OFFSPRING CANNIBALISM AND PARENTAL CARE IN THE PLAINFIN MIDSHIPMAN FISH

OFFSPRING CANNIBALISM AND PARENTAL CARE IN THE PLAINFIN MIDSHIPMAN FISH

BY
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TITLE: Offspring cannibalism and parental care in the plainfin midshipman fish

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Thesis abstract

Cannibalism of offspring is a common yet seemingly paradoxical phenomenon observed across a wide variety of taxa. Behavioural ecologists have been particularly interested in understanding this behaviour within the context of parental care. This is because, superficially, offspring cannibalism appears counterproductive to the goals of a caring parent, which are often achieved by nurturing and protecting their young. Despite the prevalence of offspring cannibalism in many taxa, we still have a poor evolutionary understanding of this intriguing behaviour. Many hypotheses have been proposed explaining why parents may terminate and consume offspring, however, empirical tests of these hypotheses frequently return mixed results, and few examples exist in which multiple hypotheses for cannibalism have been tested using the same model study system. Over the course of my PhD thesis, I tested different cannibalism hypotheses in a novel study organism, the plainfin midshipman fish (*Porichthys notatus*), a species of paternal care-giving toadfish that frequently engages in offspring cannibalism. I first characterized the factors that underlie male reproductive success in this species to provide a broader understanding of their reproductive ecology and a background for the cannibalism studies in my later chapters. I showed that both male size and nest size are important correlates of male reproductive success (e.g. mate attraction, egg acquisition, and rearing success) in this system, and that morphological, physiological, and ecological variables can also have an impact on reproductive success (Chapter 2). Next, I conducted a series of field and laboratory studies to uncover the factors that select for offspring cannibalism in this species. In particular, I investigated whether offspring cannibalism serves to replenish dwindling energy reserves and/or occurs when paternity over a brood is likely to be low. I showed that although plainfin midshipman males endure a long and energetically taxing parental care period, they cannibalize offspring most frequently early in the breeding season, the time period when the males are in their best body condition but when male-male competition is also at its peak (Chapter 3). Using a direct comparison of males that had recently cannibalized offspring versus those that had not, I showed that the males with the lowest energy reserves were actually the least likely to have offspring in their digestive tracts, suggesting that offspring cannibalism is not driven by immediate energetic need (Chapter 4). I then tested whether males assess their paternity using direct or indirect offspring cues, and showed that males use the act of a nest take-over as reliable indirect cues of their paternity over a brood (Chapter 5). Altogether, my results further our understanding of the reproductive ecology of the plainfin midshipman fish, and provide a foundation upon which to assess and test between cannibalism hypotheses in this species and others. My thesis work demonstrates the advantage of studying multiple cannibalism hypotheses in a single study system, and through this approach we can gain a more accurate evolutionary understanding of how the phenomenon of offspring cannibalism is maintained in different species.

Acknowledgements

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Declaration of academic achievement

This dissertation is organized in a sandwich format as approved by McMaster University. It consists of six chapters and two appendices. **Chapter 1** provides an overview of relevant theory and outlines the aims of my dissertation research. **Chapter 2** is a manuscript currently under review. **Chapters 3**, **4**, and **5** are published manuscripts. **Chapter 6** is a general discussion of my results from Chapters 2-5. Appendix **Chapter 7** is a manuscript in preparation and appendix **Chapter 8** is a published manuscript. Both appendix chapters are thematically connected to my main data chapters.

Chapter 1: General Introduction
Author: Aneesh P. H. Bose

Chapter 2: Male quality and resource quality as factors affecting male reproductive success in an intertidal fish
Authors: Aneesh P. H. Bose, Karen M. Cogliati, Nick Luymes, Andrew H. Bass, Margaret A. Marchaterre, Joseph A. Sisneros, Benjamin M. Bolker, and Sigal Balshine
Publication: Submitted to Behavioral Ecology (BEHECO-2017-0223).
Comments: A. Bose, K.C., and S.B. conceived and designed the studies. A. Bose, K.C. S.B. and N.L. collected data from the field. A. Bass, M.M., and J.S. assisted with data

collection and field work permissions. A. Bose conducted the statistical analyses with input from B.B., and wrote the paper with input from all co-authors.

Chapter 3: Factors influencing cannibalism in the plainfin midshipman fish

Authors: Aneesh P. H. Bose, Karen M. Cogliati, Holly S. Howe, and Sigal Balshine

Publication: Animal Behaviour, 96, 159-166. Reprinted with permission.

Comments: A.B. and S.B. conceived the study. A.B., K.C., and H.H. collected data from the field. A.B. conducted the statistical analyses and wrote the paper with input from K.C. and S.B.

Chapter 4: Cannibalism, competition, and costly care in the plainfin midshipman fish, *Porichthys notatus*

Authors: Aneesh P. H. Bose, Grant B. McClelland, and Sigal Balshine

Publication: Behavioural Ecology, 27, 628-636. Reprinted with permission.

Comments: A.B. and S.B. conceived the study. A.B. collected data from the field. A.B. conducted the laboratory analyses with assistance from G.M., conducted the statistical analyses, and wrote the paper with input from G.M. and S.B.

Chapter 5: Impacts of direct and indirect paternity cues on paternal care in a singing toadfish

Authors: Aneesh P. H. Bose, Henry H. Kou, and Sigal Balshine

Publication: Behavioural Ecology, 27, 1507-1514. Reprinted with permission.

Comments: A.B. and S.B. conceived the study. A.B. and H.K. collected data from the field and laboratory. A.B. conducted the statistical analyses and wrote the paper with input from H.K. and S.B.

Chapter 6: General Introduction

Author: Aneesh P. H. Bose

Appendix Chapter 7: Offspring cannibalism is related to low paternity in an intertidal fish

Authors: Aneesh P. H. Bose, Malcolm Lau, Karen M. Cogliati, Bryan Neff, and Sigal Balshine

Publication: In-preparation for submission to Behavioural Ecology and Sociobiology.

Comments: A.B., K.C., B.N., and S.B. conceived the study. A.B. and K.C. collected data from the field. M.L. conducted the genetic paternity testing. A.B. conducted the statistical analyses and wrote the paper with input from K.C., M.L., B.N., and S.B.

Appendix Chapter 8: A test of male infanticide as a reproductive tactic in a cichlid fish

Authors: Shagun Jindal, Aneesh P. H. Bose, Constance M. O'Connor, and Sigal Balshine

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Comments: S.J. and A.B. contributed equally to this manuscript and are therefore co-first authors. A.B. and C.O. conceived the study. S.J. collected the behavioural data in the laboratory. A.B. conducted the statistical analyses and wrote the paper with input from S.J., C.O., and S.B.

Chapter 1: General introduction

> "Cannibalism has generally been regarded as 'evil', but in nature this concept is meaningless. Virtually all natural behaviour is conducted in the evolutionary interests of survival and procreation. If cannibalism is considered to be the epitome of bestiality, this may be unfair to animals when one considers the number of cannibalistic species."
>
> **David Soulsby 2013 in *Animal Cannibalism: The Dark Side of Evolution***

1.1 Theoretical background and motivation for thesis

Cannibalism is extremely common in the animal kingdom (Fox 1975; Polis 1981), described in at least 2,000 species to date that range from insects and molluscs to fishes and mammals (Soulsby 2013). Considerable variation in form and type of cannibalism has also been documented in nature, as preying upon members of the same species can occur across an individual's lifecycle and between strangers and relatives alike (Polis 1981; Smith and Reay 1991). Researchers studying cannibalism typically classify the phenomenon into specific forms (Smith and Reay 1991), depending on the relative ages of the cannibal and prey (e.g. adult versus adult or adult versus egg) and the genetic relatedness between the cannibal and prey (e.g. kin versus non-kin). One particularly common form of cannibalism is the consumption of offspring by adults, a form of infanticide. Many early researchers tended to view cannibalism and infanticide as an oddity or a 'social pathology' that develops in populations under stress or high densities (e.g. Hauschka 1952; Calhoun 1962; Lorenz 1966; Chardine and Morris 1983; Dolhinow et al. 1977) However, Hrdy’s seminal work revealed that infanticide can increase the reproductive success of the perpetrator especially when the victim is unrelated (Ebensperger 1998; Hrdy 1974; 1977a,b; 1979; Palombit 2015). When a cannibal consumes non-kin offspring they can gain nutritional benefits, eliminate future competitors, and gain reproductive access to mates (Hrdy 1979; Ebensperger 1998; Palombit 2015).

Perhaps more perplexing is filial cannibalism, or cannibalism perpetrated by the genetic parent (Polis 1981; Elgar and Crespi 1992). Filial cannibalism gives the superficial appearance of being highly costly and maladaptive and was long thought to be a laboratory artefact and abnormal behaviour (Manica 2002a). Rohwer (1978) was the first to suggest that filial cannibalism could provide the cannibal parent with a net reproductive benefit despite the direct fitness costs associated with consuming their own offspring. He proposed that parents could maximize their lifetime reproductive success by ingesting some offspring, thereby gaining a source of energy that could be translated into alternate reproductive attempts.

Both filial cannibalism and non-kin cannibalism can occur when adults provide care for offspring, because adults in the position of the caregivers (henceforth called ‘parents’) are not always genetically related to the offspring. Although parental care benefits parents by improving offspring’s fitness, care also incurs considerable costs that can diminish the parent’s ability to produce future offspring (Trivers 1972; Williams 1966; Clutton-Brock 1991; Alonso-Alvarez and Velando 2012). As such, parents are expected to dynamically adjust their level of care depending on a wide variety of factors including the degree of relatedness between the parent and the offspring, the quality and quantity of young, the parent's own physiological condition, and the prevailing ecological and social conditions; all of these factors alter the reproductive value of current offspring relative to the parent's expected future reproduction (Williams 1966; Sargent and Gross 1985; Gross 2005). Offspring cannibalism is thought to be one way in which parents can alter their level of parental care and investment (Sargent 1992; Sargent et al. 1995; Kondoh and Okuda 2002; Payne et al. 2004; Klug and Bonsall 2007). A longstanding research goal for many evolutionary biologists has therefore been to uncover what conditions favor and facilitate adaptive cannibalism of offspring by caregiving individuals.

A parent is expected to cannibalize offspring when the benefits of doing so outweigh the long-term costs, thereby enhancing the parent's lifetime reproductive success. By cannibalizing offspring, a parent can trade a portion of its investment into current offspring against alternate future reproductive efforts. In order to describe this trade off, a conceptual difference is drawn between the cannibalism of an entire brood of offspring versus the cannibalism of only a portion of the brood (Manica 2002a). While whole brood cannibalism can only be viewed as an investment into future reproduction, partial brood cannibalism can be viewed as an investment into the remaining current offspring and/or future reproduction (Sargent 1992). Whole-brood cannibalism (or brood termination) is expected to occur when the costs of providing care are higher than the benefits (Clutton-Brock 1991), for example when broods are small and of low reproductive value. Partial-brood cannibalism (or brood reduction) should occur when the adult can still benefit from continuing to care for, or sparing, some offspring (Sargent 1992), such as when too many offspring have been produced than can be raised given current environmental conditions.

Many hypotheses have been proposed to explain the widespread occurrence of parent-offspring cannibalism (Sargent 1992; Sargent et al. 1995; Kondoh and Okuda 2002; Klug and Bonsall 2007). One of the most commonly tested hypotheses is the 'energy-reserves hypothesis', which states that cannibals exploit offspring as a nutritional resource (Rohwer 1978). Parents may consume offspring to replenish low energy reserves and offset the energetic costs associated with the physical demands and restricted foraging opportunities during parental care (Rohwer 1978). For example, parents in poor body condition were more likely to engage in filial cannibalism in the freshwater goby, *Rhinogobius sp.* (Okuda et al. 2004), and increasing food availability during parental care via supplemental feeding treatments reduced offspring cannibalism in the scissortail sergeant, *Abudefduf sexfasciatus* (Manica 2004). Another commonly tested hypothesis is the 'low parentage hypothesis', which can be divided into two non-mutually exclusive parts. First, parents may cannibalize to avoid high costs of investing into care for non-kin offspring. For example, parental care-giving male bluegill sunfish, *Lepomis machrochirus*, engage in partial brood cannibalism after detecting evidence that they had been cuckolded (Neff 2003a). Second, adults may consume non-kin offspring because the net benefits of cannibalism are higher when inclusive fitness costs are non-existent or negligible. For example, female crayfish, *Orconectes limosus*, prefer to consume non-kin over kin juveniles even after the juveniles have reached independence (Mathews 2011). Additional cannibalism-facilitating factors beyond these two main hypotheses include poor offspring quality, offspring overcrowding, and alternative mating opportunities for the cannibal (Manica 2002a).

To more quantitatively investigate the research conducted to date on parent-offspring cannibalism, I conducted a survey of the available empirical literature on this topic. I used a Web of Science topic search employing the keywords: parent* AND care AND cannibal*. My search yielded a list of 126 published and online-accessible research articles documenting parent-offspring cannibalism. I started my list in 1978, the year Rohwer published the first adaptive explanation for cannibalism (Table 1.1). My search revealed that research output on parent-offspring cannibalism has been fairly consistent since the mid 1990s and has perhaps been garnering increased attention in the last decade (Figure 1.1). The search also revealed the widespread nature of parent-offspring cannibalism across taxa, but showed that this behaviour has been most extensively studied in fishes (Figure 1.2). This is likely because the frequency of cannibalistic behaviour among care-giving fishes is high (Klug and Bonsall 2007). In fact, 53 of the 85 species (62%) in the literature survey were fishes. Parent-offspring cannibalism has been documented in many other taxa as well including mammals, birds, amphibians, reptiles, and insects (Figure 1.2). Although a number of adaptive hypotheses exist to explain why cannibalism might occur, some have been studied to greater degrees than others; the two best studied are the energy-reserves hypothesis and the low parentage hypothesis (Figure 1.3). Interstingly, the more thoroughly investigated hypotheses have revealed both considerable supporting as well as opposing evidence. To better understand the factors that underlie parent-offspring cannibalism, we need to address the following two limitations in the current literature. First, more research is needed to expand the list of species in which cannibalism hypotheses are tested. Second, there are very few study species in which multiple hypotheses have been systematically tested, making it more challenging to compare hypotheses. These two limitations have motivated my doctoral thesis.

1.2 Aims of the thesis

In my PhD research, I have endeavored to understand the factors that select for parent-offspring cannibalism in a care-giving and intertidally-breeding marine teleost, the plainfin midshipman fish, *Porichthys notatus* (Figure 1.4). The specific aims of my thesis were:

1) To further describe the reproductive ecology of the plainfin midshipman fish, specifically focusing on male traits and ecological factors that influence reproductive success for care-giving males breeding in the intertidal zone.
2) To characterize the prevalence of parent-offspring cannibalism in wild breeding plainfin midshipman males and to identify ecological correlates of this behaviour.
3) To investigate multiple adaptive hypotheses for parent-offspring cannibalism in a single species, the plainfin midshipman fish, while incorporating both experimental and correlational approaches to study these ideas in both the laboratory and field.

Table 1.1 A list of the adaptive hypotheses for parent-offspring cannibalism. Examples for or against each hypothesis are presented from the empirical literature. Cases where cannibalism could be identified as whole-brood or partial-brood are labeled as such.

Hypothesis	Theoretical prediction
1. Energy reserves	Parents in good body condition or provided with a supplementary diet are less likely to cannibalize offspring
Supporting evidence: *Fishes:* Bluegill sunfish, *Lepomis macrochirus* (Neff 2003b, partial); Cardinal fish, *Apogon doederleini* (Okuda and Yanagisawa 1996, full); Cardinal fish, *Apogon lineatus* (Kume et al. 2000, partial); Common goby, *Pomatoschistus microps* (Kvarnemo et al. 1998, partial); Damselfish, *Stegastes rectifraenum* (Hoelzer 1992, partial); Freshwater goby, *Rhinogobius sp*. (Okuda et al. 2004, partial); Long-snout clingfish, *Diademichthys lineatus* (Gomagano and Kohda 2008, partial); Painted greenling, *Oxylebius pictus* (Demartini 1987, partial); Pipefish, *Syngnathus abaster* (Cunha et al. 2016); River blennies, *Salaria fluviatilis* (Vinyoles et al. 1999); River bullhead, *Cottus gobio* (Marconato and Bisazza 1988; Marconato et al. 1993, partial); Scissortail sergeant, *Abudefduf sexfasciatus* (Manica 2004, partial); Sphynx blenny, *Aidublennius sphynx* (Kraak 1996, partial), Three-spined stickleback, *Gasterosteus aculeatus* (Candolin 2000a; Candolin 2000b) *Insects:* Assassin bug, *Rhinocoris tristis* (Thomas and Manica 2003; Gilbert et al. 2010, partial); Maritime earwig, *Anisolabis maritima* (Miller and Zink 2012, partial)	**Opposing evidence (or no evidence):** *Fishes:* Damselfish, *Stegastes leucostictus* (Payne et al. 2002, partial); Fantail darters, *Etheostoma flabellare* (Lindström and Sargent 1997, partial); Flagfish, *Jordanella floridae* (Klug and St Mary 2005; Klug 2009, full and partial); Lusitanian toadfish, *Halobatrachus didactylus* (Félix et al. 2016); Plainfin midshipman, *Porichthys notatus* (Bose et al. 2014; Bose et al. 2015, partial); Sand goby, *Pomatoschistus minutus* (Kvarnemo 1997; Klug et al. 2006, full and partial)

Table 1.1: Continued

Hypothesis	Theoretical prediction	
2. Parentage	Parents are more likely to cannibalize offspring when parentage is low	
Supporting evidence: *Fishes:* Bluegill sunfish, *Lepomis macrochirus* (Neff 2003a,b, partial); Brown trout, *Salmo trutta* (Aymes et al. 2010); Cichlid, *Lamprologus callipterus* (Sato 1994); Cichlid, *Pelvicachromis pulcher* (Nelson and Elwood 1997); Damselfish, *Acanthochromis polyacanthus*, *Chromis notatus notatus*, and *Pomacentrus nagasakiensis* (Nakazono et al. 1989; Nakazono 1993); Fathead minnows, *Pimephales promelas* (Green et al. 2008, partial); Goby, *Padogobius martensi* (Parmigiani et al. 1988); Pipefish, *Syngnathus abaster* (Cunha et al. 2016); Plainfin midshipman fish, *Porichthys notatus* (Bose et al. 2016, partial); Pygmy Sculpin, *Cottus pygmaeus* (Johnston 2000); Sand goby, *Pomatoschistus minutus* (Lindström and Hellström 1993; Lissåker and Svensson 2008, full); Scissortail sergeant, *Abudefduf sexfasciatus* (Manica 2004, partial); Silversides, *Telmatherina sarasinorum* (Gray et al. 2007); Spottail darters, *Etheostoma squamiceps* (Bandoli 2002; Bandoli 2016, partial); Three-spined sticklebacks, *Gasterosteus aculeatus* (Frommen et al. 2007; Mehlis et al. 2010, full) *Insects:* Burying beetles, *Nicrophorus spp.* (Trumbo 1994); Flour beetles, *Tribolium confusum* (Parsons et al. 2013); Hemipteran bug, *Geocoris pallens* (Law and Rosenheim 2013); Stag beetle, *Figulus binodulus* (Morih and Chiba 2009); Two spot ladybirds, *Adalia bipunctata* (Agarwala and Dixon 1993) *Arachnids:* Wolf spider, *Schizocosa ocreata* (Wagner 1995) *Crustaceans:* Amphipod, *Gammarus pulex* (Lewis et al. 2010); Copepod, *Tigriopus fulvus* (Lazzaretto and Salvato 1992); Crayfish, *Orconectes limosus* (Mathews 2011); Rock-pool amphipod, *Apherusa jurinei* (Patterson et al. 2008) *Amphibians:* Poison frogs, *Allobates femoralis* (Ringler et al. 2017); Red-backed salamander, *Plethodon cinereus* (Gibbons et al. 2003); Red-spotted newts, *Notophthalmus viridescens* (Gabor 1996)		**Opposing evidence (or no evidence):** *Fishes:* Common goby, *Pomatoschistus microps* (Svensson et al. 1998; Vallon and Heubel 2016, partial); Sand goby, *Pomatoschistus minutus* (Svensson and Kvarnemo 2007, full and partial); Lusitanian toadfish, *Halobatrachus didactylus* (Félix et al. 2016)

Table 1.1: Continued

Hypothesis	Theoretical prediction
3. Offspring quality	Adults are more likely to cannibalize small, unhealthy, or dead offspring
Supporting evidence: *Fishes:* Flagfish, *Jordanella floridae* (Klug 2009, partial); Sphynx blenny, *Aidublennius sphynx* (Kraak 1996, partial); Spottail darters, *Etheostoma squamiceps* (Bandoli 2016, partial) *Insects:* Assassin bug, *Rhinocoris tristis* (Thomas and Manica 2003; Gilbert and Manica 2009, partial) *Arachnids:* Mite, *Macrocheles glaber* (Marquardt et al. 2015) *Amphibians:* Japanese giant salamander, *Andrias japonicas* (Okada et al. 2015, partial) *Reptiles:* Colombian rainbow boas, *Epicrates cenchria maurus* (Lourdais et al. 2005, partial); Mexican lance-headed rattlesnakes, *Crotalus polystictus* (Mociño-Deloya et al. 2009, partial)	**Opposing evidence (or no evidence):** *Fishes:* Common goby, *Pomatoschistus microps* (Vallon et al. 2016)

Table 1.1: Continued

Hypothesis	Theoretical prediction
4. Offspring number	*Whole-brood cannibalism:* Small broods (of low reproductive value) are more likely to be completely cannibalized *Partial-brood cannibalism:* Large broods are more likely to be partially cannibalized than small broods because the 'per offspring' costs of cannibalism are lower
Supporting evidence: *Fishes:* Barred-chin blenny, *Rhabdoblennius nitidus* (Matsumoto and Takegaki 2016); Bluegill sunfish, *Lepomis macrochirus* (Neff 2003b, full); Cardinal fish, *Apogon doederleini* (Okuda and Yanagisawa 1996b, full); Common goby, *Pomatoschistus microps* (Kvarnemo et al. 1998; Vallon et al. 2016, full); Convict cichlid, *Cichlasoma nigrofasciatum* (Lavery and Keenleyside 1990, full); Damselfish, *Microspathodon chrysurus*, *Stegastes dorsopunicans* & *S. rectifraenum* (Petersen and Marchetti 1989; Petersen 1990; Petersen and Hess 1991, full); Egyptian mouthbrooder, *Pseudocrenilabrus multicolor* (Mrowka 1987, full); Fantail darters, *Etheostoma flabellare* (Lindström and Sargent 1997, full); Flagfish, *Jordanella floridae* (Klug et al. 2005); Sand goby, *Pomatoschistus minutus* (Forsgren et al. 1996; Lissåker et al. 2003; Pampoulie et al. 2004; Klug et al. 2006); Scissortail sergeant, *Abudefduf sexfasciatus* (Manica 2003, full); Stream goby, *Rhinogobius spp*. (Ito et al. 2016); Upland bully, *Gobiomorphus breviceps* (Stott and Pulin 1996)	**Opposing evidence (or no evidence):** *Fishes:* Bluegill sunfish, *Lepomis macrochirus* (Neff 2003b, partial); Cardinal fish, *Apogon doederleini* (Okuda and Yanagisawa 1996a, partial); Common goby, *Pomatoschistus microps* (Svensson et al. 1998; Vallon et al. 2016, partial); Damselfish, *Stegastes leucostictus* (Payne et al. 2003, full); Lusitanian toadfish, *Halobatrachus didactylus* (Félix et al. 2016); Sand goby, *Pomatoschistus minutus* (Forsgren et al. 1996; Lindström 1998; Lissåker et al. 2003, partial); Scissortail sergeant, *Abudefduf sexfasciatus* (Manica 2003, partial); Three-spined sticklebacks, *Gasterosteus aculeatus* (Mehlis et al. 2009)

Table 1.1: Continued

Hypothesis	Theoretical prediction
5. Offspring age	Young offspring are more likely to be cannibalized than older offspring
Supporting evidence: *Fishes:* Bluegill sunfish, *Lepomis macrochirus* (Neff 2003b, partial); Brook Stickleback, *Culaea inconstans* (Salfert and Moodie 2017); Cardinal fish, *Apogon doederleini* (Okuda and Yanagisawa 1996a; Okuda and Yanagisawa 1996b; Okuda et al. 1997; Takeyama et al. 2002, full and partial); Common goby, *Pomatoschistus microps* (Vallon and Heubel 2016, partial); Damselfishes, *Microspathodon chrysurus, Stegastes dorsopunicans* & *S. rectifraenum* (Petersen and Marchetti 1989; Petersen 1990, full and partial); Lizard goby, *Rhinogobius flumineus* (Takeyama et al. 2013, partial); Sand goby, *Pomatoschistus minutus* (Klug and Lindström 2008, partial); Scissortail sergeant, *Abudefduf sexfasciatus* (Manica 2002b, full)	**Opposing evidence (or no evidence):** *Fishes:* Barred-chin blenny, *Rhabdoblennius ellipes* (Takegaki et al. 2011, partial); Damselfishes, *Stegastes leucostictus* & *Hypsypops rubicundus* (Sikkel 1994; Payne et al. 2002, partial); Scissortail sergeant, *Abudefduf sexfasciatus* (Manica 2003, partial) *Insects:* Maritime earwig, *Anisolabis maritima* (Miller and Zink 2012, partial)
6. Offspring density	Offspring that are overcrowded are more likely to be cannibalized
Supporting evidence: *Fishes:* Damselfish, *Stegastes leucostictus* (Payne et al. 2002, partial); Sand goby, *Pomatoschistus minutus* (Klug et al. 2006; Lehtonen and Kvarnemo 2015a; Lehtonen and Kvarnemo 2015b, partial)	**Opposing evidence (or no evidence):**

Table 1.1: Continued

Hypothesis	Theoretical prediction
7. Alternate mating opportunities	Parents are more likely to cannibalize offspring when given ample opportunities to remate and gain alternate reproduction
Supporting evidence: *Fishes:* Cardinal fish, *Apogon doederleini* & *A. niger* (Okuda and Yanagisawa 1996b; Okuda 1999; Okuda 2000, full); Freshwater goby, *Rhinogobius sp.* (Okuda et al. 2004, partial); Lizard goby, *Rhinogobius flumineus* (Myint et al. 2011; Takeyama et al. 2013, partial)	**Opposing evidence (or no evidence):** *Fishes:* Two-spotted goby, *Gobiusculus flavescens* (Bjelvenmark and Forsgren 2003)
8. Offspring survival prospects	Parents are more likely to cannibalize when offspring survival prospects are poor
Supporting evidence: *Fishes:* Sand goby, *Pomatoschistus minutus* (Chin-Baarstad et al. 2009, full) *Mammals:* House mice, *Mus domesticus* (Maestripieri 1991; Weber and Olsson 2008); Syrian hamsters, *Mesocricetus auratus* (Beery and Zucker 2012) *Reptiles:* Long-tailed skink, *Mabuya longicaudata* (Huang 2008, full) *Birds:* Golden Eagles, *Aquila chrysaetos* (Korňan and Macek 2011)	**Opposing evidence (or no evidence):** *Fishes:* Sand goby, *Pomatoschistus minutus* (Lindström 1998; Olsson et al. 2016, partial)

Table 1.1: Continued

Hypothesis	Theoretical prediction
9. Other causes/unknown causes	Additional documented cases of offspring cannibalism

Examples:

Fishes:

Bluefin killifish, *Lucania goodei* (Fuller and Travis 2001); Cichlid, *Neolamprologus brichardi* (Von Siemens 1990); Cichlid, *Simochromis pleurospilus* (Segers et al. 2011); Cichlid, *Astatotilapia burtoni* (Renn et al. 2009); Damselfish, *Stegastes planifrons* (Sasal 2006); Dark chub, *Zacco temmincki* (Katano 1992); Fat greenling, *Hexagrammos otakii* (Munehara and Miura 1995); Pipefish, *Syngnathus typhle* (Sagebakken et al. 2010); Razorfishes, *Xyrichtys splendens*, *X. martinicensis*, *X. novacula* (Nemtzov and Clark 1994); River sculpin, *Cottus nozawae* (Goto 1993); Round goby, *Neogobius melanostomus* (Meunier et al. 2009); Sand goby, *Pomatoschistus minutus* (Lissåker 2007); Three-spined sticklebacks, *Gasterosteus aculeatus* (Pike et al. 2007)

Insects:

Burying beetles, *Nicrophorus quadripunctatus* (Takata et al. 2013); European earwig, *Forficula auricularia* (Koch and Meunier 2014)

Mammals:

Lab rat, *Rattus norvegicus* (Burn and Mason 2008); Rabbits, *Oryctolagus cuniculus* (González-Redondo and Zamora-Lozano 2008); Thick-tailed Bushbabies, *Galago crassicaudatus umbrosus* (Tartabini 1991)

Birds:

Herring Gull, *Larus argentatus* (Chardine and Morris 1983)

Chilopods:

Centipede, *Otostigmus spinosus* (Siriwut et al. 2014)

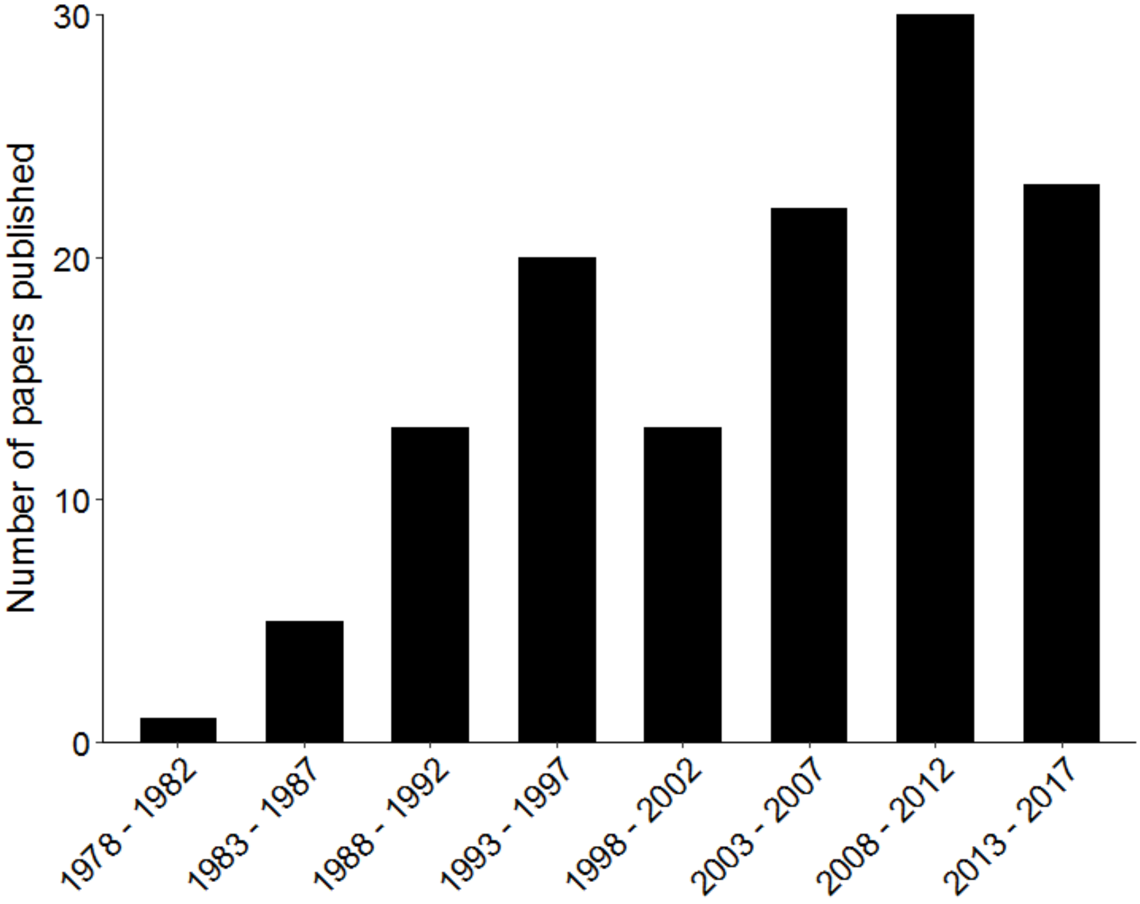

Figure 1.1 Publication output on the topic of parent-offspring cannibalism since the 1980s based on a Web of Science literature survey.

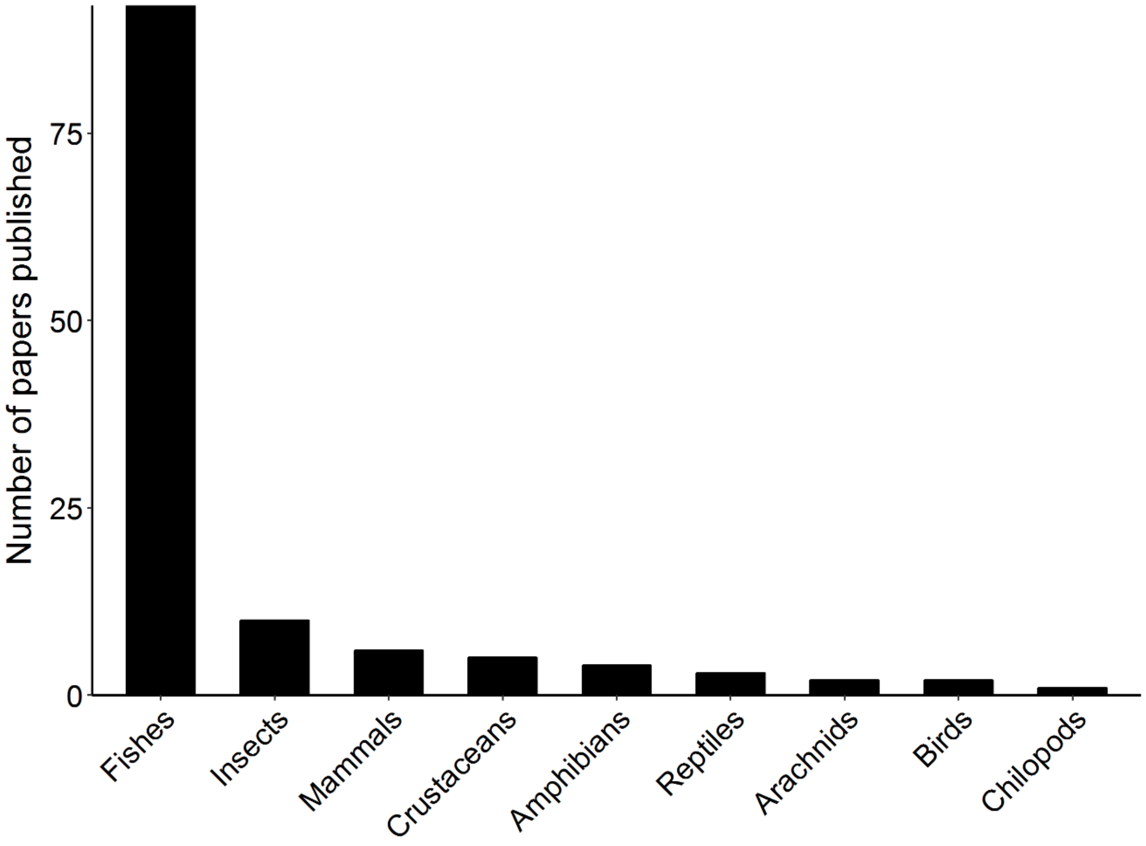

Figure 1.2 Taxonomic representation across the parent-offspring cannibalism literature based on a Web of Science literature survey.

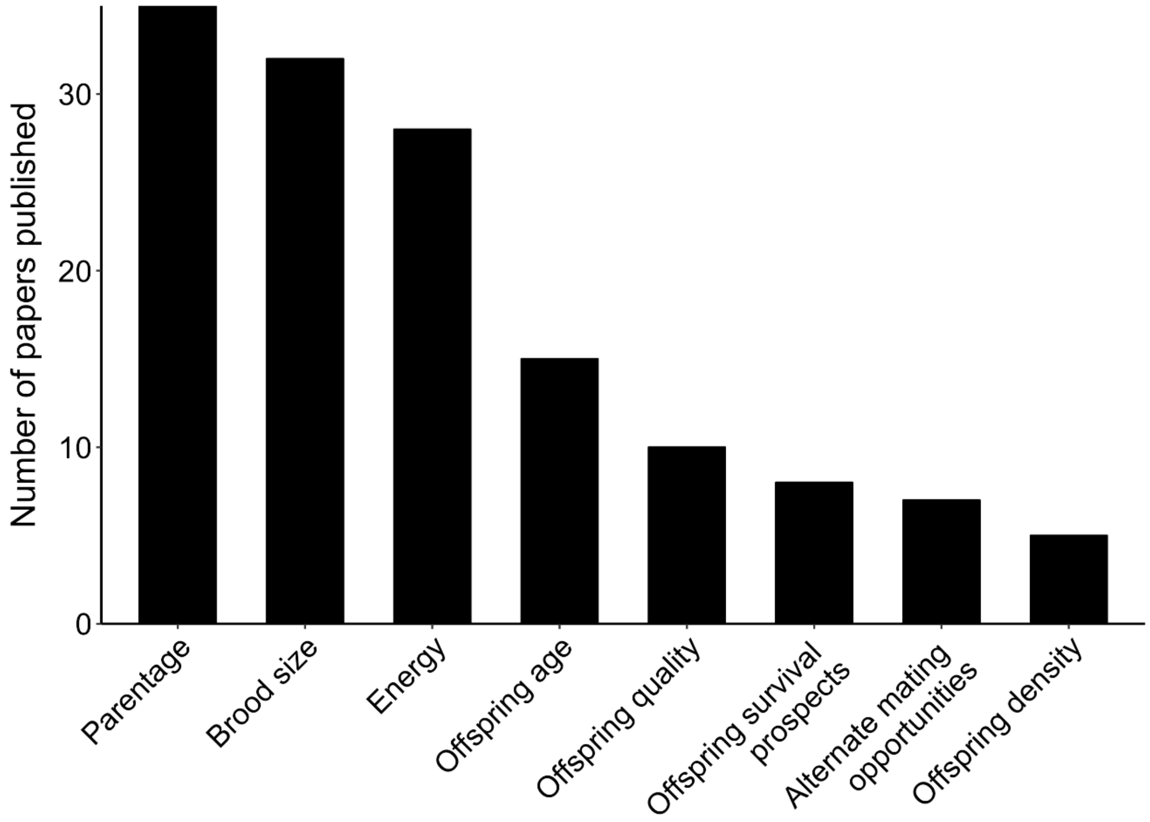

Figure 1.3 Representation of the various parent-offspring canniablsim hypotheses based on a Web of Science literature survey.

Figure 1.4 Male plainfin midshipman fish, *Porichthys notatus*, sampled at Ladysmith Inlet, Ladysmith, British Columbia (photo credit: Dr. Shyamal Bose).

