LIFE HISTORY TRADE-OFFS IN A PARENTAL CARE-PROVIDING FISH: THE ROLE OF REPRODUCTIVE VALUE, PREDATION THREAT, AND PHYSIOLOGICAL CONDITION ON BROOD ABANDONMENT DECISIONS BY PATERNAL LARGEMOUTH BASS

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THESIS

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ABSTRACT

Parental care evolved as a means to maximize reproductive success at a cost to physiological and nutritional condition of the care-providing individual, and at a cost to future reproductive potential. Parental investment decisions are rooted in tradeoffs between these factors, and when the cost of care-provision is outweighed by potential future reproductive potential, a parent may forfeit current investment in an effort to maximize future reproductive success. Few studies have approached parental care decisions using offspring abandonment as a direct and ultimate fitness affect, and fewer yet have adopted a holistic approach to test how physiological and environmental conditions compare in influencing the decision by a parent to abandon their brood. I performed two separate, yet complementary, studies to test for the effects of several factors on brood abandonment decisions in largemouth bass (*Micropterus salmoides*); a model parental care-providing species. First, I tested whether mating success and simulated brood depredation affect the decision by paternal largemouth bass to abandon a brood. Second, I used a multivariate approach to jointly test for the influence of nutritional condition, hormonal stress, androgen concentration, and oxidative stress of brood-guarding paternal largemouth bass, and the threat of brood depredation (i.e., brood predator density), on brood abandonment decisions. Together, my results suggested a threshold for brood loss at which paternal largemouth bass were more likely to abandon what remains of a depredated brood, and that a high threat of depredation and reduced androgen concentration also influenced the decision by paternal bass to abandon care. My findings have implications for science-based management of a highly sought-after sportfish, as well as offer a novel approach for testing the inter-related effects of various abiotic and biotic factors on parental care decisions across taxa.

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CHAPTER 1.
GENERAL INTRODUCTION

Reproduction is inherently costly to an individual. The greatest costs of reproduction are associated with strategies involving parental care as a mechanism by which parental individuals optimize lifetime reproductive success (i.e., fitness), incurring direct and indirect costs to future reproductive value (i.e., potential future offspring), and potentially to the individual itself (Williams 1966, Trivers 1972, Stearns 1992). Such investment, as described by Trivers (1972), yields a reduction in capacity to invest in future progeny. Specifically, investment in current progeny comes at a direct cost to future fitness - the result being a discrete 'tradeoff' associated with any parental decision (Smith & Wooton 1995, Gross 2005).

Early research into life history tradeoffs were initially explored as competition between biological function within an individual for a limited resource, notably energetic reserves (Calow 1979, Agrell et al. 1998, Ricklefs & Wikelski 2002). By this definition, finite somatic reserves are allocated to reproductive effort, a tradeoff *in lieu* of self-maintenance and growth, which is a cost especially relevant to species whose fitness increases with size (Ketterson & Nolan Jr. 1992, Steinhart et al. 2008). For example, long-lived individuals within a population of *Drosophila* experienced a decrease in fecundity at a younger age than do their shorter-lived cohorts, yet both insects exhibit equal fitness (Zera & Harshman 2001). Lactating red deer (*Cervus elaphus*), however, demonstrated lower overwinter survivorship than non-lactating females due to investment of body fat and protein into milk production (Zera & Harshman 2001). Smallmouth bass (*Micropterus dolomieu*) voluntarily cease foraging during reproductive periods, losing up to about 3 percent of body mass in the first eight days of parental care - the duration of which can last six weeks (Gillooly & Baylis 1999). As demonstrated by lower survival by lactating deer

and the loss of mass in smallmouth bass, somatic reserves are a limiting resource for reproduction, and need to be allocated accordingly to balance current versus future reproductive output.

The novel application of physiological techniques towards research of life history tradeoffs has demonstrated that parental care decisions are mediated by more than energetics. Tradeoffs are comprised by genotypic and phenotypic variation, hormones, and other physiological functions (Zera & Harshman 2001, Badyaev & Uller 2009). Plasticity in physiological responses, coupled with environmental variability, provide an evolutionary pressure selecting for a wide range of mechanisms responsible for mediating life history tradeoffs, the bulk of which are only recently being quantified in terms of parental care decisions (Ketterson & Nolan Jr. 1992, Zera & Harshman 2001, Ricklefs & Wikelski 2002, Monaghan et al. 2009). In addition, brood size, brood depredation, and physiological condition of a parental individual all play an important role in parental care decisions. To date, the consequences of life history tradeoffs on reproductive success have received less attention, and testing the extent to which these factors influence parental care decisions can have important consequences for improving our understanding of life history tradeoffs.

BROOD SIZE

One biotic variable that is of particular importance to the level of effort invested by a parent is the reproductive value of their current brood, because brood size is the basis against which life history tradeoffs are frequently weighed. Previous research on birds and fishes has demonstrated that parental expenditure is often adjusted in accordance with brood size. For example, defensive behavior and egg fanning increase with brood size in smallmouth bass and

bluegill (*Lepomis macrochirus*). Convict cichlids (*Cichlasoma nigrofasciatum*) will extend the

duration of parental care when their brood is experimentally supplemented with un-related fry

(Carlisle 1985, Ridgway 1989, Lavery & Keenleyside 1990, Coleman & Fischer 1991, Neff &

Knapp 2009). In contrast, individuals have been observed to decrease parental expenditure when

reproductive value is perceived as low. For example, common eider (*Somateria mollissima*), a

species of arctic-nesting sea duck, are more likely to abandon a nest when clutch size is below

average, and bluegill reduce nest defense when cuckolder-fertilized eggs are detected (Jennions

& Polakow 1998, Neff & Gross 2001, Burgeon et al. 2006). Fowl, passerines, centrarchids, and

cichlids exhibit thresholds at which a loss in current reproductive value, potentially via

unfavorable temperature, storm events, natural death, or depredation, will result in brood

abandonment – a tradeoff in favor of investing towards future reproductive events (Ketterson &

Nolan Jr. 1992, Ackerman & Eadie 2003, Burgeon et al. 2006, Gwinn & Allen 2010, Lunn &

Steinhart 2010). Together, past studies have demonstrated that an adjustment of parental care

activities in relation to either real or perceived reproductive value is common across taxa.

Parents will frequently engage in more costly behavior if warranted by tradeoffs related to

rewards such as increased fitness.

BROOD DEPREDATION

As with changes in brood size, adjustment of parental investment in accordance with

varying threat of offspring depredation has also been documented in care-providing species.

Agonistic brood defense behaviors come at high energetic expense as well as with inherent risk

of physical harm to the parental individual (Coleman & Gross 1991). In circumstances where

offspring are at risk of depredation, arctic shorebirds (order Charadriiformes) use several nest

defense strategies ranging from harassment to injury imitation in attempt to distract a potential predator (Smith & Wilson 2010). Centrarchid and cichlid fishes exhibit similar behaviors, such as jaw displaying and predator chasing (Carlisle 1985, Ridgway 1989, Gravel & Cooke 2009). Nest guarding behavior can also be adjusted according to shifts in predator densities, as demonstrated by smallmouth bass engaging predators more frequently in lakes with higher predator burdens (Gravel & Cooke 2009, Steinhart et al. 2008). In contrast, feeding rates and incubation times are decreased in European starlings (*Sternus vulgaris*) when experimentally presented with artificial predators near a nesting site, resulting in lowered reproductive success (Cyr & Romero 2007). Investment is also adjusted when predation pressure is relieved, as demonstrated in passerine birds. In response to experimental exclusion of predators, females of several passerine species increase parental investment through the production of clutches containing more eggs of larger size, while males increase time spent foraging and feeding their respective mate and progeny (Fontaine & Martin 2006). While external stressors such as predation risk are documented to influence parental care decisions, life history tradeoffs may also be mediated via physiological cues to the parental individual.

PHYSIOLOGICAL CONDITION OF CAREGIVER

Behavior is heavily influenced by changes in the physiological state of an individual, connecting the physical environment to decisions influencing life history tradeoffs (Ricklefs & Wikelski 2002). For instance, brood depredation, environmental condition, predator burden, and anthropogenic disturbance are documented to elicit a stress response in care-providing individuals (e.g., Calow 1979, Ostrand et al. 2004, Suski & Philipp 2004, Cyr & Romero 2007, Badyaev & Uller 2009, Hanson & Cooke 2009). The initiation of an endocrine stress response,

as measured by release of the stress hormone cortisol, can result in behavioral modifications in attempt to alleviate the perceived stress, potentially at a tradeoff to offspring care (Barton & Iwama 1991, Schreck et al. 2001). Investment in the stress response comes at a tradeoff to other physiological functions, resulting in a marked impairment of the immune system and potential disruption of reproductive hormones (Wendelaar-Bonga 1997, Ricklefs & Wikelski 2002, Alonso-Alvarez et al. 2004, Romero 2004, O'Conner et al. 2009). Despite evidence that some species can block cortisol reception during reproduction, thereby suppressing any subsequent behavioral effects, changes in concentration of a widely accepted suite of stress indicators (e.g., cortisol, glucose, and plasma ion concentration) have been correlated with decreased reproductive success, particularly in nesting starlings (Wingfield & Sapolsky 2003, Cyr & Romero 2007). A strong connection between physiological state and decreased fitness, however, is not well documented in other taxa (Dey et al. 2010). The direct influence of a stress response on fitness in fish and other species is uncertain and may be confounded by the aforementioned suppression of endocrine receptors. Alternatively, reproduction under stressful conditions may be selected for in some species, as is the case for those with semelparous reproductive strategies, short life expectancies, or single breeding bouts per season (Wingfield & Sapolsky 2003).

One specific physiological factor previously demonstrated to have a large influence on reproductive behaviors is the presence and concentration of reproductive hormones (Ketterson & Nolan Jr. 1992). Male steroids, primarily testosterone (T) and 11-keto testosterone (11-KT, the primary androgen in teleost fishes), are integral to gamete production and maintaining aggressive behaviors beneficial to territoriality, courtship, and nest guarding (Ketterson & Nolan Jr. 1992, Kime 1993). Heightened aggression, as shown in several avian and reptilian species, influences males to participate in risky behavior with over-aggression resulting in a hastened decrease in

nutritional condition, increased stress, and a subsequent decrease in fitness (Marler & Moore 1988, Dufty Jr. 1989, Ketterson & Nolan Jr. 1992). Androgens have also been shown to increase with the frequency of interactions between a male and potential nest predators, an interaction that has been previously cited to increase stress in birds and fish (Wikelski et al. 1999, Cyr & Romero 2007, O'Connor et al. 2011). The relationship between androgens and male parental behavior in bluegill, however, is not consistent with the previous notion that androgens are drivers of aggression. Defensive behavior by bluegill towards a brood predator is negatively correlated with T and 11-KT, and nest tending (egg fanning, perimeter patrol) was not correlated with either androgen (Neff & Knapp 2009). A similar response was observed in smallmouth bass when injected with an androgen blocker. Treated males responded to simulated brood predation in a less aggressive manner, but at no significant cost to fitness (Dey et al. 2010). While aggression may be beneficial to parental males in systems of high predation potential, maintenance of androgens may instigate a negative tradeoff with fitness because expenditure on heightened aggression can be unnecessarily costly.

