cooke, 2009), and potential loss of body mass (Cooke et al., 2002). Reduction of ghrelin levels would allow parental male bass to enter the catabolic state described above as plasma ghrelin levels have been shown to be positively related to lipid deposition in fish (Riley et al., 2005). High plasma ghrelin levels are simply incompatible with the requirements of parental care (specifically the need for energy utilization and decreased foraging) of male bass.

As the brood develops to the free swimming fry stage, the fry develop the ability to swim and spread out across a large area which the male patrols to defend the brood, thereby increasing the probability that a male will encounter a suitable forage item (Friesen

and Ridgway, 2000). Increases in blood-borne nutritional factors such as dietary minerals indicate that males begin to forage at this time (Mackereth et al., 1999; Steinhart et al., 2005; Hanson and Cooke, 2009). The timing of the increase in minerals derived from foraging corresponds to the timing of the increase in plasma ghrelin during the free swimming fry stage (Fig. 1). Mustonen et al. (2002) attributed similar increases in appetite hormone levels following spawning in burbot as a mechanism to increase appetite and foraging behavior in spawned individuals to replenish exhausted energy stores. In the current study, we also believe increases in ghrelin production would be necessary to induce hunger in previously fasted individuals thereby increasing foraging behavior following parental care required to enter into an anabolic state to replenish endogenous energy reserves. Additionally, it has been theorized that complete over depletion of energy reserves during a single parental care period can be linked to individual mortality during the following winter (Mackereth et al., 1999). As such, it may be necessary for bass to resume foraging and lipid deposition as soon as the brood becomes independent to ensure survival through the year and the possibility of future reproductive opportunities.

Previous studies have noted that the structure and function of ghrelin is highly conserved among vertebrates (Kaiya et al., 2008) and multiple researchers have used exogenous injections of rodent ghrelin to induce physiological changes and voluntary feeding in teleost fishes (Unniappan and Peter, 2004; Volkoff et al., 2005). In the current study, treatment fish were subjected to exogenous injections of rodent ghrelin at the egg stage of brood development that resulted in artificially increased plasma ghrelin concentrations (382.92 ± 182.49 pg/mL hours after injection) which persisted for at least 1 week until the brood developed to the egg sac fry stage (Fig. 2). However, ghrelin injected fish did not feed at elevated levels 24 h after injection when compared to controls (Fig. 4), though studies that have documented the orexigenic effects of ghrelin in fish have either measured voluntary foraging in the first hour after exposure (Matsuda et al., 2006; Miura et al., 2007) or did not show an effect at a longer time scale (Jönsson et al., 2007). Additionally, ghrelin treated fish showed no significant differences in plasma values of multiple nutritional and energetic indicators of fasting (Congleton and Wagner, 2006; Hanson and Cooke, 2009). The only differences in plasma levels of nutritional indicators and dietary minerals were related to fasting during the time elapsed from the egg to egg sac fry stage of brood development, which is consistent with previous work documenting fasting induced changes in nutritional condition across parental care in smallmouth bass (Hanson and Cooke, 2009). In effect, even though the hormonal cue to increase foraging and switch to an anabolic state was present in the blood stream at levels similar to those at the end of parental care, no physiological indicators of feeding were noted indicating that the action of the hormone was potentially blocked. This resistance to the action of ghrelin could be reinforced through receptor insensitivity (growth hormone secretagogue receptor type 1a [GHS-R1a]) in the hypothalamus as receptor expression has been shown to be positively related to ghrelin levels (Camiña, 2006). Lending support to the potential for receptor insensitivity, chronic infusions with ghrelin (a time period of elevated ghrelin lasting multiple days, similar to the week long elevation in plasma ghrelin levels noted in the current study) have been shown to stimulate sustained increases in food intake in species in multiple taxa (Riley et al., 2005; Strassburg et al., 2008). Additionally, as food intake in fish is regulated through multiple neuroendocrine pathways working both agonistically and antagonistically (Volkoff et al., 2005), other endocrine factors, such as leptin and growth hormone, may be involved in induction of an anorexic state during parental care. Functionally, it appears that individual parental bass regulate foraging and energy utilization through a combination of cessation of production of ghrelin coupled with redundant receptor insensitivity, though further research is required to determine the potential for receptor insensitivity and the role of redundant neuroendocrine pathways.

Ultimately, anorexia during the parental care period may be an adaptive behavior that prevents loss of offspring through brood predation. Multiple studies have noted that swimming performance and digestion are temporally incompatible due to constraints imparted through competing requirements for blood flow (Thorarensen et al., 1993; Alsop and Wood, 1997; Farrell et al., 2001). Digestion of food items requires a shift in blood flow to the viscera from the swimming musculature that can often last for over 24 h after ingestion (Axelsson et al., 1989; Axelsson and Fritsche, 1991; Thorarensen et al., 1993; Thorarensen and Farrell, 2006). This increase in blood flow is required for a myriad of processes required for catabolism of food items and results in an increase in metabolic rate referred to as specific dynamic action (SDA) (reviewed in McCue, 2006). In rainbow trout (*Oncorhynchus mykiss*), Alsop and Wood (1997) showed that, as fish exhibit an absolute maximum oxygen consumption, increases in SDA following feeding reduce the portion of the scope for activity that can be devoted to swimming metabolism due to digestive requirements reducing the amount of oxygen available to swimming muscles, thereby reducing critical swimming speed. Similarly, in smallmouth bass, Beamish (1974) noted that increases in oxygen consumption due to digestion of a 4% ration mirrored increases in oxygen consumption required to swim at up to 2.5 body lengths per second. In the current study, we noted that repeated burst swimming activity decreased in fish that were fed to satiation 24 h earlier when compared to unfed controls (Fig. 3A). Repeated burst swimming, as evaluated in the current study, is a measure of the maximum swimming performance of the animal consisting of repeated anaerobic muscular activity (the burst swim event; Beamish 1978) resulting in complete exhaustion. As such, both decreased blood flow to swimming musculature coupled with the metabolic demands of SDA would impact the maximum swimming performance of the individual by impairing the ability to maintain swimming metabolism and preventing further anaerobic swimming activity (Alsop and Wood, 1997). During parental care, parental bass regularly engage in burst swimming to chase potential brood predators from the vicinity of the nest (Hinch and Collins, 1991; Cooke et al., 2002). Failure to vigorously defend the brood in this manner results in reproductive failure as the male will often abandon a brood that has been severely depredated (Philipp et al., 1997; Steinhart et al., 2004). We also noted that the amount of prey consumed was negatively related to changes in aggression towards a simulated brood predator (Fig. 4). Even though supplemental foraging at high levels could mitigate the energetic decline experienced by adult males, this would occur at a potential cost to offspring survival and individual fitness. Given that parental care aims to maximize offspring survival at a cost to the condition of the parent (Trivers, 1972; Gross and Sargent, 1985; Gross, 2005), the ultimate cost of supplemental foraging to offspring survival negates any benefits to individual fitness incurred through engaging in parental care. As such, voluntary anorexia would be a required component of parental care by a male bass to successfully raise a brood.

In conclusion, voluntary anorexia during the parental care period in smallmouth bass appears to be an adaptive behavior aimed at avoiding decreases in swimming ability and parental aggressiveness that are needed to defend the brood and that could lead to potential decreases in reproductive success and fitness if impaired. This behavior seems to be modulated through a combination of declines in production of ghrelin coupled with a decrease in receptor sensitivity to this appetite hormone. Further research into the mechanism of induced voluntary anorexia during parental care should focus on the role of complementary appetite hormones such as leptin or endocrine factors such as cortisol, as well as studies at the level of the receptor and genes.

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