1.3 Structure of the thesis

During my PhD, I investigated the function and significance of parent-offspring cannibalism in the plainfin midshipman fish and the possible mechanisms that select for this behaviour. My research furthered our understanding of the basic reproductive ecology of this species. In **Chapter 2** of this thesis, I used a combination of long-term field data and controlled laboratory experiments to examine the factors related to reproductive success in male plainfin midshipman fish. I compared the contributions of male quality and resource quality to male reproductive success based on three measures of reproductive success: 1) number of female mates attracted, 2) number of eggs acquired, and 3) number of offspring reared. This second chapter also introduces the reproductive ecology of my study species and provides a biological context for my following chapters on parent-offspring cannibalism. In **Chapter 3**, I intensively surveyed a single spawning population of plainfin midshipman fish to monitor the frequency of offspring cannibalism in wild caregiving males, and to track the prevalence of cannibalism across the breeding season. Here, I simultaneously monitored metrics of parental body condition and male-male competition and used these data to examine two adaptive hypotheses for parent-offspring cannibalism, the energy-reserves hypothesis and the low parentage hypothesis. In **Chapter 4**, I more thoroughly tested the energy-reserves hypothesis. All previous tests of the energy-reserves hypothesis have taken one of two forms. Researchers have either correlated coarse measures of body condition (e.g. Fulton's condition factor) with the degree or occurance of cannibalism or they have experimentally starved parents or supplemented their diets and then subsequently monitored their cannibalistic behaviours. In this chapter, I took a more fine-scale and direct approach by relating cannibalism to a suite of endogenous energy stores such as liver glycogen, liver lipids, and muscle proteins in caregiving males. In **Chapter 5**, I experimentally investigated, both in the field and laboratory, how plainfin midshipman males assess their paternity over offspring in a nest. I manipulated direct and indirect offspring cues in the nests of breeding males and then monitored for evidence of dynamic adjustments in parental care. In **Chapter 6**, I review and synthesize my findings across the main dissertation data chapters and propose avenues of future research.

I have also included two additional studies in the appendix of my thesis, which are thematically connected to my PhD work. In **Chapter 7**, I provide a more rigorous genetic analysis of the low parentage hypothesis, where I conducted paternity tests to ascertain the relatedness between males (cannibals and non-cannibals) and the offspring found in their nests. This work has not yet been submitted for publication as we are currently completing one last additional genetic analysis to include in the manuscript. In **Chapter 8**, I further investigate the low paternity hypothesis by testing whether offspring cannibalism imparts reproductive benefits to the perpetrator by speeding up or enhancing their future reproduction. In this now published study, I used the group-living social cichlid fish, *Neolamprologous pulcher* not plainfin midshipman to test this hypothesis.

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Chapter 2: Male and resource quality as factors affecting male reproductive success in an intertidal fish

2.1 Abstract

A male's reproductive success often depends on both his phenotypic quality and the quality of the resources he controls. An important and longstanding challenge for evolutionary biologists has been to disentangle these two often-correlated factors. Here, we present the results of a large multi-year, multi-population field study along with complementary laboratory experiments aimed at disentangling the effects of male quality and nest quality in driving male reproductive success in the plainfin midshipman fish, *Porichthys notatus*. We show in the field that both male size and nest size are important correlates of reproductive success in this paternal care-giving species. Females in the laboratory prefer large males when their nest size is held constant, but females show no detectable preference when nest size is varied and male size is held constant. We also explored a suite of additional male and nest traits – including male body condition, sonic organ investment, nest species richness, and nest density. Our results highlight how male and resource quality are multivariate concepts that incorporate information from the male phenotype, the ecological environment, and even the social environment and ultimately shape mating systems by influencing an animal's choice of mating partners and nesting sites.

2.2 Introduction

A longstanding challenge for evolutionary ecologists has been to untangle the relative contributions of individual quality and resource quality to reproductive success (Dugatkin and Fitzgerald 1997; Oliveira et al. 2000; Zabala and Zuberogoitia 2014). Determining the relative fitness contributions of individual quality versus resource quality is often complicated by the indirect nature of measuring reproductive success. Strictly, lifetime reproductive success is the number of offspring an individual produces over their lifetime that manage to survive to reproduce themselves (Hamilton 1964; Williams 1966; Grafen 1988). Lifetime reproductive success is notoriously difficult to accurately measure in the wild, especially for long-lived organisms (Newton 1989; Jensen et al. 2004; Rouan et al. 2009). Researchers will therefore often measure proxies or ‘components’ of reproductive success that are more easily quantified in the field over realistic time spans (Howard 1979; Clutton-Brock 1988). Such components may include mate attraction (e.g. number of successful courtship attempts), fertilization success (e.g. number of young acquired), and rearing success (e.g. number of hatchlings raised to independence) quantified over a single season. However, because selection may act differentially on each component of reproductive success and may change across an individual’s life span, to gain a more comprehensive understanding of reproductive success in a given species, it is important to study multiple components concurrently (Burger 1982; Clutton-Brock 1988; Kelly 2008).

When making mating decisions, individuals integrate information on both the phenotypic quality and the resource quality of their prospective mates (Møller and

Jennions 2001; Candolin 2003; Lehtonen et al. 2007). However, the ‘quality’ of a mate or resource is unlikely to be determined by a single variable (Parker 1974; Johnstone 1996; Candolin 2003; Arnott and Elwood 2009). In a meta-analysis investigating the relative importance of male resource-holding potential and resource value on reproductive success, Kelly (2008) showed that body size is most commonly studied as the primary male-quality trait of interest, followed by traits such as song repertoire size or plumage colouration, then male weaponry. Individually, however, these variables that are meant to describe male quality tend to account for little variation in male reproductive success (Kelly 2008). Thus, more work is certainly needed to understand the relative fitness contributions of male quality and resource quality and the various male traits and resource characterisitcs that underlie ‘quality’.

Here, we investigate the contributions of male quality and nest quality to male reproductive success in the plainfin midshipman fish, *Porichthys notatus*. During late spring and early summer both sexes of this marine toadfish make an extensive shoreward migration from the ocean depths of ~300m to breed in the shallow rocky intertidal zones of western North America (Arora 1948; Miller and Lea 1972; Warner and Case 1980; Sisneros et al. 2004). Males excavate nesting cavities beneath rocks by removing mouthfuls of sediment and then remain in these nests for at least 60 days despite the ebbing and flooding of the tides. However, not all intertidal rocks are suitable for nesting beneath and so large rocks are highly sought-after resources and males compete intensely for nest ownership (Bose et al. 2014). Males are confined to their nests during the breeding season, acoustically courting and luring gravid females, spawning with them in the nesting cavity, and providing sole paternal care for offspring for ~60 days (Arora 1948; Ibara et al. 1983; Brantley and Bass 1994; Cogliati et al. 2013). It is particularly challenging to tease apart the relative importance of male quality versus nest quality in influencing male reproductive success, because the largest midshipman males often possess the largest nests (Demartini 1988; Bose et al. 2014) – a common phenomenon across many animal taxa (Andersson 1994; Oliveira et al. 2000; Candolin and Voigt 2001; Kelly 2008).

To better understand the mechanisms that underlie variation in reproductive success in the plainfin midshipman, we were guided by Kelly (2008) who recommended that: 1) all three pairwise relationships between male quality, resource quality, and reproductive success be assessed; 2) correlational field studies involve large sample sizes and be complemented by controlled manipulative experiments; 3) relevant spatiotemporal variables (i.e. ecological covariates) be accounted for; and 4) accurate measures of reproductive success be obtained. With these recommendations in mind, we conducted three studies on the plainfin midshipman fish to assess the relationships between individual quality, resource quality, and reproductive success. In Study 1, we used a large-scale dataset based on multi-year, multi-site field sampling to compare the relative importance of male body size (individual quality) and nest size (resource quality) on male reproductive success while accounting for spatiotemporal covariates (e.g. time in the breeding season, site differences, and year effects). In this first study, we quantified reproductive success using three components: number of mates attracted, number of eggs acquired, and rearing success. In Study 2, we conducted a series of complementary controlled laboratory experiments to test female choice for male size while holding nest size constant, and for nest size while holding male size constant. Finally, in Study 3, we explored other traits, beyond male and nest size, that could influence male and resource quality. Specifically, we investigated how reproductive success varied with male traits like body condition, liver, gonad, and sonic muscle investment, and how reproductive success varied with ecological traits like nest density and the number of other intertidal species sharing the nest space with the care-giving males.

2.3 Methods

Overview of plainfin midshipman reproductive ecology

The plainfin midshipman fish is distributed along the west coast of North America, from Sitka, Alaska to Magdalena Bay, Mexico (Walker and Rosenblatt, 1988). At the onset of the breeding season (late April) reproductive males migrate from the deep ocean to the rocky intertidal zone, where they excavate nesting cavities in the soft sediment beneath large intertidal rocks (Arora 1948). Rocks suitable for nesting vary in abundance and density across intertidal beaches (Cogliati et al. 2014a) and are in short supply (DeMartini 1988, 1991), leading to strong competition between males for nests (Bose et al. 2014). As a result, take-over events in which nests change male ownership are commonly observed, especially early in the breeding season (Bose et al. 2014). A size assortative relationship is also observed in the early breeding season in which the largest males acquire the largest nests (DeMartini 1988, Bose et al. 2014).

Once a male acquires a nest, he will produce long-duration (> 1 hour), low-frequency (fundamental ~100Hz) courtship vocalizations by rapid contractions of the sonic muscles attached to his swim bladder walls (Bass and Marchaterre 1989; Sisneros and Bass 2003). These songs act as locational beacons that attract gravid females to the nests (Ibara et al. 1983; Brantley and Bass 1994). Once a female has chosen a male she will lay her entire brood of eggs in a monolayer (ranging between 100 – 300 eggs, DeMartini 1990; Bose et al. 2014) on the roof of the nest (Arora 1948). The space available for egg-attachment is constrained not only by the underside surface area of the intertidal rock, but also by the presence of other intertidal organisms such as sessile invertebrates that share and compete for nest space (DeMartini 1991). Male *P. notatus* often spawn with multiple females, sometimes simultaneously, and acquire temporally overlapping cohorts of embryos across the first two months of the breeding season (Arora 1948; Cogliati et al. 2013, 2014b, 2015). The number of eggs in a nest is on average ~500 but can exceed 3000 (DeMartini 1988; Cogliati et al. 2014a).

After spawning, males remain in their intertidal nests providing paternal care for the embryos, which take ~30 days to hatch and then require another ~30 days to absorb their yolk sacs and become free-swimming (Arora 1948; Cogliati et al. 2013; Bose et al. 2014, 2015). Thus, depending on how many distinct cohorts of embryos a male possesses, and when in the breeding season he acquires them, a male may remain in his nest for over three months providing parental care and will lose energy reserves while doing so (Bose et al. 2015).

Study 1: Field studies on male quality and nest quality

Intertidal nest surveys

Between May 4 and July 25 of 2010, 2011, 2013, and 2015, we surveyed a total of 727 plainfin midshipman nests found in the intertidal zones of nine field sites in British Columbia, Canada, Washington State, and California, USA (see Supplementary Table 2.1 for detailed locations of the sites). We located each nest by gently overturning intertidal rocks to uncover the male and embryos within the nesting cavity beneath. If more than one male was present at a nest, we classified the nest owner as the largest and most centrally-located male. This classification was based on previous studies that have shown that the largest males secure nests while smaller males resort to cuckoldry tactics (Lee and Bass 2004). We recorded how many females were present in each of 652 nests and used this metric of *mate attraction* as one component of reproductive success (range = 0 – 4 females per nest). This method of measuring mate attraction takes a snapshot approach wherein we only recorded the females present at time of nest observation. However, we also digitally photographed (Olympus digital cameras TG-820, 12.0 megapixels; TG-850, 16.0 megapixels) each brood and used ImageJ (v1.45, http://rsbweb.nih.gov/ij/) to quantify the number of embryos in all 727 nests. Plainfin midshipman males often care for broods comprised of multiple overlapping age cohorts contributed to by multiple females over the breeding season (Arora 1948; Demartini 1988; Cogliati et al. 2013). We classified all the embryos in each nest into either pre- or post-hatch developmental stages. To quantify the *number of eggs acquired* by each male, we counted the embryos in nests that contained only pre-hatch offspring (N = 302 nests, range = 4 – 3425 embryos per nest). To quantify *rearing success*, we counted the embryos in nests that contained only post-hatch offspring (N = 102 nests, range = 2 – 1152 embryos per nest).

We also measured the standard length of each male to the nearest 0.1cm and used this metric as our primary measure of male quality. We measured the surface area available for egg laying within each nest as our primary measure of nest quality using one of two methods. In 2010 and 2011 we multiplied the length of each nesting cavity's major axis by its perpendicular axis (measured to the nearest cm). In 2013, we digitally photographed the nesting cavity from above, using reference points to delineate the edge of the cavity, and later used ImageJ to calculate its area. In 2015, we employed both methods throughout the field season.

We conducted all analyses (including those listed below in Studies 2 and 3) in R (v 3.3.1, R Core Team 2016). We compared the relative influence of male size and nest size on our three components of reproductive success: mate attraction, egg acquisition, and rearing success. We fit the following models for each component. The number of females found in a nest was fit with a generalized linear mixed effects model assuming a Poisson error distribution (GLMM, lme4 package, Bates et al., 2015). The number of embryos in each nest was fit with a linear mixed effects model (LMM) for egg acquisition (pre-hatch embryo number) and rearing success (post-hatch embryo number) separately. In both cases the number of embryos was cube root transformed to improve normality of the model residuals based on a Box-Cox analysis. We included male standard length and nest surface area in the models as well as their interaction, dropping the interaction whenever it was not significant. We included three further fixed effects in each model:

Julian date (i.e. time in the breeding season mean-centered and divided by its standard deviation), sampling year, and a factor specifying which of the two nest size measurement methods was employed for a given nest. We included field site in the models as a random intercept term (Bolker et al. 2009). Male standard length and nest surface area were mean-centered and divided by their standard deviation so that we could directly compare their parameter estimates and thus assess their relative importance (Schielzeth 2010; using the glht function from the multcomp package for comparisons, Hothorn et al. 2008). Because of the probable correlation between male size and nest size in the models described above, we calculated variance inflation factors (VIFs) for all variables of interest. We merely used the VIFs to assess the extent of any multicollinearity, which was deemed to be minimal (all VIFs ≤ 1.48, Zuur et al. 2010). When the interaction between male size and nest size was significant, suggesting that the effect of male size on reproductive success changed across the size range of nests, we investigated this interaction more closely. We focused on nests either at the small end or the large end of the spectrum, by centering nest size on a value either -2 or +2 standard deviations from the population mean, and then calculated 95% confidence intervals for the effect of male size and nest size.

Study 2: Laboratory experiments on male quality and nest quality

Do females prefer large males and large nests?

In order to assess the degree of female preference for male size and nest size, we conducted choice experiments in the laboratory. For a more detailed description of the experimental setups, see Supplementary Materials. In-brief, we collected adult fish from nests in Ladysmith Inlet, British Columbia, Canada during the summer of 2016. Two males were placed on opposite sides of a glass aquarium, each with his own artificial nest (constructed from bricks and ceramic tiles). Following a three-day acclimation period, we introduced a gravid female to the center of the aquarium. We then monitored which male the female chose to spawn with. We checked for the presence of eggs every day for three days and removed the spent female if a spawning occurred. If no spawning had occurred after three days, we removed the still-gravid female and introduced a new gravid female. If after a total of six days (and two gravid females) no spawning had occurred, we removed both males and the female and started a new trial with entirely new fish. In the first experiment, females were provided a choice between two males of different sizes (mean difference in standard length ± std. dev. = 5.6 ± 1.9 cm, and percentage difference in length = 22.7 ± 6.3%) each holding an identically sized nest (with 620 cm^2 of roof space for egg-laying), representing a nest size commonly found at our study sites (mean ± std. dev. of nest sizes in the field = 587 ± 347 cm^2, N = 727). In a second experiment, females were provided a choice between two size-matched males (mean difference in standard length ± std. dev. = 0.7 ± 0.6 cm, percentage difference in length = 3.0 ± 2.7%), one with a large nest (620 cm^2 of roof space) and one with a small nest (approximately 33% smaller: 410 cm^2 of roof space). In total 19 females were offered the differently sized males with identical nests, and 23 females were offered similar sized males with differently sized nests. To determine if females preferred one male over the other, we conducted exact binomial tests. To compare the strength of female preference for male size versus nest size, we conducted a chi-square test on the proportions of females that chose to spawn with the large male versus the large nest.

What nest characteristics do males prefer?

To assess male nest preferences, we conducted two additional laboratory experiments during the summers of 2014 and 2015 using adult males collected from nests in Ladysmith Inlet, British Columbia. For a more detailed description of the experimental setups, see Supplementary Materials. In-brief, we housed each male in a separate experimental tank, each containing two artificial nests (constructed from bricks and concrete tiles) placed 30 cm apart. Males were given a 30-min acclimation period before being given 24 h to choose between the nests. We considered the male to have made a choice if he was sitting fully within one of the nests. In the third experiment, we offered the males a choice between two nests of differing size (one with 360 cm^2 and one with 220 cm^2 of roof space). Lastly, in the fourth experiment, we offered the males two equally sized nests (each with 360 cm^2 of roof space) but one nest had a single, small entrance and was therefore more enclosed (safer) while the other nest had two large entrances and was considerably more open (vulnerable). In total, 94 males were offered the differently sized nests, and 48 males were offered the nests differing in the degree of openness/enclosure. Note that 24 males participated in both experiments, first participating in the third experiment then moving on to the fourth. To see if males preferred one nest type over the other, we fit nest choice with a generalized linear mixed effects model specifying a binomial error distribution (GLMM). Male standard length (mean-centered and standardized) was included as a fixed effect, year as a random effect, and we tested whether the intercepts of these models differed significantly from zero (a value that would indicate a 50% chance of picking either nest type).

Study 3: Exploration of additional traits influencing male quality and nest quality

During our field surveys described in Study 1, we also measured a suite of phenotypic traits that we considered to be important indicators of individual quality in addition to male size. We measured the body mass of each male nest-owner to the nearest 0.1g and used this to calculate male body condition using the residuals from a regression of ln body mass versus ln standard length (Blackwell et al. 2000). We also dissected a subset of the males (N = 257 for a series of other studies) and weighed their livers, testes, and swim bladders (with sonic muscles attached) to the nearest 0.01g. We could then calculate these males' relative investment into these organ structures, by taking the residuals from a regression of ln organ mass versus ln body mass (Warren and Iglesias 2012). To quantify male energy reserves, we calculated a hepatosomatic index (HSI) using liver mass. To quantify male spawn-readiness, we calculated a gonadosomatic index (GSI) using testes mass. Amorim et al. (2009) suggest that sonic muscle mass may signal male quality in a closely related species, the Lusitanian toadfish, *Halobatrachus didactylus*, and so we calculated a sonic muscle somatic index (SMSI) using the mass of each male's sonic apparatus (swim bladder with attached sonic muscles) as a proxy for male courtship ability.

We explored the effects of these additional male traits on reproductive success by re-fitting the same models described in Study 1, but included male body condition, HSI, GSI and SMSI (all mean-centered and divided by their standard deviation) as additional fixed effects. We assessed mate attraction using the 193 nests for which we had both dissection data and counts of females. As our sample size was restricted to only the nests for which males were dissected, we did not have enough data to independently assess egg acquisition versus rearing success, and therefore we pooled all nests in our analysis whether they included pre-hatch embryos only, post-hatch embryos only, or both. We therefore assessed total brood size as a second component of reproductive success using 257 nests for which we had dissection data as well as embryo counts. We present no p-values for our exploratory analyses; instead, the model results are illustrated graphically as coefficient plots.

We measured several nest characteristics in addition to nest size that we considered to be important correlates of nest quality. We calculated species richness scores for each nest using the digital images taken of each nest to identify all macro-organisms sharing the nest surface with the embryos. These organisms were identified down to the lowest possible taxonomic grouping and categorized as space competitors or non-space competitors based on whether or not they compete with midshipman embryos for space in the nest (see Demartini 1991). We also determined local nest density around each focal nest by measuring the distances to the three closest neighbouring nests (to the nearest cm, and up to 250cm). We used these distances to calculate a nest density index ranging from 1 to 5, where a score of 1 indicated that the nest was relatively isolated and a score of 5 indicated that the nest was part of a dense cluster of nests (see Supplementary Table 2.2 for additional details).

We explored the effects of these additional nest traits by re-fitting the same models described in Study 1, but included space competitor richness, non-space competitor richness, and nest density scores as additional fixed effects. We specifically explored the linear and quadratic effects of local nest density (a 5-level ordered factor). We assessed mate attraction using the 339 nests for which we had additional nest quality data and also counts of females. Again, we assessed total brood size using the 339 nests for which we had additional nest quality data as well as embryo counts. Unfortunately, we did not have enough overlap in data to combine both exploratory analyses together (additional male quality and nest quality traits).

All procedures used in these studies were approved by the McMaster University Animal Research Ethics Board (AUP number 13-12-52), and are in line with the guidelines set by the Canadian Council on Animal Care (CCAC).

2.4 Results

Brood sizes across the 727 sampled nests were on average (± std. dev.) 565 embryos (± 465, range: 2 – 3425 embryos, including both pre-hatch and post-hatch embryos). Based on an estimated maximum fecundity of 300 eggs per gravid plainfin midshipman female (KC personal observations), we conservatively estimate that males in our dataset attracted an average of two females to their nests leading up to the time of sampling, and this number ranged from one to 12 females per male nest owner.

Study 1: Both larger males and larger nests have higher reproductive success

More females were attracted to larger nests (GLMM, standardized nest surface area, est. ± se = 0.25 ± 0.10, z = 2.74, N = 652, p = 0.006, Fig. 2.1A) but not to larger males (GLMM, standardized male body length, est. ± se = -0.018 ± 0.086, z = -0.21, N = 652, p = 0.83, Fig. 2.1B). However, a direct comparison of the model coefficients showed that the relative influence of nest size and male size on mate attraction did not differ significantly (glht, difference in model coefficients, est. ± se = -0.27 ± 0.15, z = -1.84, N = 652, p = 0.065). The number of eggs received by a male increased with both nest size (Fig. 2.1C) and body size (Fig. 2.1D), though the effect of body size also interacted with nest size (LMM, interaction term, est. ± se = 0.32 ± 0.11, t = 2.91, N = 302, p = 0.004). For small nests (i.e. nest size centered on mean – 2 std. dev.), nest size was more strongly correlated with embryo number than was male size (Fig. 2.2A). However, for large nests (i.e. nest size centered on mean + 2 std. dev.), both male size and nest size were similarly correlated with embryo number (Fig. 2.2A). The number of young successfully reared (post-hatch embryos) also increased with both nest size (Fig. 2.1E) and male size (Fig. 2.1F), but the effect of body size again depended on nest size in a very similar pattern (LMM, interaction term, est. ± se = 0.44 ± 0.19, t = 2.33, N = 102, p = 0.022; Fig. 2.2A and B). Note, that we found the same pattern of results when pooling all nests together (N = 727 nests) and investigating total brood size, as opposed to subsampling and investigating egg acquisition (N = 302 nests with eggs only) separately from rearing success (N = 102 nests with hatched embryos).

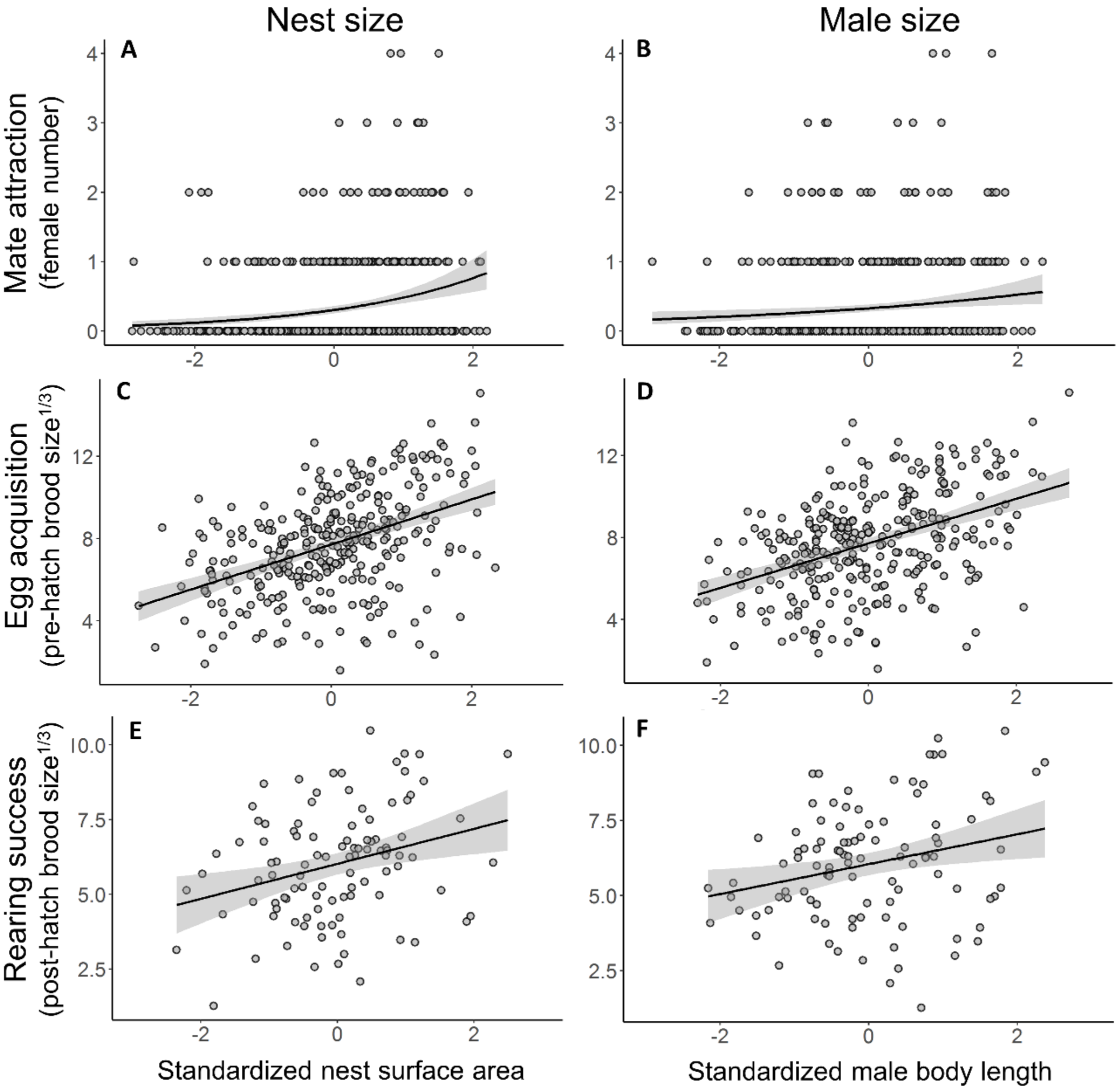

Figure 2.1 The number of females found per nest plotted against (A) nest surface area and (B) male standard length. Number of eggs acquired plotted against (C) nest surface area and (D) male standard length. Number of embryos successfully reared plotted against (E) nest surface area and (F) male standard length. These plots depict all data pooled across years and sites from Study 1.

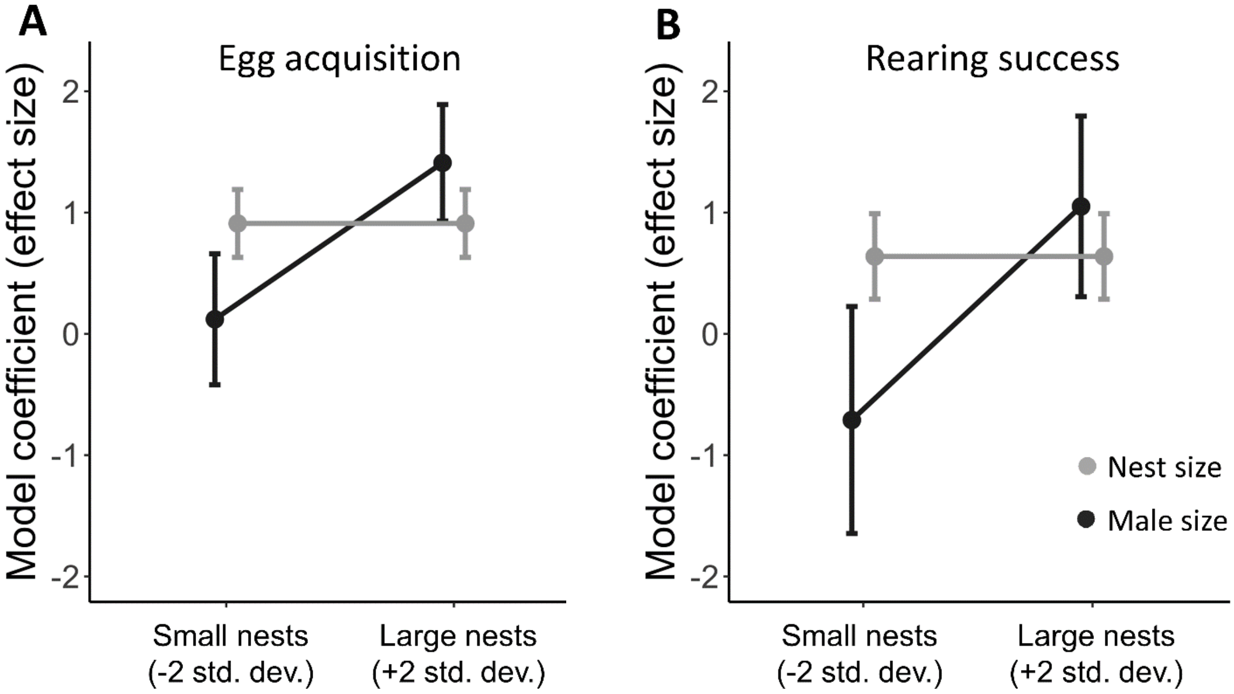

Figure 2.2 Interaction effects found in Study 1. The effect of male size relative to nest size on two components of reproductive success, (A) egg acquisition and (B) rearing success, across a wide range of nest sizes measured in the field. Error bars represent 95% confidence intervals.

Study 2: Females prefer larger males

Ten of the 19 females that were offered two differently sized males chose to spawn with one of the males. Of these ten females, nine preferred the larger male and one preferred the smaller male (exact binomial test, p = 0.02, Fig. 2.3A). Fourteen of the 23 females to which we offered two size-matched males in differently sized nests chose to spawn with one of the males. We did not detect any preference for larger nests in these trials (nine females chose the larger nest and five chose the smaller nest; exact binomial test, p = 0.40, Fig. 2.3B). Female preference for large males (9 out of 10 females) was not significantly different from their preference for large nests (9 out of 14 females; chi square test, $\chi^2 = 2.06$, p = 0.33).

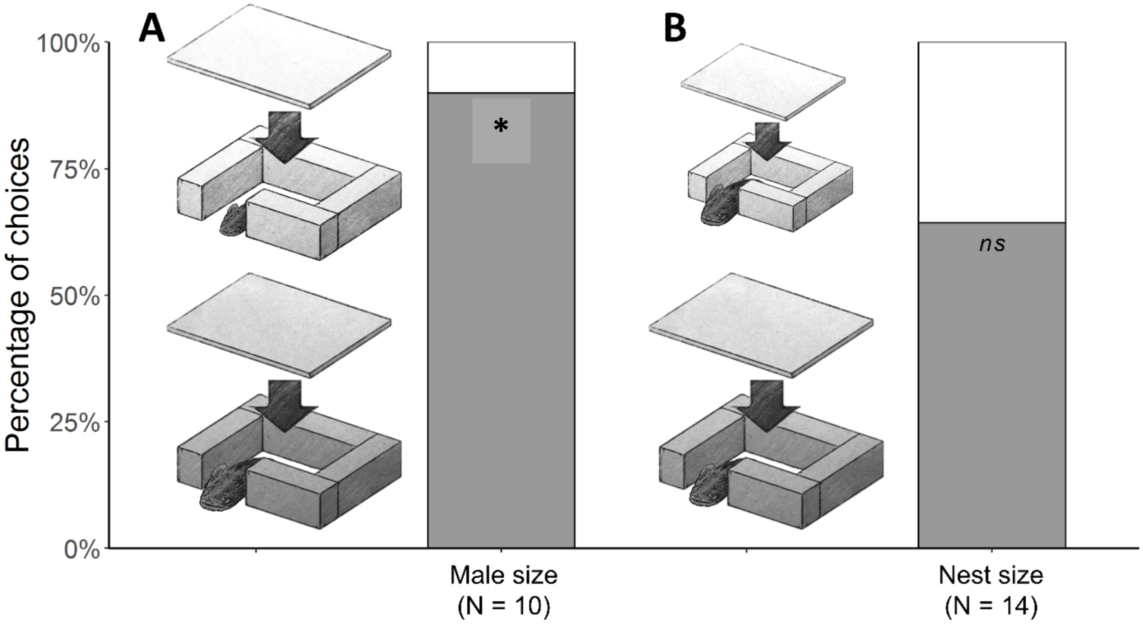

Figure 2.3 (A) Female choice between a large male (grey) versus a small male (white) in size-matched nests (95% binomial confidence interval = 0.555, 0.997). (B) Female choice between size-matched males in a large nest (grey) versus a small nest (white) (95% binomial confidence interval = 0.351, 0.872). Picture inlays illustrate the nest designs; fish are not drawn to scale.

Study 2: Males prefer larger and more enclosed nests

Sixty-eight of the 94 males offered a large versus small nest made a choice after 24 hours, and 47 (72%) of these males preferred the larger of the two nests (GLMb, est. ± se = 0.81 ± 0.26 log odds, z = 3.07, p = 0.002, Fig. 2.4A). Male body size had no detectable effect on nest choice (GLMb, est. ± se = 0.048 ± 0.26 log odds, z = 0.18, p =

0.86). Thirty-one of the 48 males offered an enclosed versus open nest had made a choice after 24 hours, and 29 of these males (94%) preferred the more enclosed of the two nests (GLMb, est. ± se = 3.38 ± 1.30 log odds, z = 2.60, p = 0.009, Fig. 2.4B).

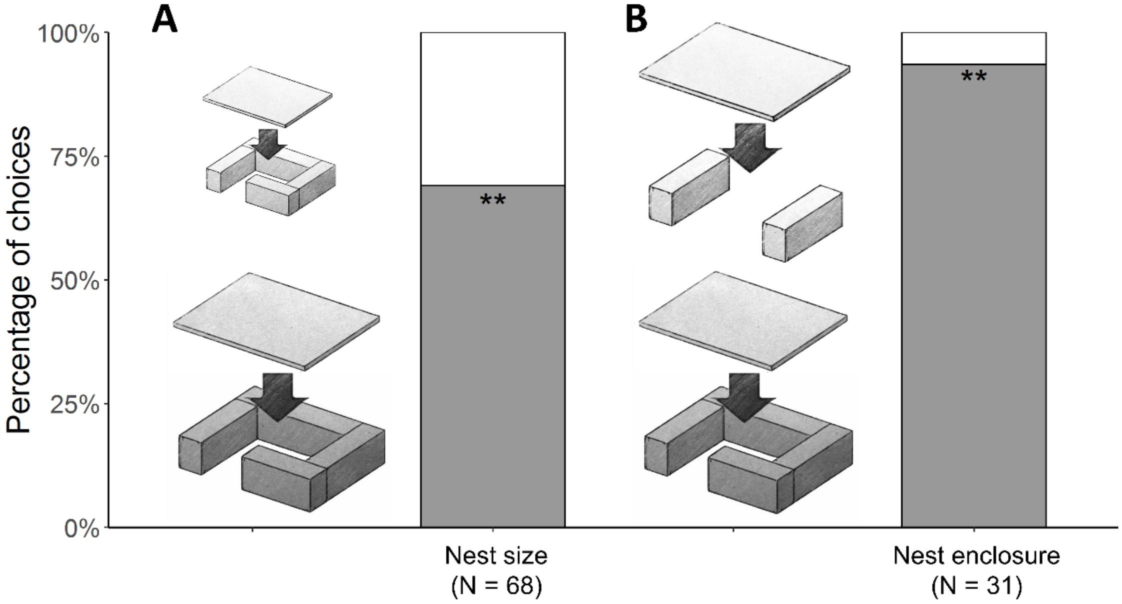

Figure 2.4 Male choice (A) between a large nest (grey) versus a small nest (white) and (B) between an enclosed nest (grey) versus an open nest (white).

Study 3: Additional male quality and nest quality traits

Males that had heavier swim bladders for their body size (including attached sonic muscles used for acoustic courtship) had attracted more females (SMSI, Fig. 2.5A). Males with lower gonadal investment for their body size (GSI) also had larger broods in their nests (Fig. 2.5B). The number of females found in nests decreased with Julian date over the breeding season, as did average brood sizes (Fig 2.5A & B).

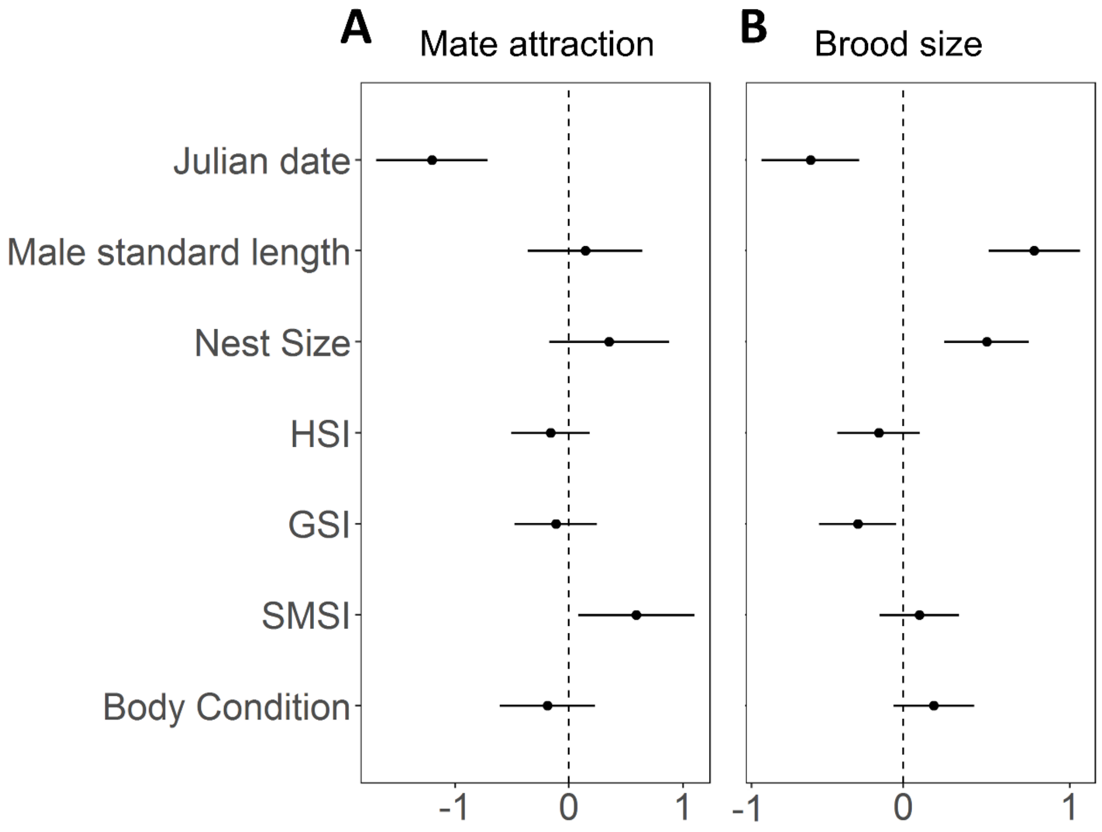

Figure 2.5 Coefficient plots depicting model estimates (dots) and 95% confidence intervals (solid bars) for additional putative male quality traits (all continuous predictors were mean-centered and divided by their standard deviation).