Recent research has demonstrated oxidative stress to also be a quantifiable measurement of individual condition. Oxidative stress is the product of cellular and molecular degradation by circulating or tissue-specific free radicals (reactive oxygen species, ROS). ROS are produced endogenously as a byproduct of ATP synthesis, and actively scavenge electrons from lipids and proteins, causing structural and functional damage to cell walls, organelles and DNA (Wiersma et al. 2004). Organisms produce natural enzymatic defenses to ROS damage, and acquire exogenous micro- and macro-molecule defenses via diet. The combination of endogenously and exogenously derived ROS defense comprises an organism's total antioxidant capacity (TAC) (Constantini & Verhulst 2009). Oxidative stress, as defined by an imbalance between ROS

production and the capacity of an individual to scavenge free radicals, is correlated with cellular senescence (i.e., aging), and emerging research has cited oxidative damage as a pertinent cost of reproduction (Alonso-Alvarez et al. 2004, Monaghan et al 2009). Preliminary studies have demonstrated that male zebra finches (*Taeniopygia guttata*) experience a decrease in TAC with increasing clutch size (Alonso-Alvarez et al. 2004). Although it is important to note that decreased TAC alone does not indicate oxidative stress (Wiersma et al. 2004, Constantini & Verhulst 2009), greater susceptibility to oxidative damage due to diminished TAC is assumed to be a tradeoff between TAC and parental expenditure associated with large clutch size, and may affect future reproductive success (Monaghen et al. 2009). Conversely, reproduction is likely to incur a greater cost of fitness in species that maintain TAC during reproduction. Further research into the life history effect of oxidative stress is required before definitive trends can be established, particularly whether depressed TAC results in greater rates of oxidative damage (Monaghan et al. 2009).

PARENTAL BEHAVIORS IN LARGEMOUTH BASS

Research on the subject of parental care pertains primarily to behavioral or physiological changes (e.g., increased nest vigilance, stress response) of an individual. In understanding the full effect of life history tradeoffs, however, it has been suggested that ultimate success (i.e., abandon a brood or maintain parental care) is a more robust measure of the influence of parental decisions on fitness than are changes in behavior (Burgeon et al. 2006, Ricklefs & Wikelski 2002). Although the effects of brood size, predation pressure, and physiological characteristics on parental behaviors have been well described for individuals, no studies to date have considered interacting effects of these variables in mediating life history tradeoffs, particularly

with regard to offspring abandonment. As a result, there is a critical need to test these factors concurrently to better understand the relative influence of different parameters on mediating parental care decisions – an investigation for which care-providing fishes are ideally suited as a study subject (Gross 2005).

Centrarchid fishes are particularly well-suited for studies testing for the influences of parental care behaviors on fitness and reproductive output. In particular, largemouth bass (*M. salmoides*) represent an ideal model organism to ask questions of interacting biotic and abiotic factors and whether they influence fitness (Gross 2005). Largemouth bass reproductive behavior begins as water temperatures approach 15° C, when male largemouth bass prepare a nest in shallow littoral substrate (< 2m in depth), court a female, and subsequently spawn. Following fertilization, the female will depart, leaving the male to provide sole parental care until offspring reach independence; generally four to six weeks post-fertilization. During this time, the male intently tends the nest, cleaning and oxygenating eggs by fanning with his pelvic and caudal fins, while providing defense against potential brood predators (e.g., bluegill, pumpkinseed [*L. gibbosus*]). Parental care in largemouth bass is energetically costly; nest defense requires twice the energetic investment compared to non-nesting conspecifics (Hanson & Cooke 2009). If nest vigilance (i.e. predator defense) is not maintained, depredation of a brood is observed to result in abandonment by the male, and a subsequent forfeiture of fitness for that reproductive event (Lunn & Steinhart 2010, Steinhart et al. 2005). Furthermore, the male largemouth bass ceases actively foraging for the entirety of the care period, instead relying on somatic reserves (Cooke et al. 2002, Hanson et al 2009). The costs and tradeoffs associated with the level of investment by male largemouth are well studied. The influence of male stress physiology, brood predator densities, and brood size on changes in behavior have been documented (e.g., Philipp et al. 1997,

Steinhart et al. 2005, Wagner et al. 2006, Hanson et al. 2008), yet the direct effect of these variables on influencing fitness of an individual are largely unknown.

Largemouth bass invest in costly care to maximize reproductive success, yet fitness of an individual may be negatively affected by recruitment variation (Gross and Kapuscinski 1997, DeVries et al. 2009). Recruitment in black bass is currently understood to be driven by a combination of environmental factors (e.g., temperature, productivity), population structure (e.g., competition among cohorts, compensatory mortality), and decreased nest success by premature abandonment (DeVries et al. 2009). This highlights the importance of reproductive output in influencing population regulation for this species. In a well-studied population of smallmouth bass in Ontario, nearly 55% of the young of year recruits could be attributed to only five percent of the male population that raised a brood to independence (Gross & Kapuscinski 1997). Modeling by Gwinn and Allen (2010), however, suggested brood abandonment may have no effect on recruitment of bass in southern latitudes. Given that only a small proportion of a nesting population successfully recruits young, and the uncertainties in factors influencing recruitment in largemouth, a better understanding of abandonment decisions can be crucial in elucidating fitness at the population level. Furthermore, a better understanding of brood abandonment can provide insight for fishery management decisions and contribute to knowledge of life history tradeoffs in terms of parental care theory.

Based on this background, I used two separate, yet complementary, experiments to test for significant drivers of premature brood abandonment decisions in largemouth bass. My objectives for the first experiment, and Chapter 1 of this thesis, were to test whether:1) mating success (i.e., number of offspring received); 2) magnitude of brood loss; and 3) remaining brood size following experimental brood removal affect the decision by male largemouth bass to

abandon their brood. My objectives for the second experiment, and Chapter 2 of this thesis, were to use a novel, holistic approach to test whether: 1) male nutritional condition; 2) male stress condition; 3) male androgen concentration; 4) male oxidative stress; and 5) the perceived threat of brood depredation, affect the decision by paternal largemouth bass to abandon their brood.

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CHAPTER 2.
THE INFLUENCE OF BROOD LOSS ON NEST ABANDONMENT DECISIONS IN *MICROPTERUS SALMOIDES*, A PARENTAL CARE-PROVIDING TELEOST

ABSTRACT

Reproductive value (i.e., the number of offspring a parent receives in mating) is indicative of the potential fitness contribution of a brood. A change in reproductive value, such as occurs following brood depredation, induces a modification in parental behavior, generally as measured by decreased investment by the care-providing individual. However, it is unknown whether a threshold exists for brood loss on the decision by parental fish to abandon parental care altogether. Largemouth bass *Micropterus salmoides* broods in Lake Opinicon, Ontario, Canada, were experimentally devalued to test whether reproductive value and simulated brood depredation affect the decision by a male bass to continue provision of care for a brood, or to abandon the brood premature to fry reaching independence. The most important factor for predicting brood abandonment was the number of offspring remaining following brood devaluation, indicating that parental males re-assess the reproductive value of a brood following brood loss. Also, paternal largemouth bass with smaller brood sizes before devaluation were more likely to abandon their brood. Across initial brood sizes, a greater magnitude of brood loss following devaluation also increased abandonment rates. These results indicate a threshold for depredation and remaining brood size on parental care decisions, have broad implications for explaining the drivers behind parental care tradeoffs, and offer insight into science-based management of largemouth bass as a sportfish.

INTRODUCTION

Parental care evolved as a strategy to maximize fitness of the care-providing parent by increasing the likelihood of offspring survival, particularly via the defense of one's brood from predators. Parental care decisions are based upon tradeoffs between current and expected future reproductive output. The investment of finite resources into the care of a current brood comes at an immediate and direct cost to the parent via energetic expenditure and risk of physical harm, as well as at a delayed and indirect cost via reduced potential for future offspring (Williams, 1966). As a result, parental investment varies based on the perceived reproductive value of a brood (*i.e.* the potential contribution of a brood to an individual's fitness), the energetic requirement to raise that brood, the parent's expected future reproductive success, and potentially, past investment into a brood (Williams, 1966; Trivers, 1972; Gross, 2005). When the reproductive value of a brood is outweighed by the perceived cost of providing the level of investment necessary to raise that brood to independence, or the value of a brood becomes reduced through depredation, individual parents may reduce their level of investment into the brood. Ultimately, if reproductive value is reduced below a particular threshold, parental individuals may abandon their offspring in favor of investing toward future reproductive output. Such parental decisions have been observed in the bi-parental care-providing cichlid *Aequidens coeruleopunctatus* (Kner, 1863). Following a reduction in reproductive value, females will partake in less risky brood defense (Carlisle 1985), and paternal males may abandon their brood in favor of attempting another bout within the same reproductive season (Jennions & Polakow, 1998). Patterns of brood abandonment are also observed across other taxa, with water fowl, passerine birds, and centrarchid sunfishes all exhibiting a tendency towards offspring abandonment following depredation (Verboven & Tinbergen, 2002; Ackerman & Eadie, 2003; Steinhart et al., 2008). For iteroparous species that are limited to a single reproductive event within a given season, the

decision to abandon a brood may be more risky from a fitness standpoint owing to uncertainties in future survival, somatic growth, and future reproductive opportunities (Wingfield & Sapolsky, 2003; Gross, 2005; Steinhart et al., 2008).

Largemouth bass *Micropterus salmoides* (Lacépède 1802) are an ideal model species for studies involving parental care decisions (Gross, 2005). Native to North America, largemouth bass begin reproductive behavior in spring as water temperatures approach 15° C, with males constructing shallow bowl-shaped nests in littoral substrate (Heidinger, 1975). Following nest construction, male largemouth bass court females and subsequently fertilize eggs as they are deposited into the nest. Following egg deposition, the female departs the nest, leaving the male to provide sole parental care for the next 3-5 weeks, until offspring reach independence. During this time, paternal largemouth bass partake in energetically costly parental care by fanning the nest using movements by the pectoral and caudal fins to increase oxygenation and by actively chasing brood predators from the vicinity of the nest (Ridgway, 1989; Hinch & Collins, 1991; Cooke et al., 2002, Gravel & Cooke, 2009). Despite the increased energetic investment accrued during care for the brood, paternal largemouth bass refrain from actively foraging, instead relying on endogenous energetic reserves. This results in an overall decline in somatic condition throughout the parental care period that could potentially effect survival and future reproductive capacity of the parental largemouth bass (Gillooly & Baylis, 1999; Hanson & Cooke, 2009; Hanson et al., 2009).