Common space competitors in our plainfin midshipman nests included colonial tunicates (e.g. *Botrylloides violaceous*, *Didemnum vexillum*), encrusting bryozoans (e.g. *Schizoporella japonica*), encrusting sponges (e.g. *Halichondria panacea*, *Haliclona permollis*, *Clathria pennata*), and egg masses of other organisms including dorids (e.g. *Doris montereyensis*) and snails (e.g. *Nucella lamellosa*). Nests that had more species of space competitors had fewer females (Fig. 2.6A). Densely clustered nests on the beach contained more females than nests that were more isolated (Figure 2.6A). We did not find a significant influence of any of the additional nest quality traits measured on brood size (Fig. 2.6B).

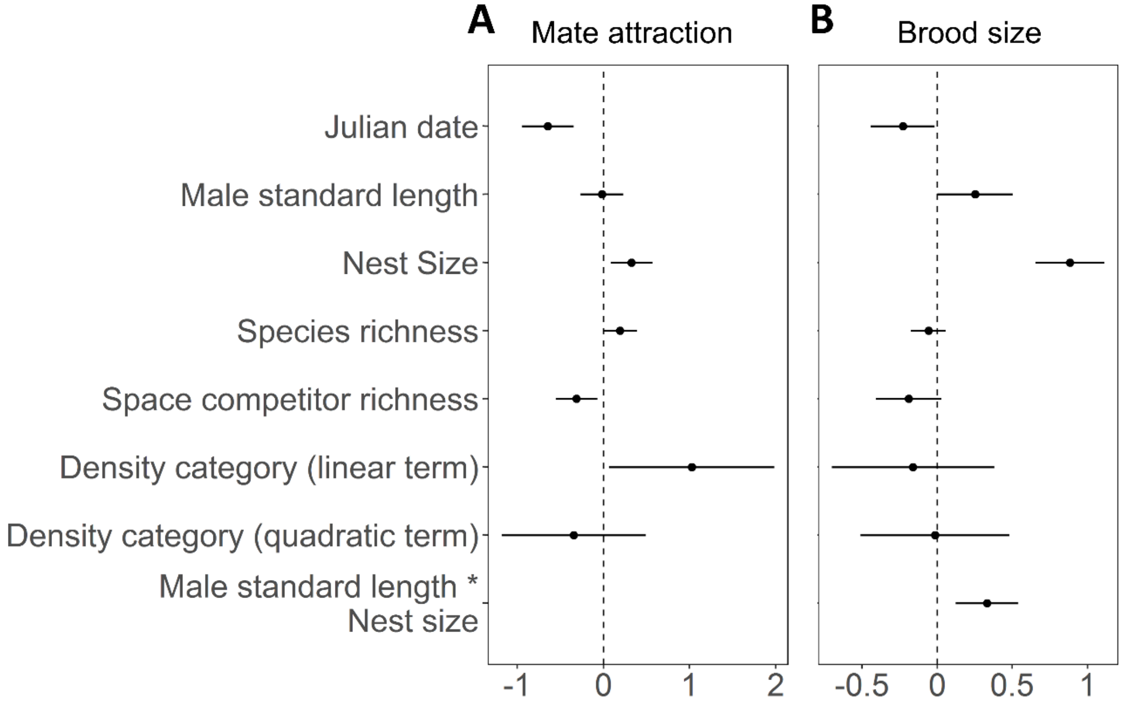

Figure 2.6 Coefficient plots depicting model estimates (dots) and 95% confidence intervals (solid bars) for additional nest quality traits (all continuous predictors were mean-centered and divided by their standard deviation). Non-significant interactions were dropped from the model.

2.5 Discussion

To evaluate the relative impacts of male and nest quality on reproductive success in a wild fish, *P. notatus*, we combined large-scale field-based surveys with laboratory-based controlled experiments. Both male size and nest size positively correlated with male reproductive success in the field. Our laboratory studies provided complementary results indicating that males preferred the larger of two nests, and that females prefer the larger of two males. Our data also suggest that the size of a male's sonic apparatus, the richness of space-competitor organisms within a nest, and the local density of nesting males also influence reproductive success and may be important variables of interest for future work. Our results fit in with and extend previous studies, providing a clearer picture of how the ecological and social landscape in which *P. notatus* breeds influences nest-site and mate choices, thereby shaping the mating system in general.

Study 1: Male reproductive success increases with both male size and nest size

In *P. notatus*, intertidal rocks are a critical resource needed for reproduction. Males compete intensely with one another over rock (and nest) ownership, and a positive relationship is commonly documented between male size and nest size (Demartini 1988; Demartini 1991; Bose et al. 2014). In many taxa, body size correlates with a male's resource holding potential permitting the largest males to secure the highest-quality territories (e.g. green frogs, *Rana clamitans*, Wells 1977; damselflies, *Megaloprepus coerulatus*, Fincke 1992; Azorean rock-pool blennies, *Parablennius sanguinolentus parvicornis*, Oliveira et al. 2000). Our results extend previous results by showing that the relationships between body size, nest size, and reproductive success hold true across numerous breeding populations of one marine fish species, and that the relative importance of male size and nest size can depend on their interaction.

More females were attracted to larger nests. Although the difference in the effect of nest size and male size on reproductive success did not reach statistical significance ($p=0.065$), nest size appeared to have a stronger effect than male size in attracting females. Theory suggests that the relative importance of resource quality versus male quality on mating success depends on whether female choice and offspring fitness are predominantly affected by male traits, nest characteristics, or both (Searcy 1979; Pomiankowski 1988; Møller and Jennions 2001; Candolin 2003). Females may prefer to spawn in large nests if such nests contain larger broods that will subsequently receive more paternal care (Coleman et al. 1985), or if nest size correlates with the nest builder's health or phenotype (e.g. blue tits, *Cyanistes caeruleus*, Tomás et al. 2006). However, it is important to consider here that *P. notatus* nests are also often small, confined spaces (Demartini 1991). Adult *P. notatus* take up a considerable amount of space within nesting cavities (see Bass 1996; McIver et al. 2014). Thus, the positive relationship between female number and nest size may in part be driven by the small nests not containing enough space to simultaneously hold multiple females.

Our males' egg acquisition and rearing success increased with both male size and nest size. Interestingly, for the small nests in our dataset, nest size was more strongly related to these components of reproductive success than male size. This may be because the dimensions of small nests impose an upper limit on brood size regardless of the size of male occupying the nest. Other studies have also found that nest size can dictate brood size or maximum reproductive success of the nest owners (e.g. Marsh Tits, *Parus palustris* and Willow Tits, *Parus montanu*, Karlsson and Nilsson 1977; sand gobies, *Pomatoschistus minutus*, Lindström 1992a), and so nest size may be an important ecological constraint in many taxa that breed in small spaces. In large nests, however, both male size and nest size were positively related to embryo number to similar degrees.

Thus, although our correlational analyses were able to reveal that both the size of a male and the size of his nest correlate with reproductive success, we could not differentiate the relative contributions of each to male reproductive success.

Study 2: Females prefer larger males and males prefer larger nests

Experimental studies have a marked advantage over correlational studies, because they allow researchers to independently manipulate variables that tend to be highly correlated under natural conditions (Kelly 2008). In our series of two-choice experiments, females preferred large males, but had no clear preference for large nests. However, given our limited sample size, we could not detect any difference in the strength of female preference for male size versus nest size, and so additional testing with more spawning trials in the laboratory would be valuable. Male body size is a reliable predictor of a male's ability to win contests in a variety of taxa (e.g. sand gobies, *P. minutus*, Lindström 1992b; orb-web spiders, *Metellina mengei*, Bridge et al. 2000; Magellanic penguins, *Spheniscus magellanicus*, Renison et al. 2002; red deer, *Cervus elaphus*, Clutton-Brock et al. 1979) and could therefore be a reliable cue of a male's ability to defend a nest or territory (Lindström and Pampoulie 2005). Male body size is also a reliable predictor of nest ownership in *P. notatus* (Lee and Bass 2004). Nest take-overs are a frequent occurrence in the *P. notatus* breeding season, and successful nest take-overs are associated with embryo cannibalism by the usurping male (Bose et al. 2014) and a decline in offspring survival (Bose et al. 2016). Thus, large males may represent a safer option for a female to entrust her eggs, if they are more likely to retain resource ownership over the extended (~60 day) parental care period, as found in other species (e.g. sand gobies, *P. minutus*, Lindström and Pampoulie 2005; *Lamprologus callipterus*, Maan and Taborsky 2008).

Male *P. notatus* preferred larger and more enclosed nests. Preferences for nest size in nature is likely to reflect a tradeoff between the benefits of having space for many offspring and the costs of maintaining and defending a large preferred nest (Kvarnemo 1995; Mainwaring et al. 2014). It has been proposed that male sand gobies, *P. minutus*, choose smaller and easier-to-defend nests when egg-predation risk is high, but prefer larger nests when the risk is low (Björk and Kvarnemo 2012). Interestingly, we did not detect any effect of male size on their choice of nest size suggesting that males pursue large nests regardless of their own body size. Perhaps, the benefits of owning a large nest outweigh the costs associated with defending it because in the wild, small nests constrain the reproduction of all males alike. Future studies could test a greater diversity of nest sizes and do so under different predation regimes to more explicitly test size-assortative nest choice in *P. notatus*.

Study 3: Additional male and nest quality traits

Male plainfin midshipman fish generate an advertisement call typically referred to as a ‘hum’ to attract gravid females by rapidly contracting the sonic muscles attached to their swim bladder walls (Cohen and Winn 1967; Ibara et al. 1983; Bass and Marchaterre 1989; Brantley and Bass 1994; McKibben and Bass 1998). Because males with larger swim bladders and sonic muscles for their body size attracted more females, the overall size of this organ may influence characteristics of the male song. While the fundamental frequency of a male's advertisement hum appears to be unrelated to his body size (McIver et al. 2014), and thus to sonic muscle or swim bladder size (Brantley et al. 1993), females will choose the louder of two simulated advertisement calls (McKibben and Bass 1998). Males inflate their swim bladder during advertisement calling (Bass et al. 2015), likely as an adaptation to enhance sound amplitude that would increase with increasing swim bladder volume (see Russel et al. 1999). Furthermore, call loudness scales with sonic muscle mass in other fish species (e.g. Lusitanian toadfish, *Halobatrachus didactylus*, Vasconcelos and Ladich 2008; weakfish, *Cynoscion regalis*, Connaughton et al. 1997). Therefore, if males with large swim bladders have louder advertisement calls, that could, in part, explain the results we observed here. It still remains to be tested whether other spectral or temporal song characteristics correlate with swim bladder size in this species. Interestingly, although large swim bladder and sonic muscle investment correlated with more females per nest, this trait did not translate to significantly larger broods. Perhaps after entering a nest, females use additional information to assess males before engaging in spawning. In the dark conditions of male's nests, females might use mechanosensory information from their lateral lines to further assess male size and quality as has been shown in Atlantic mollies, *Poecilia mexicana* (Plath et al. 2004) and himé salmon, *Oncorhynchus nerka* (Satou et al. 1994). The mechanosensory lateral line of midshipman is also sensitive to the frequency content of male calls (Weeg and Bass 2002). Hence, the lateral line might play a role in mate assessment based on advertisement calls that the males sometimes continue to produce up to one minute after female entry into the nest (Brantley and Bass, 1994).

Females were found in greater numbers in densely clustered nests, yet these densely-clustered nests did not have correspondingly larger broods relative to more isolated nests. Breeding under conditions of high nest density may present both costs and benefits. For example, as density increases, females may be more efficient at comparing males and so might make better or more efficient mating decisions (e.g. common yellowthroat warblers, *Geothlypis trichas*, (Taff et al. 2013). High density can also reduce a female's search time before she encounters a suitable male mate (e.g. bushcrickets, *Xederra charactus*, Lehmann 2007). However, if high density provides more opportunity for males to interfere with one another's reproduction, then high density may reduce the overall success of many males in the population and potentially counteract the benefits of nesting in dense areas (Kokko and Rankin 2006). The community of intertidal organisms living in a nest also influenced the quality of that nest. Fewer females were found in nests that contained many species of space-competitor organisms. Species that are space-competitors represent an interesting and novel angle from which to study territory quality because they reduce the effective size, and thus quality, of a nest without changing its physical dimensions (Hastings 1988; Demartini 1991).

In this study, we attempted to disentangle the relative influence of male quality and nest quality on male reproductive success in the plainfin midshipman fish. We did not find that one variable was consistently more important than the other, but rather we showed that their interactions need to be carefully considered. We also highlight the importance of considering morphological, ecological and physiological traits when studying reproductive success and including multiple metrics of reproductive success whenever possible. We suggest that future studies focus on whether females use auditory cues only to assess their potential male mates or whether they use multimodal cues (including other sensory information) while in the dark conditions of the male nest. Furthermore, future studies should also consider paternity loss while assessing male reproductive success in *P. notatus* as cuckoldry and nest takeovers are known to contribute to variable brood paternities in the wild (Brantley and Bass 1994; Cogliati et al. 2013; Bose et al. 2014; Cogliati et al. 2014a,b; Fitzpatrick et al. 2015). Finally, we underscore the utility of combining correlational analyses from the field with controlled laboratory experiments to investigate the typically highly correlated variables that underlie reproductive success.

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2.8 Supplementary materials

Supplementary Table 2.1: Geographic locations of intertidal field sites used to survey plainfin midshipman nests. BC – British Columbia, Canada; WA – Washington State, USA; CA – California, USA.

Sampling region	GPS coordinates
Ladysmith Inlet, BC (Site A)	49°1'N, 123°50'W
Ladysmith Inlet, BC (Site B)	49°0'N, 123°49'W
Nanoose Bay, BC	49°15'N, 124°10'W
Deep Bay, BC	49°27'N, 124°43'W
Mill Bay, BC	48°37'N, 123°31'W
Boundary Bay, BC	49°2'N, 122°53'W
Dabob Bay, WA (Site A)	47°45'N, 122°50'W
Dabob Bay, WA (Site B)	47°42'N, 122°53'W
Tomales Bay, CA	38°10′N, 122°54′W

Supplementary Table 2.2: Criteria for assigning nest density index scores based on the distances of each focal nest to its three nearest neighbouring nests (measured center to center).

Nest density index score	Criteria
(1) Low density	All three neighbouring nests > 200cm **OR** Average distance to the three nearest neighbouring nests > 200cm
(2) Medium – Low density	All three nearest neighbouring nests between 100 – 200cm *and* average distance < 200cm **OR** One neighbouring nest < 100cm *and* average distance > 200cm
(3) Medium density	One neighbouring nest < 100cm *and* average distance < 200 cm
(4) Medium- High density	Two neighbouring nests < 100cm **OR** One neighbouring nest < 100cm *and* average distance < 100 cm
(5) High density	All three nearest neighbouring nests < 100cm

Supplementary Methods: Laboratory choice trials

Female preference for male size and for nest size

We collected adult gravid females and adult male midshipman fish from Ladysmith Inlet, British Canada (49°01′N, 123°83′W), Canada between April 30 and May 28, 2016. The fish were transported to the outdoor aquatic unit at the University of Victoria, British

Columbia, Canada. The glass aquaria (L 2’11” x W 11” x H 2’), which housed the two males in each choice trial were 150L, lined with a pea gravel substrate, held at 13 ± 1°C, and fitted to a recirculating system supplying filtered seawater. During acclimation, the males were separated from one another with an opaque plastic barrier. Each male was provided with an artificial nest on his side of the tank. In both experiments 1 and 2, the walls of the artificial nests were constructed from bricks while the roof was constructed from an acrylic plastic sheet and a ceramic tile (12” x 12”). Males were observed producing courtship vocalizations nocturnally while in these conditions suggesting that they were engaged in natural breeding behaviours (Bose A, personal observations).

Male preference for nests

We collected adult male midshipman fish from Ladysmith Inlet, British Columbia, Canada (49°01′N, 123°83′W) between May 24 and June 9, 2014 as well as April 25 and June 8, 2015 and transported them to the Pacific Biological Station, in Nanaimo, British Columbia, Canada. Each male was housed separately in large 300L outdoor fiberglass holding tanks. Each tank was lined with a sand substrate, held at 18 ± 1°C, and fitted with a flow-through system supplying filtered seawater. The walls of the artificial nests in experiments 3 and 4 were built from bricks and the roof from a 12” x 12” concrete garden tile. We initially placed the males under a brick ‘start shelter’ (~ 8” x 8”) in each tank for the 30-minute acclimation period. In experiment 4, the dimensions of the entranceway to the safer nest were ~ 2” x 2.4”, while the dimensions of both entranceways to the vulnerable nest were ~ 2” x 7.9”. We commonly observed the males inspecting their surroundings and even producing courtship vocalizations nocturnally while in these tanks (Bose A, personal observations).

Chapter 3: Factors influencing cannibalism in the plainfin midshipman fish

3.1 Abstract

Cannibalism of young is a common yet seemingly paradoxical phenomenon observed across a wide variety of taxa. Understanding this behaviour in the context of parental care remains a challenge for evolutionary biologists. A common adaptive explanation for the consumption of offspring is that it serves to increase the current or future reproductive success or survival of the cannibalistic parent by replenishing energy stores and facilitating continued care for any remaining young. Another explanation is that cannibalism may be a competitive response to cuckoldry or lowered certainty of parentage. We tested these ideas using the plainfin midshipman fish, *Porichthys notatus*, a species with an extended period of male-only parental care and documented offspring cannibalism. We found that the occurrence of cannibalism was not linked to the deterioration of body condition, but instead was most frequent during periods of high intrasexual competition and nest take-overs. Our results suggest that cannibalism is not driven by the energetic demands of parental care, but instead by competition among males for nests and females, and the resulting low paternity stemming from both nest take-overs and cuckoldry.

3.2 Introduction

Cannibalistic behaviour has been observed in a vast range of taxa throughout the animal kingdom (Polis 1981; Smith and Reay 1991; Elgar and Crespi 1992; Soulsby 2013). Predation upon conspecific offspring has been widely documented across species, even for those that provide a high degree of parental care (Polis 1981; Elgar and Crespi 1992). In most caregiving species, parents invest considerable time and resources into offspring production, protection and growth, so cannibalism of offspring appears counterproductive to the goals of a caregiving parent. Evolutionary theory suggests that even with filial cannibalism, the acute loss of current reproductive success associated with the consumption of one's own offspring can be offset by future fitness benefits to the cannibal (Rohwer 1978; Polis 1981; Smith and Reay 1991; Elgar and Crespi 1992; Manica 2002). For example, by recouping energy stores through filial cannibalism, the cannibal can prevent starvation, increase its ability to attract better or more mates, or ensure continuity of care (Rohwer 1978; Sargent 1992). While recouping energy presents one explanation for filial cannibalism, both empirical and theoretical studies suggest that the occurrence of offspring cannibalism across species may be driven by a variety of differing selective factors (e.g. Manica 2002; Manica 2004; Klug et al. 2006; Gray et al. 2007; Klug and Bonsall 2007). Thus, the evolutionary function and origins of this behaviour remain an active area of study.

Starvation or dwindling energy reserves is one of the most studied driving factors for a parent to cannibalize their own offspring (Manica 2002). This energy-based hypothesis predicts that cannibalism will increase as parental body condition deteriorates

(Rohwer 1978; Sargent 1992). The energy recouped through filial cannibalism can be invested into future reproductive attempts, or into the continued care for the remaining offspring (Rohwer 1978; Sargent 1992). This hypothesis has been tested empirically in a number of different species. Kvarnemo et al. (1998) showed that supplemental feeding in the laboratory could decrease egg cannibalism in the common goby, *Pomatoschistus microps*. However, supplemental feeding had no effect on either the number of eggs cannibalized in threespine stickleback, *Gasterosteus aculeatus* (Belles-Isles & FitzGerald 1991), or in the fantail darter, *Etheostoma flabellare* (Lindström and Sargent 1997). In addition, field studies have also shown that supplemental feeding cannot abolish filial cannibalism in Cortez damselfish, *Stegastes rectifraenum* (Hoelzer 1992), or a species of Mediterranean blenny, *Aidablennius sphinx* (Kraak 1996). Therefore, empirical support for the energy-based hypothesis is currently mixed (Manica 2002; Klug and Bonsall 2007).

In contrast to filial cannibalism, nonkin cannibalism of another individual's offspring can be viewed as a form of competition. For example, a male can increase his own condition and fitness at the expense of a rival's reproductive success by eating the offspring sired by a competitor (Bertram 1975; Polis 1981; Smith and Reay 1991). For numerous species of fishes, nest take-overs have been documented, wherein a competitive individual displaces a resident for its nest or territory, gaining control over it (Coleman and Jones 2011). As offspring in a newly acquired nest or territory are typically sired by previous residents, the take-over victor will suffer no direct fitness costs by consuming them (Sargent 1989; Coleman and Jones 2011). In the same vein, an association between low paternal certainty and offspring cannibalism has been shown in several fish species. For example, in the scissortail sergeant, *Abudefduf sexfasciatus*, the near proximity of potential cuckolder males resulted in increased cannibalism of eggs from a caregiving male's brood (Manica 2004). Male caregivers of both bluegill sunfish, *Lepomis macrochirus* (Neff 2003a) and threespine stickleback (Frommen et al. 2007) are able to use direct offspring cues to assess their level of paternity over a brood and cannibalize more often when nonkin offspring are present. Even male *Telmatherina sarasinorum*, a species that does not provide parental care, will cannibalize their broods more often if cuckolders are present during spawning (Gray et al. 2007). Still other studies have been unable to show a relationship between paternal certainty and offspring cannibalism (e.g. common goby, *P. microps*: Svensson et al. 1998; sand goby, *Pomatoschistus minutus*: Svensson and Kvarnemo 2007).

Hypotheses for cannibalism as an energy-replenishing tactic and as a competitive tactic are not necessarily mutually exclusive. However, their relative importance within a single study system has not been previously assessed. We tested these two hypotheses in the plainfin midshipman fish, *Porichthys notatus*. This species is a useful model to investigate cannibalism of offspring for a variety of reasons. First, nest-guarding males have a protracted and a presumably energetically taxing parental care period (Sisneros et al. 2009; Craig et al. 2014), which may select for cannibalism as an energy-replenishing strategy. Second, these males compete intensely with each other over nesting sites and access to mates (Cogliati et al. 2013; Cogliati, Balshine, et al. 2014). In combination with the expression of alternative male tactics, males consequently have surprisingly low levels of paternity in broods that they care for in the wild (on average 52%; Cogliati et al. 2013). This could select for cannibalism as a competitive strategy. Third, male midshipman fish have been documented with conspecific offspring in their digestive tracts (Sisneros et al. 2009; Cogliati et al. 2015), however, the driving factors behind this cannibalism remain unknown.

We examined cannibalism across the long midshipman breeding period, which spans over 3 months (May–July) of care (Cogliati et al. 2013). We explored whether the prevalence of cannibalism changed across the breeding season and whether cannibalism was linked to patterns of energy loss or competition. If caregiving males cannibalize as a result of depleting energy reserves, then we predicted that cannibalism would increase over the breeding season as paternal body condition deteriorates (Sisneros et al. 2009). Alternatively, if cannibalism is a competitive tactic, then we predicted that it would be most frequent early in the season, when male–male competition is most intense (Cogliati et al. 2013). Consequently, the plainfin midshipman system provides the chance to explore cannibalism in a multifaceted way, allowing us to consider both energy-based and competition hypotheses in one species.

3.3 Methods

Study Species

The plainfin midshipman is a marine toadfish (family Batrachoididae) distributed along the west coast of North America, from California to Alaska (Arora 1948; Miller and Lea, 1972; Walker and Rosenblatt 1988). Two alternative male reproductive tactics have been well described in this species (Brantley et al. 1993; Brantley and Bass 1994; Bass et al. 1996; Lee and Bass 2004; Cogliati et al. 2013). At the onset of the breeding season, large nest-guarding males (also known as type I) migrate to the intertidal zone, where they excavate a nesting cavity in the soft sediment beneath large rocks (Arora 1948). As the tides retreat, these guarder males do not leave their nests, even as the small pools of remaining water become hypoxic (Craig et al. 2014). Males can endure these hypoxic conditions through metabolic suppression, a switch to anaerobic pathways (Craig et al. 2014) and a well-adapted system for acid–base regulation (Perry et al. 2010). The guarder males produce an acoustic signal with a specially adapted swim bladder encased in a sonic muscle capable of generating long-duration, low-frequency vibrations (Bass and Marchaterre 1989; Sisneros and Bass 2003) to attract gravid females to their nests (Ibara et al., 1983; Brantley et al. 1993; Brantley and Bass 1994). Typically, the largest males acquire the largest nests and attract the most females (Demartini 1988; Fitzpatrick et al. 2015). Females deposit their entire clutch of eggs (typically 150–300 eggs; APH Bose & KM Cogliati, personal observations; Demartini 1988), in a monolayer on the underside of the rock, which is the roof of the nesting cavity (Arora 1948). Nest-guarder males care for offspring by cleaning, digging and maintaining the nest, fanning the eggs during high tide, hydrating the eggs during low tide, and defending them against egg predators and male competitors (Arora 1948). Offspring take approximately 60 days to develop into free-swimming juveniles, but because males often continue attracting females, and care for young of various developmental stages, the care period can be longer than 60 days for males of this species (Cogliati et al. 2013), beginning in late April and continuing until early August (Crane Jr. 1981; Demartini 1988; Cogliati et al. 2013).

While guarder males care for offspring and court females, sneaker males (also known as type II males) are also present in the population. These smaller males do not build nests, court or provide parental care. Instead, they attempt to fertilize eggs through stealth and sneaking behaviours, whereby they release their sperm while a guarder male spawns with a female (Brantley and Bass 1994). Interestingly, guarder type I males are sometimes behaviourally flexible and have been observed to cuckold other guarder males (Lee and Bass 2004; Cogliati et al. 2013; Cogliati et al. 2014). Presumably, this occurs when these males have no eggs in their own nest. If detected, cuckoldry attempts by type I guarder or type II sneaker males or the mere presence of other males in the nest will decrease the nest-guarding male's certainty of paternity over his brood.

Field Observations

A total of 166 plainfin midshipman nests were located between May and July 2013 in the intertidal zone of Crescent Beach (South Surrey, BC, 49°04′N, 122°88′W), a long rocky shoreline that supports a large population of spawning plainfin midshipman. It is a productive, well-sheltered spawning ground with many large rocks that are used as nesting sites, and large nearby eelgrass beds that likely serve as a nursery habitat for newly hatched juveniles. We sampled nests during three periods over the breeding season (23–26 May, 22–26 June, 19–24 July), which corresponded to the early, mid and late breeding season, respectively.

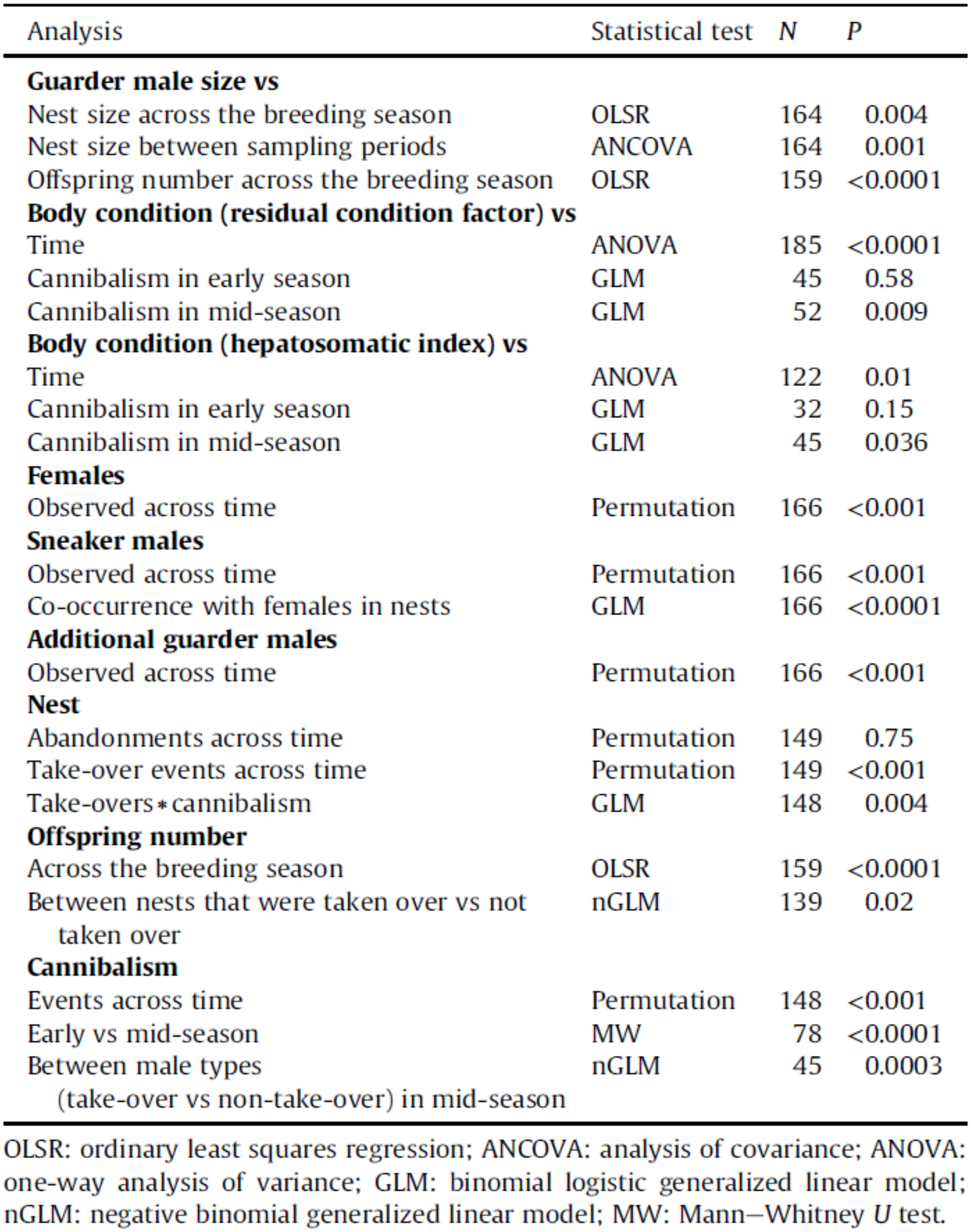
During each period, we checked nests using a short 2-day protocol (consecutive days) to minimize the likelihood of nests gaining new eggs between sampling days or losing nests from unanticipated factors such as predation. On day 1, we laid out 20 m transects parallel to the shore and located nests by overturning all suitable rocks (i.e. rocks that were sufficiently large and not too embedded into the substrate; see (Cogliati, Mistakidis, et al. 2014) within 2 m of either side of these transects. Each nest that contained a guarding male and offspring was considered an active nest and marked with a labelled numbered tent peg. A digital photograph was taken of such nests (Olympus digital camera TG-820, 12.0 megapixels). For each nest, we recorded the number, sex and tactic type (guarder versus sneaker) of each fish, tagged all fish with a nontoxic injectable elastomer (Northwest Marine Technology, Inc., Shaw Island, WA, U.S.A.) for future identification, and weighed (total mass to the nearest 0.1 g) and measured (total and standard length to the nearest 0.1 cm) them. On the next day, day 2, we returned to these nests, lifted the rock and took a second photograph. We noted whether the resident guarder male from the previous day was still in the nest, if the nest had been abandoned, or if a new untagged guarder male was in the nest (classified as a 'nest take-over'). If the original guarder male from day 1 as well as a new untagged guarder male were both present, we noted the presence and position of the second male, and classified these events as 'attempted take-overs in progress'. All digital photographs from day 1 and day 2 samplings were analysed using ImageJ software (v1.45, http://rsbweb.nih.gov.libaccess.lib.mcmaster.ca/ij/) to quantify the number of offspring in each nest and the size of the nest (total surface area available for egg laying, cm2).

We determined cannibalism by the guarder male in two ways: (1) by comparing photographs of nests taken on day 1 versus day 2 and noting whether offspring had disappeared; (2) by examining stomach contents of a sample of the fish for the presence of embryos. Thus, every fish in our data set was marked as either a cannibal (showing evidence of recent cannibalism) or a noncannibal (showing no evidence of recent cannibalism).

Data and Statistical Analyses

In total, we sampled 166 nests and collected data on the presence or absence of guarder males, sneaker males, females and any additional guarder males that appeared to be cuckolding or attempting a take-over in these 166 nests. Sample size varied between analyses depending on the measures included in the models (e.g. number of nests, number of fish of a particular sex or tactic, number of fish dissected to examine stomach contents, etc.) and the number of fish sampled (i.e. we were unable to obtain some measures from some individuals; a summary of sample sizes used in each analysis is given in Table 3.1).

Table 3.1 Summary of sample sizes used in each analysis.



Analysis	Statistical test	*N*	*P*
Guarder male size vs			
Nest size across the breeding season	OLSR	164	0.004
Nest size between sampling periods	ANCOVA	164	0.001
Offspring number across the breeding season	OLSR	159	<0.0001
Body condition (residual condition factor) vs			
Time	ANOVA	185	<0.0001
Cannibalism in early season	GLM	45	0.58
Cannibalism in mid-season	GLM	52	0.009
Body condition (hepatosomatic index) vs			
Time	ANOVA	122	0.01
Cannibalism in early season	GLM	32	0.15
Cannibalism in mid-season	GLM	45	0.036
Females			
Observed across time	Permutation	166	<0.001
Sneaker males			
Observed across time	Permutation	166	<0.001
Co-occurrence with females in nests	GLM	166	<0.0001
Additional guarder males			
Observed across time	Permutation	166	<0.001
Nest			
Abandonments across time	Permutation	149	0.75
Take-over events across time	Permutation	149	<0.001
Take-overs*cannibalism	GLM	148	0.004
Offspring number			
Across the breeding season	OLSR	159	<0.0001
Between nests that were taken over vs not taken over	nGLM	139	0.02
Cannibalism			
Events across time	Permutation	148	<0.001
Early vs mid-season	MW	78	<0.0001
Between male types (take-over vs non-take-over) in mid-season	nGLM	45	0.0003

OLSR: ordinary least squares regression; ANCOVA: analysis of covariance; ANOVA: one-way analysis of variance; GLM: binomial logistic generalized linear model; nGLM: negative binomial generalized linear model; MW: Mann–Whitney *U* test.

We assessed how a number of variables changed across the breeding season, combining linear and generalized linear models for estimating parameters with permutation tests based on the same models to obtain accurate *P* values. For count responses (e.g. number of sneaker or additional cuckolding guarder males found in nests), we used a generalized linear model (GLM) with a negative binomial response, appropriate for overdispersed data (Bolker 2008; MASS package, version 7.3.31, Venables and Ripley 2002). For binary responses (e.g. the occurrence of nest take-over events or cannibalism), we used a binomial GLM (logistic regression). In both cases we compared the fit of the model that included a parameter for sampling time with a null model that excluded it, and repeatedly estimated the change in deviance between models (Δdev) for 1000 random permutations of the data. The *P* value was computed as the fraction of Δdev values for permuted data that were greater than or equal to the observed Δdev.

We assessed changes in body condition over the breeding season using two common indices: (1) a residual condition factor (RCF), which uses residuals from a regression of ln total body mass (g) against ln standard length (cm) (Fechhelm et al. 1995; Blackwell et al. 2000); and (2) a hepatosomatic index (HSI), calculated for all dissected fish ($N = 123$), by regressing ln liver mass (g) against ln eviscerated body mass (total body mass − digestive tract mass) and using the residuals as an approximation of the HSI. We used ANOVAs to compare body condition (RCF and HSI) across sampling periods (early, mid, late season) and binomial logistic generalized linear models (GLMs) to test for a relationship between cannibalism and body condition (RCF and HSI).

To assess how competition changed over the breeding season, we used several indicators of male–male competition. First, we used the permutation tests described above to investigate whether the number of sneaker males or additional guarder males in nests (count response variable) or the frequency of nest take-over events (binary response variable) changed over the season. Second, we explored the intensity of male competition for nests by testing the strength of the correlation between guarder male size (standard length) and nest size (surface area) within each sampling period using ordinary least squares regression. We also tested for an interaction between guarder male size and sampling period using an ANCOVA. If competition for nests is strong, as suspected, then we predicted a size-dependent distribution of males among nests.

To examine whether the incidence of cannibalism (i.e. proportion of fish within the population that show evidence of cannibalism) varied across the breeding season, we used the permutation tests described above (binary response variable). We also used binomial logistic GLMs to test for a relationship between nest take-over events and cannibalism. All analyses were performed in R version 2.15.1 (R Development Core Team, 2012) and significance was assessed at $\alpha = 0.05$.

Ethical Note

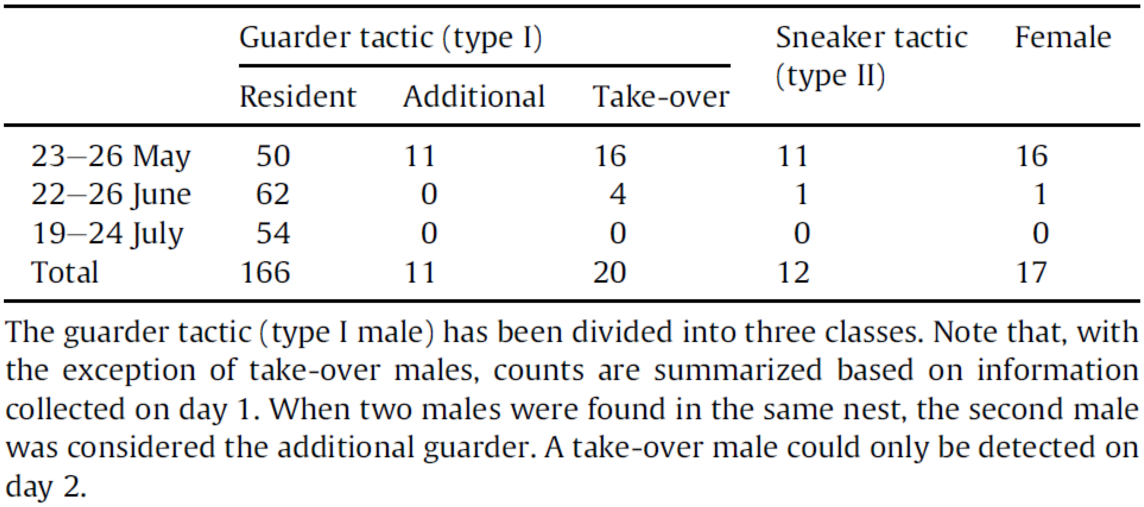
Plainfin midshipman fish are a common intertidal species and are not considered threatened or endangered. All animals were collected in accordance with the Canadian Department of Fisheries and Oceans (DFO scientific license number XR 14 2013). Marking involved injecting a nontoxic elastomer into the fin tissue between the dorsal fin rays. We also cut a small piece of fin tissue from the caudal fin to use for genetic testing in other studies, but this cut also served as an additional mark on each fish. The fin tissue regenerates in about 2 weeks, and removal of a small amount of fin tissue does not influence fish behaviour (APH Bose, KM Cogliati, HS Howe, and S Balshine personal observations). The fish were kept wet during handling, and marking and tissue collection procedure took no longer than 1 min. It is exceedingly unlikely that our sampling methods caused fish to abandon their nests; fewer than 4% of our sampled nests were found to be empty (a presumed abandonment) on day 2. On day 2, fish that were to be euthanized were first anaesthetized in a bath of (>0.1%) benzocaine followed by cervical severance, and their liver and digestive tracks were weighed for this study. These and other harvested tissues were used in a number of other studies. The procedures used in this study were approved by the McMaster University Animal Research Ethics Board (AUP number 10-11-70), and are in line with the guidelines set by the Canadian Council on Animal Care (CCAC).

3.4 Results

Across the 166 nests sampled, we found 209 males, of which 79% were guarder males, 6% were sneaker males and 5% were additional guarder males intruding on the nest and not associated with an active nest of their own. The remaining 10% of males were take-over guarder males that had replaced a previous nest owner and had taken up residence in that nest (Table 3.2). Larger guarder males guarded larger nests or rocks with more surface area (cm^2; ordinary least squares regression, OLSR: $t_{162} = 2.95$, $R^2 = 0.05$, P

= 0.004) and their nests contained more offspring (OLSR on log offspring number: $t_{157} = 5.85$, $R^2 = 0.18$, $P < 0.0001$). Nests that changed ownership (i.e. a take-over event, $N = 20$) contained fewer offspring than did nests where the original guarder male remained as resident (negative binomial GLM: $z = -2.4$, $N = 119$, $P = 0.02$).

Table 3.2 Summary of plainfin midshipman, *Porichthys notatus*, found in nests at Crescent Beach, South Surrey, BC, in the 2013 breeding season.



	Guarder tactic (type I)			Sneaker tactic (type II)	Female
	Resident	Additional	Take-over		
23–26 May	50	11	16	11	16
22–26 June	62	0	4	1	1
19–24 July	54	0	0	0	0
Total	166	11	20	12	17

The guarder tactic (type I male) has been divided into three classes. Note that, with the exception of take-over males, counts are summarized based on information collected on day 1. When two males were found in the same nest, the second male was considered the additional guarder. A take-over male could only be detected on day 2.

Body Condition Decreased across the Breeding Season

Both measures of body condition showed that guarder males sampled early in the season were in better condition than those sampled late in the season (ANOVA: RCF: $F_{2,182} = 20.12$, $P < 0.0001$; HSI: $F_{2,119} = 4.64$, $P = 0.01$).

Competition was Most Intense Early in the Breeding Season

Females and sneaker males were more common early in the breeding season than late in the breeding season (permutation test with females: $\Delta\mathrm{dev} = 24.13$, $P < 0.001$; Fig.

3.1a; permutation test with sneakers: Δdev = 16.5, $P < 0.001$; Fig. 3.1b). The presence of sneaker males in a nest was strongly correlated with the presence of females (binomial GLM: $z = 4.78$, $P < 0.0001$). Nests containing two or more guarder males were significantly more common early in the breeding season than late in the breeding season (permutation test: Δdev = 23.02, $P < 0.001$; Fig. 3.1c). The frequency of nest abandonment (the discovery of an empty nest on day 2 where a guarder male had originally been found on day 1) did not change over the season (permutation test: Δdev = −0.792, $P = 0.75$). Nest take-overs occurred more frequently early in the breeding season than late in the season (permutation test: Δdev = −19.5, $P < 0.001$), and all three cases of 'attempted take-overs in progress' (~2% of nests observed on day 2) were found early in the season.

There was a strong positive correlation between male body size and nest size early in the season ($t_{48} = 4.88$, $R^2 = 0.33$, $P < 0.0001$; Fig. 3.2), and a weaker but still significant correlation in mid-season ($t_{58} = 2.85$, $R^2 = 0.12$, $P = 0.006$), but no correlation between male body size and nest size late in the season ($t_{52} = -1.32$, $R^2 = 0.03$, $P = 0.19$). This led to a significant interaction between guarder male size and sampling period (ANCOVA: $F_{2,158} = 7.16$, $P = 0.001$). Taken together these results suggest that there is more competition among males early in the season than late in the season.

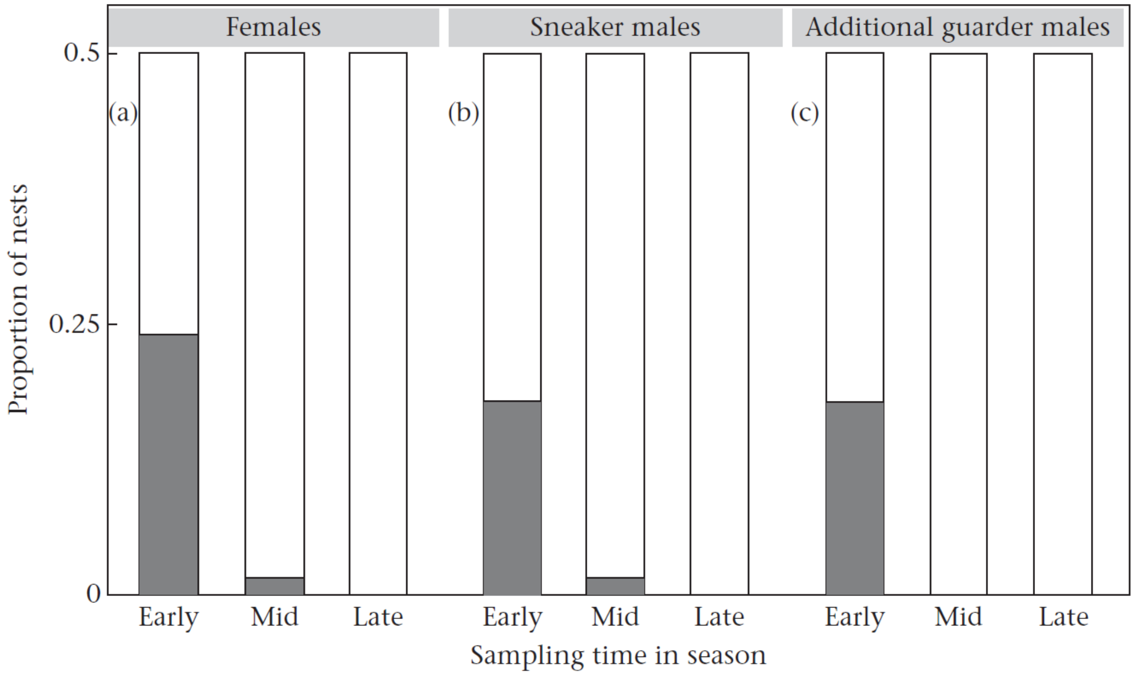


Figure 3.1 Proportion of plainfin midshipman (a) females, (b) sneaker males and (c) additional guarder (type I) males observed in nests of guarder males over the breeding season (early: May; mid: June; late: July). Grey bars: fish types found in the nest; white bars: fish types absent from the nest.

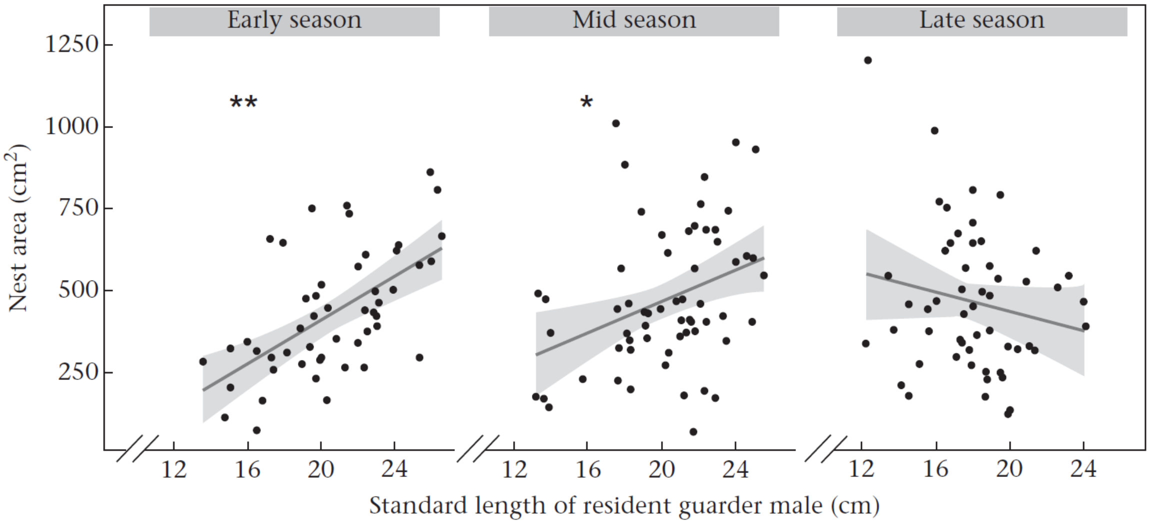


Figure 3.2 Standard length of plainfin midshipman guarder males plotted against nest area during the breeding season (early: May; mid: June; late: July). Confidence intervals were calculated based on the variance within each month and are presented in each panel. *P < 0.01; **P < 0.001.

Higher Prevalence of Cannibalism Early in the Breeding Season

None of the females ($N = 10$) or sneaker males ($N = 10$) internally inspected had offspring in their digestive tracts, regardless of when they were collected in the breeding season. Only guarder males ($N = 123$ males whose digestive tracks were inspected) were observed to have cannibalized offspring and cannibalism was significantly more common in the early part of the breeding season. Cannibalism declined significantly over time (permutation test: $\Delta\text{dev} = -49.6$, $P < 0.001$; Fig. 3.3a). Cannibalism was observed in 58% of sampled males in May and 27% of sampled males in June and ceased altogether at the end of the season (0% of the sampled males in July had cannibalized young).

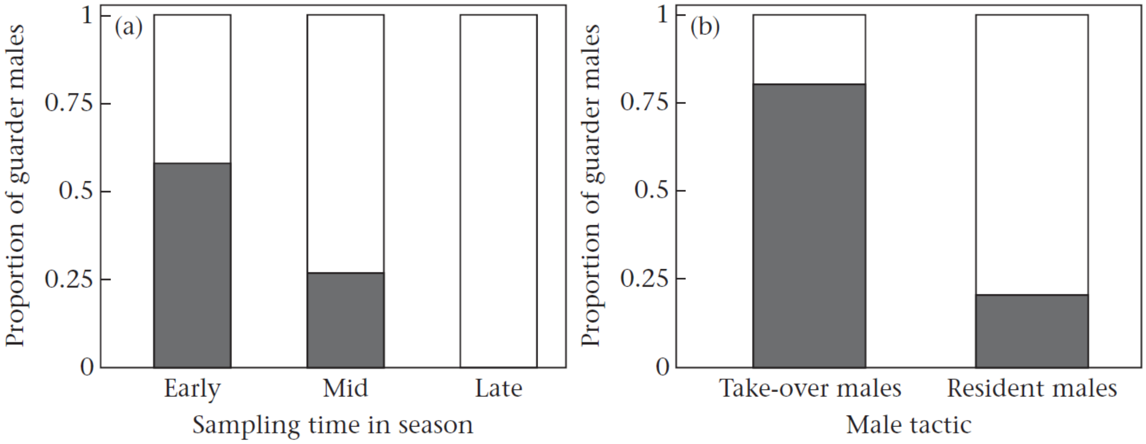


Figure 3.3 (a) Proportion of plainfin midshipman guarder males that cannibalized eggs in their nests plotted against time within the breeding season (early: May; mid: June; late: July). (b) Proportion of guarder males that cannibalized eggs in their nests as a function of take-over status. Grey bars: cannibalistic males; white bars: noncannibalistic males.

On average, males consumed 9.3 ± 2.1 offspring (mean ± SE; range 0–53) in the early season, 3.6 ± 1.6 offspring (range 0–58) in the mid-season and 0 offspring in the late season. This decrease in number of offspring consumed was significant (Mann–

Whitney *U* test: $Z = 3.99$, $N_1 = 33$, $N_2 = 45$, $P < 0.0001$). Take-over events were highly correlated with cannibalism (80% of take-over males cannibalized; binomial logistic GLM: $z = 2.85$, $P = 0.004$; Fig. 3.3b). Overall, take-over events accounted for 38% of all the cannibalism observed over the season. During the early season (May), cannibalistic take-over males had similar numbers of offspring in their guts (8.25 ± 2.1, range 2–17) compared to non-take-over males that cannibalized (15 ± 3.5, range 1–53; negative binomial GLM: $z = -1.58$, $P = 0.11$). However, during the mid-season (June), take-over males had significantly more offspring in their guts (23.7 ± 8.9, range 1–58) than did non-take-over males (3.0 ± 1.7, range 1–13; negative binomial GLM: $z = 3.61$, $P = 0.0003$). Cannibalism and nest take-overs did not occur in the late season (July).

Early in the season, no differences in body condition were detected between fish that cannibalized and those that did not (binomial logistic GLM on RCF: $z = 0.58$, $P = 0.56$; HSI: $z = 1.42$, $P = 0.15$). However, by the mid-season, cannibals were in better condition than noncannibals (binomial logistic GLM on RCF: $z = 2.60$, $P = 0.009$; HSI: $z = 2.09$, $P = 0.036$). No cannibalism occurred during the late season, when recorded body conditions were lowest.

3.5 Discussion

Despite a prolonged and costly period of care, our results suggest that plainfin midshipman males do not engage in cannibalism to counteract the energetic demands of parental care. Male body condition clearly deteriorated as the season progressed, and males were in the poorest condition at the end of July. Therefore, if cannibalism were a strategy used primarily to regain energy lost by prolonged parental care, then we would expect to observe an increase in cannibalism across the parental care period. Furthermore, we found that cannibals in the mid-season were in better condition than noncannibals, which is in opposition to expectations of the energy-based hypothesis. We found that prevalence of offspring cannibalism was high in the early season (observed in 58% of nesting males), but declined across sampling periods. The most frequent and severe (in terms of number of offspring consumed) cannibalism was observed in the early season, which then declined in the middle of the breeding season, and completely ceased by the end of the season. This pattern mirrors the decline in both the degree of male–male competition and the frequency of nest take-over events observed across the breeding season. Cannibalism therefore appears to be associated with a competitive environment when gravid females are more plentiful in the spawning grounds and males are still competing for nests and eggs.

Based on a number of indices, we show that mating competition is most intense early in the season. We found that guarder male size was strongly correlated with nest size early in the season, less so in the mid-season and not at all late in season. Early in the breeding season, females, sneaker males and additional 'non-nest owner' guarder males were more common in nests. A high level of competition will impose several costs on nesting midshipman males, including the energetic costs of defending their nest against competitors and a lowered certainty of paternity arising from more frequent cuckoldry attempts. Indeed, Cogliati et al. (2013) showed that paternity levels are lowest early in the season, further corroborating the hypothesis that there is more competition for fertilization early in the breeding season. Evidence from other species indicates that the level of paternity influences the amount of parental care provided to a brood (e.g. Neff 2003b). Furthermore, if males are capable of kin recognition among their offspring, then selective consumption of unrelated young will incur no direct fitness costs.

While the termination of care through whole-brood cannibalism has typically been reported to occur early rather than late in the breeding period (Manica 2002), more variation exists among species in the timing of partial-brood cannibalism (e.g. Belles-Isles and FitzGerald 1991; Marconato et al. 1993; Neff 2003a). Several additional explanations should be considered with respect to the observed seasonal decline in cannibalism that we observed in the plainfin midshipman fish. Such a seasonal decline could also arise if males place greater value on their broods as they develop from eggs into hatched embryos (Rohwer 1978). We also found that females became scarce or absent late in the season, leading to an increasingly male-biased operational sex ratio. Therefore, early in the season, if a male cannibalized from his nest when it was at maximum capacity with offspring, he would still have a chance to acquire replacement offspring, but this would not occur late in the season. In the convict cichlid, *Cichlasoma nigrofasciatum*, experimental reductions in brood size can incite filial cannibalism, but only early in the breeding cycle when parents are still able to prepare for a subsequent brood (Lavery and Keenleyside 1990). Increased brood investment later in the season may be favoured because alternative mating opportunities are less probable (Kondoh and Okuda 2002), or if body condition deteriorates considerably, lowering an individual's expected future reproductive success (i.e. residual reproductive value; Pianka 1976).

Finally, density-dependent offspring survival may also influence the likelihood of cannibalism as shown in the sand goby, *P. minutus* (Klug et al. 2006), where cannibalism is used to reduce brood density, thereby improving the survival of the remaining offspring in the nest. While such impacts of offspring density on survival have not been studied in the plainfin midshipman, we think that it is an unlikely driver of cannibalism in this system. Midshipman females deposit new eggs in the nest in a single tightly packed monolayer (Arora 1948) and will fill in any gaps among the offspring already present on the nest surface even when free space is accessible elsewhere in the nest (APH Bose, KM Cogliati, HS Howe, and S Balshine personal observations). Finally, cannibalism may reflect selective consumption of dead or unhealthy eggs and so may be adaptive if it protects the remaining offspring within a brood from spread of infection (Kraak 1996). Although we did not explicitly test this idea in the present study, visual inspection and DNA testing of consumed offspring suggest that guarder males do not specifically engage in cannibalism of dead or unfertilized eggs (Cogliati et al. 2015).

We also observed more frequent changes in nest ownership (more take-over events) early in the breeding season. This, combined with the strong tendency for take-over fish to be cannibalistic, could also contribute to our observation of more frequent cannibalism early in the season. By being cannibalistic, the new take-over guarder male can gain an energy benefit at the expense of the previous nest owner. Take-over males can also be more certain that they are not related to the eggs in their newly acquired nest compared to a nest owner who has occupied the nest for a long time. As the season progressed, we found that take-over males had on average more offspring in their guts than did non-take-over males. It is possible that our brief 2-day sampling methodology underestimated the rate of take-over events. It is entirely possible that certain males in our data set had taken over a nest prior to our sampling, yet were categorized as resident, non-take-over males. If this is the case, then competitive cannibalism of unrelated offspring, as facilitated by nest take-overs, could have driven an even larger proportion of overall cannibalism than the 38% we allocated above. However, the extent to which take-over events contribute to overall rates of cannibalism has yet to be tested explicitly.

Furthermore, nests that experienced a take-over event contained, on average, fewer offspring than nests where the original nest owner remained, indicating that small broods might have lower value to original male caregivers. If a small brood results in a lowered motivation to defend for a caregiver, this could lead to an increased likelihood of abandonment if challenged by another male. Brood size is typically a strong predictor of parental expenditure (Gross 2005). For example, bluegill sunfish, *L. macrochirus*, vary their parental effort according to brood size (Coleman et al. 1985), and Galilee St Peter's fish, *Sarotherodon galilaeus*, caregivers are more likely to abandon small broods than large ones (Balshine-Earn and Earn 1998).

More nest take-overs were observed early in the midshipman breeding season. For a number of reasons, we do not think that the temporal patterns of cannibalism, take-overs and abandonment were the result of sampling-related nest disturbance. First, we sampled in an identical fashion across the breeding season, but the rate of take-overs ceased. Second, we observed more, presumably, cuckolder or take-over males in nests early in the season than later in the season. Third, Cogliati et al. (2013), used microsatellite paternity analyses to reveal a genetic signature of nest take-overs occurring early in the season. Taken together these results suggest that it is unlikely that our disturbance caused undue nest abandonment. Available nesting sites are limited (Demartini 1988; APH Bose, KM Cogliati, HS Howe, and S Balshine, personal observations) and, therefore, are a valuable resource for a guarder male, making abandonment a costly act. Indeed, abandonment occurred at an extremely low frequency of less than 4% of all our sampled nests.

All incidences of cannibalism observed in the present study involved partial-brood cannibalism. Cannibals in our sample never consumed all of the offspring in a nest, even if they had taken over a nest and were presumably not related to any of the offspring. In fact, both take-over males and cuckolded males have been shown to provide alloparental care to unrelated offspring (Cogliati et al. 2013; Cogliati, Balshine, et al. 2014). Several potential explanations exist for why alloparental care is observed in the plainfin midshipman. First, parental care in the plainfin midshipman is largely nondepreciable with the possible exception of egg fanning (Cogliati et al. 2013). Thus, the costs of caring for a larger brood may not be much higher than caring for a small brood, promoting alloparental care (Wisenden 1999). Second, males may be willing to care for unrelated offspring if these eggs serve to attract more mates. In many fish species females engage in mate choice copying (Dugatkin 1992). For example, female blennies, *A. sphinx* (Kraak 1996), threespine sticklebacks, *G. aculeatus* (Ridley and Rechten 1981), and sailfin mollies, *Poecilia latipinna* (Witte and Ryan 2002) all prefer to nest with males that have already obtained eggs from another female. Third, unrelated offspring may serve as a potential ‘insurance policy’, providing an accessible food source for the guarder male distinct from his own offspring. These explanations are not mutually exclusive and have yet to be tested empirically.

Collectively, our study has described cannibalism in a system with intense male–male competition and prolonged costly parental care. While the energy-based hypothesis of offspring cannibalism has received the most attention across animal taxa, in plainfin midshipman, we show that poor parental body condition does not correlate with cannibalism. Instead the prevalence of cannibalism was related to the degree and timing of intense male intrasexual competition. We suggest that cannibalism may help a male remain in excellent competitive condition at a time when females are readily available for spawning and the fitness rewards are likely greatest. Cannibalism may also be a product of compromised paternity resulting from prevalent cuckoldry by both guarder and sneaker males, although whether guarder males are capable of differentiating kin and nonkin offspring remains to be tested. Cannibalism may in fact be selected for by high certainty of nonpaternity that would accompany a nest take-over event. In addition, we acknowledge that we did not directly observe egg-eating behaviour by type I guarder males, a gap that future work should strive to fill. Eggs loosened in the process of fanning and caring for the brood may contribute to the levels of cannibalism we report here, but the extent to which this occurs also remains to be investigated. Future work should also focus on experimentally manipulating body condition, degree of mating competition and brood paternity to further test our findings. It would also be valuable to assess the extent to which cannibalism of offspring can augment body condition, affect competitive abilities, facilitate continued parental care, or enhance offspring survival through density-dependent effects. Finally, the present work highlights the importance of monitoring cannibalistic behaviours across an entire breeding season, as ecological driving forces for this behaviour may change temporally.

3.6 Acknowledgments

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Chapter 4: Cannibalism, competition, and costly care in the plainfin midshipman fish, *Porichthys notatus*

4.1 Abstract

Caring for offspring is energetically expensive, and when the costs of care are sufficiently high, the use of cost-reducing strategies can be favored. Such strategies may include the avoidance of nest construction through nest take-over and the restoration of depleted energy reserves through offspring cannibalism. Despite extensive theoretical and empirical work on parental care, neither the actual energetic costs of care nor the putative benefits of cost-reducing strategies have been systematically measured. Using plainfin midshipman fish, *Porichthys notatus*, we assessed how energy reserves of caring parents varied with duration of care, offspring cannibalism, and nest take-overs. We show that liver glycogen and lipid contents declined by 58% and 18.7%, respectively, that liver investment (measured via a hepatosomatic index) declined by 32.6%, and that muscle protein content declined by 8.8%. Other measures of body condition and energy reserves, such as hepatic glucose and adenosine triphosphate, remained stable over the extraordinarily long care period (3 months). Experimentally starved fish showed depletions of energy stores similar to caring fish. Fish that took over nests or that cannibalized eggs both had higher glycogen reserves than fish that did not adopt these behaviours. These findings show that even when parental care is energetically costly, starvation may not be the dominant driving factor behind parent–offspring cannibalism.

4.2 Introduction

Parental care can be costly when the energy and resources put into care trade-off against alternative investments, such as those made into somatic growth, which can diminish future reproductive success (Williams 1966). Although parental care enhances offspring fitness, caring parents may have less energy for growth and bodily maintenance and suffer reduced fecundity, mating opportunities, and/or survival (Alonso-Alvarez and Velando 2012). Investments made into current offspring may diminish resources available for future offspring, and parents should balance the benefits of providing care against the associated costs (Trivers 1972; Gross 2005; Smiseth et al. 2012). Although the costs of parental care have been well studied theoretically, few empirical studies have investigated the direct costs of care on parental energy reserves (Alonso-Alvarez and Velando 2012).

It has been long argued that parents can offset some of the high costs of care through offspring cannibalism, even when the offspring consumed are genetically related to the cannibal (i.e., filial cannibalism, Rohwer 1978; Sargent et al. 1995). Offspring cannibalism by caring-giving individuals is a taxonomically widespread phenomenon (Polis 1981; Soulsby 2013) and can be categorized into distinct types including filial versus nonfilial or partial-brood versus whole-brood cannibalism. Each of these types of cannibalism differ in terms of the selection pressures that drive them, how they influence the investment of parental resources, and the adaptive benefits that they confer (Smith and Reay 1991). Offspring cannibalism, both filial and nonfilial, is known to occur in birds (Stanback and Koenig 1992), mammals (Elwood 1992), and insects (Bartlett 1987;

Thomas and Manica 2003) and is thought to occur for a variety of reasons including food shortage, mating competition, selective termination of low-quality young, and low certainty of parentage (Polis 1981; Smith and Reay 1991; Manica 2002). All types of parent–offspring cannibalism have also been observed among teleost fishes (Smith and Reay 1991; FitzGerald and Whoriskey 1992). As in other taxa, a large number of fish studies have focused on offspring cannibalism in the context of parental care, in which cannibalism of a portion of the brood may serve as a means to replenish dwindling energy reserves that would otherwise compromise the quality or quantity of care. According to this "energy-based hypothesis," the recouped energy may then be allocated towards future reproductive efforts or toward the care for any remaining unconsumed offspring (Klug and Bonsall 2007). Such energy-driven cannibalism is clearly profitable when targeting non-kin offspring, but it can also be adaptive even when targeting kin offspring if the future benefits of the energetic gains outweigh the immediate loss of progeny (Rohwer 1978). Although cannibalism may serve to recoup energy, nest take-overs may provide a means to preserve energy, by bypassing the costs associated with nest construction. However, in previous studies, the lack of comprehensive energy reserve measures of offspring cannibalism or nest take-overs has not permitted an unequivocal testing of the energy-based hypothesis.

To date, most studies have tested the energy-based hypothesis using coarse measures of body condition or manipulations of food availability, and results from these studies have provided mixed support for the hypothesis (Manica 2002). For example, negative correlations between body condition and cannibalism have been uncovered in the bluegill sunfish, *Lepomis machrochirus* (Neff 2003), the freshwater goby, *Rhinogobius* sp. (Okuda et al. 2004), and the assassin bug, *Rhinocoris tristis* (Thomas and Manica 2003). In female earwigs, *Anisolabis maritima*, filial cannibalism appears as a last resort to sustain a female through the nesting period (Miller and Zink 2012). Increased food availability via supplemental feeding (mitigating the energy depletion during care) has reduced but not abolished cannibalism in the Cortez damselfish, *Stegastes rectifraenum* (Hoelzer 1992), sphinx blenny, *Aidablennius sphynx* (Kraak 1996), common goby, *Pomatoschistus microps* (Kvarnemo et al. 1998), and scissortail sergeant, *Abudefduf sexfasciatus*, (Manica 2004). However, such feeding studies have had no effect on cannibalism in the three-spined stickleback, *Gasterosteus aculeatus* (Belles-Isles and FitzGerald 1991), fantail darter, *Etheostoma flabellare* (Lindström and Sargent 1997), or beaugregory damselfish, *Stegastes leucostictus* (Payne et al. 2002). No link between energy depletion and observations of filial cannibalism could be made for orangutans, *Pongo abelii* (Dellatore et al. 2009) and house finches, *Carpodacus mexicanus* (Gilbert et al. 2005). Additionally, in stark contrast to the predictions of the energy-based hypothesis, Klug and St. Mary (2005) showed that male flagfish, *Jordanella floridae*, placed on an enhanced diet cannibalized more than fish placed on a restricted diet. In many systems, the importance of cannibalism as a means for restoring energy remains unclear, possibly because studies are often conducted in artificial environments and have assumed the energetic costs of care but not obtained any direct measures of energy stores.

Adequately testing the energy-based hypothesis requires several steps. First, the energetic costs associated with care should be demonstrated. Second, the available energy reserves of individuals that engage in cannibalism should be measured and compared with those that do not cannibalize. Measures of energy reserves should be taken soon after cannibalism occurs, but before nutrients from the meal can be absorbed and stored. In this study, we implemented these 2 steps and investigated the energetic costs of parental care and how these costs are linked to cannibalism and nest take-overs using a wild fish, the plainfin midshipman, *Porichthys notatus*. This species is well suited for testing the energy-based hypothesis. Males dig and maintain nests under rocks, providing sole parental care in the form of embryo fanning and brood defense (Arora 1948) for up to 3 continuous months (Cogliati et al. 2013). Guarding males do not leave their nests during the care period, and must therefore rely on food items found within their nests (Sisneros et al. 2009; Bose et al. 2014; Cogliati et al. 2015). We tested 3 distinct predictions pertaining to the energy-based hypothesis. First, if parental care is costly then declines in energy reserves should reflect the length of time a parent provides care. Second, individuals that take over a nest should have higher energy reserves than those that have invested energy into building their own nest and caring for offspring. Third, if a dwindling energy reserve triggers cannibalism, then cannibals should have lower energy reserves than noncannibals.

4.3 Methods

Field sample collections

In 2013, we located and marked 122 plainfin midshipman nests along Crescent Beach, British Columbia, Canada (49°02′N, 122°52′W). A plainfin midshipman nest is a small, excavated cavity beneath an intertidal rock that contains a guarding male and a monolayer of eggs, which are deposited on the nest ceiling. We found and sampled these nests during the early, mid, and late parts of the breeding season (23–26 May; 22–26 June; 19–24 July). Nests were photographed using an Olympus TG-820 digital camera. Males were measured (total and standard lengths to the nearest 0.1cm and total mass to the nearest 0.1g) and uniquely marked with nontoxic injectable elastomer (Northwest Marine Technology, Inc., Shaw Island, WA) before the rocks were carefully repositioned. On the following day, we checked if the male from the previous day still remained in the nest, or whether a new unmarked male was present, and the nest was rephotographed. Nest photographs were later used for offspring quantification in the software ImageJ (v1.45, http://rsbweb.nih.gov.libaccess.lib.mcmaster.ca/ij/). New untagged males that had replaced the original males were classified as nest "take-over" males, whereas original males were categorized as "remaining resident" males. We examined the stomach contents of a subset of fish from each sampling period, to determine whether take-over males or remaining resident males had recently cannibalized. Fish were sacrificed with an overdose of benzocaine. During dissection, a caudal vein blood sample was taken from each fish and frozen in liquid nitrogen. All organs were weighed (to the nearest 0.01g) and both liver and muscle samples were frozen in liquid nitrogen to later assess energy stores.

Of the 122 males sampled over the season, 50 were randomly targeted for detailed energetic analyses. Twenty fish were selected from the May sampling period so that 10 of these had embryos in their digestive tracts, hereafter called cannibals, and 10 had empty digestive tracts, hereafter called noncannibals. Another 20 males were selected from the June sampling period (10 cannibals and 10 noncannibals). Only 10 fish were selected from the July sampling period (all of these were noncannibals because we did not find evidence of cannibalism in any fish sampled during July, see Bose et al. 2014). Of the 40 fish analyzed from the May and June sampling periods, 11 were take-over males.

Starvation sample collections

Wild caring males eat little over the breeding season (Cogliati et al. 2015). Thus, we also held 12 males under controlled food deprivation for 82 days to serve as a reference against which to compare changes in body condition and energy reserves due to caring. Midshipman males can care for 90 days or longer (Cogliati et al. 2013). The males for this experiment were collected from Ladysmith Inlet, British Columbia, Canada (49°1′N, 123°50′W) in early May 2013. They were measured and weighed (as above) and housed individually in 300-L outdoor aerated tanks supplied with filtered 12 °C seawater, lined with a sand substrate, and provided with shelter. Fish were monitored daily and experienced no mortality. After 82 days in these tanks, all 12 fish were remeasured, euthanized, and dissected. All organs were weighed (to the nearest 0.01g) and blood plasma, liver, and muscle samples were preserved as previously described.

Measures of body condition and energy reserves

To measure body condition, we employed 2 commonly used indices: 1) a relative condition factor (RCF), based on residuals from a regression of ln body mass against ln standard length (Blackwell et al. 2000) and 2) a hepatosomatic index (HSI), based on the residuals of ln liver mass regressed against ln eviscerated body mass. A gonadosomatic index (GSI) was also calculated for each fish using residuals of ln gonad mass against ln eviscerated body mass, as well as a gut investment index using the residuals of ln empty gut mass against ln eviscerated body mass.

Frozen liver samples were measured for water, glycogen, free glucose, adenosine triphosphate (ATP), total lipids, and total protein contents. Frozen muscle samples were analyzed for water and total protein contents. Frozen plasma samples were analyzed for ammonia content. A full description of the lab protocols used can be found in the Supplementary Materials.

Total lipids and glycogen per whole liver were also determined for each fish by multiplying total liver dry weight (g) by either mass-specific lipid (mg/g dry weight) or glycogen content (μmol/g dry weight). These reserves were then converted into a condition index using residuals similar to RCF and HSI as described above.

Egg digestion study

We used the presence or absence of embryos within the digestive tract as an indicator of whether offspring cannibalism had recently occurred. However, this indicator offers only minimal information regarding the rate of cannibalism that occurs in the field or the rate at which nutrients from a meal are accessed. To gain more insight into this, we estimated gastrointestinal evacuation rates by conducting an egg-feeding study between 17 May and 14 June 2013. Thirty males were collected from Ladysmith Inlet, held in aerated tanks of seawater, and fasted for 42h. Each male was then fed 5 eggs. Six randomly selected fish were sacrificed and dissected at one of five times: 6-, 12-, 24-, 36-, or 48-h postfeeding. Fish body size did not differ between time point groups ($F_{(4,24)}$ = 0.11, P = 0.98). To track the progression of digestion, a digestion index was created using a 4-level scale. Fully intact undigested eggs were given a score of 1; mild digestion (loss of spherical shape but yolk still present) was given a 2; major digestion (loss of shape and yolk) was given a 3; and passed from the track entirely was given a 4. Gut content mass (g) was also recorded for each fish by weighing the full gut mass and subtracting the empty gut mass once the contents had been removed (see Cogliati et al. 2015 for details).

Data and statistical analysis

All analyses were performed in R version 3.1.0 (R Core Team 2014). We compared overall body condition (RCF) and body size (based on standard length in cm) between those males that were no longer present in their nests on the second day of sampling with those males that remained in their nests using exact Wilcoxon rank sum tests (“exact RankTests” package, Hothorn and Hornik 2015). We also tested whether males that took over the ownership of a nest were larger than the males that they replaced with a 1-sample *t* -test using difference scores in their standard lengths.

We tracked how measures of body condition and energy reserves changed across the breeding season in wild fish, and also compared these measures with those from the lab-held fish that were experimentally starved for 82 days. Only remaining resident males that were noncannibals were considered for these analyses. For each measure of body condition and energy reserves that we quantified, we ran a linear model (LM) that included sampling time (i.e., May, June, July, and "starved") as a predictor. All models also included a parameter for fish size (standard length, cm) unless the analysis was conducted on an index that already accounted for body size. Both plasma ammonia concentration and liver glucose content were ln-transformed to meet parametric assumptions.

We then compared cannibals with noncannibals. We used a Wilcoxon rank sum test to test whether the nests of cannibal males experienced a larger reduction in relative brood size than the nests of noncannibal males. This analysis only considered nests sampled in May and June, the 2 months in which cannibalism was observed. We compared energy reserves between cannibals and noncannibals, and between take-over males and remaining resident males. We ran LM that included sampling time (i.e., May, June, July, and "starved") and either cannibal status (i.e., cannibal or noncannibal) or take-over status (i.e., take-over male or remaining resident male) as predictor variables. Fish size (standard length, cm) was also included in all models, except for the aforementioned indices. Nest take-overs and cannibalism were only detected in the first 2 sampling periods of the breeding season, and so only fish sampled from these first 2 periods could be included in these analyses. All take-over males were excluded from the comparison of cannibals to noncannibals. All fish sampled during the first 2 periods were included in the comparison of take-over males to remaining resident males. Muscle water and protein contents were measured from noncannibal males only. Liver glucose content was ln-transformed to meet parametric assumptions for both the cannibalism and take-over comparisons, and liver protein content was ln-transformed for the take-over comparison only. A ln-transformation could not resolve the heteroskedasticity in total glycogen per liver for the take-over comparison, and so a generalized least squares (GLS) regression was used in this case to accommodate the uneven variance ("nlme" package, Pinheiro et al. 2014). Lastly, each model also included an interaction term between sampling period and either cannibal status or take-over status, removing the term if it was nonsignificant.

Finally, a cumulative link mixed model (CLMM) for ordinal data ("ordinal" package, Christensen 2014) was used to correlate the digestion index scores with elapsed time in the egg digestion study. Change in gut content mass with time was analyzed with a LM including elapsed time (hours) and fish size (body mass, g) as predictors.

Ethical Note

Plainfin midshipman fish are neither threatened nor endangered (Collette et al. 2010). All animals were collected and handled quickly in accordance with the Canadian Department of Fisheries and Oceans protocols/rules/guidelines (Scientific license XR 14 2013). All procedures were approved by the McMaster University Animal Research Ethics Board, DFO's Animal Care Committee (AUP number 13-003), and are in line with the guidelines set by the Canadian Council on Animal Care (CCAC).