Abandonment of a brood prior to offspring independence has been observed in largemouth and smallmouth bass *M. dolomieu* (Lacépède 1802) and attributed to acute changes in water temperature (Steinhart et al., 2005; Suski & Ridgway, 2007; Landsman et al., 2011), energetic and physiological costs associated with increased vigilance under high densities of brood

predators (Gillooly & Baylis, 1999; Steinhart et al., 2005; Gravel & Cooke, 2009), and angling-induced reduction in physiological condition of the male (Suski et al., 2003; Steinhart et al., 2005; Wagner et al., 2006). Brood abandonment in largemouth and smallmouth bass is also more likely to occur due to a decrease in reproductive value following brood depredation, whether that depredation occurred naturally or because the parental male was removed from a nest during capture by an angler (Philipp et al., 1997; Suski et al., 2003; Steinhart et al., 2005, Lunn & Steinhart 2011). Philipp et al. (1997) demonstrated that the occurrence of brood loss by depredation during angling events increased the rate of brood abandonment among parental largemouth and smallmouth bass, and parental largemouth bass that experienced a 50% reduction in brood size abandoned their brood up to 70% of the time (Suski et al., 2003). Despite the potential for negative fitness and recruitment effects of brood abandonment in bass, few studies have tested for the magnitude of decrease in reproductive value that is needed for parental largemouth bass to abandon their brood. The goals of my study were to test whether nest abandonment decisions in largemouth bass are influenced by: 1) initial brood size; 2) magnitude of brood loss; and 3) the size of the remaining brood following depredation.

MATERIALS AND METHODS

SNORKEL OBSERVATIONS

This study was conducted on Lake Opinicon, an 890 ha mesotrophic lake in eastern Ontario, Canada (44°33'N, 76°19'W). From early May to mid-June 2011, after water temperatures reached 13° C, daily snorkel surveys were performed in the littoral zone to locate nesting largemouth bass. Upon discovery of a largemouth bass nest containing eggs, male total length (L_T) was visually estimated to the nearest 2 cm (Suski & Philipp, 2004), depth of the nest

in the water column was recorded to the nearest 0.25 m, and a uniquely numbered polyvinyl chloride (PVC) tag was placed near each nest for identification. Brood size, a categorical ranking of the quantity of eggs within a nest, was visually estimated from 1 (few eggs) to 5 (many eggs) upon discovery of each nest. In Lake Opinicon, a BS estimate of 1 produces 850 ± 80 (mean $\pm$ SE) swim up fry, and a BS of 5 produces $25\ 500 \pm 1\ 500$ swim up fry (Kubacki et al., unpublished) (see Appendix A). Brood size was subsequently verified by at least two additional snorkelers to ensure precision of estimates (Suski et al., 2003; Suski & Philipp, 2004). Estimates of brood size from largemouth bass nests have previously been demonstrated to correlate positively with the number of eggs in the a nest, as well as the number of swim-up fry produced at each nest in Lake Opinicon, thus an estimation of brood size from 1 - 5 provides an accurate ranking of the reproductive value, and the expected fry production, for each parental male (Kubacki, 1992; Parkos et al., 2011).

The number of potential brood predators (bluegill *Lepomis macrochirus* Rafinesque 1819 and pumpkinseed *L. gibbosus* (L. 1758)) within a 2 m radius of each nest was quantified by instantaneous counts upon the snorkeler's approach to and departure from the nest. The mean of these counts provides an estimate of brood predator burden in proximity to a nest (adapted from Gravel & Cooke, 2009). The relative spawn date for the nests used in this study was determined by the first date of observed egg deposition, and ended with the last date of observed egg deposition.

Following the brood predator counts, nests were randomly assigned to a treatment of 0, 25, 50, 75, or 100% brood removal (herein termed devaluation), and stratified by brood size. Devaluations were conducted by a snorkeler removing the designated percentage of eggs from the nest using a turkey baster, with the devalued eggs being placed in a jar and removed from the

vicinity of the nest. Control nests (*i.e.* 0 % devaluation) were treated similarly to devalued nests

with no eggs removed. To minimize potential influence of offspring age and past investment on

parental decisions, only nests containing eggs ≤ 3 d old were included in the study.

To quantify abandonment rates, a second snorkel survey was conducted 24 hours

following brood devaluation. Males that were present on a devalued nest were deemed to have

maintained parental care following devaluation; nests with no male present were deemed to have

been abandoned (Philipp *et al*,. 1997; Hanson et al., 2007).

DATA ANALYSIS

A one-way analysis of variance (ANOVA) was used to compare L_T, brood size, nest

depth, relative spawn date, and predator burden across devaluation categories. Means were

separated using a Tukey's *post-hoc* test where appropriate (JMP version 7.0, SAS Institute Inc.,

www.sas.com; Zar, 1999). A chi-square test was used to test whether brood devaluation resulted

in different abandonment rates between treatment levels (JMP version 7.0, SAS Institute Inc.,

www.sas.com). To quantify the influence of independent variables and treatment on brood

abandonment (a binary response, *i.e.* abandon or continue guarding), data were analyzed using

fixed-effect generalized linear models (GLMs) via the GLIMMIX procedure using the default

binary logit link function (Wagner et al., 2006) (SAS version 9·2, SAS Institute Inc.,

www.sas.com;). The GLIMMIX procedure allows for robust modeling of non-continuous

explanatory variables (*e.g.* the ordinal variables brood size and proportion of a nest devalued)

against non-Gaussian dependent distributions, in particular, the binary response (SAS Institute,

2006). Biologically significant models that may explain patterns in brood abandonment were

developed *a priori* and contained the covariates relative spawn date, nest depth, predator burden,

proportion of brood removed (percent devalued), change in brood size (*i.e.* the relative change in brood size following devaluation), and remaining brood size (*i.e.* the brood size remaining following devaluation). Because male L_T was positively correlated with brood size (linear regression, $P < 0.0001$, $R^2 = 0.2890$), it was not included in the analysis.

Model fit was verified by the Pearson's chi squared/degrees freedom ratio (χ^2/d.f.), which, as χ^2/d.f. approaches 1·00, signifies a lack of residual overdispersion. Model rank was determined using a Wald-type Z-test when χ^2/d.f. was within limits of variance; a Wald-type *t*-test was used when χ^2/d.f. signified overdispersion (*i.e.* χ^2/d.f. > 1·50; Greenwood, 2008; Bolker et al., 2009). Statistical significance for all tests was concluded at $\alpha \leq 0.05$ and all means are reported as ± standard error (SE) where appropriate. Following model generation, the relationship between nest abandonment and remaining BS was visualized using logistic regression, plotting the probability of abandonment as predicted by the model against the expected number of fry produced at each nest.

RESULTS

Largemouth bass spawning began on 9 May and lasted through 5 June 2011. A total of 123 nests were located and included in this study, of which 45 males (37 %) abandoned within 24 h following devaluation treatments. Male L_T, brood size, and nest depth did not vary across treatments (Table 2.1), nor did predator burden (range = 0 – 18 brood predators; Table 2.1). Despite random assignment of treatments, there was some minor variation in spawn date among treatment groups (ANOVA, d.f. = 4,118, $P = 0.0021$, Table 2.1). The distribution of spawn date for nests included for treatment was contained within the first two weeks of the spawn period, thus this variation is unlikely to impact further results.

No model containing relative spawn date, nest depth, or predator burden as covariates explained any significant variability in abandonment ($P > 0.05$, Table 2.2). Brood loss did induce nest abandonment in largemouth bass, with the rate of brood abandonment increasing with the proportion of brood devalued (Chi-square test, $\chi^2 = 50.389$, d.f. = 4, $P < 0.0001$; Table 2.1; Fig. 2.1). At the extremes, all controls that experienced 0 % devaluation maintained parental care beyond the 24 h follow-up snorkel survey, while all males that experienced 100 % devaluation abandoned their broods (Fig. 2.1). Although a decrease in brood size is the mechanism by which a parent must re-assess the reproductive value of a brood, the model based solely on the percent of brood devalued failed to significantly predict brood abandonment ($P = 0.7075$, Table 2.2).

The generation of competing models identified three variables that explained significant variation in brood abandonment, with remaining brood size following devaluation being the best predicting model (Table 2.2; $P < 0.0001$). Largemouth bass with a remaining brood size of 3 (expected fry production of 7 900 ± 310) or greater had less than a 1.0 % probability of abandoning their brood within 24 hours of brood devaluation ($R^2 = 0.4111$; Fig. 2.2).   The likelihood of abandoning a brood increased markedly when brood size was reduced below an expected fry value of 2 400 ± 163 (BS = 2), and a threshold for 50 % probability of premature abandonment is estimated at an expected fry value of 780 (BS < 1) ($R^2 = 0.4111$; Fig. 2.2). Also, male largemouth bass showed an increasing likelihood of brood abandonment with the magnitude of decrease in brood size ($P = 0.0005$; Table II). Modeling further indicated that initial brood size, prior to devaluation, influenced nest abandonment decisions in male largemouth, with the likelihood of brood abandonment declining with increasing initial brood size ($P = 0.0199$, Table II).

DISCUSSION

Care-providing organisms adjust investment into their offspring dependent upon the perceived fitness contribution of a brood. Parents may also choose to abandon their brood in favor of investing toward potential future reproductive efforts (Carlisle, 1982; Beauchamp & Kacelnik, 1990; Gross, 2005; Bourgeon et al., 2006). In the current study, abandonment decisions were based on the initial level of mating success (brood size and subsequent fry production) and the level of brood devaluation. That is, although brood devaluation did induce abandonment in parental largemouth bass, the treatment alone did predict the likelihood that a male will abandon his brood. Rather, brood abandonment by parental bass was best explained by the number of offspring remaining in the nest following devaluation. When remaining BS indicates fry production to be $> 2\,400$ (BS = 2), paternal largemouth bass are unlikely to forfeit parental care activities. The likelihood for brood abandonment, however, increases exponentially to 50 % abandonment when remaining fry production $= 780$ (BS < 1). This marked increase in the rate of abandonment represents a threshold at which further investment into the remaining brood outweighs potential fitness benefits, thus evoking the decision by parental male largemouth bass to abandon what remains of the devalued brood.

The influence of remaining brood size on abandonment confirms that parental largemouth re-assess the reproductive value of their brood following depredation, and adjust parental investment according to the expected fitness contribution of their remaining offspring. This finding is in accordance with results from brood manipulation experiments that induced premature nest abandonment in smallmouth bass (Lunn & Steinhart, 2010) and the great tit *Parus major* (Verboven & Tinbergen, 2002); both species based abandonment decisions primarily on the number of offspring remaining following brood removal. Ackerman and Eadie

(2003) also demonstrated an abandonment threshold in mallards *Anas platyrhynchos*, which experienced an exponential increase in the likelihood of abandonment when clutch size was devalued to 60 % or 40 % of initial size, dependent upon the time within breeding season at which the devaluations occurred. The current study, however, only included nests that contained offspring aged ≤ 3 d old, thereby minimizing the potential influence of offspring age and past investment on parental decisions (Cooke *et. al.*, 2002). Together, these results demonstrate that care-providing individuals are capable of re-assessing the reproductive value of their brood following disturbance to a nest, thereby basing the decision to abandon a brood on the remaining reproductive value.