4.4 Results

Body size and condition do not predict loss or retention of nest ownership

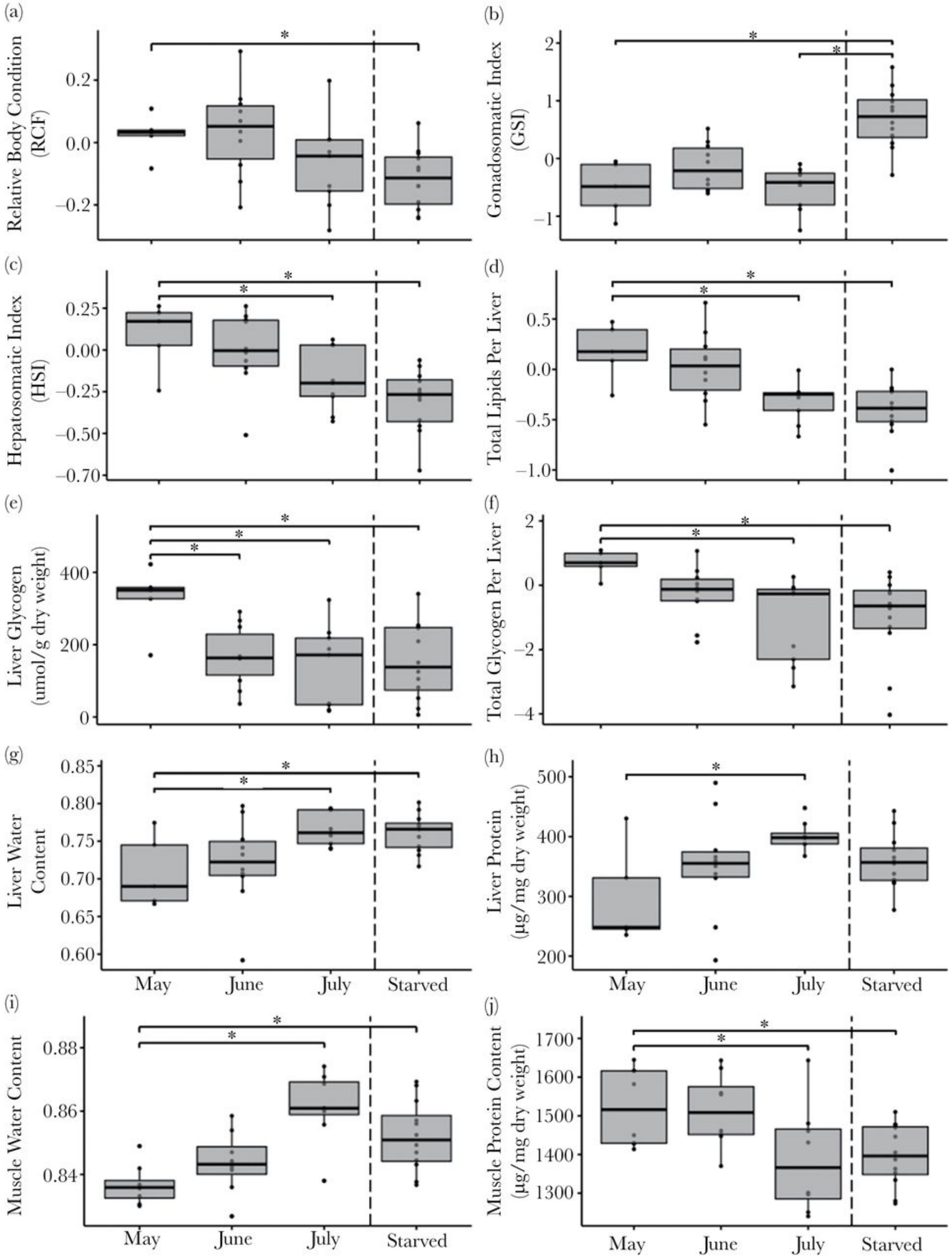
Throughout the breeding season, we observed numerous occurrences of changes in nest ownership (see Bose et al. 2014). Such events indicate the take-over of an occupied nest, where the previous resident was ejected, or the take-over of an empty nest, where the previous resident had already abandoned. Early in the season (May), 32% of nests experienced a change in nest ownership between the 2 consecutive sampling days, 7% in the midseason (June), and 0% in the late season (July). Additionally, the proportion of nests found to be empty on the second sampling day were 4%, 5%, and 2% for the early, mid, and late seasons, respectively.

No significant difference could be detected in the overall body condition (RCF) of males that were no longer in their nests on the second sampling day and the males that remained in their nests (Wilcoxon rank sum, May: $W = 223$, $N = 46$, $P = 0.71$; June: $W = 207$, $N = 56$, $P = 0.93$; July: $W = 26$, $N = 48$, $P = 0.92$). Furthermore, no differences in body size could be detected between these 2 groups of males (May: $W = 299$, $N = 46$, $P = 0.18$; June: $W = 250.5$, $N = 56$, $P = 0.39$; July: $W = 23.5$, $N = 48$, $P = 1$).

Energy reserves decline across the breeding season

Both body condition indices and energy reserve measures indicated deteriorating condition across the season (Figure 4.1, Supplementary Tables 4.1 and 4.2). Significant declines in liver investment ($P = 0.02$, Figure 4.1c), weight-specific liver glycogen content ($P = 0.003$, Figure 4.1e), total glycogen per whole liver ($P = 0.007$, Figure 4.1f), total lipids per whole liver (*P* = 0.003, Figure 4.1d), and muscle protein content (*P* = 0.016, Figure 4.1j) were observed over the season. The males sampled at the end of the breeding season also had higher liver water (*P* = 0.02, Figure 4.1g), liver protein (*P* = 0.005, Figure 4.1h), and muscle water (*P* < 0.0001, Figure 4.1i) contents relative to males sampled early in the season, and they also tended to have smaller gastrointestinal tracts for their body size (*P* = 0.07, Supplementary Table 4.2). There were no changes in plasma ammonia levels across the season (Supplementary Table 4.2). We found that liver glycogen and lipid contents declined by 58% and 18.7%, respectively, that HSI declined by 32.6%, and that muscle protein content declined by 8.8% over the season.

Figure 4.1 (next page) Seasonal variation in (a) relative body condition (RCF), (b) gonadosomatic index (GSI), (c) hepatosomatic index (HSI), (d) total lipids per whole liver, (e) weight-specific liver glycogen content, (f) total glycogen per whole liver, (g) liver water content, (h) weight-specific liver protein content, (i) muscle water content, and (j) weight-specific muscle protein content from nesting and forced starved male midshipman fish. The dashed line separates the fish sampled over the breeding season from the fish held under food deprivation. Brackets with * indicate a significant difference between 2 groups at P < 0.05.


(a)
Relative Body Condition (RCF)
0.2
0.0
−0.2
*
(b)
Gonadosomatic Index (GSI)
2
1
0
−1
*
*
(c)
Hepatosomatic Index (HSI)
0.25
0.00
−0.25
−0.50
−0.70
*
*
(d)
Total Lipids Per Liver
0.5
0.0
−0.5
−1.0
*
*
(e)
Liver Glycogen (umol/g dry weight)
400
200
0
*
*
*
(f)
Total Glycogen Per Liver
2
0
−2
−4
*
*
(g)
Liver Water Content
0.85
0.80
0.75
0.70
0.65
0.60
*
*
May
June
July
Starved
(h)
Liver Protein (µg/mg dry weight)
500
400
300
200
*
May
June
July
Starved
(i)
Muscle Water Content
0.88
0.86
0.84
*
*
May
June
July
Starved
(j)
Muscle Protein Content (µg/mg dry weight)
1700
1600
1500
1400
1300
*
*
May
June
July
Starved

Starved fish have significantly reduced energy reserves

After 82 days, the food-deprived reference males had lost 16.8±0.6% (mean ± standard error [SE]) of their initial body mass. They were also in lower body condition and had smaller hepatic energy reserves when compared with wild fish sampled early in the breeding season (Figure 4.1, Supplementary Table 4.3). Specifically, relative to early season fish, these starved fish had lower RCF ($P = 0.038$, Figure 4.1a), smaller livers ($P = 0.001$, Figure 4.1c) and digestive tracts ($P = 0.036$), lower weight-specific liver glycogen content ($P = 0.004$, Figure 4.1e), total glycogen per whole liver ($P = 0.009$, Figure 4.1f), total lipids per whole liver ($P < 0.001$, Figure 4.1d), and muscle protein content ($P = 0.019$, Figure 4.1j, Supplementary Table 4.3). The starved fish also had significantly higher water content in their livers ($P = 0.023$, Figure 4.1g) and muscles ($P < 0.0001$, Figure 4.1i), and also higher gonadal investment ($P < 0.001$, Figure 4.1b) relative to early season fish. Except for higher liver-free glucose, few differences could be detected between the starved fish and late season (July) fish from the wild that had presumably been caring for 3 months (Supplementary Table 4.4).

Nest take-over males have greater energy reserves

Take-over males were not significantly different in body size (standard length) than the males that they replaced (t -test, $t = -1.5$, degrees of freedom = 17, $P = 0.15$). However, take-over males had higher weight-specific liver glycogen content and total glycogen per whole liver than males that had remained on their nests, but these measures were significantly different only later in the season (glycogen content LM, interaction effect, est. ± SE = 154.9±70.3, $t_{(35)} = 2.2$, $P = 0.03$, Figure 4.2a; total glycogen GLS, interaction effect, est. ± SE = 0.72±0.32, $t_{(35)} = 2.2$, $P = 0.03$). Take-over males also had higher gonadal investment compared with males that remained the resident of their nests (LM, est. ± SE = 0.61±0.16, $t_{(36)} = 3.79$, $P < 0.001$, Figure 4.2b). All other measures of body condition and hepatic energy reserves were nonsignificant (all $P > 0.11$).

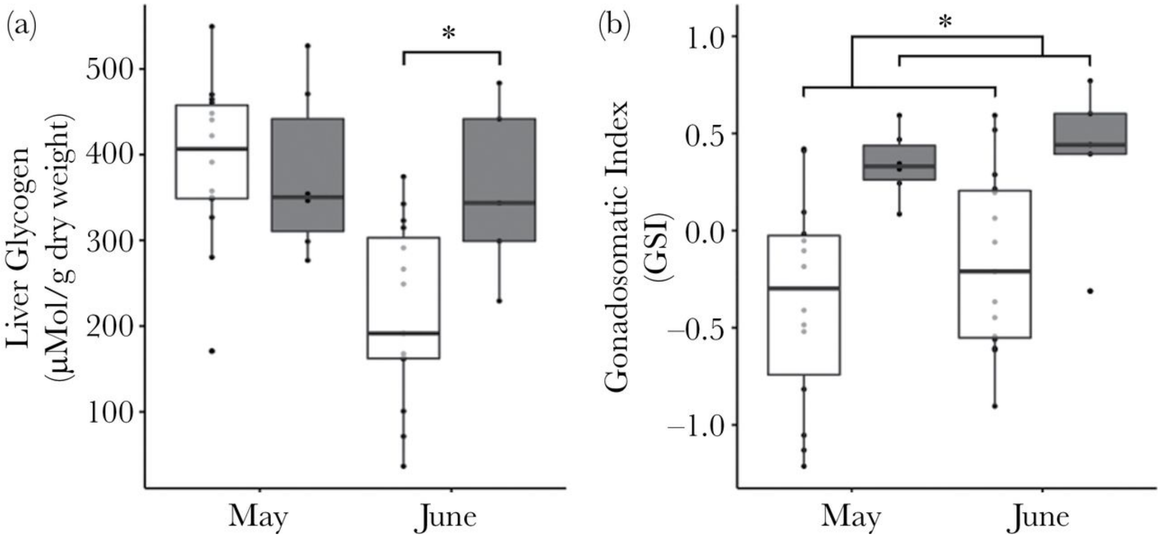


Figure 4.2 Variation in weight-specific liver glycogen content (in µmol/g dry weight) and gonadosomatic index (GSI) between male midshipman fish that took over a nest (nest take-over male shown in dark gray boxes) and males that remained in their nest (remaining resident male, white boxes). Brackets with * indicate a significant interaction (a) or a main effect (b) at $P < 0.05$.

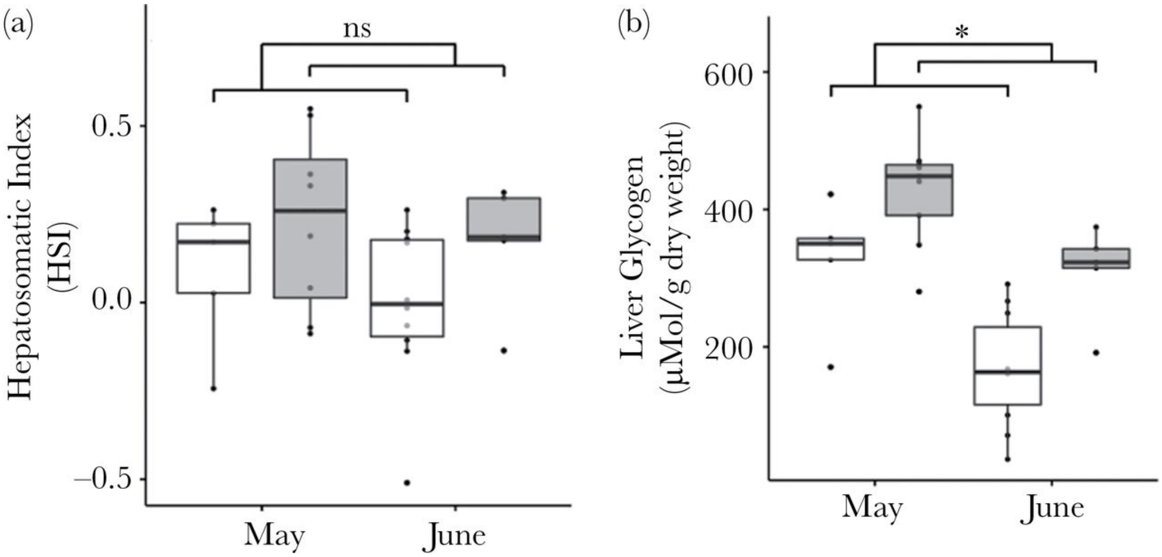


Figure 4.3 Variation in a body condition index (HSI) and weight-specific liver glycogen content (in µmol/g dry weight) between midshipman fish found to have recently cannibalized (light gray boxes) and fish with empty guts (noncannibals, white boxes). Brackets with * indicate a significant main effect at $P < 0.05$.

Cannibals do not have lower energy reserves than noncannibals

Cannibalistic males were found to have an average of 17.8±3.5 (mean ± SE) eggs in their guts. Additionally, the nests of cannibal males suffered a greater reduction in brood size between the 2 sampling days than the nests of noncannibal males (proportion of brood disappearing overnight, mean ± SE = 23.1±1.2% for cannibals vs. 6.3±0.3% for noncannibals; Wilcoxon rank sum, $W = 48$, $N = 40$, $P = 0.002$).

Cannibals appeared to have higher HSIs (LM, est. ± SE = 0.16±0.09, $t_{(25)} = 1.79$, $P = 0.09$) compared with noncannibals though this difference did not reach significance (Figure 4.3a). However, cannibals had higher weight-specific liver glycogen content (LM, est. ± SE = 112.4±33.7, $t_{(25)} = 3.33$, $P = 0.003$; Figure 4.3b) and total glycogen per whole liver (LM, est. ± SE = 0.634±0.266, $t_{(24)} = 2.38$, $P = 0.03$). All other measures of body condition and hepatic energy reserves were nonsignificant (all $P > 0.17$).

Gastric evacuation rates

Extent of egg digestion was strongly related to time elapsed since feeding in the egg digestion study (CLMM, $z = 4.6$, $N = 138$, $P < 0.001$, Figure 4.4). Gut content mass also declined significantly with time elapsed since feeding (LM, est. ± SE = −0.02±0.006, $t_{(26)} = -2.3$, $P = 0.03$).

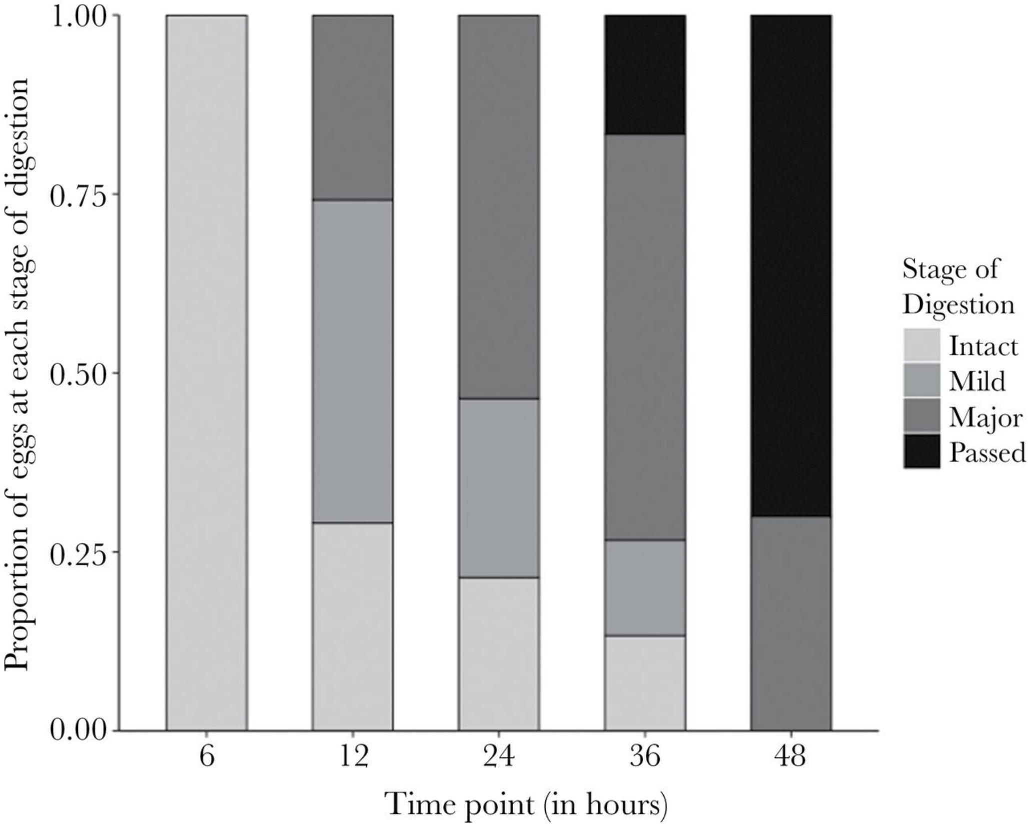


Figure 4.4 Progression of egg digestion (in hours) postfeeding in plainfin midshipman guarder males.

4.5 Discussion

We predicted that if parental care was energetically costly, then the longer an individual provides care the more their energy reserves should deteriorate. We corroborated this prediction in the plainfin midshipman, as parental condition as well as hepatic and somatic energy stores declined with time in the breeding season, a proxy for duration of care provided. We also predicted that if care is costly, then late-coming males that avoid nest building by taking over nests from previous nest owners should be in better condition than those males that have spent more time caring and building a nest. Indeed, we found that take-over males represent a subset of the population that is in better condition than males that have cared for and maintained a nest and offspring for a longer period of time. Finally, we also predicted that if cannibalism was used as a means for caregivers to replenish dwindling energy reserves, as the energy-based hypothesis suggests, then cannibals should have lower energy reserves than noncannibals. We did not find any evidence to support this hypothesis as cannibals had similar or even higher energy reserves to noncannibals.

Males that lost their nest are similar to those that retained it

Any male found to be absent from his nest on the second day of sampling could not be collected for dissection. As such, we were unable to directly compare energy reserves between these absent males and those that remained in their nests. However, we could compare relative condition factor (RCF) and skeletal body length, 2 measures that did not require dissection, between these 2 groups of males and did not detect any significant difference. Previous studies have suggested that larger and stronger males may be more likely to abandon a nest because their higher resource holding potentials enhance their probability of obtaining an alternate site (sand goby, *Pomatoschistus minutus*, Lindström and Pampoulie 2005). However, we found no evidence to suggest that either body condition or body size influenced the likelihood of a male retaining his nest. Thus, it seems unlikely that our disturbance introduced a systematic bias in the males that remained in their nests to be collected on day 2.

Parental care period is associated with declining parental energy reserves

Many animals undergo fasting during one or more parts of their life history and may rely on endogenous fuels for extended periods of time. For example, in many bird species, the defense of young is most crucial during egg incubation, and parents may forgo foraging to remain on the nest during this stage (Mrosovsky and Sherry 1980; Clutton-Brock 1991). This also appears to be true of male plainfin midshipman, as their diet during the breeding season is comprised of the few limited food items found in their nests, suggesting that they remain confined to their nests throughout the care period (Cogliati et al. 2015). Animals undergo 3 well-defined phases of starvation with predictable depletions of available endogenous fuel stores (Bar 2014). Phases I and II rely first on glycogen and then primarily lipid stores. By phase III, animals have crossed a critical body lipid threshold, and have switched to protein catabolism (Bar 2014). Liver glycogen decreased significantly between the early and mid-breeding season and then remained relatively stable throughout the remainder of the season. Liver lipid reserves declined slowly and steadily over the season, revealing a significant decrease only by the late season. Muscle protein decreased significantly between the mid- and late-breeding season. This suggests that hepatic glycogen, hepatic lipids, and somatic proteins are mobilized over the course of starvation in the midshipman fish, but specifically implicates glycogen as an important fuel source during the initial stages of starvation. Water content of the liver and muscle tissues also increased over the season, consistent with a general trend for starving animals to replace lost mass with water (McCue 2010). Taken together, we find it likely that the midshipman males in our study were in phase II of starvation after 2 months of parental care and were in phase III of starvation after 3 months.

Several previous studies have also demonstrated results generally consistent with ours. For example, in three-spined sticklebacks, *G. aculeatus*, males lose the majority of their liver glycogen and lipid reserves and also a considerable amount from their somatic muscle over the course of their approximately 3-month breeding season (Chellappa et al. 1989). *Gasterosteus aculeatus* also displays a similar pattern of fuel use, with liver glycogen being preferentially mobilized before liver lipids. Male grass goby, *Zosterisessor ophiocephalus*, also experience a large decrease in HSI and somatic body lipids between the beginning and end of their breeding season (Malavasi et al. 2004). An increase in liver protein concentration has also been documented in starving Atlantic cod, *Gadus morhua* (Black and Love 1986).

We also found that the relative mass of gastrointestinal tissues declined with duration of both parental care and the food deprivation treatment, suggesting that midshipman males can adaptively reduce investment into unused tissues. Gastrointestinal tissues have high rates of protein synthesis, fast cellular turnover, and are metabolically expensive to maintain, and a broad range of animal taxa are known to reduce digestive tract size during periods of fasting (Piersma and Lindström 1997; Zaldúa and Naya 2014).

Starved males were in a similar physiological state as the late-season males, with similar declines in energy reserves. The only notable difference was that starved males had larger gonads. We interpret the large GSI in starved fish as a result of these males not having an opportunity to spawn repeatedly over the season. Starved males also had lower liver ATP and higher liver glucose levels than did wild males, suggesting an increasing mismatch between ATP demand and supply as food deprivation is prolonged.

Interestingly, a recent diet analysis by Cogliati et al. (2015) revealed that food abundance within the guts of guarder males generally does not change throughout the season and that food availability within the nest, mostly in the form of small invertebrates, may actually increase over the season. Therefore, any decrease in body condition experienced by caregivers is unlikely to be the result of a decline in food availability as the season progresses. Overall, the similarities between laboratory starved males and those providing care in the wild further support the idea that parental care is energy demanding and restricts foraging beyond the nest.

Take-over males are in better condition than resident males that remain on nests

Males can be motivated to take-over nests when appropriate nesting sites are limited (e.g., Bessert et al. 2007) and when nest ownership provides higher reproductive output than cuckoldry strategies alone (e.g., Gomagano and Kohda 2008; Cogliati et al. 2013). Here, we show that take-over males are in better condition than guarder males that have constructed nests and likely spent time caring for offspring. Midseason take-over males had higher levels of liver glycogen (Figure 4.2a). Here, liver glycogen can be used as an indicator of recent energetic strain as we showed it to be the first hepatic fuel source to display a measurable decline in response to care duration in the plainfin midshipman. Any decrease in other fuels, such as lipids or proteins, should first be preceded by a decrease in glycogen, but not vice versa.

These late-coming take-over males are likely to be in better condition as a result of 2 non-mutually exclusive factors. First, take-over males are likely to have invested fewer resources overall into nest construction or parental care. Indeed, take-over males also had higher GSI (Figure 4.2b) suggesting that they had invested less into recent spawning. Second, take-over males may represent a sample of the guarder male population with sufficient energy to be able to out-compete already established nest owners. Interestingly however, take-over males were not larger than the males that they replaced, as has been found in other taxa (e.g., Lindström and Pampoulie 2005; Peixoto and Benson 2011). In the future, to explicitly test the importance of body condition and size on nest tenure in this species, resource contests between already caring and newly arriving males would be valuable.

Energy reserves in cannibals are not lower than in noncannibals

There are several lines of evidence to suggest that cannibalistic guarder males consume eggs from the nest directly under their care. First, all nests experienced a reduction in brood size between the 2 sampling days. This reduction is likely due to a combination of natural mortality, predation, cannibalism, and nest disturbance. However, cannibal fish were associated with nests that experienced a much larger decrease in brood size, consistent with the hypothesis that guarder males cannibalize from the nests under their care as opposed to consuming the eggs of neighbors. Second, Cogliati et al. (2015) made use of genetic paternity testing to show that some eggs consumed by males are in fact related to the cannibal. Cannibalism in this system therefore appears to be a mixture of both filial and nonfilial cannibalism. Third, recent video footage of guarder males within their nests shows the fish periodically engaging in cannibalism of offspring from the roof of their nest (Bose APH, personal observations). The captured cannibalistic behaviors are associated with a characteristic arching of the back, positioning of the nares close to the offspring, and then a forceful suction or expulsion of water from the mouth powerful enough at times to dislodge offspring from the rock surface.

The energy-based hypothesis predicts that parents in poor condition should be the most likely to cannibalize, yet our results do not support this contention; similar to take-over males, cannibals possessed higher levels of liver glycogen suggesting that they had, overall, experienced lower recent energetic strain. Offspring found within the guts of cannibal males were most likely consumed within the 24-h window prior to fish dissection, based on our gastric evacuation rate data. The majority (~90%) of offspring found within the guts of wild cannibals showed little sign of digestion (i.e., classified as either 1, intact, or 2, with mild loss of shape). We therefore assert that 1) it is unlikely for the recently consumed offspring to have contributed to the elevated liver glycogen detected in cannibals and 2) that the energy reserves of the cannibals are likely representative of their condition when they began consuming the offspring. Thus, despite the significant costs of parental care it appears unlikely that low-energy reserves drive offspring cannibalism in the midshipman fish system. Furthermore, egg cannibalism among midshipman males is most frequent early in the breeding season when energy reserves are still high (Bose et al. 2014). Interestingly, our results are consistent with several previous studies. Both Klug and St. Mary (2005) and Klug et al. (2006), respectively, recorded filial cannibalism correlating positively with either the amount of supplemental food provided to caregiving parents (flagfish, *J. floridae*) or the initial body condition of cannibal parents (sand goby, *P. minutus*). They suggest that if a decline in body condition is also associated with diminished expected future reproduction, then this should reduce cannibalism and promote investment into current offspring.

Alternate selective forces that may drive offspring cannibalism in the plainfin midshipman system are paternal uncertainty and mating competition. Cuckoldry and competition among males is likely to influence paternity, or paternity certainty, thereby decreasing the reproductive value of the offspring at hand and the optimal level of investment that a caregiver should provide (Klug et al. 2012). Future studies will investigate these possible factors. Cannibalism could also be an incidental component of nest/brood cleaning or the selective termination of unhealthy offspring. However, the high prevalence and intensity of cannibalism especially in the early season (see Bose et al. 2014) suggests that incidental offspring consumption associated with cleaning is not the primary explanation of this behavior. Furthermore, offspring found in the guts of cannibals appeared healthy. Finally, cannibalism in this system may simply represent an investment in future reproduction. Cannibalism is most common in the early season, when males have invested relatively little into the offspring, and time remains for the males to attract new females. The extent to which body condition affects reproductive success (e.g., nest tenure, offspring survival, female mate choice) still remains to be evaluated.

Few studies on reproductive costs in fish have directly quantified on-board energy reserves over an extended offspring-care period or to the level of detail of this study. Our results show that there is a considerable decline in parental energy reserves associated with progression through the care season. We show that such behavioral strategies such as nest take-overs are associated with an energetic advantage. Lastly, we refute the energy-based hypothesis, at least in this batrachoidid species by demonstrating that low-energy reserves do not drive offspring cannibalism. Thus, it is apparent that energetic need is not a ubiquitous driving factor for offspring cannibalism. Furthermore, we expect that if other species also possess comparable starvation-tolerance and similar systems of mating and parental care to the plainfin midshipman, then they too should be similarly unaffected by declining energy reserves.

4.6 Acknowledgments

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4.8 Supplementary materials

Analytical protocols

Frozen liver, muscle, and plasma samples were stored at -80°C prior to analysis. Water content of liver samples was determined after 24 hours of lyophilization (lyph-lock 6, Labconco Freeze Dry System, Kansas City, MO, U.S.A.). Protein content was measured following the method of Bradford (1976). Lipid content was measured following extraction by the method of Folch et al. (1957) and evaporated dry under a stream of nitrogen with heat (50°C). Extracted lipids were weighed and expressed per gram liver dry weight. Plasma ammonia was determined following Da Fonseca-Wollheim (1973) using a commercial ammonia kit (Cliniqa Corp., San Marcos, CA, U.S.A.).

Liver ATP, glycogen, and free glucose, were extracted and quantified following standard methods of Bergmeyer (1974) adapted to a 96-well format for the Spectramax

Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA). Frozen liver samples were powdered using a liquid nitrogen-cooled mortar and pestle. Powdered tissue was homogenized in 500µl of 6% perchloric acid using a motorized homogenizer (PowerGen 125, Fisher Scientific, Whitby, ON). 100µl of the homogenate was removed and stored at -80°C for later determination of glycogen and free glucose content. The remaining 400 µl of homogenate was centrifuged at 10 000 x g for 10 min at 4°C. The supernatant was removed and neutralized to pH 7.0 with K2CO3 (1M). Samples were then centrifuged again at 10 000 x g for 10 min at 4°C and the resulting supernatant was used for quantification of ATP content.

Final assay conditions for measuring ATP were as follows (in mM): 50 Tris (pH 8.0), 5 MgCl2, 5 glucose, 2 NAD, 1 U/ml glucose-6-phosphate dehydrogenase (G6PDH from L. mesenteriodes, Roche Diagnostics). Reactions were started with 1U of hexokinase (HK from yeast, Roche Diagnostics). The reduction of NAD+ to NADH was monitored at 340nm.

For determination of glycogen and free glucose, 50µl K2HCO3 (1M) and 100µl acetate buffer (400mM, pH 4.8) were added to 100µl crude homogenate samples of which 125µl was set aside to measure free glucose content. 7µl of amyloglucosidase enzyme (4 U/µl; Roche Diagnostics) was added to the remaining 125µl to determine glycogen content. All samples were incubated at 40°C for 2 hrs, and vortexed every 20 min. After incubation, all samples were neutralized to pH 7.0 with K2CO3 (1M) and assayed for glucose content. Assay conditions for measuring glucose were as follows (in mM): 20 imidazole, 1 ATP, 0.5 NADP, and 5 MgCl2. Reactions were started by adding 1U

G6PDH (Roche Diagnostics) followed by 1U HK (Roche Diagnostics). The reduction of NAD+ to NADH was measured at 340nm. Glycogen content was calculated by subtracting free glucose content.

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Supplementary Table 4.1 Body condition and energy reserves in males caring during the early season (May) compared to males caring during the mid-season (June). Abbreviations: RCF – relative condition factor, HSI – hepatosomatic index, GSI – gonadosomatic index, GII – gut investment index.

	May to June comparison				
Measure	May Mean ± SE	June Mean ± SE	Estimate ± SE	t, df	P
RCF	0.024 ± 0.031	0.036 ± 0.046	0.012 ± 0.066	0.18, 32	0.86
HSI	0.088 ± 0.092	-0.001 ± 0.072	-0.089 ± 0.110	-0.81, 32	0.42
GSI	-0.518 ± 0.207	-0.150 ± 0.129	0.368 ± 0.246	1.50, 32	0.14
GII	0.004 ± 0.111	0.054 ± 0.064	0.050 ± 0.098	0.52, 32	0.61
Liver Water Content	0.709 ± 0.021	0.721 ± 0.018	0.010 ± 0.023	0.46, 31	0.65
Liver Glycogen Content (umol/g)	325.6 ± 41.8	167.1 ± 26.4	-156.1 ± 57.5	-2.71, 31	**0.011**
Liver Glucose Content (umol/g)	9.57 ± 0.86	11.76 ± 2.26	7.33 ± 6.48	1.13, 31	0.27
Liver ATP Content (umol/g)	4.83 ± 0.77	4.78 ± 0.55	-0.152 ± 1.229	-0.12, 31	0.90
Liver Lipid Content (mg/g)	486.6 ± 65.5	499.8 ± 46.8	-0.025 ± 0.142	-0.18, 31	0.86
Liver Protein Content (ug/mg)	298.1 ± 37.2	350.8 ± 27.3	38.08 ± 33.6	1.14, 29	0.27
Plasma Ammonia (umol/L)	177.1 ± 56.5	135.6 ± 17.3	-0.218 ± 0.862	-0.94, 28	0.35
Total Lipids Per Liver (Index)	0.174 ± 0.129	0.024 ± 0.111	-0.150 ± 0.152	-0.99, 32	0.33
Total Glycogen Per Liver (Index)	0.684 ± 0.183	-0.274 ± 0.273	-0.957 ± 0.623	-1.54, 32	0.13
Muscle Water Content	0.837 ± 0.002	0.844 ± 0.004	0.006 ± 0.005	1.14, 31	0.26
Muscle Protein Content (ug/mg)	1522.4 ± 35.5	1514.1 ± 33.8	-8.37 ± 53.38	-0.16, 31	0.88

Supplementary Table 4.2 Body condition and energy reserves in males caring during the early season (May) compared to males caring during the late season (July). Abbreviations: RCF – relative condition factor, HSI – hepatosomatic index, GSI – gonadosomatic index, GII – gut investment index.

Measure	May to July comparison				
	May Mean ± SE	July Mean ± SE	Estimate ± SE	t, df	P
RCF	0.024 ± 0.031	-0.071 ± 0.047	-0.095 ± 0.067	-1.40, 32	0.17
HSI	0.088 ± 0.092	-0.182 ± 0.062	-0.27 ± 0.11	-2.40, 32	**0.022**
GSI	-0.518 ± 0.207	-0.515 ± 0.127	0.002 ± 0.251	0.01, 32	0.99
GII	0.004 ± 0.111	-0.182 ± 0.051	-0.186 ± 0.10	-1.87, 32	0.07
Liver Water Content	0.709 ± 0.021	0.766 ± 0.007	0.055 ± 0.023	2.43, 31	**0.021**
Liver Glycogen Content (umol/g)	325.6 ± 41.8	138.1 ± 37.8	-185.8 ± 57.8	-3.22, 31	**0.003**
Liver Glucose Content (umol/g)	9.57 ± 0.86	8.82 ± 1.14	2.87 ± 6.51	0.44, 31	0.66
Liver ATP Content (umol/g)	4.83 ± 0.77	6.02 ± 1.10	1.115 ± 1.234	0.90, 31	0.37
Liver Lipid Content (mg/g)	486.6 ± 65.5	395.6 ± 31.4	-0.239 ± 0.143	-0.09, 31	0.10
Liver Protein Content (ug/mg)	298.1 ± 37.2	401.1 ± 8.5	103.6 ± 33.9	3.06, 29	**0.005**
Plasma Ammonia (umol/L)	177.1 ± 56.5	119.1 ± 6.8	-0.276 ± 0.229	-1.21, 28	0.24
Total Lipids Per Liver (Index)	0.174 ± 0.129	-0.319 ± 0.066	-0.493 ± 0.155	-3.18, 32	**0.003**
Total Glycogen Per Liver (Index)	0.684 ± 0.183	-1.148 ± 0.436	-1.83 ± 0.63	-2.89, 32	**0.007**
Muscle Water Content	0.837 ± 0.002	0.861 ± 0.004	0.024 ± 0.005	4.81, 31	**< 0.0001**
Muscle Protein Content (ug/mg)	1522.4 ± 35.5	1388.0 ± 49.5	-134.4 ± 52.8	-2.55, 31	**0.016**

Supplementary Table 4.3 Body condition and energy reserves in males caring during the early season (May) compared to males held under food deprivation for 82 days (Starved). Abbreviations: RCF – relative condition factor, HSI – hepatosomatic index, GSI – gonadosomatic index, GII – gut investment index.

	May to Starved comparison				
Measure	May Mean ± SE	Starved Mean ± SE	Estimate ± SE	t, df	P
RCF	0.024 ± 0.031	-0.116 ± 0.028	-0.140 ± 0.064	-2.17, 32	**0.038**
HSI	0.088 ± 0.092	-0.304 ± 0.054	-0.392 ± 0.107	-3.66, 32	**<0.001**
GSI	-0.518 ± 0.207	0.697 ± 0.150	1.21 ± 0.24	5.08, 32	**<0.001**
GII	0.004 ± 0.111	-0.204 ± 0.040	-0.208 ± 0.095	-2.19, 32	**0.036**
Liver Water Content	0.709 ± 0.021	0.761 ± 0.007	0.053 ± 0.022	2.40, 31	**0.023**
Liver Glycogen Content (umol/g)	325.6 ± 41.8	153.7 ± 30.6	-174.1 ± 55.7	-3.12, 31	**0.004**
Liver Glucose Content (umol/g)	9.57 ± 0.86	31.7 ± 6.24	17.43 ± 6.29	2.77, 31	**0.009**
Liver ATP Content (umol/g)	4.83 ± 0.77	3.47 ± 0.41	-1.26 ± 1.19	-1.06, 31	0.30
Liver Lipid Content (mg/g)	486.6 ± 65.5	454.8 ± 31.0	-0.013 ± 0.138	-0.10, 31	0.93
Liver Protein Content (ug/mg)	298.1 ± 37.2	358.3 ± 13.2	59.28 ± 32.14	1.85, 29	0.07
Plasma Ammonia (umol/L)	177.1 ± 56.5	132.0 ± 14.1	-0.193 ± 0.227	-0.85, 28	0.40
Total Lipids Per Liver (Index)	0.174 ± 0.129	-0.405 ± 0.074	-0.580 ± 0.148	-3.91, 32	**<0.001**
Total Glycogen Per Liver (Index)	0.684 ± 0.183	-1.01 ± 0.39	-1.69 ± 0.61	-2.79, 32	**0.009**
Muscle Water Content	0.837 ± 0.002	0.861 ± 0.004	0.018 ± 0.005	3.78, 31	**< 0.0001**
Muscle Protein Content (ug/mg)	1522.4 ± 35.5	1398.0 ± 22.9	-124.35 ± 50.15	-2.48, 31	**0.019**

Supplementary Table 4.4 Body condition and energy reserves in males caring during the late season (July) compared to males held under food deprivation for 82 days (Starved). Abbreviations: RCF – relative condition factor, HSI – hepatosomatic index, GSI – gonadosomatic index, GII – gut investment index.

	July to Starved comparison				
Measure	July Mean ± SE	Starved Mean ± SE	Estimate ± SE	t, df	P
RCF	-0.071 ± 0.047	-0.116 ± 0.028	-0.045 ± 0.053	-0.85, 32	0.40
HSI	-0.182 ± 0.062	-0.304 ± 0.054	-0.122 ± 0.089	-1.38, 32	0.18
GSI	-0.515 ± 0.127	0.697 ± 0.150	1.21 ± 0.20	6.12, 32	**<0.001**
GII	-0.182 ± 0.051	-0.204 ± 0.040	-0.022 ± 0.079	-0.29, 32	0.78
Liver Water Content	0.766 ± 0.007	0.761 ± 0.007	-0.003 ± 0.020	-0.14, 31	0.89
Liver Glycogen Content (umol/g)	138.1 ± 37.8	153.7 ± 30.6	11.79 ± 49.96	0.24, 31	0.82
Liver Glucose Content (umol/g)	8.82 ± 1.14	31.7 ± 6.24	14.55 ± 5.63	2.58, 31	**0.015**
Liver ATP Content (umol/g)	6.02 ± 1.10	3.47 ± 0.41	-2.38 ± 1.07	-2.23, 31	**0.033**
Liver Lipid Content (mg/g)	395.6 ± 31.4	454.8 ± 31.0	0.227 ± 0.123	1.84, 31	0.075
Liver Protein Content (ug/mg)	401.1 ± 8.5	358.3 ± 13.2	-44.27 ± 29.39	-1.51, 29	0.14
Plasma Ammonia (umol/L)	119.1 ± 6.8	132.0 ± 14.1	0.084 ± 0.204	0.41, 28	0.69
Total Lipids Per Liver (Index)	-0.319 ± 0.066	-0.405 ± 0.074	-0.087 ± 0.123	-0.71, 32	0.49
Total Glycogen Per Liver (Index)	-1.148 ± 0.436	-1.01 ± 0.39	0.140 ± 0.502	0.28, 32	0.78
Muscle Water Content	0.861 ± 0.004	0.852 ± 0.03	-0.006 ± 0.005	-1.24, 31	0.23
Muscle Protein Content (ug/mg)	1388.0 ± 49.5	1398.0 ± 22.9	10.06 ± 52.29	0.19, 31	0.85

Chapter 5: Impacts of direct and indirect paternity cues on paternal care in a singing toadfish

5.1 Abstract

Effort spent on raising unrelated offspring can be costly and wasteful, and parents are expected to reduce their level of investment when they have low or uncertain relatedness to the young under their care. Although the relationship between parental certainty and parental investment is theoretically well established, empirical support has been mixed. Here, we report on a series of lab and field experiments that test whether paternal investment is reduced as paternity decreases in the plainfin midshipman fish (*Porichthys notatus*), a species of toadfish with male-only care. We explored what cues plainfin midshipman males use to assess their paternity. We show that a nest takeover, in which a male displaces another male from a nest, can be a reliable indirect cue of paternity information and leads to a drop in offspring survival. We also show that, when presented in isolation, direct cues of reduced offspring relatedness do not result in a decline in offspring survival in midshipman. Our findings help clarify what systems, species, and theoretical assumptions best reveal the link between parental investment and parentage.