Parental largemouth did demonstrate some resiliency against brood abandonment following devaluation; the predicted probability of abandonment was low when remaining expected reproductive value > 2 400 fry (BS = 2), despite this value representing a reduction in brood size of ≥ 50 % for some males (*i.e.* males with an initial brood size of 4 or greater). Resiliency against premature abandonment in largemouth bass may be attributed to the inherently high cost of reproduction for the species, particularly at northern latitudes where males are unlikely to attempt more than a single breeding event within a given reproductive season (Suski et al., 2003; Magee & Neff, 2006; Steinhart, 2008). Partial brood loss occurs naturally for centrarchids due to natural nest depredation, storm events, egg fungal infection, and potentially poor egg quality (Knotek & Orth, 1998; Steinhart et al., 2005; Steinhart et al., 2008; Gingerich & Suski, 2011). However, the capacity for brood loss before a male decides to abandon a brood is limited in largemouth bass, as demonstrated by males being more likely to abandon with increasing magnitude of change in brood size.

The magnitude of change in brood size following experimental brood devaluation was also a significant predictor of brood abandonment in largemouth bass. The probability of abandoning a brood increased from male controls that experienced no loss in brood size, to males that experienced a complete loss of brood (*i.e.* 100 % devaluation) (Fig. 2.1.). Among centrarchids, Coleman et al. (1985) observed a decreased willingness in bluegill to defend a brood following a 50 % reduction in brood size, and male largemouth and smallmouth bass abandon a brood prematurely after the occurrence of natural or experimental brood depredation (Suski et al., 2003; Gross, 2005; Steinhart et al., 2005; Hanson et al.; 2007, Lunn & Steinhart, 2010). Although the loss of reproductive value is the mechanism by which an individual is forced to re-assess the fitness benefit of continued care into a brood, it is suggested here that the remaining reproductive value is the deciding factor underlying the decision by a care-provider to abandon a brood.

The likelihood of brood abandonment in the current study was negatively correlated with the number of offspring in a brood prior to experimental devaluation. This variation in initial investment is observed in parental smallmouth bass and water fowl of the genus *Anas*, all of which engage in more frequent and costly predator defense behaviors when guarding broods of greater reproductive value (Forbes et al., 1994; Hanson & Cooke., 2009). In the current study, however, larger brood sizes had a greater capacity for depredation before being devalued below the abandonment threshold. Initial brood size likely buffers the impact of brood loss on remaining brood size, thus initial brood size and the magnitude of brood loss together, are less important than the remaining value of a brood when largemouth bass weigh the decision to abandon or maintain care of a brood.

Due to size-assortative egg deposition by females, brood size correlates positively with the L_T and age of brood-guarding largemouth bass (Philipp et al., 1997; Suski & Philipp, 2004). Although the non-invasive experimental approach of the current study precluded collection of age data from brood-guarding males, estimated male L_T was, indeed, positively correlated with brood size. As a result, nesting male largemouth with larger broods were larger and likely older, than con-specifics with smaller broods. From an energetic standpoint, larger centrarchids show increased overwinter survivorship, and larger individuals have greater somatic reserves with which to sustain parental care activities, potentially minimizing costs on future broods (Erikstad et al., 1997; Fullerton et al., 2000; Cooke et al., 2006; DeVries et al., 2009). Potential future reproductive success in iteroparous species is variable due to increased likelihood of mortality with age, thus it may prove prudent for older males to maintain parental care following a high level of brood devaluation to best maximize fitness when future reproductive opportunities are uncertain (Williams, 1966; Gross, 2005; Steinhart et al., 2008). Together, greater initial brood size, and presumably male L_T and age, likely combine to influence brood abandonment decisions in largemouth bass.

Interestingly, predator burden, spawn date, and nest depth - factors previously demonstrated to influence parental investment in centrarchid fishes – were not significant predictors of premature nest abandonment in this study (Magee & Neff, 2006; Wagner et al., 2006; Gravel & Cooke, 2009). Centrarchids increase investment accordingly with the threat of brood depredation, as demonstrated by an increase in brood defense behaviors by smallmouth bass in lakes with high brood predator densities (Gravel & Cooke, 2009). Despite variation in nest-specific predator counts in the current study, and the high energetic cost of guarding offspring from depredation by bluegill and pumpkinseed (Gillooly & Baylis, 1999; Cooke et al.,

2002), paternal bass nesting in areas of relatively higher predator densities were no more likely to abandon their brood than were males nesting in areas of lower predator burden. Also, unlike many avian species or the colonially spawning bluegill (Magee & Neff, 2006), largemouth bass generally complete only a single spawning bout per season at the northern-most latitudes of its range (Kubacki et al. 2002). With a limited window for reproduction, the relative date at which each brood was fertilized may have little influence on the parental decisions to abandon a brood (Wingfield & Sapolsky, 2003). Together, these abiotic and biotic variables not related to brood size are unlikely mediators of brood abandonment decisions in largemouth bass, when considered in conjunction with reproductive value.

Results from this study have several implications for recruitment and fitness in largemouth bass. Because largemouth offspring in the egg and fry stages are not capable of survival without parental care, premature brood abandonment almost certainly results in a complete loss of the brood (Philipp et al., 1997; Parkos et al., 2011). Furthermore, because water temperatures in the portion of the largemouth bass range where this study was conducted generally preclude a second nesting attempt within a given year, negative effects on fitness or recruitment because of brood abandonment are not likely to be remediated within the same season. Although bass recruitment can be influenced by density-dependent or environmental variables (Ludsin & DeVries, 1997; DeVries, 2009; Gwinn & Allen, 2010), evidence exists that premature brood abandonment may limit the number of offspring produced in a population, thus negatively impacting recruitment (Gross & Kapuscinski, 1997; Parkos & Wahl, 2010; Parkos et al., 2011). Brood-guarding largemouth bass are sometimes preferentially targeted by recreational anglers across North America, thereby inducing premature brood abandonment in response to offspring depredation while the male is away from the nest (Philipp et al., 1997;

Wagner et al., 2006; Hanson et al., 2007; Siepker et al., 2007). In water bodies with high predator burden, removing a brood-guarding male may result in accelerated brood depredation to the point where remaining brood size is reduced below the threshold for abandonment. Angling-induced effects on population level reproductive success may be further exacerbated by the fact that larger males are more likely to successfully recruit offspring beyond the parental care period, yet are more susceptible to capture by angling due to their heightened nest vigilance than are smaller brood-guarding con-specifics (Suski & Philipp, 2004; Parkos et al., 2011). The current study demonstrates the importance of brood loss on parental decisions in care-providing organisms and provides further evidence that parental individuals re-assess their reproductive value following brood loss, using this information as the basis for making fitness-related abandonment decisions. To minimize potential fitness and population costs of premature brood abandonment, disturbances that might allow for nest depredation need to be minimized, particularly with regard to the removal of brood-guarding largemouth bass from their nest via selective angling practices.

ACKNOWLEDGEMENTS

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CHAPTER 2 FIGURES AND TABLES

Table 2.1. Mean (± SE) male total length (L_T), initial brood size before devaluation treatment (initial BS, a categorical ranking of the number of offspring in a nest), change in brood size following devaluation(ΔBS), brood remaining following devaluation (remaining BS), and the mean number of predators present within a 2 m radius of each nest (predator burden) for brood guarding largemouth bass. Means sharing like letters are not significantly different from one another (ANOVA; Tukey's *post-hoc*). The number of males that abandoned prematurely varied significantly across treatments (Chi-square test), and the percentage of males that abandoned within each treatment is depicted in parentheses.

| | Treatment (% devalued) | | | | | |
	0	25	50	75	100	*P*
N	26	26	26	26	19	n/a
Male L_T (mm)	359·5 ± 9·1	347·8 ± 8·8	351·7 ± 9·2	352·6 ± 6·7	344·9 ± 9·6	0·8165
Spawn date	3·8 ± 0·6[a]	4·8 ± 0·9[ab]	6·7 ± 1·1[ab]	7·7 ± 0·9[b]	9·9 ± 2·0[b]	**0·0021***
Nest depth (m)	1·2 ± 0·1	1·1 ± 0·1	0·8 ± 0·1	0·8 ± 0·1	0·9 ± 0·1	0·1031
Predator burden	3·4 ± 0·8	4·2 ± 0·1	3·5 ± 0·1	2·7 ± 0·6	3·5 ± 1·0	0·8214
Initial BS	3·2 ± 0·3	3·0 ± 0·2	3·2 ± 0·3	3·1 ± 0·2	3·1 ± 0·3	0·9949
ΔBS	0 ± 0	-0·8 ± 0·1	-1·6 ± 0·1	-2·3 ± 0·2	-3·1 ± 0·3	n/a
Remaining BS	3·2 ± 0·3	2·3 ± 0·2	1·6 ± 0·1	0·8 ± 0·1	0 ± 0	n/a
Number of males abandoned	0 (0%)	6 (23%)	9 (35%)	11 (42%)	19 (100%)	**<0·0001***

See Appendix A for quantitative description of brood size (BS) classes.

Table 2.2. Generalized linear fixed models explaining variation in premature brood abandonment in largemouth bass following experimental brood devaluation. Model selection of independent variables and interactions are based on a Wald-type hypothesis test, α=0·05. Models predicting significant variation in brood abandonment are bold with an asterisk.

Model	χ^2/df	F	P
Remaining BS	0·86	28·06	**<0·0001***
ΔBS	0·99	13·00	**0·0005***
Initial BS	1·04	3·04	**0·0199***
Nest depth	1·01	2·85	0·0939
Remaining BS*relative spawn date	1·71	2·61	0·1087
Relative spawn date	1·02	1·95	0·1654
Remaining BS*nest depth	0·88	1·95	0·1657
Predator burden*initial BS	1·01	1·84	0·1260
Initial BS*relative spawn date	1·10	1·39	0·2405
Remaining BS*predator burden	0·94	1·28	0·2604
Remaining BS*predator burden*nest depth	0·98	1·17	0·2814
Remaining BS*percent devalued	0·60	1·08	0·3588
Remaining BS* ΔBS *initial BS	0·80	0·80	0·7863
Percent devalued	0·66	0·54	0·7075
Predator burden	1·02	0·46	0·4976
Predator burden*nest depth*initial BS	1·10	0·39	0·8161
ΔBS *initial BS	0·81	0·34	0·8509
Relative spawn date*percent devalued	0·69	0·19	0·9406
ΔBS *predator burden	1·01	0·05	0·8176

Initial BS= brood size, a categorical ranking of the number of eggs in a nest prior to devaluation treatment; ΔBS= the change in brood size following brood devaluation; remaining BS=the number of brood remaining following brood devaluation treatment. See Appendix A for quantitative description of BS.

Fig. 2.1. Proportion of parental largemouth bass that abandoned their brood following experimental brood devaluation. Shading represents initial brood size (BS, a categorical ranking of the number of offspring in a nest; see Appendix A for quantitative description of BS estimates) prior to brood removal. Change in BS indicates the decrease in BS following experimental devaluation. Starting at a Change in BS of 0, bars within each Initial BS grouping indicate treatment levels of 0 %, 25 %, 50 %, 75 %, and 100 % brood devaluation, respectively.
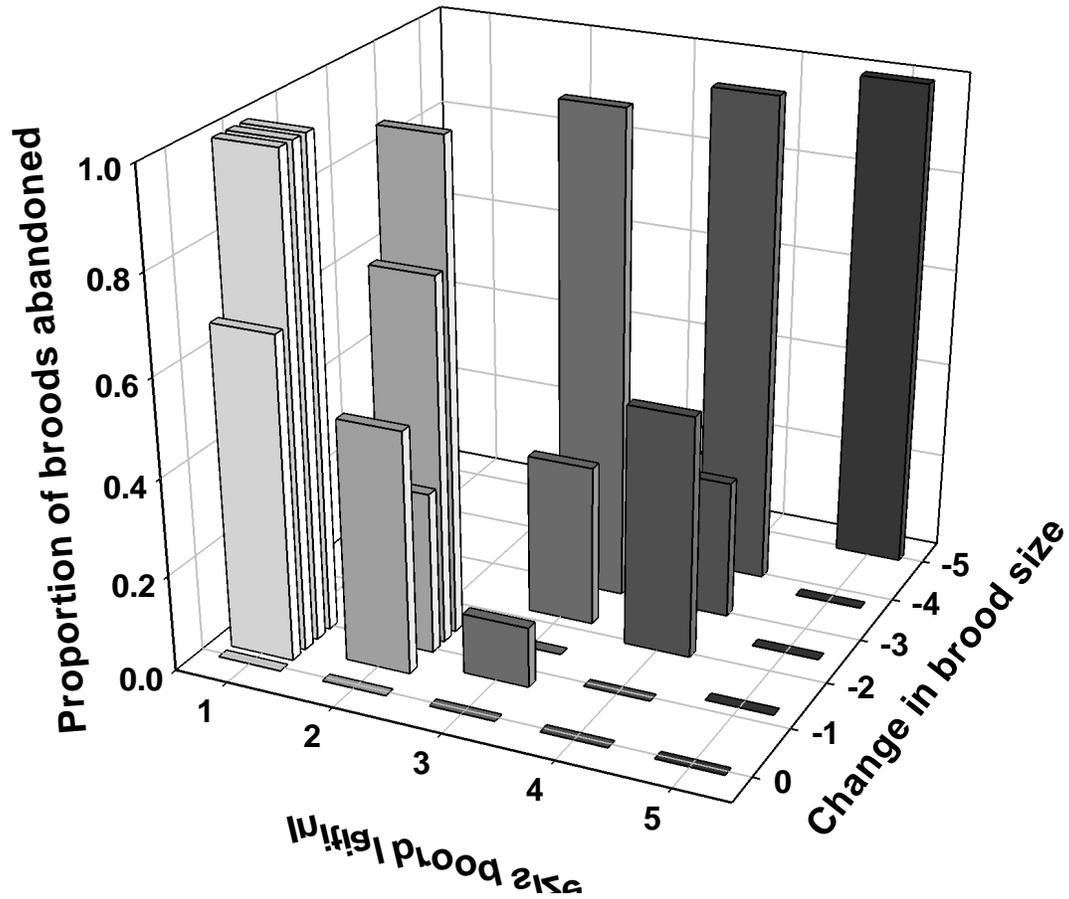
Fig. 2.2. Probability of premature brood abandonment by largemouth bass, as predicted by fixed generalized linear modeling, regressed against the number of fry expected by each male following experimental brood devaluation. The threshold for premature brood abandonment (*i.e.* 50 % probability) occurs where expected fry production is 780.
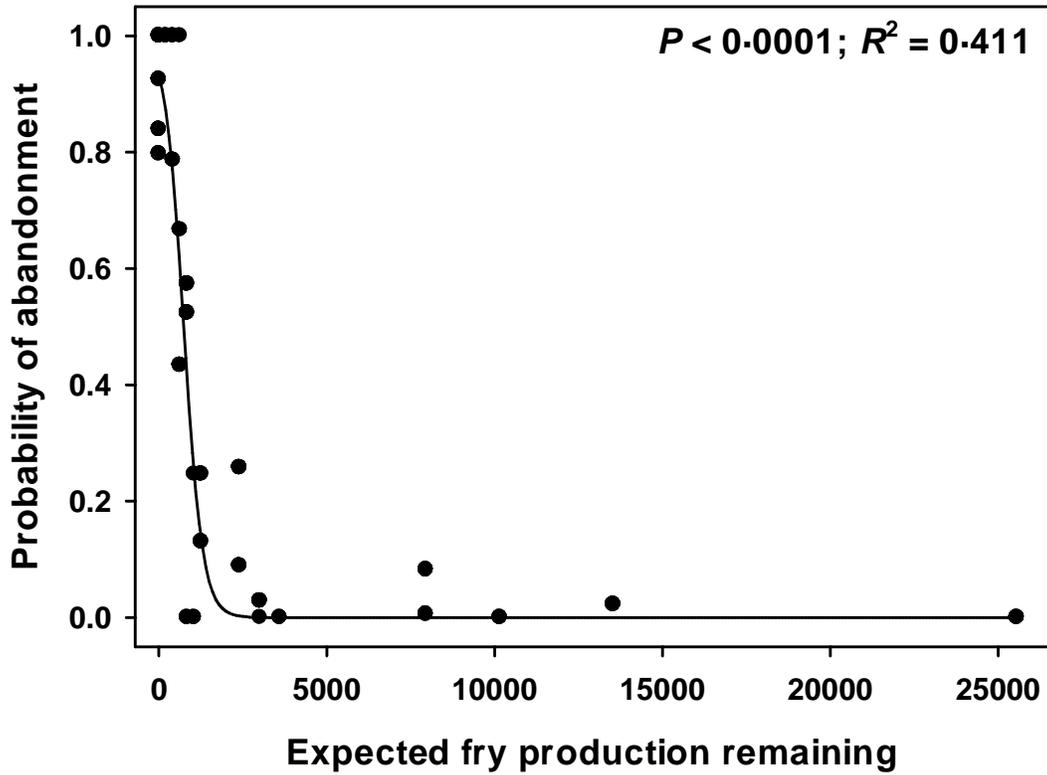
CHAPTER 3. HORMONE CONDITION AND PREDATOR BURDEN AFFECT BROOD ABANDONMENT DECISIONS IN A PARENTAL CARE-PROVIDING TELEOST

ABSTRACT

For parental care-providing organisms, reproductive success is dependent upon continued investment by the parent. Parental individuals may modify parental care behaviors based on physiological condition and the threat of depredation to a brood, thus incurring an indirect cost to their current offspring. However, how these factors compare in mediating a direct fitness cost (i.e., abandonment of a brood) is uncertain. I collected data on a free-living population of spawning largemouth bass *Micropterus salmoides* in Mills Lac, Quebec, Canada, and employed a multivariate approach to test how the threat of brood depredation, together with parent nutritional condition, stress condition, androgen condition, and oxidative stress, compare in driving the decision by a care-providing fish to abandon its current brood. My results suggest that high threat of brood depredation and low male androgen condition incur direct fitness consequences and influence the decision by a parent to abandon parental care activities.

INTRODUCTION

Reproductive success for parental care-providing individuals can be influenced by tradeoffs between the cost of care, and current and future fitness (Williams 1966; Trivers 1972). Research addressing the cost of parental care has primarily focused on brood size, brood loss, resource availability, predation risk, or endocrine-related physiological condition of a parent (Fontaine & Martin 2006; Gravel & Cooke 2009; Dassow et al. 2011; Wingfield et al. 1990; Neff & Knapp 2009), and oxidative stress has emerged as an additional potential driver of fitness (Alonso-Alvarez et al. 2004; Wiersma et al. 2004). Studies that use a holistic approach to test for the relative influence of these factors concurrently are few; most work to date has quantified these individual factors in isolation (Zera & Harshman 2001; Schreck 2010). Furthermore, few studies have correlated these factors to a direct fitness response (e.g., offspring abandonment, reproductive success) (Breuner et al. 2008), with most work extrapolating changes in behavior to a loss of 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 et al. 2003; Barton 2002; Ricklefs & Wikelski 2002). The threat of predation incurs a strong influence on parental behavior; care-providing birds, when threatened by predation, reduce foraging effort and decrease maternal investment into egg mass and clutch size (Fontaine & Martin 2006). This behavioral response is purported 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 egg-laying 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 defense behaviors (Steinhart et al. 2004; Gravel & Cooke 2009). This behavioral response is associated with increased circulating testosterone concentrations, but is mediated only by the

high brood predator environment, as no link between physiological condition and brood defense behaviors has been reported (O'Connor et al. 2011b).

In addition to mediating behavioral 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. The glucocorticoid stress response and testosterone compromise immune function (Barton 2002; Hau 2007), and testosterone-mediated male aggression has been implicated as being detrimental to parental care behaviors and longevity, yet appears to play an integral role in male reproductive strategies (Wingfield et al.1990; Magee et al. 2006). 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 et al. 2009). The energetic demand of brood defense 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 (Erikstad et al. 1997). It remains unclear, however, whether varying physiological and environmental conditions have a hierarchal effect on parental behaviors. Specifically, it is unknown how these factors compare or correlate in driving the decision by a parent to abandon its offspring, thereby incurring a potential cost to fitness.

Using largemouth bass (*Micropterus salmoides*) as a model, we employed a multivariate approach to test whether a suite of physiological and environmental factors, when examined concurrently, influenced potential fitness of an individual as determined by 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: a) a holistic approach was used to directly compare the relative influences of physiological and environmental (predator) variables on parental care decisions; b) the population examined was wild and free-swimming without environmental variable manipulation; and c) 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 (*Micropterus salmoides*) was studied in Mills Lac (45°47'46" N, 74°46'54" W), a mesotrophic lake near Kenauk, Quebec, Canada. Largemouth bass spawning begins as water temperatures approach 15° C, when males construct a saucer-shaped nest in the littoral substrate to which females are courted and 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 et al. 2002). Parental care activities include fanning eggs to prevent accumulation of sediment and maintain sufficient

oxygenation, and engagement in agonistic behaviors in defense of the brood from nest predators, here *Lepomis macrochirus* (bluegill) and *L. gibbosus* (pumpkinseed) (Hanson et al. 2009). At the northern extent of largemouth bass range, and 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, weekly snorkel surveys were performed parallel to the entire length of the shoreline of Mills Lac to locate nesting largemouth bass, and a uniquely numbered polyvinyl chloride (PVC) tag was placed approximately 1 m from each nest for identification purposes. Developmental stage of each brood (brood age) was estimated from newly fertilized eggs ($\leq$ 1 d old) to egg sac fry (approximately 7 d old), 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 was visually estimated using a proven technique (brood size, BS), and assigned a categorical ranking from 1 (few) to 5 (many) (Suski et al. 2003) (see Appendix A). 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 these 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 and Cooke 2009).