5.2 Introduction

Raising offspring is a demanding endeavor and so parents are expected to ensure that their parental efforts are not misdirected (Alonzo and Klug 2012). When parentage is low or uncertain, a caregiver is expected to reduce parental investment and preserve resources for more certain reproduction in the future (Alonzo and Klug 2012). Theory suggests that parental investment will fluctuate in response to variation in certainty of parentage but only when the following 3 conditions are satisfied: 1) parental care is costly such that investment into current offspring diminishes the ability to invest in future reproduction, 2) relatedness to the offspring varies between reproductive bouts, and 3) caregivers have access to cues that reliably predict their relatedness to the offspring (Westneat and Sherman 1993). When studying how patterns of parentage relate to parental effort, it is important to carefully consider these 3 conditions in order to determine whether a relationship can be expected. Many of the empirical studies conducted to date on this topic have been correlational in design and have revealed mixed results with some studies revealing a positive relationship between parentage and parental care (e.g., Sheldon and Ellegren 1998; Hunt and Simmons 2002; Neff 2003; Apicella and Marlowe 2007) where others have uncovered no relationship (e.g., Peterson et al. 2001; Östlund-Nilsson 2002; Härdling et al. 2007; Svensson and Kvarnemo 2007) or even found a negative relationship (Alonzo and Heckman 2010). This inconsistency may, in part, be the result of various study systems not meeting the above 3 criteria, not properly accounting for confounding variables, or not using the appropriate proxies for parental investment (Kempenaers and Sheldon 1997; Sheldon 2002; Alonzo 2010). Hence, there is currently a research need to better determine and characterize the relationship between certainty of parentage and parental investment and to identify the particular recognition mechanisms used during such parentage assessments across species (Alonzo and Klug 2012).

Recognition mechanisms employed by parents to assess their relatedness to offspring are generally categorized into the use of direct cues versus indirect cues (Sherman and Neff 2003). Direct, or phenotypic, cues are those that emanate from the offspring themselves, such as how an offspring looks or smells, and these cues are often compared for similarity with the parent (e.g., via self-referent phenotype matching, Hauber and Sherman 2001). The use of direct cues is known to occur in numerous taxa, including mammals (e.g., Belding's ground squirrels, *Urocitellus beldingi*, Mateo 2010), birds (e.g., brown-headed cowbirds, *Molothrus ater*, Hauber et al. 2000), arthropods (e.g., ladybirds, *Adalia bipunctata*, Agarwala and Dixon 1993), and fishes (e.g., bluegill sunfish, *Lepomis macrochirus*, Neff and Sherman 2003, 2005). In contrast, indirect cues are those that originate from the individual's ecological or social environment (Hauber and Sherman 2001). For example, a parent may use the presence of sexual competitors in the vicinity during mating, or during their mate's fertile period, as a cue of reduced parental certainty (Waldman 1987; Sherman and Neff 2003). The use of such indirect cues is also taxonomically widespread (e.g., dunnocks, *Prunella modularis*, Davies et al. 1992; wolf spider, *Pardosa milvina*, Anthony 2003; silversides, *Telmatherina sarasinorum*, Gray et al. 2007; poison-dart frogs, *Oophaga pumilio*, Stynoski 2009). Interestingly, Alonzo and Heckman (2010) documented a counterintuitive case in the ocellated wrasse, *Symphodus ocellatus*, wherein the degree of paternal care actually increased with risk of sperm competition. The influence of a particular cue on parental investment should depend on several factors, including the cue's reliability in predicting parentage and the costliness of losing parentage (Neff and Sherman 2002). Thus, it is informative to assess multiple potential cues, direct and indirect, within a single system in order to identify which affect parental behaviors and which do not.

The plainfin midshipman fish (*Porichtys notatus*) represents an excellent model system in which to examine how cues of relatedness might influence parental behavior. Plainfin midshipman satisfy 2 of the required conditions identified by theoretical models for parentage to influence parental investment. First, parental care in *P. notatus* is extremely costly. Males provide sole paternal care for offspring over a 3–4-month-long breeding season, and this care incurs a high physiological cost severely draining paternal energy reserves and body condition (Sisneros et al. 2009; Bose, McClelland, et al. 2015). Furthermore, caring for non-kin offspring imposes an additional cost because these offspring take up valuable space in an already space-limited nest (DeMartini 1991). Non-kin offspring within a nest therefore restrict a male's opportunity for additional or future reproduction. Second, males of this species compete intensely for reproduction (Brantley and Bass 1994; Lee and Bass 2004; Bose, Cogliati, et al. 2014) leading to highly variable levels of paternity among broods in the wild (range 0–100%, Cogliati, Neff, et al. 2013). The third condition of whether or not males have access to reliable cues of paternity loss has yet to be tested. In this study, we aimed to uncover whether nesting plainfin midshipman males have access to reliable cues of paternity loss and specifically tested whether guarder males use direct (i.e., offspring) cues and/or indirect (i.e., environmental and social) cues to inform their paternity assessments over a brood of offspring.

5.3 Methods

Study species

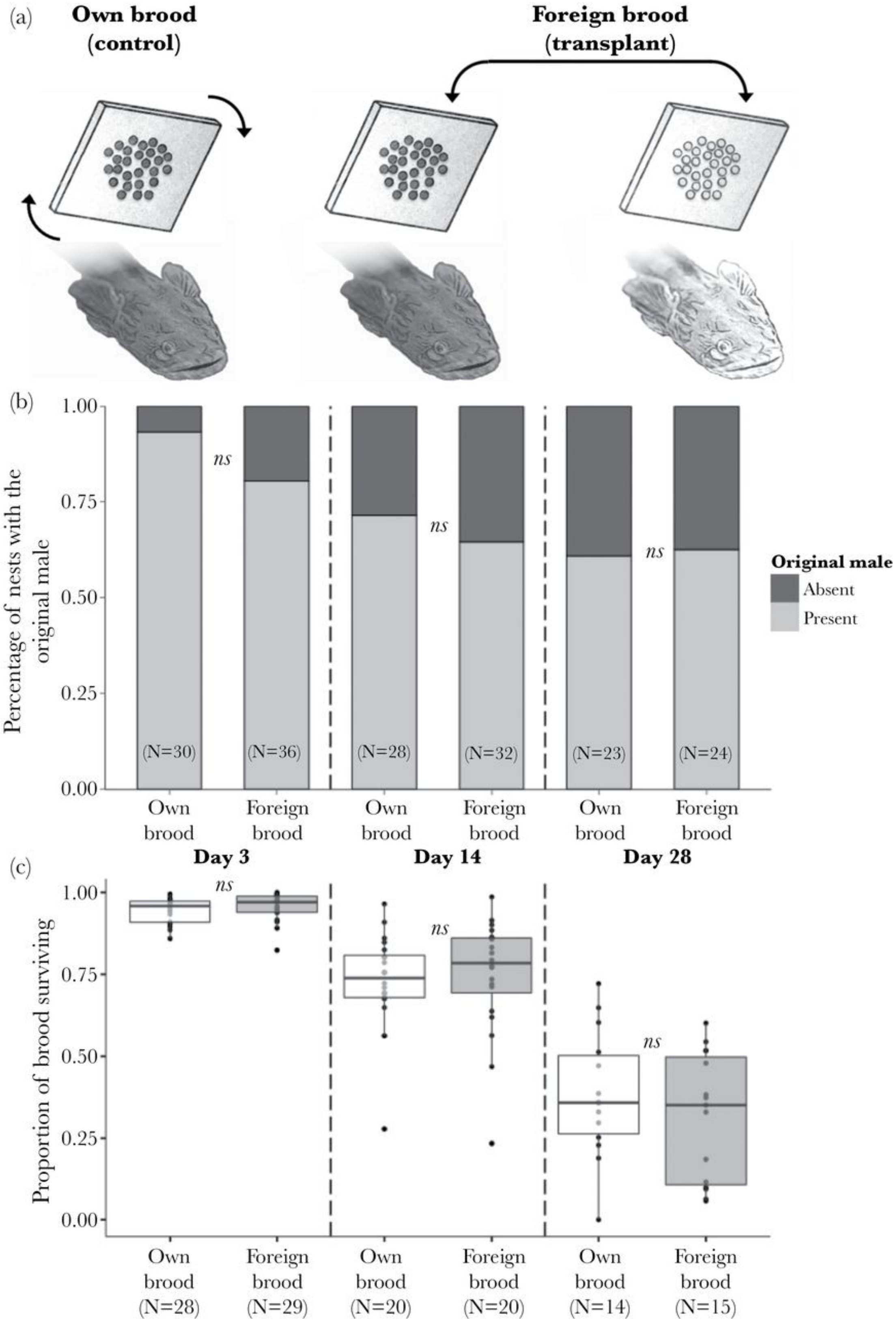
The plainfin midshipman is a marine toadfish native to the western coast of North America (Arora 1948; Miller and Lea 1972; Walker and Rosenblatt 1988). Male plainfin midshipman fish are found as one of 2 well-characterized alternative reproductive morphs (Brantley and Bass 1994; Lee and Bass 2004). Guarder males (also called Type I males) build nests within the intertidal zone by excavating nesting cavities beneath intertidal rocks. From their nests, each guarder male produces a low-frequency, long-duration acoustic signal in order to attract gravid females (Ibara et al. 1983; Brantley and Bass 1994). Females adhere their eggs to the underside of the rock, the roof of the nest (Arora 1948). Guarder males are polygynous, acquiring eggs from numerous females over the breeding season, and will simultaneously care for several cohorts of offspring at different stages of development. Eggs develop for approximately 30 days and then hatch. The hatched offspring remain adhered to the roof of the nest, absorbing a large yolk sac and develop for another ~30 days before reaching independence and will finally swim freely and leave the nest (Arora 1948; Cogliati, Neff, et al. 2013). Intense competition among guarder males for adequate nesting sites leads to high frequencies of nest takeover early in the breeding season (Cogliati, Neff, et al. 2013; Bose, Cogliati, et al. 2014). In contrast to guarder males, sneaker males (also called Type II males) do not physically compete for nests nor do they construct nests or acoustically court females. Instead, they use sneaking and satellite spawning tactics to steal fertilizations away from guarder males effectively parasitizing the guarder male's courtship and parental investment (Brantley and Bass 1994; Lee and Bass 2004).

Experiment 1: manipulation of direct cues via offspring transplants

Between 14 and 17 May 2014, 91 artificial nests were constructed using concrete tiles (12″ × 12″, 929.0cm^2) placed within the intertidal zone of a private beach located in Dabob Bay of the Hood Canal, Washington (47°76′N, 122°86′W). Such tiles serve well as artificial nests and are easier to lift and inspect than are the nests found beneath natural rocks. Guarder males readily dig nesting cavities beneath these tiles, from where they will acoustically court females, spawn, and care for offspring. Females adhere their embryos on the roof of the nest (the underside of the tile). The tile nests were checked again on 12 June 2014, and we found that 76 of them contained both a guarder male and developing offspring. The males and their broods were digitally photographed with a ruler (for later measurement of the male standard length and quantification of the brood size using the software ImageJ [v1.48]). Care giving males were each given a unique dorsal fin mark with injectable elastomer (Northwest Marine Technology, Inc.) for future identification. These 76 nests were then randomly assigned to either a control group ($N = 37$) or to a transplant group ($N = 39$). No differences in standard length of males (t -test, $t = 1.35$, df = 65.6, $P = 0.18$, mean standard length ± [standard error] SE = 23.1±0.3, range: 16.5–28.9cm) or in initial brood sizes (t -test, $t = 0.38$, df = 72.6, $P = 0.71$, mean brood size ± SE = 1234±71 embryos, range: 94–2983 embryos) were observed between the males and nests assigned to either the control or transplant treatment groups prior to the manipulation.

Nests in the transplant group were swapped with one another, controlling as closely as possible for brood size and offspring developmental stage between the swapped tiles. Only broods in which all offspring were still eggs (prehatch stages of development) were used in these transplants. Note, eggs take 30 days to hatch and hatched embryos remain adhered to the nest ceiling for an additional 30 days absorbing the large yolk sac before leaving the nests. The swapped tiles were always spatially distant (>5 m apart) from one another in the intertidal zone, making it highly unlikely that males within the transplant group could have fathered the brood of offspring on the transplanted tile they received. To control for disturbance, the tiles in the control group were lifted, rotated 180°, and then placed back on the nest cavity with their original brood intact (Figure 5.1a). Thus, the males in the transplant group each received an entire foreign brood, whereas males in the control group each received their own brood.

Figure 5.1 (next page) (a) A schematic of the design of Experiment 1. Tiles with offspring were transplanted between the nests of caregiving males (foreign brood), or lifted, rotated, and returned to the original caregiver (own brood). (b) The presence or absence of the original guarder male between treatment conditions, on days 3, 14, and 28. The dark bars refer to cases where the original guarding male was absent (presumed to have abandoned), and the light bars refer to cases where the original guarding male remained with the nest. (c) Proportion of the offspring surviving under the care of an alloparent (transplanted foreign brood) or the care of the original parent (own brood) at 3-, 14-, and 28-day postmanipulation.


(a)
Own brood (control)
Foreign brood (transplant)
(b)
Percentage of nests with the original male
1.00
0.75
0.50
0.25
0.00
ns
ns
ns
(N=30)
(N=36)
(N=28)
(N=32)
(N=23)
(N=24)
Original male
Absent
Present
Own brood
Foreign brood
Own brood
Foreign brood
Own brood
Foreign brood
Day 3
Day 14
Day 28
(c)
Proportion of brood surviving
1.00
0.75
0.50
0.25
0.00
ns
ns
ns
Own brood (N=28)
Foreign brood (N=29)
Own brood (N=20)
Foreign brood (N=20)
Own brood (N=14)
Foreign brood (N=15)

We visited these nests 3-, 14-, and 28-day postmanipulation. On each visit, we recorded the presence or absence of the marked guarder male and took additional digital photographs of the brood to quantify the number of offspring remaining. Note, the challenging time restrictions of working within a low tidal schedule meant that we did not always manage to visit every nest at every time point leading to slight variation in sample sizes between time points.

As some nests were abandoned or taken over by a new male after our manipulations, we compared the proportion of original guarder males still within their nests between the control and transplant groups. To do this, we conducted a generalized linear model (GLM) at each time point, specifying a binomial error distribution suitable for binary response data. Treatment condition (own brood, foreign brood), guarder male standard length (centimeter), and initial brood size (embryo count) were all included as predictor variables in the models. We then focused only on the nests that retained a guarder male, and compared the proportions of offspring still surviving from the original brood at each time point between the control and transplant groups. To do this, we performed a GLM at each time point, specifying a quasibinomial error distribution (accounting for overdispersion, Kabacoff 2011) suitable for proportion data. Parameters for treatment condition (own brood, foreign brood), guarder male standard length (centimeter), and initial brood size (embryo count) were included in the models.

Experiment 2: brood recognition via direct cues

In April 2015, 55 artificial nests were constructed using concrete tiles as described above in Experiment 1 within the intertidal zone of Ladysmith Inlet, British Columbia, Canada (49°01′N, 123°83′W). These nests were monitored every other day between 24 April 2015 and 27 June 2015 for the presence of a guarder male and for broods of embryos. Of these, a total of 52 guarder males and their tiles (with embryos adhered to these tiles) were transported to the Pacific Biological Station in Nanaimo, British Columbia, Canada. Each tile was digitally photographed so that brood size could be quantified. Each male was housed individually in a 300-L outdoor fiberglass holding tank, lined with a sand substrate and fitted with a flow-through system that supplied temperature controlled (18±1 °C) filtered seawater. Each male was provided with 2 artificial nests placed 30cm apart, identical in size (361.0cm^2) and in layout (rectangular with one 5×6cm entrance, Figure 5.2a). Each artificial nest was constructed from 4 bricks and a square concrete garden tile. The males in tanks swam around actively, and even sang nocturnally suggesting that the captive fish still engage in natural breeding behaviors (e.g., Brantley and Bass 1994; Bose A, personal observation). Males were initially placed in a start chamber for an acclimation period of 30min before being given 24h to choose between the 2 artificial nests within their tanks.

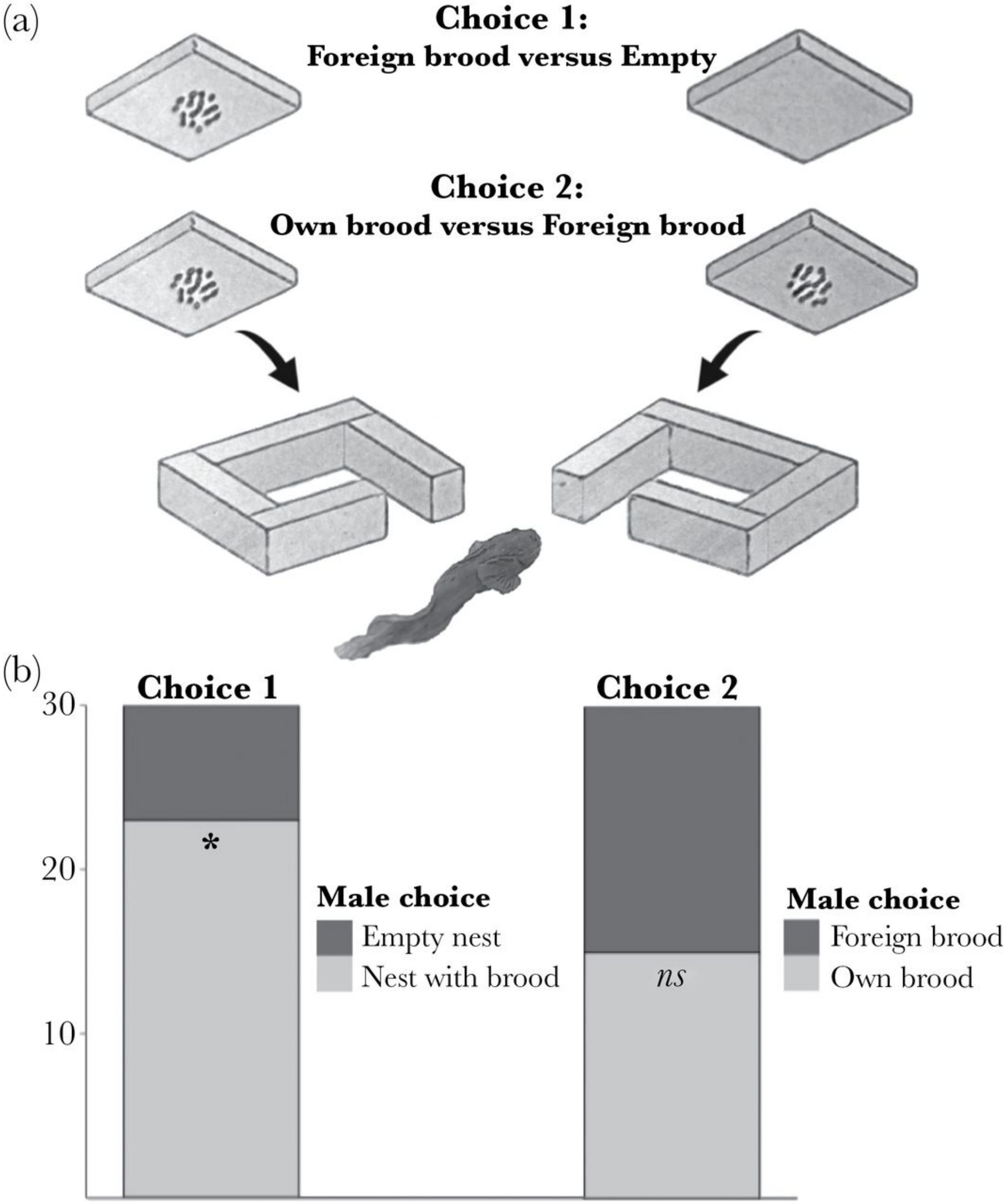


Figure 5.2 (a) A sketch of the design of Experiment 2 (not drawn to scale). Guarder males were given a choice between 2 artificial nests. In Choice 1, males were simultaneously presented with 2 nests, one that contained another male's offspring and a second nest without any offspring. In Choice 2, males were presented with 2 nests, one that contained another male's offspring and one that contained their own offspring. (b) Number of males choosing each nest type when given the choice.

Choice 1: nest with embryos versus empty nest

Thirty-eight fish were used to test whether males preferentially choose nests that already contained embryos over a nest that did not. For this test, one nest contained a brood of embryos (not belonging to the focal male, mean brood size ± SE = 461.0±79.9 embryos), whereas the other nest contained no embryos (Figure 5.2a). Embryos at both prehatch and posthatch stages of development were offered to these males over the course of the experiment. The side of the tank where the brood-bearing tile was positioned in relation to the empty tile was alternated each trial.

Choice 2: nest with own embryos versus nest with foreign embryos

Thirty fish were used to test whether males preferred and/or could distinguish between their own familiar embryos versus foreign unfamiliar embryos. For this test, one nest in the male's tank contained that guarder male's original brood from the field and the other nest in the tank contained a brood of foreign embryos. The 2 nests were matched as best as possible for brood size (paired *t* -test after ln-transformation, $t = 1.1$, degrees of freedom [df] = 29, $P = 0.28$, mean brood size ± SE = 305.3±29.1 embryos, range: 42–938 embryos) and were matched for stage of embryo development (Figure 5.2a). Embryos at both prehatch and posthatch stages of development were offered to males over the course of the experiment. The positions of the tiles within the tanks were alternated for each trial. For both choice tests, we recorded in which nest each male resided after 24h before digitally photographing the broods once again. Males were measured for standard length (centimeter; to the nearest 0.1cm) and total body mass (gram; to the nearest 0.2g). Twenty-two fish participated in both choice trials counterbalancing for order.

We tested whether guarder males were more likely to choose nests that already contained offspring as opposed to empty nests, using a binary logistic GLM specifying a binomial error distribution, including parameters for the developmental stage of the brood (prehatch, posthatch), guarder male standard length (mean-centered, centimeter), and brood size (mean-centered, embryo count).

Next, we tested whether guarder males were more likely to choose a nest that contained their own embryos versus a nest that contained unfamiliar foreign embryos, using a binary logistic GLM specifying a binomial error distribution, including parameters for the developmental stage of the brood (prehatch, posthatch), guarder male standard length (mean-centered, centimeter), and relative brood size (mean-centered, difference in embryo counts). Lastly, we compared rates of offspring mortality between the 2 broods (own vs. foreign) using a Wilcoxon signed rank test (“MASS” package, Venables and Ripley 2002) to test whether the unfamiliar foreign broods suffered higher mortality (suggestive of embryo cannibalism) than did the familiar broods over the 24-h choice period.

Experiment 3: benefits of paternal care versus allopaternal care versus no care

On 16 and 17 May 2015, a total of 60 concrete tiles (12″ × 12″, 929.0cm^2) were placed within the intertidal zone of the private beach in Dabob Bay, Washington. These artificial nests were monitored daily until they were occupied by a guarder (Type I) male and had received embryos. Within 2 days of setting out the tiles, every nest had been taken up by a guarding male, and it took on average 1 further day (range 1–5 days) for these males to acquire broods of eggs. The broods were digitally photographed for later quantification of embryo number and to provide estimates of embryo survival. These males and nests were then randomly assigned to one of 3 experimental treatments: a "paternal care" group, an "allopaternal care" group, and a "no care" treatment group (Figure 5.3a). In the paternal care treatment, the original males were left in their nests to continue caring for the brood. In the allopaternal care treatment, original males were removed from their nests and new males were permitted to take up these nests (this nearly always occurred within 24h of removing the original male, range 1–2 days). On nest takeover, the embryos were digitally photographed again to accurately quantify the starting brood size under allopaternal care. Each caregiving male in the paternal and allopaternal care treatment groups were also given a unique dorsal fin mark as described above for future identification. Lastly, in the no care treatment, original males were removed from the nest, but no new males were permitted to take up the nest. In order to hinder any further nest takeovers by a new male, *all* nests were covered with plastic mesh (mesh size ~1″ × 1″). We returned to check these nests, lifting the plastic mesh, 14- and 28-day postmanipulation to verify the presence of the marked guarder male (or in the case of no care nests, to verify that a male was still absent). We also took a digital photograph of each brood for later quantification of changes to embryo number and development. At the beginning of the experiment, there were no differences in standard length of the male care givers (t -test, $t = 1.73$, df = 19.0, $P = 0.10$, mean standard length ± SE = 19.1±0.6cm, range: 14.9–23.6cm) or initial brood sizes (Anova, $F_{2,36} = 2.85$, $P = 0.07$, mean brood size ± SE = 535.1±62.2 embryos, range: 15–1320 embryos) between treatment groups.

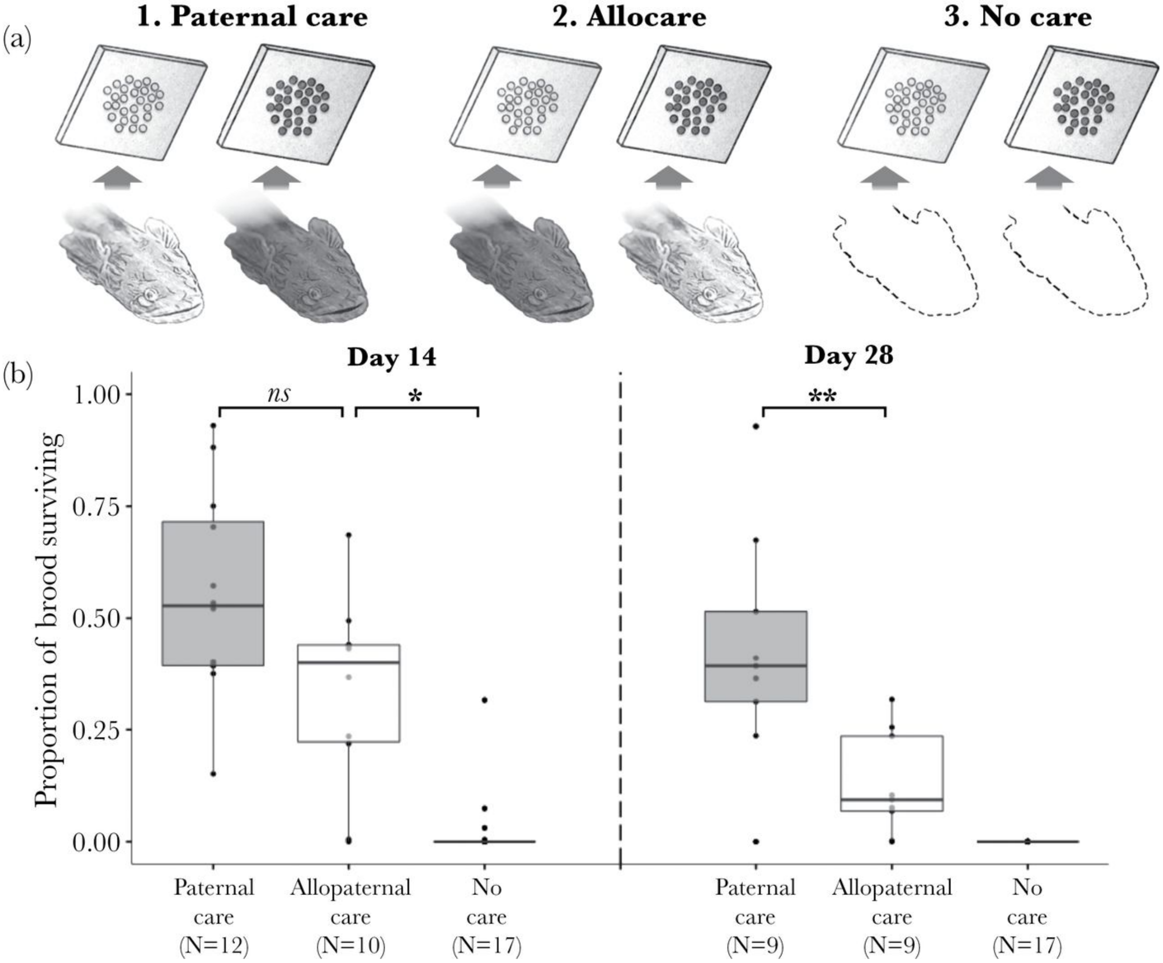


Figure 5.3 (a) A schematic of the design of Experiment 3. Offspring were either cared by the original male (paternal care), adopted by a new male (allopaternal care), or not cared by any male (no care). Nests under all conditions were covered with mesh barriers to hinder changes in nest ownership postmanipulation. Note the difference in embryo-to-male coloration between the paternal care and allopaternal care treatments (specific coloration patterns on the males are for illustration purposes only). (b) Proportion of the brood surviving under paternal care, allopaternal care, and no care at 14- and 28-day postmanipulation. Note that at day 28, the no care condition was not included in the analysis because offspring survival had dropped to zero with no variance.

We performed a GLM at each time point, specifying a quasibinomial error distribution (accounting for overdispersion, Kabacoff 2011) on the proportion of offspring still surviving from the original brood. At the 14-day time point, the model included parameters for treatment condition (paternal care, allopaternal care, no care), and initial brood size (embryo count). To test for an effect of male body size, this model was run again excluding the "no care" condition and including a parameter for male standard length (centimeter). At the 28-day time point, offspring survival was compared between treatment conditions, and parameters for male standard length (centimeter) and initial brood size (embryo count) were also included in the model.

Ethical note

Plainfin midshipman fish are neither threatened nor endangered (Collette et al. 2010). All animal collections and handling were in accordance with the Canadian Department of Fisheries and Oceans rules. Fish were collected and studied in British Columbia, Canada, on scientific license XR 121 2014 and XR 81 2015 and in Washington, on Washington State scientific collections permit 14-147. All procedures were approved by the McMaster University Animal Research Ethics Board (AUP 13-12-52), DFO's Animal Care Committee (AUP 13-12-52), and the University of Washington Institutional Animal Care and Use Committee (AUP 4079-06) and are in line with the guidelines set by the Canadian Council on Animal Care (CCAC).

5.4 Results

Experiment 1: do males adjust parental care in response to direct cues of reduced paternity?

No, when we manipulated direct cues of paternity via offspring transplants, guarder males receiving transplanted foreign embryos were not more likely to abandon than males caring for their own embryos (GLM, day 3: $z = -1.1$, df = 62, $P = 0.29$; day 14: $z = -0.17$, df = 55, $P = 0.86$; day 28: $z = 0.19$, df = 43, $P = 0.85$; Figure 5.1b). Initial brood size did not significantly predict brood abandonment at any time point (GLM, day 3: $z = -0.32$, df = 62, $P = 0.75$; day 14: $z = 0.73$, df = 55, $P = 0.46$; day 28: $z = 1.3$, df = 43, $P = 0.18$) and neither did male body size (GLM, day 3: $z = 1.7$, df = 62, $P = 0.10$; day 14: $z = 0.94$, df = 55, $P = 0.35$; day 28: $z = 0.09$, df = 43, $P = 0.93$).

Males receiving transplanted foreign embryos successfully reared similar numbers of offspring compared with males who continued to care for their own offspring (GLM, day 3: $t = 1.03$, df = 53, $P = 0.31$; day 14: $t = 1.19$, df = 36, $P = 0.24$; day 28: $t = -0.60$, df = 25, $P = 0.55$; Figure 5.1c). Initial brood size did not significantly predict brood mortality at any time point (GLM, day 3: $t = 1.69$, df = 53, $P = 0.10$; day 14: $t = 1.69$, df = 36, $P = 0.06$; day 28: $t = 0.98$, df = 25, $P = 0.34$). Male body size had no detectable influence on offspring survival at any time point (GLM, day 3: $t = -0.91$, df = 53, $P = 0.37$; day 14: $t = 1.0$, df = 36, $P = 0.30$; day 28: $t = 1.34$, df = 25, $P = 0.19$). It should be noted that although the offspring on these tiles had matured by day 28, the offspring had not yet reached the free-swimming stage of nest independence. This suggests that offspring disappearances were due to mortality rather than having matured and left the nest on their own. Interestingly, when a caregiving male was absent from a nest, it was common for the nest cavity to either have filled in completely with sediment or to be occupied by several species of crab (*Cancer gracilis*, *Hemigrapsus oregonensis*, *Hemigrapsus nudus*, *Pagurus* spp.).

Experiment 2: do males prefer nests with embryos and do they prefer their own embryos?

Yes, males were more likely to take up residence in nests containing embryos (23 males chose nests with embryos, whereas 7 males chose empty nests, Figure 5.2b, GLM, intercept: $z = -2.52$, $P = 0.012$). Of the 38 males used in this trial, 30 were found to have chosen a nest after 24h. Male choice of nest was also not influenced by the developmental stage of the offspring (GLM, $z = 1.60$, $P = 0.11$), by male size (GLM, $z = -1.05$, $P = 0.29$), or by the size of the brood (GLM, $z = 0.53$, $P = 0.59$). Although males expressed a strong preference for embryo-containing nests over empty nests, they were not more likely to choose their own brood over a foreign brood (15 males chose their own broods, whereas 15 males chose foreign broods, Figure 5.2b, GLM, intercept: $z = -0.56$, $P = 0.58$). All 30 males used in this second trial were found to have chosen a nest after 24h. Developmental stage of the offspring did not affect nest choice (GLM, $z = 0.78$, $P = 0.43$) nor did male size (GLM, $z = 1.58$, $P = 0.11$). However, although we attempted to size match to the best of our abilities, males did prefer the larger of the 2 broods (i.e., relative brood size = size of familiar brood − size of unfamiliar brood, GLM, $z = -2.44$, $P = 0.02$). Lastly, after 24h, the broods had suffered on average 6.2±1.5% mortality (i.e., offspring disappearance). However, the proportions of offspring found to be missing from own familiar broods did not differ significantly from unfamiliar foreign broods (Wilcoxon signed rank test, $V = 224$, $N = 30$, $P = 0.90$).

Experiment 3: is allopaternal care as effective as paternal care?

No, offspring survival was lower under allopaternal care than under paternal care. Although offspring survival declined in all nests over the care period, approximately 22.6% fewer offspring survived to day 14 in nests under allopaternal care compared with nests under paternal care. Although this difference was statistically significant in the model that ignored male standard length (i.e., the model including the “no care” condition, GLM, $t = 2.4$, df = 38, $P = 0.022$), it was no longer significant when male standard length was included (i.e., the model excluding the “no care” condition, GLM, $t = 1.48$, df = 21, $P = 0.16$; Figure 5.3b). By day 28, approximately 31.5% fewer offspring survived under allopaternal care than under paternal care. This difference was statistically significant (GLM, $t = 2.45$, df = 17, $P = 0.03$; Figure 5.3b). When no care was provided, survival dropped dramatically to a mere 4±2% (mean ± SE) by day 14, which was significantly lower than the offspring survival recorded under allopaternal care at that time point (GLM, $t = -4.05$, df = 35, $P = 0.0003$). By day 28, offspring survival under no care had dropped to 0±0% (mean ± SE). Initial brood size did not predict offspring survival at either time point ($P > 0.08$). Again, by day 28, the surviving offspring in these nests had not yet reached the stage of nest independence, suggesting that any offspring disappearances were due to mortality rather than fully developed offspring leaving the nest. In the absence of a caregiver, the nest cavities had often completely filled in with sediment.

5.5 Discussion

Our study shows that a manipulation of an indirect cue of paternity can influence offspring survival, whereas a manipulation of a direct cue does not have such an effect. We show that male midshipman fish do not appear to use direct cues on which to solely base their parental investment decisions. Males showed no obvious preference for their own broods in either controlled choice tests in captivity or in transplant experiments in the field. Following a nest takeover, however, offspring survival declined in comparison with nests still under the care of the original parent. We also show that the presence of a caregiving guarder male is crucial for the survival of plainfin midshipman embryos. A key role of the male guarding a brood is to actively maintain the nesting cavity and to defend the brood against egg predators.

Why are direct cues not used for offspring recognition?

Plainfin midshipman guarder males do not appear to recognize their own offspring based on our manipulations of direct cues alone. Although guarding males were more likely to choose a nest that already contained embryos over an empty nest, when they were offered a choice between their own brood versus a foreign brood, males did not preferentially choose their own broods. Moreover, male abandonment rates and the survival of offspring did not differ between foreign transplanted offspring and control offspring, which remained with their original caregiver. The lack of response to direct offspring cues might mean that 1) plainfin midshipman males *cannot* identify their own offspring based on direct offspring cues alone or 2) plainfin midshipman males *can* detect their own offspring via direct offspring cues, but do not act on these cues. The choice not to act on a detected cue may occur when alternate sources of information about parentage, which we did not manipulate, are more reliable (see "evolved predispositions" in Neff and Sherman 2002). For example, if the probability of being cuckolded decreases over the breeding season, then males might rely on cues of paternity only early in the season when paternity is naturally more variable, and would not rely on these cues (i.e., have a predisposition to ignore these cues) later on when paternity is more certain. Cuckoldry and competition in midshipman fish are indeed more common early in the breeding season (Cogliati, Neff, et al. 2013; Bose, Cogliati, et al. 2014). However, all our experimental manipulations were conducted in the early season. Therefore, guarder males at this time would be expected to be least certain about their paternity and to be most attentive to potential cues of paternity loss.

It is also possible, if not likely, that caregivers assess *multiple* cues of paternity rather than just a single cue (Neff and Sherman 2002). For example, direct cues such as offspring odor may only be reliable indicators of paternity loss when they are also accompanied by a congruent indirect cue, such as the presence of a cuckolder in the nest during spawning. We did not control the presence of sneaker males near the experimental nests in this study. Interestingly, if direct cues are unreliable unless they are supplemented by an indirect cue, then parasitic cuckolder males can benefit. Cuckolding males that stealthily and successfully steal fertilizations without being detected, and thus do not elicit a reduction in paternal care by the cuckold, would have their offspring raised by an unwitting male. This is in line with the observation that the nests of plainfin midshipman fish in the wild display wide variability in mean paternity (Cogliati, Neff, et al. 2013). Average paternity lost to other males has been estimated to be between 26% and 48% across different studies (Cogliati, Neff, et al. 2013; Cogliati, Balshine, et al. 2014). Future studies are now needed to investigate the importance of single cues of paternity in isolation versus multiple cues in combination.