Following completion of each snorkel survey, newly discovered largemouth bass with broods aged ≤ 7 d old were 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, then transferred to a 1.5 ml vial for centrifugation at 3000 rpm 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 minute holding period, the timing of which commenced upon successful hooking of the male during angling. The 10 minute holding period allowed for the standardization of handling techniques and recovery of the male largemouth 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 and 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 generally resumed paternal care behaviors 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, in press). 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 hours following angling

and blood sampling procedures; males present on their nests during this survey, as indicated by a

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 were successful in raising broods to the free-swimming fry stage, and were thus

assumed to have successfully reared broods to independence.

PHYSIOLOGICAL ANALYSES

Plasma cortisol was quantified via a colorimetric competitive enzyme-linked

immunoassay, (ELISA; Enzo Life Sciences Cortisol ELISA Kit ADI-900-071, Farmingdale,

NY), a technique previously validated for quantification of cortisol concentrations in largemouth

bass plasma (Sink et al. 2008). Quantification of plasma potassium (K^+) and sodium (Na^+)

occurred by flame atomic mass spectrometry (Cole-Palmer Instruments Model 2655-00, Vernon

Hills, IL), while chloride (Cl^-) concentration was quantified by coulormetric titration (Labconco

Co. Digital Chloridometer Model 4425000, Kansas City, MO). Plasma glucose was quantified

via a colorimetric enzymatic assay adapted from Lowry and Passonneau (1972). Plasma protein

concentration was determined by total solid refractometry (Reichert VET 360 model 137536L0, Depew, NY) (Wells & Pankhurst 1999), and plasma cholesterol was quantified with a commercially available colorimetric assay (BioAssay Systems EnzyChrom[TM] AF Cholesterol Assay Kit E2CH-100, Hayward, CA). Concentrations of 11-Keto Testosterone (11-KT), the primary androgen in teleost fishes, were determined by colorometric ELISA (Cayman Chemical 11-KT Testosterone EIA Kit 582751, Ann Arbor, MI).

Oxidative stress of each paternal largemouth was assed via two variables describing oxidative damage and antioxidant capacity. Lipid peroxidation, an indicator of oxidative damage to cell lipids, was quantified by colorimetric determination of thiobarbituric acid reactive substances (TBARS) following the reaction between malondialdehyde, the byproduct of peroxidation of polyunsaturated fatty acids, and thiobarbituric acid (Cayman Chemical TBARS Assay Kit 10009055, Ann Arbor, MI). 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, Ann Arbor, MI). All colorimetric analyses were quantified by microplate spectrometry (Molecular Devices Spectramax Plus model 384, Sunnyvale, CA).

STATISTICAL ANALYSES

Consistent with patterns commonly observed in *Micropterus* spp., male TL was correlated with the number of offspring (i.e., brood size) that a male received in mating (linear regression; $R^2 = 0.31$, $P < 0.0001$). TL was therefore not included in further analysis (Philipp et al. 1997; Gingerich & Suski 2011). Due to the complexity and potential for biological

interrelatedness among the 16 variables quantified by snorkel observation and physiological analysis (Table 1) (Zera & Harshman 2001; Travers et al. 2010), and to avoid potential pitfalls associated with stepwise regression modeling (Whittingham et al. 2006), a multivariate principal component analysis (PCA) was used to reduce variables into newly defined terms. Pre-capture and post-capture predator burdens for each nest were averaged ('mean predator burden') and included in the PCA as a third metric summarizing the predator densities experienced by each brood-guarding largemouth bass throughout the experiment. 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 (Peres-Neto et al. 2003; Gingerich & Suski 2011).

To test for factors influencing brood abandonment by paternal largemouth, PCs were compared between males that maintained parental care following sampling procedures and those that abandoned their brood using a t-test, with significance determined at $\alpha = 0.05$. The relationship between maximally loaded parameters for PCs exhibiting significant variation between males that maintained care and those that abandoned their brood were further analyzed via cubic regression of un-rotated variables, where appropriate. All analyses were performed using JMP version 7.0 (SAS Institute Inc., Cary, NC) and all means are reported ± standard error (SE) where appropriate.

RESULTS

Fifty-five brood-guarding largemouth bass were included in this study, of which, 35 % abandoned their brood within 24 hours of the standardized stressor (Table 3.1). Six PCs with eigenvalues >1.0 were retained, and together explained 72.4 % of the observed variation in the

data (Table 3.2). PC1 describes male nutritional condition, and was characterized by high

plasma protein and cholesterol, and also by high TBARS (Table 3.2). PC2 describes brood

predator burden as characterized by pre-capture, post-capture, and mean predator burden at each

nest prior to and following plasma collection (Table 3.2). Brood age and K^+ loaded positively

into PC3, with 11-KT loading negatively, thus PC3 describes a decrease in male androgen

condition with brood age (Table 3.2). PC4 was characterized by positive loadings of BS,

cortisol, and TAC, and negative loading of plasma glucose (Table 3.2). PC5 describes male

stress condition as characterized by concentrations of plasma cortisol, Cl^-, and K^+ (Table 3.2).

PC6, as characterized by positive loadings by spawn date and K^+, and negative loading by TAC,

describes the diminishing antioxidant capacity of paternal largemouth bass throughout

progression of the parental care period.

Comparison of each PC between males that abandoned their brood and those that

maintained care yielded a significant difference in PC2 ($P = 0.046$; Table 3.3, Fig 3.1(a)).

Paternal largemouth bass that abandoned their brood following the standardized disturbance had

higher PC2 scores relative to males that maintained parental care, thus males with nests located

in areas of higher predator burden (mean predator burden per 2 m nest radius $= 5.1 \pm 1.0$) relative

to nest locations with fewer predators (mean predator burden per 2 m nest radius $= 2.8 \pm 0.5$)

(Fig 3.1(a), Fig 3.2). PC3 scores were also significantly higher in males that abandoned their

broods premature to offspring independence ($P = 0.023$; Table 3.3), indicating that concentrations

of 11-KT in plasma were lower for males that forfeited parental care and that the probability of

premature abandonment increased with brood age (Fig 3.1(b)). Accordingly, cubic regression of

male 11-KT concentrations by brood age indicated a negative relationship between androgen

concentration and developmental stage of offspring ($P <0.0001$, $R^2 = 0.429$; Fig 3.3).

DISCUSSION

        The level of parental care behavior displayed by reproducing animals 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 et al. 2008); rarely have these changes in parental care behavior been correlated to a potential fitness cost. This study suggests that the perceived threat of brood depredation can induce premature brood abandonment by paternal largemouth bass. Specifically, parental largemouth bass that abandoned their broods following a standardized stressor had higher PC2 scores relative to males that continued brood investment, indicating that predator densities can incur a direct fitness cost on care-providing individuals. The indirect effect of predation pressure on parental care behaviors has previously been documented, with the occurrence of nest predators inducing a decreased rate of foraging and mate feeding in several passerine birds (Ghalambor& Martin 2002; Fontaine & Martin 2006), and decreased investment into clutch size and egg mass by female sparrows (*Melospiza melodia*) (Travers et al. 2010). In contrast, male smallmouth bass nesting in areas of high predator density engage in more frequent aggressive behaviors relative to individuals nesting in lakes of low brood predator density (Steinhart et al. 2004; Gravel & Cooke 2009), yet predator burden has not been previously 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 2005), it is unlikely that significant brood depredation occurs while a male largemouth bass is actively defending his brood, and no measurable brood loss occurred in the current study while largemouth were away from their nest during plasma collection. Thus, pre-capture and post-capture predator burdens represent the threat of predation as perceived by the brood-guarding male, independent of actual

loss in reproductive value (Gravel & Cooke in press). However, male largemouth bass may have been less willing to guard their brood following capture, resulting in heightened rates of brood depredation following observation of the nest (Suski et al. 2003). Despite uncertainty in what occurred within the 24 hour post-capture period, the study at hand provides evidence that the threat of brood depredation alone, when used as a basis for brood abandonment decisions, can have a direct negative effect on an individual's potential fitness.

The androgen 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 et al. 2006). The exact function of male androgens in parental care behavior across taxa has not been clearly defined, and is likely to vary with species and life history stage (Dufty Jr. 1989; Wingfield et al. 1990; Hau 2007), but is believed to play a role in spermatogenesis, sexually dimorphic trait development, and nest construction in teleost fishes (Borg 1994). Brood-guarding largemouth bass with lower concentrations of 11-KT in this study, and older broods, exhibited increased rates of premature brood abandonment, suggesting androgens play a role in parental decisions. Similarly, Dey et al.(2010) showed that aggression in brood-guarding smallmouth bass was significantly attenuated following experimental injection of an androgen receptor antagonist (cyproterone acetate), suggesting androgens to be important for brood defense behaviors. In contrast, Neff and Knapp (2009) demonstrated that both 11-KT and T had no influence on nest-tending behaviors in paternal bluegill, and also that11-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 and circulating concentrations of T. Together, these studies indicate that androgen concentration can either increase or decrease nest defense behaviors by a care-providing fish, yet

no influence of androgens has been observed to result in a direct decrease in reproductive success. The trend towards lower 11-KT concentrations for largemouth bass that abandoned a brood here offers novel evidence that androgen concentration likely does 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.

As described in PC3, largemouth bass circulating 11-KT did decrease with brood age in the current study, similar to androgen profiles in male smallmouth bass and bluegill (Magee et al. 2006; O'Connor et al. 2011a), and also correlated with K^+. Despite a positive relationship between K^+ and 11-KT concentrations, no other stress correlates were significant or correlated with 11-KT, thus this is not a likely indicator of a correlation between stress and androgen condition. However, parental care theory suggests that the relative value of a brood increases as the offspring approach independence (Trivers 1972; Östlund-Nilsson 2002). To control for the influence of brood age on male decisions, largemouth bass were included in the current study only if their offspring were non-mobile (i.e., egg sac fry ≤ 7 d old) and fully dependent upon paternal care for protection from depredation. Brood abandonment at this stage would result in complete forfeiture of reproductive value for the paternal largemouth bass, thus the relative fitness cost of brood abandonment on male fitness is the same across these early brood stages. Cooke et al. (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). In contrast, nutritional condition did not vary between males that abandoned and males that maintained care in the current study, and plasma metabolites did not correlate with either brood age and 11-KT concentration in PC3. Thus, it is unlikely that

decreasing nutritional condition with brood age was a limiting factor for maintaining parental care in the current study. Similar to this study, Neff and Knapp (2009) observed androgen concentrations in paternal bluegill to also decrease with early brood development, then for males that re-nested within the same season, increase again as fry neared independence. Together, these studies demonstrate that high circulating androgen concentration may be critical for maintaining male investment into offspring, and potentially plays a role as an endocrine control for limiting energetic investment into a brood.