Are indirect cues important for offspring recognition?

Guarder midshipman males do use indirect cues to inform their assessment of paternity over offspring. The act of taking over another male's nest provides a male with a reliable indirect cue of paternity, and so males would be able to follow a simple behavioral rule: "assume that offspring in a newly acquired nest are non-kin." Our observation that allopaternal care following a nest takeover was associated with lowered offspring survival is consistent with take-over males adhering to such a rule. The higher rates of offspring mortality observed under allopaternal care could have been driven by a number of different factors, including cannibalism by the alloparent, deficient fanning and cleaning of the young by the alloparent, and/or a decrease in defense against embryo predation. Several potential egg predators observed around the intertidal zone would have been small enough to still access the nest through the mesh barrier, including *H. oregonensis*, *H. nudus*, and *Pagurus* spp. Consistently, Bose, Cogliati, et al. (2014)

recorded a strong likelihood for recent take-over males to have engaged in recent partial-brood cannibalism. Across disparate taxa, adopted offspring often receive less care than own offspring (e.g., African lions, *Panthera leo* L., Bertram 1975; fathead minnows, *Pimephales promelas*, Sargent 1989; Australian social spiders, *Diaea ergandros*, Evans 1998; spottail darters, *Etheostoma squamiceps*, Bandoli 2002).

Interestingly, nest takeovers in our study were not associated with complete termination of the offspring present in the nest. In a recent genetic study, Cogliati, Neff, et al. (2013) found that the oldest offspring in midshipman nests were commonly unrelated to the caregiver. The adoption of non-kin offspring has been documented in many animal taxa including mammals and birds (Riedman 1982), arthropods (e.g., Thomas and Manica 2005; Requena et al. 2013), and fishes (e.g., Rohwer 1978; Porter et al. 2002). Take-over males may be selected to still provide care for non-kin offspring if the alloparent receives a fitness benefit for continuing with care. For example, females may prefer to mate with males that are already caring for offspring (Coleman and Jones 2011). Females may have this preference if the presence of young in a male's nest indicates that he is a high-quality mate or parent, or if laying eggs where other offspring already exist dilutes their predation risk (Kraak 1996). This may explain take-over males' apparent tolerance for some non-kin offspring in their nests. It may also explain why the males in our nest-choice trials rejected empty nests in favor of nests that had eggs, and also preferred to take up nests that had larger broods. However, whether female plainfin midshipman fish display a preference for laying eggs where other eggs already exist still requires explicit testing.

In this study, we used a combination of lab and field studies to manipulate both direct and indirect cues of paternity loss in the nests of breeding guarder plainfin midshipman males. We expected guarder males to reduce parental effort in response to cues of lost paternity loss because 1) plainfin midshipman naturally and commonly experience lost brood paternity due to high rates of nest takeovers and cuckoldry (Brantley and Bass 1994; Cogliati, Neff, et al. 2013; Bose, Cogliati, et al. 2014) and 2) paternal care for non-kin offspring is costly, because care is prolonged and physiologically demanding (Bose, Cogliati, et al. 2014; Bose, McClelland, et al. 2015) and because nest space for eggs is limited (DeMartini 1991). We show that the act of taking over a brood from another male can be a reliable indirect cue of relatedness to that brood and that offspring survival was reduced following such nest takeover events. We also show that direct offspring cues are either undetectable by guarder males or ignored as unreliable sources of paternity information when presented in isolation.

5.6 Acknowledgments

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Chapter 6: General discussion

6.1 Thesis aims revisited

During my PhD, I aimed to: 1) describe further the reproductive ecology of the plainfin midshipman fish, specifically assessing various male traits and ecological factors that influence reproductive success in this paternal care-giving species; 2) describe the prevalence of offspring cannibalism in wild breeding plainfin midshipman males and identify environmental correlates of this behaviour; and 3) investigate multiple adaptive hypotheses for offspring cannibalism in this species. I achieved these goals by incorporating both experimental and correlational approaches in the laboratory and the field. In this final discussion chapter, I summarize my main findings, place my findings in a theoretical and empirical context, and propose possible directions for future research.

6.2 Thesis summary

The plainfin midshipman fish is studied for a variety of reasons in various branches of biology. It is a model system for studying fish bioacoustics and auditory neurobiology (e.g. Bass and Marchaterre 1989; Sisneros et al. 2004; Feng and Bass 2016), as well as for studying the ecology and evolution of male alternative reproductive tactics (e.g. Bass and Marchaterre 1989; Brantley and Bass 1994; Cogliati et al. 2014; Fitzpatrick et al. 2015). Numerous diet analyses of marine mammals and birds have established the important role plainfin midshipman fish play in coastal ecosystems (e.g. Elliott et al. 2003; Guertin et al. 2010; Luxa and Acevedo-Gutiérrez 2013). However, surprisingly little research has focused on understanding the factors that underlie reproductive success and parental care in this species. This is surprising because there are many unique aspects to their breeding biology. These include an unusually long parental care period (~3 months in total, Arora 1948; Cogliati et al. 2013; Bose et al. 2014) likely owing to their large egg sizes (Sargent et al. 1987), and an extensive annual vertical migration to breeding grounds in the highly dynamic intertidal zone (Hubbs 1920; Arora 1948; Miller and Lea 1972). Although early research such as Hubbs (1920), Arora (1948), and Crane Jr. (1965), provided us with descriptions and accounts of reproductive behaviours in the plainfin midshipman fish, it was not until later research conducted by Dr. Edward DeMartini (in the late 1980s) and Dr. Karen Cogliati (in the early 2010s) that we began to gain an understanding of the ecological factors that influence reproductive habits in this fish.

In my thesis, I have built upon the work of these two previous researchers to further characterize the reproductive ecology of the plainfin midshipman fish. In **Chapter 2**, I provided a detailed assessment of how a suite of male traits and nest traits correlate with male reproductive success. I showed in the field that both male and nest size are important correlates of male reproductive success. I also showed in the laboratory that females prefer spawning with large males over small males, but I could not detect any clear female preference for spawning in large nests over small nests. Males did show a preference for taking up large nests. Finally, in addition to male size and nest size, I identified several traits that appear to influence male reproductive success. In particular, I showed that males with a large swim bladder sonic apparatus attracted more females.

Furthermore, densely clustered nests attracted more females than isolated nests, and nests containing many space-competitor species attracted fewer females than nests with fewer space-competitor species.

Prior to my thesis work, the occurance of offspring cannibalism in the plainfin midshipman fish was only beginning to be recognized. Both Sisneros et al. (2009) and Cogliati et al. (2015) described a surprisingly high rate of offspring cannibalism in this species, but we still lacked an understanding of the factors that might select for this behaviour. Parent-offspring cannibalism is a particularly widespread phenomenon taxonomically (Polis 1981; Elgar and Crespi 1992; Manica 2002), yet we have a poor evolutionary understanding of this behaviour and few cases exist where multiple hypotheses have been tested in a single system (Klug and Bonsall 2007). This gap in our knowledge motivated my research in **Chapters 3**, **4**, and **5**. In **Chapter 3**, I intensively surveyed a wild spawning population of plainfin midshipman fish across their breeding season to characterize temporal patterns in cannibalism, identify environmental correlates of cannibalism, and direct my later research questions regarding specific cannibalism hypotheses. I showed that parent-offspring cannibalism occurred frequently in the early breeding season (~60% of males sampled in the early season had recently cannibalized embryos), but this behaviour declined as the season progressed and ceased altogether in the late season. This temporal pattern in cannibalism mirrored several other temporal patterns observed. First, the decreasing rates of cannibalism across the breeding season necessarily followed offspring age such that younger offspring were more likely to be consumed than older offspring. Second, female abundance declined across the breeding season, suggesting that cannibalism occurred when offspring were most likely to be replaced because reproductive females were still present in the intertidal zone. Third, caregiving male body condition also declined across the breeding season, suggesting that cannibalism occurred primarily when males were in their *best* body condition. Fourth, incidents of nest takeover also declined across the breeding season suggesting that cannibalism occurred when male-male competition was most intense in the early season. In **Chapter 4**, I tested the energy reserves hypothesis by comprehensively characterizing the endogenous energy stores of caregiving males. I demonstrated that males who had recently cannibalized offspring had greater energy stores than males who had not recently cannibalized. Furthermore, I showed that males who had recently taken over a nest were in better energetic condition than males who had spent time maintaining a nest and caring for offspring. Lastly, in **Chapter 5**, I investigated several possible cues that males may use to assess their paternity over a brood of offspring. I showed that males are unlikely to use direct offspring cues and instead appear to use indirect cues, such as a successful nest takeover, to gauge their paternity over a brood and adjust their parental investment and offspring cannibalism behaviours accordingly. In the remainder of this Discussion chapter, I discuss my work in the context of other research in the field and propose topics where future research is needed.

6.3 Evaluating the energy reserves hypothesis in the plainfin midshipman

Rohwer (1978) proposed that parents may receive a net reproductive benefit by cannibalizing offspring if alternative food sources are unavailable. Offspring cannibalism could minimize deterioration of body condition that would otherwise compromise the quality or quantity of care that a parent could provide. Thus, even filial cannibalism can be favoured if the future benefits of immediate energy gain outweigh the costs of current progeny loss. This ‘energy-reserves hypothesis’ has since been evaluated in a variety of taxa, however largely mixed results have been uncovered (Manica 2002; Klug and Bonsall 2007). My primary contribution to the empirical literature was to evaluate this hypothesis from a mechanistic and physiological perspective. All previous studies had taken one of two possible approaches: either artificially manipulating the diet of caregivers and thus sacrificing some ecological validity, or correlating cannibalism with coarse metrics of body condition that are indirect and sometimes poor measures of endogenous energy stores (Peig and Green 2009). In **Chapter 4** I used natural variation in caregiver body condition and correlated cannibalism with fine-scale measures of energy reserves including liver glycogen, lipid stores, and muscle protein content. This mechanistic approach allowed me to critically evaluate if offspring cannibalism was associated with the metabolic transition to a particular phase (i.e. severity) of starvation. Such an approach has also never been undertaken in the literature, and I hope these tools will be used in future studies to critically assess the energy-reserves hypothesis. Using this approach is especially important for future research because my **Chapter 4** results were in direct opposition to the predictions of the energy-reserves hypothesis. I found cannibals actually had greater energy stores than non-cannibals, suggesting that offspring are not consumed for the purpose of replenishing dwindling energy supplies.

Overall, my results from **Chapter 3, 4** and **7** converge and suggested that the energy-reserves hypothesis is unlikely to explain why caregiving male plainfin midshipman fish engage in offspring cannibalism. In **Chapter 3**, I showed that males primarily engage in offspring cannibalism early in the breeding season when they are in their best overall condition. In **Chapter 4**, I showed that cannibal males are in better condition than noncannibal males. In **Chapter 7**, I showed that the number of offspring a male consumes increases with body condition. Interestingly, Mehlis et al. (2009) and Gomagano and Kohda (2008) found similar results in three-spine sticklebacks, *Gasterosteus aculeatus*, and long-snout clingfish, *Diademichthys lineatus*, respectively, where males in better condition consumed more offspring. The authors suggested that partial-brood cannibalism allows males to maintain sufficient body condition to defend and retain a nest. Thus, a form of the energy-reserves hypothesis might still be supported in the plainfin midshipman fish if it can be shown that males consume offspring in the early season to pre-emptively offset the energetic costs of the parental care period to come. This idea could be tested in the plainfin midshipman fish by experimentally manipulating the predicted future energetic costs of parental care, perhaps by increasing the abundance of competitors and egg predators thereby raising the need for nest defense males, or by providing alternate food sources thereby reducing foraging costs, or by adjusting dissolved oxygen levels thereby increasing the demand for embryo fanning.

In the discussion section of **Chapter 4** I briefly mention two non-mutually exclusive explanations for why the cannibals in my sample were in better condition than the non-cannibals, and I will further expand on these explanations here. First, it is possible that in my comparison of cannibals versus non-cannibals, some of the males classified as cannibals had incidentally consumed one or two offspring while conducting natural cleaning and nest maintenance behaviours. Video footage taken of males engaging in parental care within their nests revealed that males spend a considerable amount of time fanning and flushing embryos with water from their mouths (A Bose personal observations). It is conceivable that the embryos become dislodged and are consumed as a result of this activity. Thus, if some males had incidentally consumed offspring during nest maintenance without any energetic need to do so, I would have still labelled them as cannibals and thereby inflated my measures of energy reserves for cannibal fish. However, in **Chapter 7**, I analyzed a much larger sample of males than in Chapter 4 and showed that the number of offspring consumed increased with a coarse metric of male body condition, which is a result that is not easily explained by the incidental consumption of a few eggs during nest maintenance. Further studies would be required to investigate whether male body condition is related to vigour of nest maintenance.

A second possible explanation for why the cannibals in my sample were in better condition than the non-cannibals is nest take-overs. Although I removed all *known* take-over males from my comparison of cannibals versus non-cannibals, it is still possible that some of the cannibal males in my sample had taken over their nest, but did so before my observation of their nest began. Thus, any signature of low energy reserves could have been masked by take-over males in good body condition consuming non-kin offspring. Future studies could eliminate the potentially confounding effects of reduced paternity by performing genetic paternity tests and statistically controlling for relatedness or by monitoring parental care and cannibalism under laboratory conditions where paternity can be carefully controlled.

6.4 Evaluating the low paternity hypothesis in the plainfin midshipman

Cannibalism as a means of investing into future reproduction is expected to occur in situations when the parent's current parental investment is low (Sargent 1992). When males suffer paternity loss, they are expected to reduce their paternal investment into a brood (Owens 1993; Westneat and Sherman 1993) and should therefore be more likely to cannibalize offspring (Neff 2003a; Gray et al. 2007). However, to date the empirical work linking paternity to paternal investment (Kempenaers and Sheldon 1997; Sheldon 2002; Alonzo 2010; Griffin et al. 2013), or paternity to offspring cannibalism (e.g. Svensson and Kvarnemo 2007; Félix et al. 2016) has yielded mixed results. While males in many species reduce their paternal care in response to cues of paternity loss, many others appear highly tolerant to paternity loss and do not alter their behaviour (Alonzo 2010). Theory predicts that males should be able to dynamically adjust their paternal investment in relation to paternity when three assumptions are satisfied (outlined below, Westneat and Sherman 1993; Griffin et al. 2013). I have evaluated each of these three assumptions in the plainfin midshipman fish over the course of my PhD research, and my work strongly suggests that paternal care should vary positively with brood paternity in this species.

The first assumption that needs to be satisfied for paternal investment to vary with paternity is that paternal care must be costly, such that investment in current offspring diminishes the ability of the male to invest into future reproduction. In **Chapter 4**, I revealed that the energetic costs of parental care in the plainfin midshipman fish are high. Although males consume small amounts of food items found around their nests while caring (Cogliati et al. 2015), I showed that by the third month of parental care, males are emaciated and have begun to catabolize muscle proteins (i.e. have entered phase III starvation). However, measuring parental investment in the wild is notoriously difficult (Smiseth et al. 2012), and although I did not directly measure parental investment (i.e. the fitness costs associated with parental expenditure), it is probable that the severity of the energetic costs diminishes a male's likelihood of surviving to the next breeding season. It would be useful for future work to identify reliable biomarkers associated with poor body condition that can be sampled non-invasively (e.g. blood hematocrit, plasma ammonia, or plasma glucose). Researchers could measure these markers for a large number of males at the end of their parental care period to quantify variation in end-of-season body condition. Then, by implanting males with acoustic telemetry tags, these fish could be monitored while they over-winter in the deep ocean. End-of-season body condition could be related to a male's probability of surviving over winter, returning to a spawning ground the next year, and their reproductive success in the subsequent breeding season.

The second assumption that needs to be satisfied for paternal investment to vary with paternity is that paternity needs to vary between reproductive bouts. Cogliati et al. (2013, 2014) revealed that brood paternity is highly variable across nests in this species. In **Chapter 7**, I contributed further genetic paternity data, and revealed that brood paternity is variable across nests, ranging from 0 – 100%. Furthermore, in **Chapter 2** I showed evidence that nest size can constrain the total number of embryos a male acquires. This space limitation in the nest implies that non-kin offspring should be particularly costly to the caregiver, because they are occupying valuable space that could otherwise be used for kin offspring.

The third assumption that needs to be satisfied for paternal investment to vary with paternity is that caregivers need to have access to cues that reliably predict their relatedness to the offspring. Over my body of work, I have demonstrated multiple times that nest takeovers can be a reliable indirect cue of offspring paternity. In **Chapter 5**, I demonstrated that offspring survival decreases when under the care of a take-over male (i.e. an alloparent) compared to the care of a genetic parent. In **Chapter 3**, I revealed that males that had recently taken over a nest were more likely to also have recently cannibalized offspring, indicating take-overs may trigger cannibalistic behaviour. In **Chapter 7**, I showed that the most cannibalistic males in the population also had exceptionally low brood paternities, suggestive of nest takeovers. Interestingly, in **Chapter 8**, I showed that offspring cannibalism also frequently occurs following group takeovers in the African cichlid, *Neolamprologous pulcher*. Offspring cannibalism following the takeover of a new nest or territory may be a taxonomically widespread phenomenon as it has also been recently reported in poison frogs, *Allobates femoralis* (Ringler et al. 2017).

By evaluating each of the three assumptions required for males to dynamically adjust their paternal investment in response to paternity loss, my PhD work has shown that the plainfin midshipman fish is an excellent system in which to study the relationship between paternity and paternal care. While I have shown that males base parental investment decisions on indirect cues such as nest takeovers, my next step will be to investigate whether males cannibalize more offspring when confronted with other indirect cues such as the presence of cuckolder males in the vicinity of their nest during spawning.

6.5 Evaluating other cannibalism hypotheses in the plainfin midshipman

During my PhD, I focused my efforts on evaluating two cannibalism hypotheses in particular, the 'energy-reserves hypothesis' and the 'low paternity hypothesis'. However, many other, non-mutually exclusive hypotheses remain to be tested in the plainfin midshipman fish. In fact, my results from **Chapter 3** lend preliminary support to two additional hypotheses for parent-offspring cannibalism that both warrant further study.

First, offspring cannibalism in the plainfin midshipman fish coincides with the period of the breeding season when females are most abundant. Kondoh and Okuda (2002) suggest that offspring cannibalism can be favoured when mating opportunities are plentiful and consumed offspring can be quickly replaced. This hypothesis has since garnered support in a freshwater goby (*Rhinogobius flumineus*, Okuda et al. 2004; Myint et al. 2011; Takeyama et al. 2013) and mouthbrooding cardinal fishes (*Apogon doederleini* and *A. niger*, Okuda and Yanagisawa 1996; Okuda 1999). Testing this hypothesis in the plainfin midshipman could involve an experimental approach wherein male parental care and cannibalism would be monitored under various regimes of female availability.

Second, because plainfin midshipman offspring take ~2 months to develop during a 3-month breeding season and the frequency of cannibalism declines across the season, it follows that cannibalism primarily targets younger rather than older offspring. In fact, the majority of offspring found in the stomachs of caregiving males were unhatched embryos, under 30 days old (A Bose personal observations). A similar preference to cannibalize younger, pre-hatch offspring as opposed to older, post-hatch offspring has also been found in bluegill sunfish, *Lepomis macrochirus* (Neff 2003b). Vallon and Heubel (2016) suggest that older offspring should be more valuable to a caregiver, because less parental effort is required to raise them to independence. These researchers showed that when given the choice, male common goby, *Pomatoschistus microps*, prefer to consume younger rather than older offspring, and this relationship has been observed in other fishes as well (e.g. sand goby, *Pomatoschistus minutus*, Klug and Lindström 2008). Plainfin midshipman males are often found caring for broods comprised of multiple age cohorts of offspring (Demartini 1988; Brantley and Bass 1994; Cogliati et al. 2013; Figure 6.1). Testing the hypothesis that younger offspring are preferentially consumed over older offspring in the plainfin midshipman fish should involve a careful assessment of which cohorts, if any, are preferentially eaten while controlling for possible confounds such as cohort paternity or quality.

Other factors that may influence offspring cannibalism in the plainfin midshipman fish include brood size, age of parent, and offspring mortality risk. Brood sizes in plainfin midshipman range from ~10 to over 3000 embryos (**Chapter 2**), so the costs of cannibalizing individual offspring from a small brood will be higher than the costs of consuming individual offspring from a large brood (Manica 2002), although the benefits would presumably be identical. Caregiving males also vary widely in body size (standard length range = 10.7 – 30.5 cm), and presumably also in terms of age, suggesting that male midshipman are iteroparous and breed over multiple seasons. If cannibalism represents an investment in future reproduction, then older parents should also be comparatively less cannibalistic than younger parents because current offspring represent a larger portion of the older parent's reproductive value (Williams 1966; Clutton-Brock 1984). Offspring cannibalism is also expected to increase when the risk of offspring mortality is high. Cannibalism can either divert energy from doomed offspring to more certain future broods (e.g. predation risk, Lindstrom 1998; Huang 2008) or it can serve to improve the survival probability of any remaining offspring (e.g. fungal infection risk at high offspring densities, Lehtonen and Kvarnemo 2015). In general, offspring cannibalism is thought to be affected by any factors that influence the reproductive value of current offspring relative to a parent's residual reproductive value (Manica 2002), which generates a wealth of possible research avenues for future cannibalism studies.

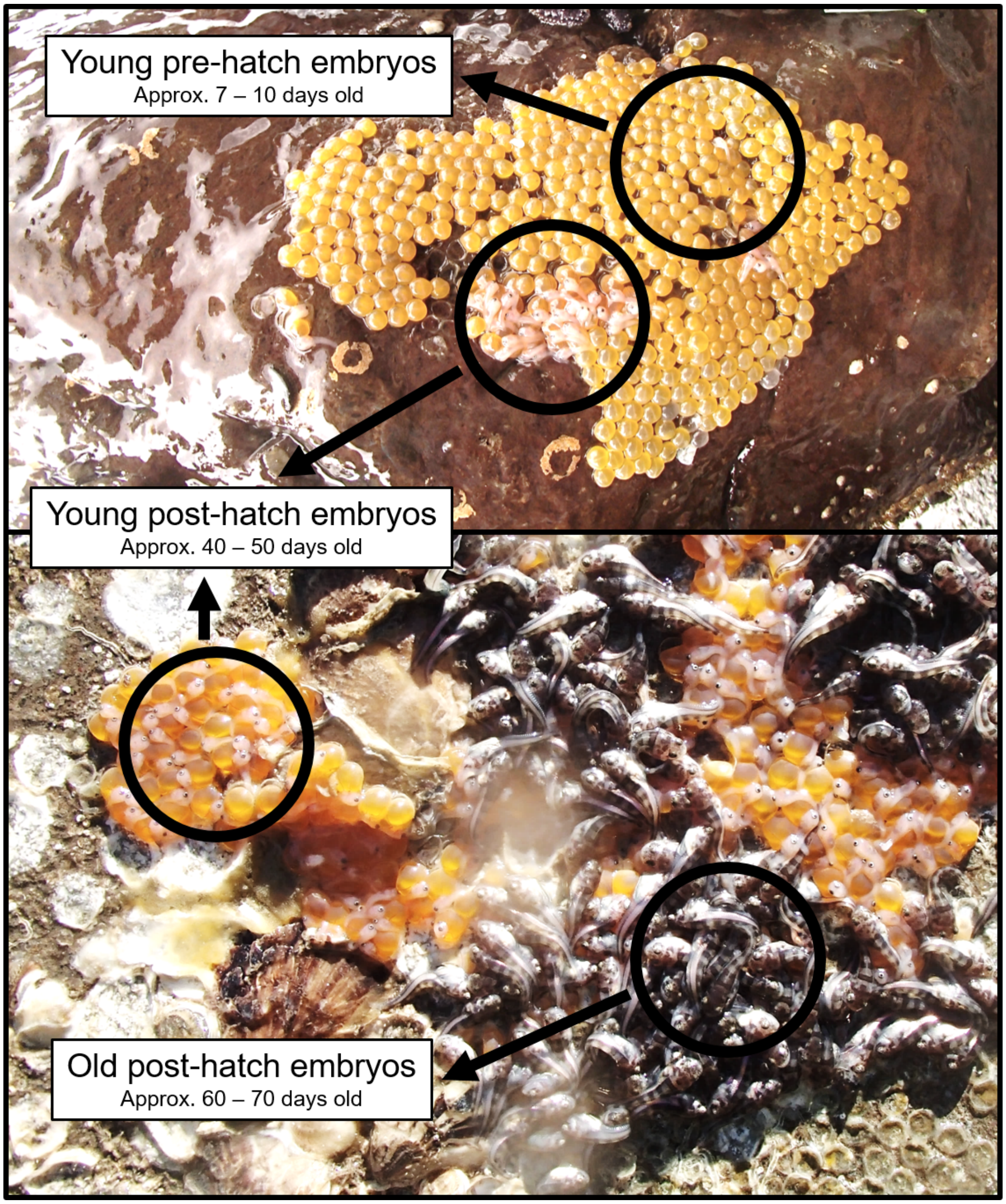

Figure 6.1 Digital photographs of plainfin midshipman nests depicting multiple developmentally distinct cohorts of offspring.

6.6 Non-cannibalism topics of future research with the plainfin midshipman

Over the course of my PhD, my thesis work has also raised several questions related to female mate choice and male alternative reproductive tactics, which would be fruitful topics of future research.

i. Do females prefer to spawn in nests with offspring already present?

In **Chapter 5** I showed that offspring survival decreased following a nest takeover in comparison to when the original parent remained with their brood. However, an interesting finding was that not all the original offspring were consumed or died following the nest takeovers. One month after the takeovers occurred, ~15% of the original offspring were still alive under the care of the new male, who had by that time acquired new offspring of his own. Cogliati et al. (2013) also found genetic evidence of nest takeovers in the wild, showing that the oldest offspring in many nests were often unrelated to their caregivers. Why do plainfin midshipman males retain and provide alloparental care for some original offspring, despite the presumed costs of allowing non-kin offspring to occupy valuable nest space? One possible explanation for the continued care by males is that females might prefer to spawn in nests where offspring are already present. Females that spawn with a male that is already caring for offspring may gain a number of benefits, including diluting the predation risk to her offspring, ensuring additional parental care for her offspring if care increases with brood size, or increasing the probability of spawning with a high-quality male (Kraak 1996). Future studies should investigate this possibility and test whether female mate choice is influenced by the presence of offspring in a male's nest. This could be accomplished by conducting a series of controlled female choice trials in the laboratory, where gravid females choose between two size-matched males in individual nests, one possessing non-kin embryos and the other defending an empty nest. Revealing such a benefit during mate choice could explain why takeover males appear to spare some of the original brood.

ii. Do males engage in nest piracy as an alternative reproductive tactic?

The fact that following a nest takeover event, male plainfin midshipman fish will provide alloparental care for some of the previous nest resident's offspring, opens the exciting possibility for an additional male alternative reproductive tactic: nest piracy. Nest piracy occurs when some males, typically the largest and more competitive males, benefit by repeatedly taking over nests, spawning with females, and then abandoning those offspring to be cared for by other males (van der Berghe 1988). This tactic has been described in the peacock wrasse, *Symphodus tinca* (van der Berghe 1988) and African cichlids, *Telmatochromis temporalis* (Mboko and Kohda 1999) and *Telmatochromis vittatus* (Ota and Kohda 2006). A piracy tactic may confer fitness benefits to the largest plainfin midshipman males in a population that are able to takeover and spawn in multiple nests, and then exploit the parental efforts of subsequent males after abandoning the nest. Non-pirate take-over males may use the pirate's sired offspring to attract new females for themselves. Our field observations of breeding plainfin midshipman fish in **Chapter 3** revealed that nest ownership changes frequently early in the breeding season, but interestingly, no consistent body size differences could be detected between new nest residents (i.e. take-over males) and the original nest owners. If nest take-overs were solely the result of competitive contests, I would expect take-over males to be consistently larger than the previous nest residents. However, such a relationship might be obscured if the largest males willingly abandon nests that are then taken up by smaller males. Testing for a nest piracy tactic would require tracking the movements, spawning behaviours, and reproductive success of males in and around nests within the intertidal zone over the course of a breeding season. This could perhaps be achieved by incorporating acoustic telemetry, radio telemetry, or passive integrated transponder (PIT) tracking with genetic paternity testing of offspring in nests.

6.7 Conclusions

Throughout my PhD thesis, I have taken an integrative research approach to uncover factors that affect reproduction, parental care, and offspring cannibalism in the plainfin midshipman fish. I have done this by combining correlational and experimental work in both the field and laboratory involving ecological, genetic, physiological, and behavioural techniques. My thesis work has several important implications. First, I conducted a detailed study to disentangle the factors that affect male reproductive success, a challenging topic in evolutionary ecology research. By showing that resource quality (i.e. nest size) can constrain the effect of male quality (i.e. male size) and influence components of male reproductive success, I showed the importance of exploring interactions between factors expected to influence reproductive success. I suggested that the effects of male quality and resource quality should not be assumed to be independent of one another. Second, I carefully tested two hypotheses: that parent-offspring cannibalism is used to replenish depleted parental energy reserves and that paternal care should decrease with paternity loss. Throughout my work, I found that offspring cannibalism is not driven by immediate energetic need. I also showed that the plainfin midshipman fish meets the theoretical requirements for males to dynamically adjust their paternal care in response to paternity loss, making this species a useful system for future research on paternity and paternal care. Lastly, my work uncovered multiple avenues for future cannibalism research in the plainfin midshipman fish, highlighting the usefulness of studying multiple cannibalism hypotheses in a single study system.

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Appendix

Chapter 7: Offspring cannibalism is related to low paternity in an intertidal fish

7.1 Abstract

Parental care is costly and theory suggests that caregivers should reduce parental investment or even cease caring altogether when the costs are too high relative to the benefits. Brood cannibalism is one tactic by which parents can divert energetic investment away from low-quality current offspring and towards potentially higher-quality future offspring. Here, we investigated whether partial brood cannibalism is a response to low genetic relatedness between caregiver and offspring in the plainfin midshipman fish, *Porichthys notatus,* a marine toadfish. We first characterized the prevalence of partial brood cannibalism in nesting *P. notatus*, and show that that about half of all nesting males in our population had embryos in their stomachs. The number of embryos consumed decreased over the breeding season and, surprisingly, males in better body condition consumed more embryos. Males that had recently consumed a large number of embryos (i.e. males that exhibited high degrees of brood cannibalism) had low nest paternities suggesting that they had recently taken over their nests. However, the consumption of a small number of embryos appeared to occur independent of mean nest paternity. We discuss our results in terms of the costs and benefits of alloparental care and the factors that underlie partial brood cannibalism.

7.2 Introduction

Parental care and offspring cannibalism frequently co-occur. These apparently opposing behaviours appear together in a surprisingly large number of species in a wide range of phylogenetically distinct taxa (Polis 1981; Elgar and Crespi 1992; Klug and Bonsall 2007). While parental care promotes the survival and development of progeny (Smiseth et al. 2012), cannibalism results in their termination, and therefore these two behaviours (care and cannibalism) often appear to work in opposition to one another. Yet cannibalizing some or all offspring can be adaptive, providing fitness benefits to parents under specific circumstances (Elgar and Crespi 1992; Manica 2002; Payne et al. 2004; Klug and Bonsall 2007). While cannibalism of entire broods can be viewed as a decision by the parent to focus and invest into future reproduction, cannibalism of partial broods can be viewed either an investment to enhance future reproduction or as a means to fuel care for the remaining current offspring (Rohwer 1978; Sargent 1992). Theory suggests that parents should completely cannibalize all their current offspring when they are not worth the parental effort needed to raise them (Clutton-Brock 1991; Gross 2005). That is, parents should terminate care whenever the reproductive value of a current brood is too low to be worth the fitness costs associated with parental effort. The phenomenon of ending care by complete-brood cannibalism has been well-studied in fishes. It is common for small broods to suffer a higher risk of total cannibalism compared to large broods, as seen in fathead minnows, *Pimephales promelas* (Sargent 1988), damselfishes, *Stegastes dorsopunicans* and *Microspathodon chrysurus* (Petersen 1990), sand gobies, *P. minutus* (Forsgren et al. 1996), and bluegill sunfish, *Lepomis macrochirus* (Neff 2003). Frommen et al. (2007) and Mehlis et al. (2010) both showed that male three-spined sticklebacks, *Gasterosteus aculeatus* are more likely to completely cannibalise broods under conditions of low rather than high paternity.

While we have better understanding of complete brood cannibalism, the driving factors behind partial brood cannibalism are far less clear (Smith 1992; Manica 2002). One hypothesis suggests that caregivers may cannibalize a portion of their young as a means for energy acquisition (Rohwer 1978). Support for this idea has been found in some fishes such as three-spined sticklebacks, *G. aculeatus* (Mehlis et al. 2009) and bluegill sunfish, *L. macrochirus* (Neff 2003), insects such as assassin bugs, *Rhinocoris tristis* (Thomas and Manica 2003) and maritime earwigs, *Anisolabis maritima* (Miller and Zink 2012), and in some reptiles, such as Colombian rainbow boas, *Epicrates cenchria maurus* (Lourdais et al. 2005). A second hypothesis states that when offspring survival is density dependent, parents cannibalize some of their young when resources are limited so as not to jeopardize the whole brood (e.g. Bartlett 1987; Payne et al. 2004). Support for this hypothesis comes from burying beetles, *Nicrophorus vespilloides* (Bartlett 1987), beaugregory damselfish, *Stegastes leucostictus* (Payne et al. 2002), and sand gobies, *Pomatoschistus minutus* (Lissåker et al. 2003). A third hypothesis posits partial-brood cannibalism tagets offspring with low reproductive value (Hoelzer 1988). For example, male sphynx blennies, *Aidublennius sphynx*, are known to cannibalize dead or diseased offspring from their broods (Kraak 1996), and male assassin bugs, *R. tristis*, selectively consume offspring with the highest probability of being parasitized by wasps (Thomas and Manica 2003). A fourth hypothesis states that large broods are more susceptible to partial brood cannibalism because the cost of consuming individual eggs decreases proportionally with brood size (Hoelzer 1995); however, empirical support for this relationship has been scarce (Manica 2002). In fact, overall empirical support for adaptive hypotheses regarding partial brood cannibalism has been more mixed than for complete brood cannibalism, without considerable support for any one hypothesis (Manica 2002).

Genetic relatedness strongly affects the reproductive value of offspring, and so paternity is expected to strongly influence parental care (Westneat and Sherman 1993) and brood cannibalism (Manica 2002). However, to date the empirical support for a relationship between paternity and paternal care has been remarkably ambivalent (Sheldon 2002; Alonzo 2010). For example, a review by Alonzo (2010) revealed that paternal care decreases in response to lowered paternity in roughly only half of the documented studies. Theory suggest that parents should reduce parental investment in response to lower certainty of parentage when the following three conditions are met: 1) care imposes costs on parental residual reproductive value; 2) parentage either varies between offspring within a reproductive bout or varies between reproductive bouts; and 3) caregivers have access to reliable sources of information to predict their relatedness to the offspring (Westneat and Sherman 1993). Using a meta-analysis approach Griffin et al. (2013) provided support for these conditions showing that the species most likely to reduce parental investment in the face of reduced paternity cues are those that have both costly parental care and high variance in paternity. However, the relationship between partial brood cannibalism and brood paternity varies among studies that seemingly satisfy these theoretical conditions (e.g. Neff 2003; Manica 2004; Gray et al. 2007; Svensson and

Kvarnemo 2007; Lissåker and Svensson 2008; Mehlis et al. 2010). To date relatively few such studies have been conducted and certainly, further empirical study of the relationship between paternity and partial brood cannibalism is needed.

In this study, we investigated the prevalence of partial brood cannibalism across populations of plainfin midshipman fish, *Porichthys notatus*. We studied how brood cannibalism relates to male body size, body condition, and brood size, and then more specifically investigated how it relates to average brood paternity. *P. notatus* is a convenient species to study the relationship between paternity and paternal care because care in this species is costly (Bose et al. 2014; Bose et al. 2015; Bose et al. 2016) and males experience high variance in paternity between reproductive bouts (ranging from 0-100% paternity) and low overall nest paternity (52 ± 4%, mean ± se, Cogliati et al. 2013). Some of this variance in paternity results from aggressive nest take-overs (Cogliati et al. 2013; Bose et al. 2014). Nesting males appear to use the act of taking over a nest as a reliable cue of brood paternity (Bose et al. 2016) and embryos are more frequently cannibalized early in the breeding season, when nest take-overs are most prevalent (Bose et al. 2014; Cogliati et al. 2015). Males also engage in intense competition over egg fertilization (i.e. sperm competition, Fitzpatrick et al. 2015), frequently employing cuckoldry tactics (Brantley and Bass 1994; Lee and Bass 2004; Cogliati et al. 2013). In this study, we use genetic paternity analyses to investigate whether males engage in offspring cannibalism more frequently when their overall brood paternity is low and thus when the putative benefits of offspring care are low.

7.3 Methods

Study Species

The plainfin midshipman fish, *Porichthys notatus*, is a marine toadfish distributed along the west coast of North America (Arora 1948; Miller and Lea 1972; Walker and Rosenblatt 1988). At the onset of the breeding season (late April – early August) male fish migrate from depths to the shallow intertidal zone and excavate nesting cavities beneath large intertidal rocks. From these nests, males acoustically court females (Ibara et al. 1983) that may lay a monolayer of eggs on the roof or underside of their selected mate's nest (Arora 1948; Demartini 1988). Suitable nesting sites are limited (Demartini 1988) and males compete intensely with one another over nest-ownership often resulting in nest take-over events wherein one male is forcefully ousted from his nest by another male, which then acquires any present embryos (Bose et al. 2014). Cogliati et al. (2013) used a paternity threshold of less than 10% to indicate whether a nest had been taken over by a new male fish. Male plainfin midshipman fish also engage in cuckolding behaviours via sneak or satellite spawning (Brantley and Bass 1994). Indeed, adult males express one of two well-characterized fixed alternative reproductive tactics (Brantley and Bass 1994; Bass et al. 1996; Lee and Bass 2004; Cogliati, Balshine, et al. 2014). Type I or conventional males, also called guarder males, are large, build and fight for nests, court females, and care for offspring, while Type II or parasitic males, also called sneaker males, are small, do not build or fight for nests, court females, or care for offspring (Brantley and Bass 1994). Both sneaker and guarder males can gain reproduction through cuckoldry (Cogliati et al. 2013; Cogliati, Balshine, et al. 2014), but while sneaker males are obligate cuckolders, guarder males only use cuckolding when they have no nest of their own or have no offspring in their nest (Brantley and Bass 1994; Lee and Bass 2004; Cogliati, Balshine, et al. 2014).