Interestingly, several variables previously shown to influence parental care behaviors and decisions in fishes were not significant drivers of brood abandonment decisions for largemouth bass. Principal components described by brood size, nutritional condition, stress condition, and oxidative stress did not differ significantly between paternal largemouth bass that abandoned their broods prematurely and those that maintained parental care. Resource limitation is often cited as a 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 et al. 2002). Poor body condition can hinder parental behaviors 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 et al. 2002; Hanson & Cooke 2009). For paternal bass in the current study, plasma protein correlated negatively with brood age in PC2, although this relationship did not load maximally. No significant variation in the PC comprised of plasma metabolites (PC1), however, was observed

between males that abandoned their brood and those that maintained care. This may be attributed to the limited time of parental care under scrutiny (i.e., all broods were ≤7 d old at the time of plasma sampling), or potentially because male largemouth, if emerging from overwinter in poor body condition, may opt out of attempting a reproductive bout altogether (Fullerton et al. 2000). 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, particularly when to other environmental and physiological variables.

No difference between the primary or secondary stress response (i.e., cortisol and subsequent plasma ion concentration), nor a difference in oxidative stress, were measured when comparing PCs for paternal bass that abandoned their brood and those that maintained parental care. The stress response is important in ensuring the survival of an individual, but during reproduction, 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 et al. 2008; Schreck 2010). In largemouth bass, however, implanting of parental males with exogenous cortisol did not induce changes in parental behavior, thus the stress response has not been linked to parental care decisions in largemouth bass (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 behavioral changes associated with maintaining homeostasis, thereby avoiding any cost to the current brood. The attenuation of a stress response in brood guarding bass is further substantiated by the current study in that variation in stress condition was not detected between paternal largemouth bass that abandoned their brood, and those that maintained parental care. The evidence against stress-mediated behavioral 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.

In addition to parameters associated with stress and nutrition, oxidative stress did not differ between males that maintained parental care following sampling procedures, and those that forfeited their brood. TBARS, a measure of lipid peroxidation, and TAC, a measure of free-radical scavenging capacity, did not load into either of the PCs that differed with male abandonment. TBARS 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 incurs a negative impact on 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 et al. 2009). Exercise, such as increased muscle activity by paternal bass into brood fanning and predator chasing (Cooke et al. 2002), increases the production of damaging ROS as a byproduct of aerobic respiration (Leeuwenburgh & Heinecke 2001), yet oxidative stress is not observed to occur in bass (Wilson et al. 2012). While the occurrence of ROS alone does not constitute oxidative stress, 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 et al. 2009). TAC did diminish with brood age in the current study, yet did not load into any significant PC, nor did this decrease in TAC correlate with increasing oxidative damage. In accordance with previous findings on the effect of oxidative stress on parental care, reproduction in largemouth bass is unlikely to incur an oxidative cost, and does not induce premature brood abandonment by parental males when compared to other variables.

The influence of stress, predation risk, resource limitation, and androgen condition on reproductive success of care-giving animals 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 et al. 2009). The current study holistically compared how predator burden, stress, oxidative stress, nutritional condition, and androgen concentration in a care-provider compare in their influence on potential fitness decisions, using brood abandonment as a direct fitness affect. Results indicate that androgen condition and predator burden, when compared directly to other factors known to influence parental care, are most important in driving the decision by paternal largemouth bass to forfeit a brood. The framework put forth here addresses the lack of empirical evidence as to how different factors, together, affect fitness of an individual (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 (Breuner et al. 2008).

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CHAPTER 3 TABLES AND FIGURES

Table 3.1. 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 (d)	8	1	31	0.71
Brood size	2.8	1	5	0.71
Brood age (d)	3.5	1	7	0.31
Pre-capture pred. burden	3.9	0	17.5	0.61
Post-capture pred. burden	3	0	18	0.60
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	1.40
Plasma glucose (mmol/l)	3.8	2.1	8.1	0.17
Plasma protein (g/ml)	0.05	0.04	0.09	0.02
Plasma cholesterol (mg/dl)	503.2	327.7	667	11.42
Plasma 11-KT (ng/ml)	1021.15	27.3	2854.6	100.00
Plasma TAC (mM)	0.32	0.07	0.45	0.02
Plasma TBARS (μM)	340.3	115.25	882	21.70

TL, male total length; 11-KT, 11-Keto Testosterone; TAC, total antioxidant capacity; TBARS, lipid peroxidation

Table 3.2. Principal components retained (i.e., PCs with eigenvalue > 1.0) following orthogonal rotation. Principal components are described by factors that loaded maximally (values ≥ 0.4 or $\leq$ -0.4) and are presented in bold.

|	PC1	PC2	PC3	PC4	PC5	PC6
Spawn date	0.07	0.09	0.20	-0.37	-0.14	**0.62**
Brood size	0.22	-0.27	-0.19	**0.66**	-0.05	0.10
Brood age	-0.02	-0.06	**0.87**	0.01	-0.08	-0.06
Pre-capture pred. burden	0.10	**0.70**	0.35	-0.13	0.18	-0.14
Post-capture pred. burden	-0.18	**0.80**	-0.23	0.06	-0.07	0.07
Mean pred. burden	-0.05	**0.98**	0.08	-0.05	0.08	-0.05
Plasma Cl$^-$	0.09	0.08	0.07	0.14	**0.82**	-0.05
Plasma Na$^+$	0.13	-0.07	-0.07	0.20	0.18	**0.78**
Plasma K$^+$	-0.10	0.08	**0.60**	-0.12	**0.44**	0.32
Plasma glucose	0.21	-0.08	0.03	**-0.66**	0.01	0.12
Plasma cortisol	0.05	-0.02	0.25	**0.41**	**-0.62**	-0.04
Plasma total protein	**0.90**	-0.02	0.18	-0.16	0.08	0.08
Plasma cholesterol	**0.89**	0.00	-0.03	0.16	-0.13	0.04
Plasma 11-KT	-0.12	-0.10	**-0.82**	0.15	0.09	-0.08
Plasma TAC	-0.01	0.24	-0.12	**0.44**	0.37	**-0.56**
Plasma TBARS	**0.87**	-0.13	-0.06	-0.08	0.11	0.07
% Var. explained	18.2	17.1	12.7	9.3	8.6	6.5
Eigenvalue	2.9	2.7	2	1.5	1.4	1.1

11-KT, 11-Keto Testosterone; TAC, total antioxidant capacity; TBARS, lipid peroxidation

Table 3.3. Description of each principal component (PC) based upon maximally loaded variables. PC scores were compared between male largemouth bass that abandoned their brood premature to offspring independence, and those that maintained parental care, using a student's *t*-test.

	Description	*P* value
PC 1	Nutritional condition	0.549
PC 2	Predator burden	0.046*
PC 3	Androgen condition and brood age	0.023*
PC 4	Stress and oxidative stress	0.503
PC 5	Stress and ion imbalance	0.856
PC 6	Antioxidant capacity over time	0.852

*Denotes PCs that significantly differ between males that abandoned their brood and those that maintained paternal care, α = 0.05.