Field collections

In 2010, 2011 and 2013, between May and July, we located 299 plainfin midshipman nests in the intertidal zones of British Columbia, Canada (see Cogliati, Mistakidis, et al. (2014) for more detailed information on field sites). Nests were located by gently lifting intertidal rocks to expose any fish in their nesting cavity. We identified active nests if we found an excavated nesting cavity, a guarder male, and a brood of eggs beneath an intertidal rock. All fish located in these nests were measured for standard length (to the nearest 0.1cm) and body mass (to the nearest 0.1g), broods of embryos were digitally photographed and the embryos were later counted from the images using the software ImageJ (v1.45). Male body condition was calculated using the residuals from a regression of ln(total body mass) against ln(standard length). We also removed and preserved in 70% ethanol a small (~2x2cm) clip from each male's caudal fin for microsatellite genotyping, and also sampled ~40 embryos from every developmentally distinct cohort in the nest, preserving these embryos in ethanol for paternity testing. To quantify the number of embryos consumed, the males were euthanized using an overdose of benzocaine (>250 mg/L for 3 min) followed by cervical severance and dissected to inspect stomach contents. A previous study quantified digestive tract evacuation rates reporting that embryos found in the guts were almost always consumed within the 24-

hour period prior to dissection (Bose et al. 2015). Embryo counts were not possible via gastric lavage techniques and all of the euthanized males were used in a number of other studies (Cogliati et al. 2013; Bose et al. 2014; Cogliati, Balshine, et al. 2014; Cogliati, Mistakidis, et al. 2014; Bose et al. 2015).

Lab Analyses

We categorized the dissected guarder males into three groups based on the number of embryos that we found in their guts: 'Non-cannibals' were males that were found to have eaten no embryos, 'low cannibals' had eaten a few embryos (1-4 embryos), and 'high cannibals' had eaten a large number of embryos (≥ 5 embryos). We chose the threshold criterion that differentiated 'high' vs. 'low' levels of cannibalism based on results from Bose et al. (2014), which found that guarder males had on average 4 ± 10 embryos (mean ± SD) in their guts across the whole breeding season (range = 0 – 58).

We haphazardly chose a subset of males that fit into the 'non' (N = 5), 'low' (N = 8), and 'high' (N = 7) cannibalism groups (total N = 20). The chosen males all needed to have embryos that were at least two weeks of age in their nests to ensure that the embryos were sufficiently developed to sample enough tissue to extract DNA (extracting sufficient quantities of DNA from younger eggs is exceedingly difficult, M Lau and K Cogliati personal observations). We extracted DNA from the fin clips of these 20 males and from a total of 586 offspring under their care following methods outlined in Suk et al. (2009). Briefly, the mean number of offspring extracted per male was 27.9 ± 15.3 (mean ± SD, range = 13 – 77). Males were found to be caring for an average of 1.5 ± 0.7

developmental cohorts, and we randomly selected offspring from each cohort to extract DNA from (17 ± 7, mean no. offspring extracted per cohort ± SD, range = 5 – 27). The males and offspring were genotyped using five polymorphic microsatellite loci identified by Suk et al. (2009). We carried out PCR amplification using a T1 thermocycler (Montreal Biotech Industries), followed by fragment analysis at the NAPS unit in the University of British Columbia. Microsatellite alleles for each locus were scored for each individual based on characteristic peaks. We used the 2-sex model developed by Neff et al. (2000a,b; Neff 2001) to estimate mean nest paternity for each male (see Cogliati et al. 2013 for further details). We calculated the mean nest paternity for each nest by averaging the paternities for each cohort of offspring and weighting them by their respective cohort size (i.e. embryo counts).

Statistical analyses

All analyses were conducted in R (v3.3.1). We first used the complete dataset (N = 309 guarder males) to investigate whether the observed degree of embryo cannibalism (i.e. the number of embryos found in the digestive tracts) was related to guarder male size, body condition, or brood size. To do this we fit a generalized linear mixed effects model specifying a negative binomial error distribution to account for overdispersion (nbGLM; glmmADMB package, Skaug et al. 2014). The number of offspring consumed was included as the response variable, while male standard length, body condition, brood size, Julian date, and year were included as fixed effects, and field site was included as a random intercept.

To test whether mean paternity of males differed across our cannibalism groups we conducted a linear model. We included mean nest paternity in the model as the response variable, cannibalism group as the fixed factor, and male body condition as a covariate. Due to heteroskedasticity between the cannibalism groups we used a randomization test to construct a null distribution against which we would test our observed test-statistic and obtain accurate p-values (Good 2005). We first tested whether mean nest paternity differed among the cannibalism groups. We did this by repeatedly extracting the t-statistic from each pairwise comparison between the groups for 10,000 random permutations of the data (rearranging the labels for mean nest paternity among samples). We also tested for an effect of the covariate, body condition, by repeatedly extracting its t-statistic from the linear model over the same 10,000 random permutations of the data. P-values were computed as the fraction of t-statistics for the permuted data that were greater than or equal to the observed t-statistics.

7.4 Results

Of the 299 guarder males sampled, the mean number of embryos consumed was 5.0 ± 10 (mean ± SD, range = 0 – 58). We found that ‘non-cannibals’ comprised 54% of all sampled males (or N = 161 males had zero embryos in their guts), ‘low cannibals’ comprised 20% of all sampled males (or N = 61 males had 1-4 embryos in their guts, mean ± SD = 1.5 ± 0.5 embryos), and ‘high cannibals’ comprised 26% of all males (or N = 77 males had ≥ 5 embryos in their guts, mean ± SD = 24.9 ± 14.9). We found that the number of embryos detected in male guts decreased as the breeding season progressed (nbGLM, $z = -6.29$, $p < 0.0001$). Males in better body condition consumed more embryos ($z = 3.56$, $p < 0.001$), however we detected no effect of male body length ($z = 1.16$, $p = 0.25$; mean standard length ± SD = 20.7 ± 3.4cm, range: 12.2 – 29.5cm) or brood size ($z = -1.88$, $p = 0.06$; mean embryo number ± SD = 530 ± 426 embryos, range: 7 – 3147 embryos) on the number of embryos detected in the gut contents.

Of the 20 males whose nests were genotyped, five males classified as non-cannibals because they had consumed zero embryos, eight males were classified as low cannibals because they consumed between one and four embryos (mean ± SD, 1.5 ± 0.5 embryos, and the seven males were high cannibals because they had consumed over five embryos (mean ± SD, 24.9 ± 14.9 embryos). On average, these 20 males had sired a mean (± SD) of 38.4 ± 34.1% of the offspring in their nests (range = 0 – 100%), suggesting that many of these males were take-over males. Males in the high cannibalism group were on average related to only 7 ± 17% (mean ± SD) of the embryos in their nests, and many of them fall below the 10% threshold that identifies cases of nest take-over (see Cogliati et al. 2013). In contrast, the males in the low cannibalism group were on average related to 55 ± 35% (mean ± SD) of the offspring under their care, while males in the non-cannibalism group had a mean paternity of 58 ± 23%. The males in the high cannibalism group were on average less related to offspring under their care than were males in the low cannibalism group (permutation test, $t = -2.95$ $p = 0.011$), and males in the no-cannibalism group (permutation test, $t = -2.81$, $p = 0.013$; Fig. 7.1). Males in the low cannibalism and in the non-cannibalism group did not differ from each other in terms of how related they were to the young in their nests (permutation test, $t = -0.19$, $p = 0.85$).

We found that male body condition did not relate to mean nest paternity in this smaller dataset (permutation test, t = -0.85, p = 0.40).

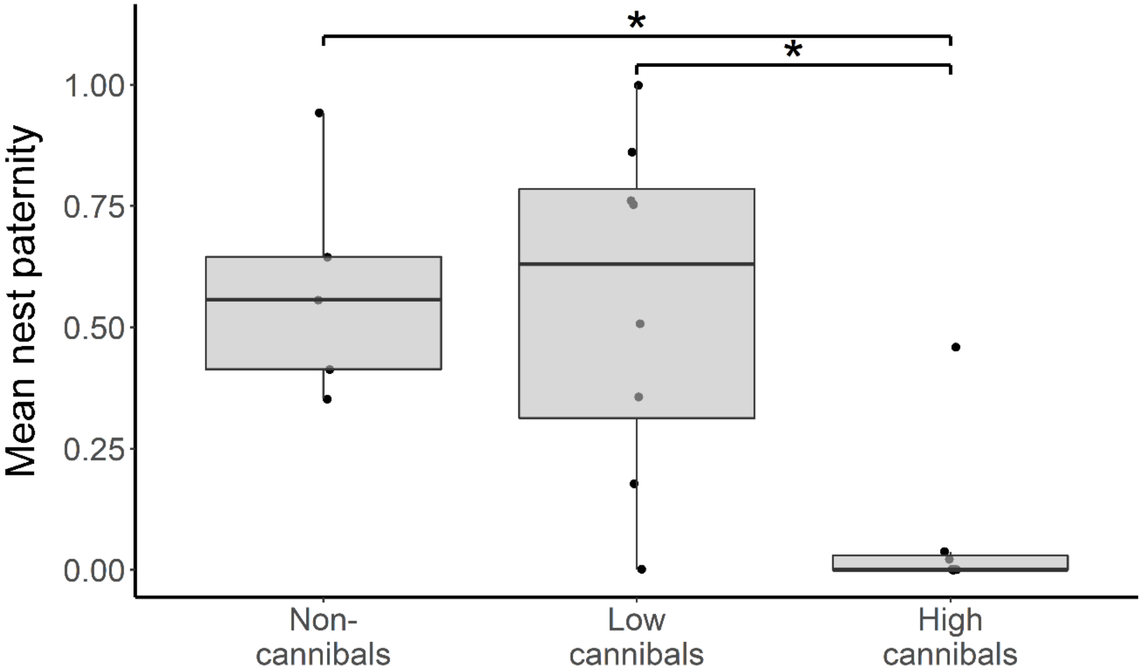

Figure 7.1 Mean nest paternity across offspring cannibalism groups in breeding *Porichthys notatus*. Groups indicate the degree to which males engaged in partial brood cannibalism (Non-cannibals consumed 0 embryos, low cannibals consumed 1-4 embryos, and high cannibals consumed 5+ embryos). High degrees of embryo cannibalism are associated with low mean nest paternity. * denotes $p < 0.05$.

7.5 Discussion

Our results show that high degrees of embryo cannibalism by guarder male *P. notatus* are correlated with low nest paternity. In the majority (6 out of 7) of genotyped nests where the males ate five or more embryos (the high cannibalism group) mean paternities were low (median: 0%, range: 0 – 58.1%), and suggestive of nest take-over events. Bose et al. (2014, 2016) showed that these take-over males are highly cannibalistic towards newly acquired broods, and that offspring survival decreases dramatically under the care of an allocaring take-over male. Taken together, our results suggest that males do not tolerate large numbers of non-kin offspring in their nests and will consume them. *P. notatus* nests are thought to be extremely space-limited (Demartini 1988; Demartini 1991), so any non-kin offspring in a nest will be taking up valuable room that could otherwise be used for direct offspring. However, it is noteworthy that take-over males typically do not cannibalize the entire brood of non-kin embryos that they acquire during the take-over. Cogliati et al. (2013) showed that it is most often the oldest cohort of embryos within a nest that are unrelated to the guarder male caregiver, suggesting that some non-kin offspring are spared. Similarly, Bose et al. (2016) showed that even a month after a take-over event, a nest might still retain some of the non-kin embryos under the care of the alloparent. These results suggest that retaining at least some non-kin offspring may confer a benefit to a take over male, perhaps in terms of future mate attraction or embryo dilution effects (Jamieson 1995; Wisenden 1999; Lindström et al. 2006; Matsumoto and Takegaki 2013). These possible benefits associated with retaining non-kin embryos still require explicit testing in *P. notatus*. Interestingly, Sargent (1989) describes an analogous situation in the fathead minnow, *P. promelas*, in which alloparental males reduce their adopted broods down to a size that still attracts females to their nests, but permits enough nest space for the new eggs to be laid.

Our results also indicate that a low degree of embryo cannibalism, i.e. the consumption of only 1-2 embryos at a time, occurs independently of mean nest paternity.

Whether males are selectively consuming individual non-kin offspring from within their brood or from elsewhere remains to be explicitly tested. However, previous studies have suggested that *P. notatus* may not have the capability to discriminate kin from non-kin offspring (Bose et al. 2016). Furthermore, a previous diet analysis revealed that while offspring cannibalism is largely directed towards non-kin embryos, filial cannibalism does occur as well (Cogliati et al. 2015). Hence low levels of offspring cannibalism may occur due to a number of different reasons. For example, partial-brood filial cannibalism has been suggested to be a means of removing non-viable eggs from the nest, a phenomenon that occurs in sphinx blennies, *Aidublennius sphinx* (Kraak 1996) and scissortail sergeants, *Abudefduf sexfasciatus* (Manica 2004). Low levels of cannibalism could also be incidental during routine nest cleaning and maintenance. Direct observations of *P. notatus* guarder males within their nests indicate that they spend a considerable amount of time engaged in nest maintenance and egg cleaning (A Bose personal observations).

Consistent with previous studies, we found that energy demands do not correlate with cannibalism of offspring in *P. notatus* (Bose et al. 2014, 2015). In fact, the males that had consumed the most embryos had the highest body condition scores, most likely because these were successful nest usurpers that had spent relatively less time providing costly parental care. Similarly, brood size did not influence brood cannibalism. Theory suggests that partial brood cannibalism should be more prevalent in larger broods because the costs of losing offspring are proportionally smaller for large broods (Sargent 1992; Manica 2002). This prediction, however, has been difficult to support empirically; many previous studies have also failed to detect a correlation between brood size and cannibalism by caregivers (e.g. fathead minnows, *Pimephales promelas*, Sargent 1988; scissortail sergeant, *A. sexfasciatus*, Manica 2003, 2004; maritime earwig, *A. maritima*, Miller and Zink 2012; Lusitanian toadfish, *Halobatrachus didactylus*, Félix et al. 2016).

In this study, we used genetic paternity testing to show that high levels of cannibalism occur when mean nest paternity is low. These findings suggest that embryo cannibalism is linked to nest take-overs by new and unrelated males and are in-line with theory that individuals should reduce their level of care into offspring when genetic relatedness is low or uncertain (Owens 1993; Westneat and Sherman 1993). Future work is now needed to investigate 1) whether offspring cannibalism is directed at specific unrelated offspring cohorts within a nest or brood, and 2) whether offspring cannibalism increases or parental effort decreases in response to cues of paternity loss such as cuckoldry and sperm competition.

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Appendix

Chapter 8: A test of male infanticide as a reproductive tactic in a cichlid fish

8.1 Abstract

Infanticide and offspring cannibalism are taxonomically widespread phenomena. In some group-living species, a new dominant individual taking over a group can benefit from infanticide if doing so induces potential mates to become reproductively available sooner. Despite widespread observations of infanticide (i.e. egg cannibalism) among fishes, no study has investigated whether egg cannibalism occurs in fishes as a result of group takeovers, or how this type of cannibalism might be adaptive. Using the cooperatively breeding cichlid, *Neolamprologus pulcher*, we tested whether new unrelated males entering the dominant position in a social group were more likely to cannibalize eggs, and whether such cannibalism would shorten the interval until the female's next spawning. Females spawned again sooner if their broods were removed than if they were cared for. Egg cannibalism occurred frequently after a group takeover event, and was rarer if the original male remained with the group. While dominant breeder females were initially highly aggressive towards newcomer males that took over the group, the degree of resistance depended on relative body size differences between the new pair and, ultimately, female aggression did not prevent egg cannibalism. Egg cannibalism, however, did not shorten the duration until subsequent spawning, or increase fecundity during subsequent breeding in our laboratory setting. Our results show that

infanticide as mediated through group takeovers is a taxonomically widespread behaviour.

8.2 Introduction

One of the most dramatic examples of sexual conflict in the animal kingdom is infanticide perpetrated by newly dominant male lions, *Panthera leo*. When male lions take over a pride of females, they will kill cubs and the termination of maternal provisioning allows females to mate again sooner (Bertram 1975; Pusey and Packer 1994; Palombit 2015). Infanticide defined as the direct killing of, or lethal curtailment of investment into, conspecific young (Hrdy and Hausfater 1984) therefore grants 'takeover' males sexual access to females, increasing the males' reproductive success, albeit at a cost to the females (Hrdy 1974; Palombit 2015). The sexual selection hypothesis for infanticide predicts that offspring will be killed when: (i) an individual in a dominant breeding position is replaced by a new, unrelated, individual; (ii) infanticide elicits a state of receptivity in the potential mates of the perpetrator who have had their care terminated; and (iii) subsequent reproduction between the perpetrator and the mates is enhanced either through shortened interbirth/interhatch intervals, increased fecundity, or both. The costs to the original parents are also thought to have driven the evolution of numerous counter-strategies, including female aggression against usurping males (Ebensperger 1998; Ebensperger and Blumstein 2008; Palombit 2015).

The sexual selection hypothesis for infanticide was originally formulated to describe the behaviour of Hanuman langurs, *Semnopithecus entellus* (Hrdy 1974), and the hypothesis has received support from a number of mammalian studies (Ebensperger and Blumstein 2008; Palombit 2012). Mammals are particularly apt for illustrating the adaptive benefits of infanticide, because the costs associated with female lactation are so prohibitive that they generally preclude the raising of multiple broods simultaneously, and these costs can be avoided by eliminating dependent offspring (Ebensperger 1998; van Schaik 2000). The sexual selection hypothesis has received far less research attention in non-mammalian species (Palombit 2015; but see also Veiga 2000) and therefore garnered only limited empirical support across taxa. This is despite widespread understanding that parental care imposes reproductive costs to caregiving individuals (Williams 1966; Gross 2005). Although parental care has been shown to reduce female breeding frequency by increasing interspawn intervals in numerous fish species (*Oreochromis mossambicus*, Smith and Haley 1987; *Haplochromis argens*, Smith and Wootton 1994; *Sarotherodon galilaeus,* Balshine-Earn 1995), no study has yet tested the sexual selection hypothesis for infanticide in fishes.

Here, we used the cooperatively breeding, group-living cichlid fish, *Neolamprologus pulcher*, to conduct the first test of the sexual selection hypothesis for infanticide in fishes. In fishes, infanticide is always committed through cannibalism (Dominey and Blumer 1984) and so we henceforth refer to infanticide as egg cannibalism in this study. We first examined whether the cessation of parental care would shorten interspawn intervals and/or enlarge subsequent broods by conducting brood removals in the early stages of parental care. We next staged scenarios in which breeding groups were taken over by a new, unrelated dominant male to test whether egg cannibalism subsequently occurred. We then tested whether female aggression towards newcomer males was effective in preventing egg cannibalism, and whether egg cannibalism provided the takeover male with a reproductive benefit. We predicted that: (i) social groups of *N. pulcher* would spawn again sooner when their broods were removed, thereby ending parental care early, compared to when their broods were cared for; (ii) new takeover males would cannibalize any eggs currently receiving care from the social group; (iii) maternal aggression would counter the takeover male's cannibalistic efforts, particularly when the female was well matched in terms of body size to the takeover male; and (iv) egg cannibalism would benefit the takeover male by shortening the interval until subsequent spawning and/or by increasing female fecundity in the following breeding event.

8.3 Methods

Study species

Social groups of *N. pulcher* in the wild consist of a dominant breeding male and female and 0–20 subordinate helpers ordered in a size-based dominance hierarchy (Balshine-Earn et al. 1998). Average group size in the wild varies between 7 (Balshine et al. 2001) to 9 (Heg et al. 2005). While both breeders and helpers maintain the territory, defend it from competitors and predators, and care for offspring, the breeder female tends to be the most active in all these respects (Balshine et al. 2001; Desjardins et al. 2008). Males typically disperse further than females and have shorter tenure in the dominant position than do females, resulting in males generally having fewer reproductive opportunities over their lifetimes than females (Stiver et al. 2004). From time to time, a new breeding male from outside of the social group (often a neighbour) will attempt to take over a recently vacated breeding position (often vacant due to predation) or to challenge the current breeding male for their position (Stiver et al. 2004; Stiver et al. 2006). Like many cichlids, *N. pulcher* breed regularly throughout the year and groups tend to produce eggs once per month (Nakai et al. 1990; Desjardins et al. 2011).

Does egg removal expedite subsequent reproduction?

Between April 2011 and September 2012, 10 social groups of *N. pulcher* were held in the laboratory at McMaster University, Hamilton, Canada in 189-litre aquaria. The aquaria were maintained at 25 ± 2°C with a 13 : 11 h light–dark cycle and contained crushed coral substrate. Housing conditions were environmentally controlled and kept stable across the entirety of the observation period. Each tank was also given two halved flowerpots, which are used for shelter and breeding. Each flowerpot provided ample surface area for egg attachment ensuring that spawning was never space-limited. Fish were fed Nutrafin® cichlid food flakes daily and the groups were breeding consistently in the laboratory (i.e. at least once every two months). For each of these groups, two broods were haphazardly chosen for complete removal immediately upon their discovery, and another two broods were allowed to be raised by their social group. We recorded the dates of subsequent spawning in order to calculate interspawn intervals ($N = 40$ interspawn intervals calculated, $N = 10$ groups). When the broods were clearly visible, we also recorded brood size ($N = 26$ brood sizes recorded).

All statistical analyses were performed in R (v. 3.2.3, R Core Team 2015). To assess whether brood removal influenced a social group's interspawn interval or brood size, we fit two linear mixed-effects models (LMMs), including either interspawn interval (in days) or brood size (egg number) as the response variable, treatment (removed versus not removed) as a fixed effect and GroupID as a random intercept (data provided in electronic supplementary material).

Does group takeover incite egg cannibalism and expedite subsequent reproduction?

We conducted this experiment between April 2015 and March 2016 at McMaster University using 39 social groups of *N. pulcher* held in 189-litre aquaria. Each social group consisted of a dominant male and female breeding pair plus three or four subordinate helpers. Aquarium conditions were environmentally controlled and kept stable as described above, but in this experiment each social group was fed a precise diet of 75 mg of Nutrafin® cichlid food flakes daily.

Here, we simulated the natural situation often observed in the wild in which a neighbouring male takes over a social group. Under natural conditions, it is common for the dominant male breeding position to be filled by a closely neighbouring male, who either actively competes for the position by ousting the original male through a takeover or waits for the position to become vacant through predation (Stiver et al. 2006; O'Connor et al. 2015). For example, Stiver *et al*. (2006) showed in the wild that 71% of experimentally vacated male breeder positions were filled by neighbouring males taking over the group. We therefore held a neighbouring adult male in an end-compartment of the same aquarium as our social groups, where he was visible to the social group but separated by a removable clear barrier. Each social group was inspected daily for eggs. When a brood of eggs was detected, it was photographed, and the group was haphazardly assigned to either the takeover ($N = 25$) or control ($N = 14$) condition. In takeover groups, the dominant breeding male was removed. The group was permitted 15 min to recover from this disturbance before the barrier was removed, thereby allowing the neighbouring male to 'take over' the social group. In control groups, the dominant male was removed, but after 15 min, he was returned to his social group while the neighbouring male and barrier were removed. We video-recorded all behaviours for 1 h, and then photographed the broods after this hour and again after 24 h. We quantified brood size from the digital photos using ImageJ (v 1.48), and used these numbers to calculate the proportion of each brood that survived to the 1 h and 24 h time points. We inspected all groups daily for the next two months and recorded any subsequent spawning events.

From the recorded videos, a single observer scored the first 20 min of interactions between the original breeding pair in control groups, and between the dominant female and the new male in takeover groups. All aggressive and submissive behaviours performed and received were scored (Hick et al. 2014), and used to calculate a resistance index for each female as a measure of her ability to dominate the male: Resistance Index $= (\text{Aggression}_{\text{given}} + \text{Submission}_{\text{received}}) - (\text{Aggression}_{\text{received}} + \text{Submission}_{\text{given}})$. Interactions with helpers were rare and therefore were not analysed. The observer also recorded whether or not egg cannibalism occurred, the identities of all egg cannibals and how many eggs were cannibalized over the entirety of the 60-min trial.

Prior to the manipulation, group size did not differ between takeover and control groups, nor did brood sizes, nor the body sizes of dominant females, original dominant males and isolated/takeover males (all $p > 0.22$). We fit a linear model (LM) to test how female resistance differed between the takeover and control groups and how it correlated with brood size and male–female size disparity (% difference in standard length). Next, we tested whether groups differed in terms of the proportions of the brood surviving to the 1 h and the 24 h time points. For each time point, we fit a generalized linear model, specifying a quasi-binomial error distribution (GLMqb) suitable for overdispersed data and proportion data (Kabacoff 2011). We specified treatment group, initial brood size and female resistance index as independent variables. All two-way interactions were tested and removed when non-significant. We then employed a hurdle model to test how takeovers affected subsequent reproduction. We fit a binary logistic regression to compare groups for their likelihood of having a subsequent spawning within the two-month post-manipulation monitoring period. We then focused only on trials in which a subsequent spawning occurred ($N = 33$), and fit two linear models to see how takeovers affected the number of days until the next spawning (log-transformed), and if takeovers affected subsequent brood size. For both these models, treatment group was specified as the independent variable (data provided in electronic supplementary material).

8.4 Results

Removing broods shortens interspawn intervals

In 2011 and 2012, the 10 *N. pulcher* social groups studied spawned on average every 28.0 ± 14.9 days (mean ± s.d.). Groups that had their broods removed re-mated sooner (every 16.9 ± 7.2 days) compared with groups that cared for their broods (every 39.0 ± 12.1 days; LMM, $t = 7.7$, $p < 0.0001$, marginal $R^2 = 0.55$, conditional $R^2 = 0.64$; Figure 8.1). However, subsequent brood sizes were not influenced by whether the previous brood was removed (84.1 ± 65.8 eggs) or cared for (49.8 ± 26.5 eggs; LMM, $t = -1.6$, $p = 0.13$, marginal $R^2 = 0.09$, conditional $R^2 = 0.18$).

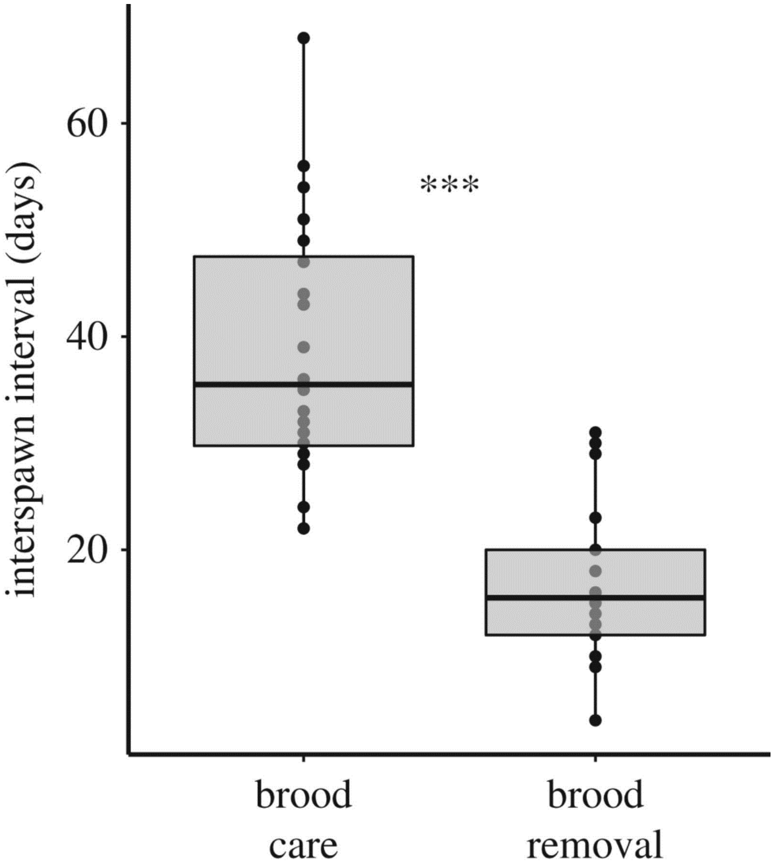

Figure 8.1 Interspawn intervals shorten when broods are removed. *** indicates $p < 0.0001$.

Group takeovers are associated with female aggression and egg cannibalism

More aggression was observed in the takeover groups (mean ± s.d., 67.1 ± 42.4 aggressive acts/20 min) than in control groups (13.3 ± 12.0 aggressive acts/20 min). While female resistance was not related to brood size (LM, $t = -0.16$, $N = 39$, $p = 0.88$), it was correlated with male–female size disparity, but only in the takeover groups (LM, interaction, $t = -2.80$, $N = 39$, $p = 0.008$, adjusted $R^2 = 0.42$; Figure 8.2).

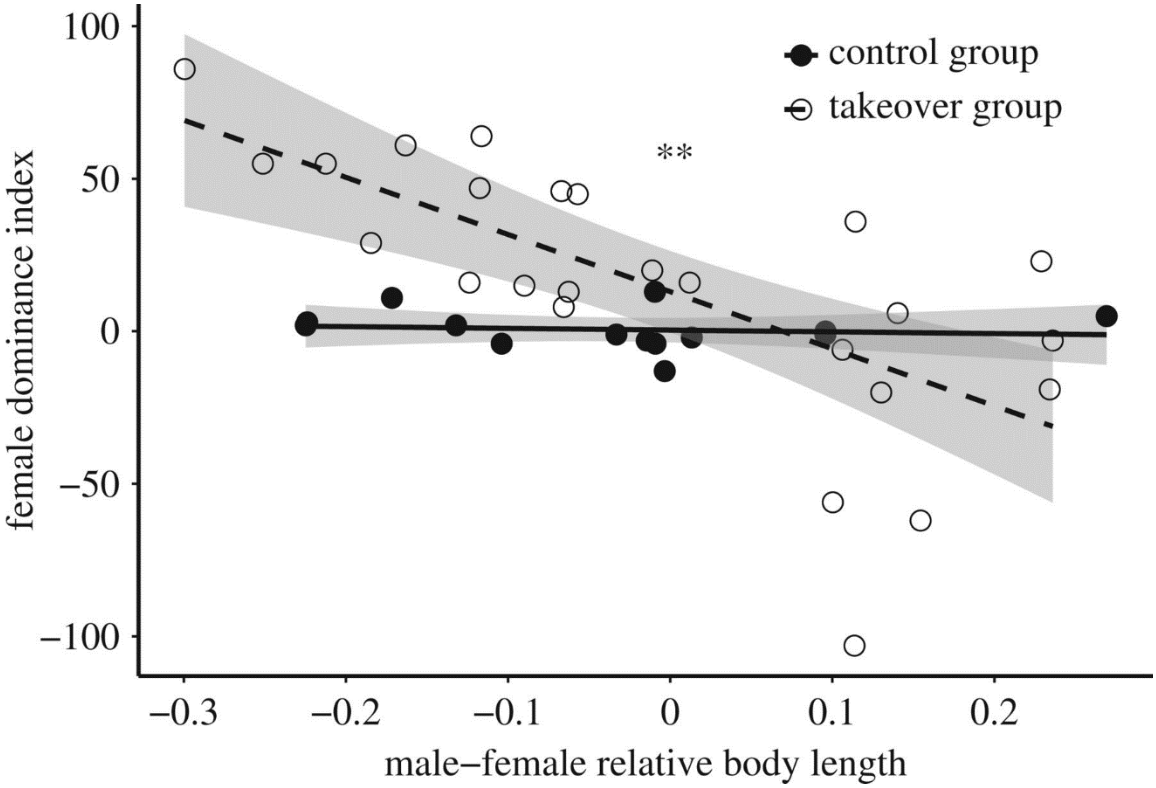

Figure 8.2 Female resistance to male takeover was high when females were larger than the takeover males, but diminished when the takeover males were larger (dashed line). This pattern was not observed in the control groups (solid line). 95% confidence intervals shown. ** indicates $p < 0.01$.

Over the 1 h trials, egg cannibalism was directly observed in *both* control and takeover groups. Over the first hour of the trials, group takeovers resulted in the cannibalism of 177 eggs (in total), while controls resulted in the cannibalism of 58 eggs (in total). Cannibalism was directly observed in seven of the 25 takeover trials (all committed by the takeover male) and in five of the 14 control groups (four of these were committed by the control breeder male, and once by the control breeder female). After 1 h, there were no differences between the control and takeover groups in terms of the proportions of the broods surviving (GLMqb, $t = -0.84$, $N = 39$, $p = 0.41$; Figure 8.3*a*), though larger broods were more likely to have a higher proportion of offspring surviving after 1 h (GLMqb, $t = 2.05$, $N = 39$, $p = 0.048$). After 24 h, however, far fewer offspring remained in the takeover groups than the control groups (GLMqb, $t = -2.77$, $N = 39$ $p = 0.009$; Figure 8.3*b*). Female resistance was not related to the proportion of offspring surviving at either time point (both $p > 0.2$).

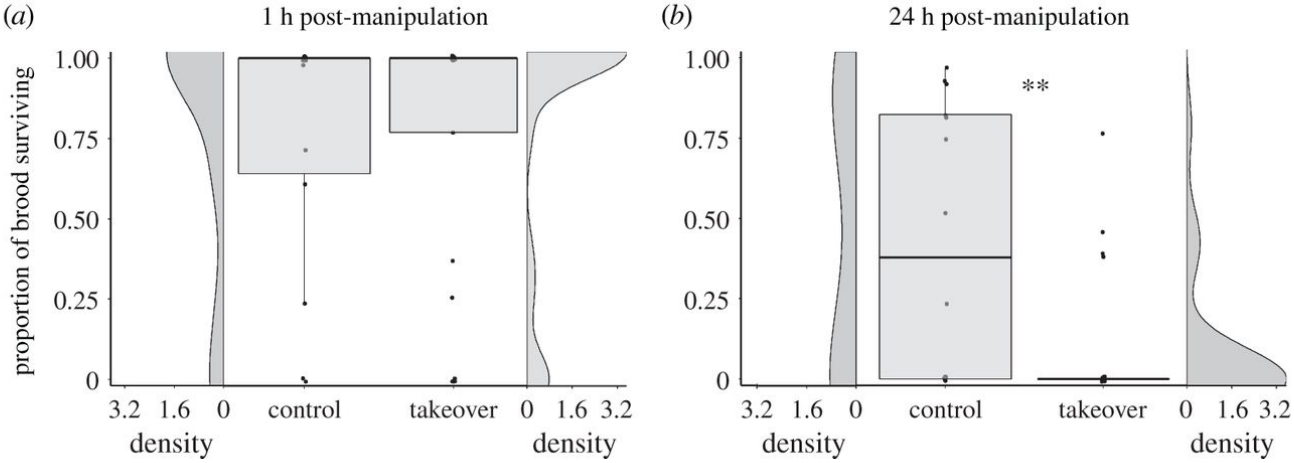

Figure 8.3 Proportion of brood surviving at (*a*) 1 h and (*b*) 24 h post-manipulation. Kernel density plots are also presented to better visualize the shape of the skewed data in each boxplot. ** indicates $p < 0.01$.

Group takeovers and egg cannibalism are not associated with expedited reproduction

Thirty-three of the 39 social groups used in our experiment spawned again within two months. Takeover events did not increase the likelihood of a second spawning occurring in this timespan (binary logistic regression, $z = -1.02$, $N = 39$, $p = 0.31$), or reduce the days until the second spawning (LM, $t = 0.22$, $N = 33$, $p = 0.82$, multiple $R^2 = 0.002$). The mean (±s.d.) interspawn interval was 24.2 ± 13.6 days for control groups and 24.2 ± 13.0 days for takeover groups. The subsequent brood size also did not differ between treatments (62.7 ± 20.1 eggs for control groups and 52.2 ± 27.7 eggs for takeover groups; LM, $t = -1.18$, $N = 33$, $p = 0.25$, multiple $R^2 = 0.04$).

8.5 Discussion

Infanticide and cannibalism following group takeovers have been documented in numerous mammalian and avian species that display parental care (Hrdy 1974; Ebensperger 1998; Ebensperger and Blumstein 2008; Palombit 2012). However, to the best of our knowledge this phenomenon had not yet been studied in any fish species. With hierarchical group living and offspring care, *N. pulcher* provides an ideal model to investigate this phenomenon. Furthermore, though egg cannibalism is widespread among fishes (Smith and Reay 1991; Manica 2002; Deal and Wong 2016), it has not been well studied in *N. pulcher*, and never in the context of group takeovers.

Group takeovers were associated with high levels of egg cannibalism. Egg survival after 24 h was much lower in the takeover condition compared to the control condition. Thus, it appears that takeover males benefit more by cannibalizing eggs immediately rather than allowing those offspring to augment the group in the future as helpers (e.g. Heg et al. 2005; Brouwer et al. 2005). Intriguingly, we showed that removing broods could shorten interspawn intervals in *N. pulcher*, illustrating that cannibalistic takeover males could *potentially* benefit in this respect. However, in our takeover experiment we found no evidence that takeovers and egg cannibalism in *N. pulcher* sped up future reproduction or led to larger subsequent broods. We speculate that the abundant risk-free food provided in our laboratory may have influenced this result. Throughout the study, all groups were fed a standardized diet that was probably generous in comparison to what these fish manage to acquire in the wild (Wong and Balshine 2011). Stronger resource limitation or riskier foraging situations, such as those experienced naturally in the wild, may lead to more pronounced trade-offs between current parental care and egg production. In support of this idea, breeding in the wild occurs every one to two lunar cycles (Wong and Balshine 2011), while breeding in the laboratory can occur twice per month, suggesting that egg production is resource-limited. A critical future test will be to combine group takeovers in the wild with a manipulation of the takeover male's ability to cannibalize eggs.

We were surprised to also observe filial egg cannibalism occurring in control trials. Some egg cannibalism does occur naturally in this cooperative breeding fish (von Siemens 1990). Previous reports of egg cannibalism in *N. pulcher* have been in relation to dominant females cannibalizing the non-kin broods of subordinate females as a means of suppressing subordinate reproduction (Heg and Hamilton 2008). However, egg cannibalism in this species has never been directly observed in the wild. Therefore, we cannot rule out that filial cannibalism is not a natural component of *N. pulcher* parental care. Furthermore, our handling disturbance may also have induced some filial cannibalism in our control groups, and indeed also in our takeover groups.

Females were aggressive towards takeover males, yet they did not ultimately prevent cannibalism; the majority of takeovers resulted in complete brood mortality. In many species, maternal aggression has been hypothesized to be a female counter-tactic to male infanticide. However, support for this idea is mixed, as maternal aggression often appears to delay, rather than prevent, infanticide (Palombit 2015; Ebensperger and Blumstein 2008). If maternal aggression in *N. pulcher* were indeed a tactic meant to prevent egg cannibalism, then we predicted female aggression towards the takeover male to be positively related to brood size. However, we did not detect this relationship in our data. Aggression may instead be a way to establish dominance and familiarity between the new pair of fish. The use of aggression in establishing dominance hierarchies has been well documented in this species (Wong and Balshine 2011). Alternatively, it is possible that female aggression is still an effective cannibalism-prevention tactic in the wild. In our laboratory conditions, takeover males did not have the opportunity to retreat away from a contest (confined within the aquaria), and so females may not have been able to truly expel the new male from the territory.

In this study, we staged group takeovers in *N. pulcher* to assess the role of egg cannibalism and infanticide as an adaptive reproductive tactic. Conventional parental investment theory predicts that infanticide would occur because the putative costs of killing unrelated young are low. Here, we tested an alternative, but non-mutually exclusive explanation, that the benefits can also be high. While takeovers were associated with high offspring mortality, we were unable to show that egg cannibalism shortens interspawn intervals or increases subsequent brood sizes. We urge that future work be undertaken in the field to further test this hypothesis under more naturalistic conditions. Our study demonstrates that infanticide following group takeovers extends to fishes and suggests interesting new avenues for research on the adaptive benefits of infanticide.

8.6 Acknowledgments

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