Fig. 3.1. Principal component (PC) scores describing predator burden (PC 2, a), and male 11-KT concentration over time (PC 3, b), compared between male largemouth bass that abandoned their brood prematurely (Y) and males that maintained parental care following blood sampling protocol (N) using a *t* test ($\alpha = 0.05$).
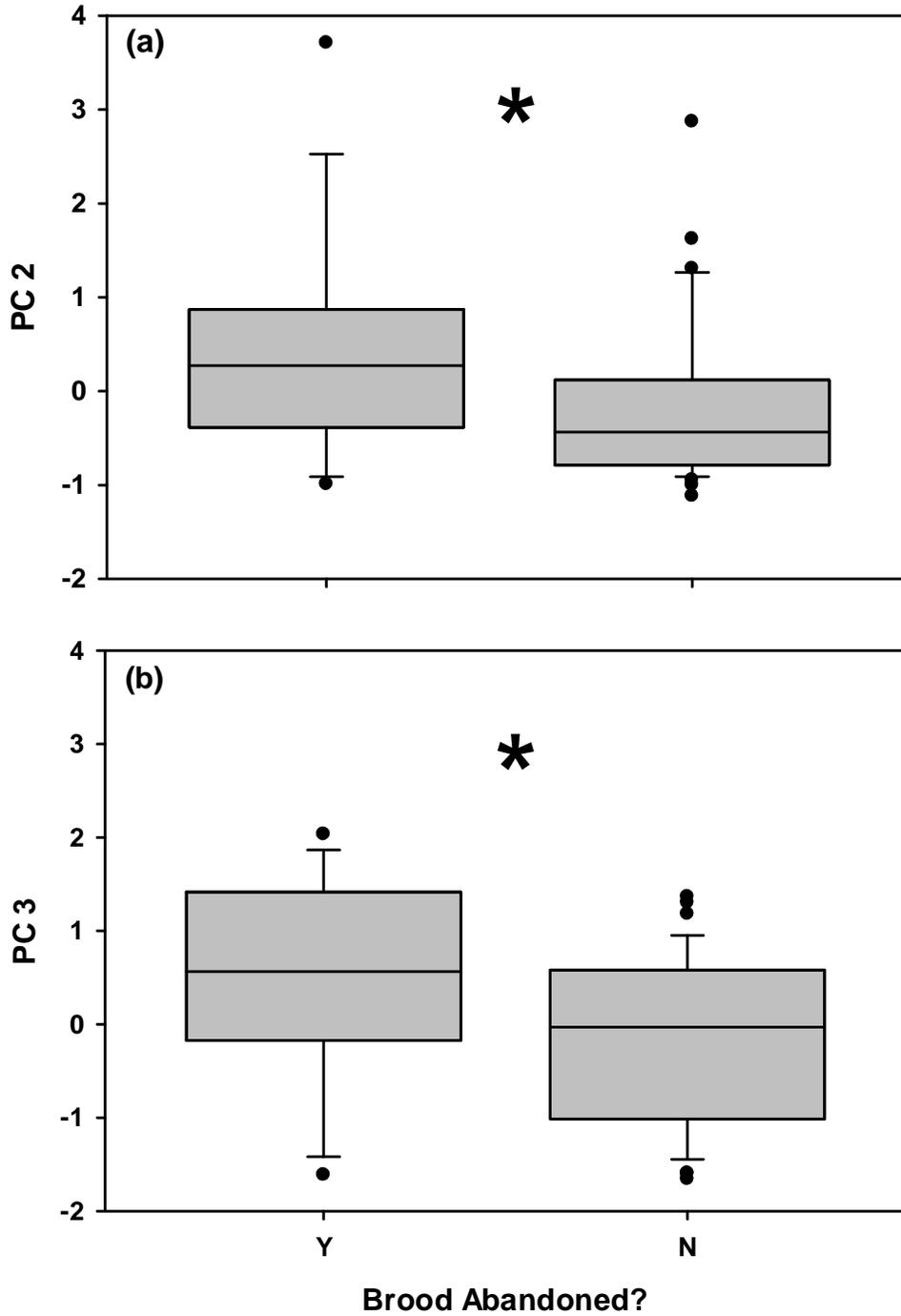
Fig. 3.2. Mean values for the number of brood predators, bluegill and pumpkinseed, within a 2 m radius of each largemouth bass nest during parental care (Pre-capture), upon the male's return to the nest following plasma sampling protocol (Post-capture), and the mean predator burden at each nest across throughout the study (Mean), represented by males that abandoned parental care (Y) and those that maintained parental care (N).
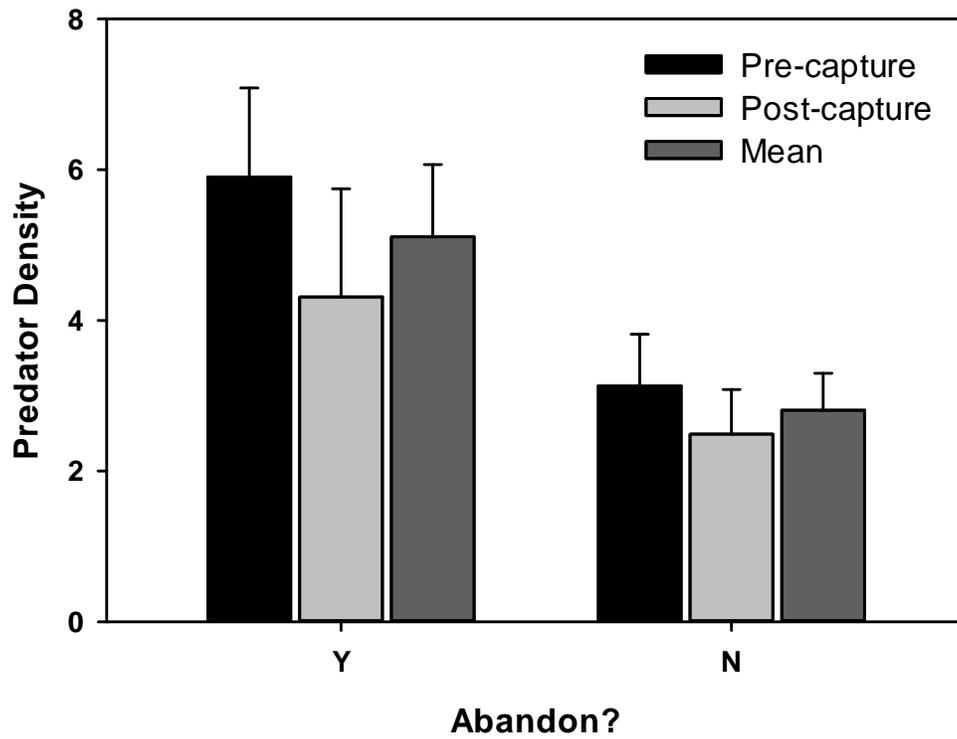
Fig. 3.3. Change in paternal largemouth bass circulating androgen concentration (11-Keto testosterone, 11-KT) with increasing brood age. Closed circles indicate males that maintained parental care; open circles indicate males that abandoned. Significance for cubic regression and associated R^2 for all males combined are reported in the figure.
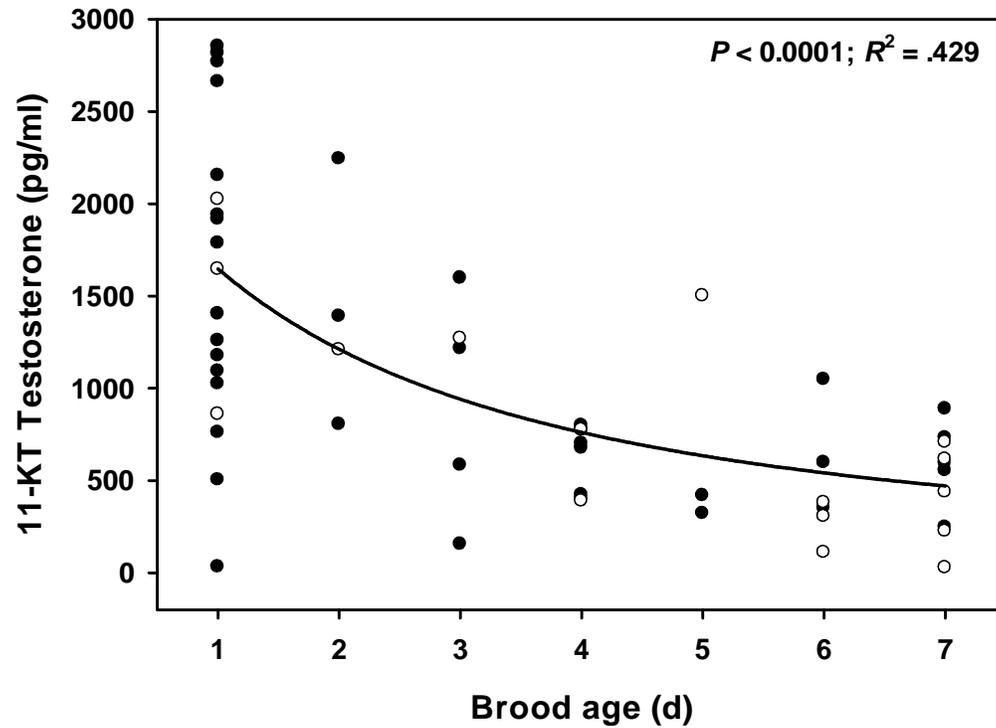
CHAPTER 4.
CONCLUSION: STRESS, OFFSPRING, OR NEIGHBORHOOD: WHAT DRIVES BROOD ABANDONMENT DECISIONS IN LARGEMOUTH BASS?

The introduction of life history and parental care theories has brought forth competitive and compelling hypotheses describing constructs on the fitness contribution of an individual. Resource availability and body condition have long been described as limiting to parental investment (Williams 1966, Trivers 1972), but the advance of wide-spread and cost-effective laboratory capabilities has ushered in a new era of competing hypotheses, allowing for quantification of a range of physiological correlates. A disconnect exists, however, between the physiology-based costs of parental care (i.e., oxidative stress and hypothalamic-pituitary-interrenal axis-mediated stress) and direct consequences of physiological condition on fitness (Burgeon et al. 2006). Although correlations and competition exist among the environment, behavior, and physiological response of a care-provider, few studies have attempted a holistic approach to test for the fitness effects of these variables, together. The complementary studies put forth here offer a multi-faceted approach to describing parental decisions, using brood abandonment by paternal largemouth bass as a direct, and ultimate, fitness cost.

The combined results of my studies describe the effect of brood loss and predator density on the reproductive success of male largemouth bass. I demonstrated that, following a reduction in brood size, abandonment decisions are based primarily on the number of offspring remaining, independent of the proportion of the brood lost. Predator densities, independent of depredation, were significant drivers in brood abandonment decisions when compared to male stress, nutritional condition, and oxidative stress. My findings have significant implications towards the sound management of parental care-providing species, with a particular emphasis on largemouth

bass as a highly sought-after sportfish. For male largemouth bass nesting in lakes of high predator burden, the threat of brood depredation (i.e., population densities of nest predators) is likely perceived as a heightened cost to parental care (Gravel and Cooke 2009), and males are more likely to abandon following a natural (e.g., storm event) or anthropogenic (e.g., angling) disturbance. If brood-guarding largemouth bass are targeted by anglers, this not only constitutes a disturbance, but also results in brood depredation while the male is away (Suski et al. 2003, Philipp et al. 2007). The threat of depredation to a brood, confounded by brood loss sustained during an angling event, is likely to result in high rates of brood abandonment by male largemouth bass in lakes of high predator burden. To ensure reproductive success and recruitment at the population level, it is suggested that management actions be considered that restrict angling of brood-guarding largemouth bass in lakes characterized by high *Lepomis* spp. or other brood predator densities.

Results of my studies have further implications for parental care across taxa. The response by largemouth bass to a heightened threat of brood depredation offers potential effects on reproductive success of care-providing species due to shifting community structure following the introduction of invasive species, and potentially climate change (Steinhart et al. 2005). My findings also offer evidence that male androgen condition can directly affect parental care decisions in wild-living animals, and may potentially benefit reproductive success of an individual, as evidenced by resiliency of males with higher circulating 11-KT to brood abandonment. My collective studies provide the framework for a holistic approach to quantifying the factors which ultimately incur a fitness cost to an individual, and it is suggested that this approach be used across taxa to best understand the relative costs of parental care.

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APPENDIX A.
BROOD SIZE ESTIMATES

Table A.1. The mean number of fry produced for estimated largemouth bass brood size (BS) in Lake Opinicon, Ontario, Canada in 1992 and 1993. Snorkel surveys were performed to assign a brood size estimate to newly discovered largemouth bass nests. Fry were collected upon reaching the swim-up stage, and subsequently enumerated (Kubacki et al. unpublished data).

BS estimate	N nests	Mean fry produced	SE
1	12	840	80
2	11	2400	160
3	14	7900	300
4	12	13500	600
5	9	25500	1500

Fig. A.1. Cubic regression analysis of largemouth bass brood size estimated during snorkel survey, plotted against the mean number of swim-up fry produced by each brood size class. $R^2 =$ 0.996 (Kubacki et al. unpublished data). Error bars indicate standard error (SE).
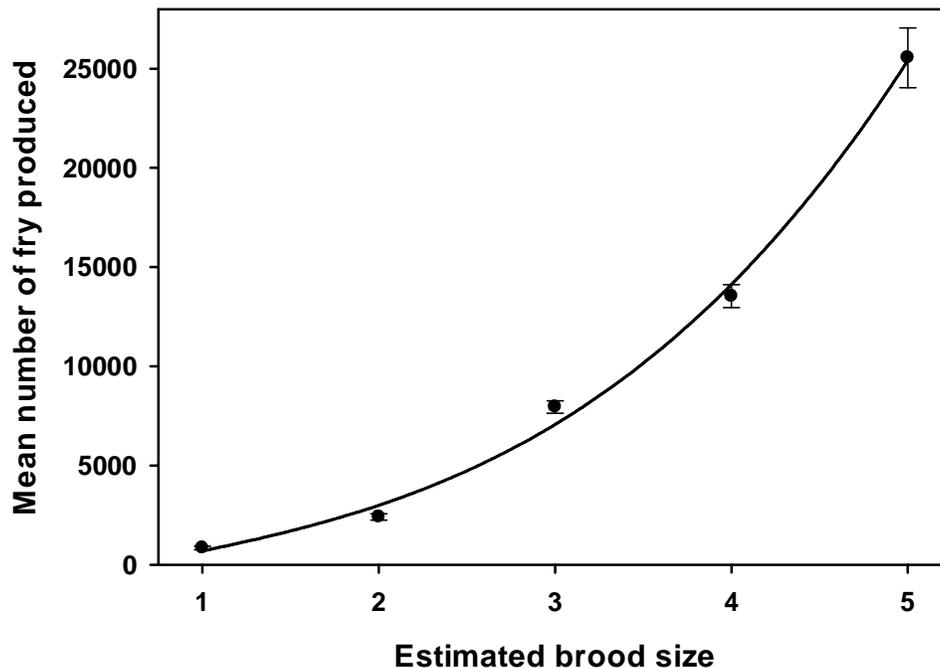
REFERENCE

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