THE REPRODUCTIVE ECOLOGY OF PLAINFIN MIDSHIPMAN

THE REPRODUCTIVE ECOLOGY OF PLAINFIN MIDSHIPMAN: VARIATION ACROSS TIME AND SPACE IN A SPECIES WITH ALTERNATIVE REPRODUCTIVE TACTICS

BY

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements for the Degree

Doctor of Philosophy

McMaster University

DOCTOR OF PHILOSOPHY (2013) McMaster University
(Psychology, Neuroscience & Behaviour) Hamilton, Ontario

TITLE: The reproductive ecology of plainfin midshipman: variation across time and space in a species with alternative reproductive tactics

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NUMBER OF PAGES: xv, 177

ABSTRACT

Alternative reproductive tactics (ARTs) have been described across taxa numerous times, and are especially common in fishes. The advent of molecular techniques has enhanced our understanding of such mating systems, yet these techniques have only been applied to relatively few species. Furthermore, ecological variation has long since been recognized as an important factor influencing mating patterns and sexual selection, yet it is often ignored. In my thesis, I conducted field studies to investigate the reproductive ecology of a species with well characterized ARTs, the plainfin midshipman (*Porichthys notatus*), and to explore how these mating patterns varied across time and space. This species is characterized by two male tactics: guarders and sneakers. I show that both tactics are successful at gaining fertilization. Indeed, I found evidence for several behavioural adaptations by the guarder male tactic to gain fitness, including guarding, nest takeovers, and plastic cuckoldry behaviours. I also documented the lowest paternity for guarder males for a species with obligate male parental care. Paternity increased over the course of the breeding season, which was likely a result of a higher occurrence of nest takeovers early in the season. In a cross-population analysis of plainfin midshipman, I found significant differences in nest availability and density between two genetically distinct populations, but this did not translate into significant differences in mating patterns. Taken together, my thesis helps elucidate the reproductive ecology of a classic species with ARTs, and has important implications

for our understanding of ecological influences on mating patterns and sexual selection.

ACKNOWLEDGMENTS

First and foremost, I sincerely thank my co-advisors, Dr. Sigal Balshine and Dr. Bryan Neff, for all of their support and feedback over the years. To Sigal, thank you for always encouraging me to step out of my comfort zone even though I was reluctant, and for your constant enthusiasm and passion for research. To Bryan, thank you for your carefully thought out comments, and for always finding the time to help explain and clarify challenging concepts. Thank you both for being great mentors. To my committee members, Dr. Paul Faure and Dr. Jim Quinn, thank you for your helpful comments and feedback regarding my research over the years.

I have a special thank you to Dr. Benjamin Bolker, for always managing to find the time in your busy day to help me sort out messy field data, challenging statistical models, and all the woes of R. Despite having been stuck numerous times in “statistical spirals”, I learned a great deal as a result, and I am immensely grateful. A large component of my thesis was based on genetic analyses, and it would not have been possible without the help of Dr. Shawn Garner. I arrived in the Neff lab in September of my first year without knowing how challenging genetic work could really be. Shawn, not only did you show me the ropes, you seemed to *always* find the time to help me troubleshoot at every step of the way (literally every step), from figuring out how to get DNA from VERY young eggs, to why my microsatellite peaks were a mess, and running me through how to use genetic programs and writing excel macros while you were no longer even a member of the Neff lab. THANK YOU!

Thank you to all the field assistants and past students who helped collect data for this project: Dr. Julie Marentette, Ahdia Hassan, Celia Chui, Allison Mistakidis, Jennifer Reynolds, Natalie Sopinka, Dr. John Fitzpatrick, and Matt Taves. I also thank folks in BC who have helped make this project possible: Mr. and Mrs. Balshine, Capt. Bill and Rubie Cogswell, Mr. and Mrs. Weatherell, and especially Mr. and Mrs. Flader. To the undergraduate thesis and project students: Amanda Pereira, Claire Danukarjanto, Adrienne Lau, Malcolm Lau, Holly Howe, Cayli Hunt, Celeste Bouchard, Michael Barone, Ryan Caldwell, and Michael Roubakha. Thank you for all of your hard work on the project.

Thanks to all my colleagues and ABEL lab mates, both past and present, for all the support, feedback, and discussion over the years: Aneesh Bose, Dr. Lynda Corkum, Cody Dey, Dr. Zac Durisko, Dr. Constance O'Connor, Dr. Adam Reddon, Natalie Sopinka, and Dr. Maz Wong. In addition to these colleagues and lab mates, I have a special thanks to a few that I was honoured to get to know on a more personal level. To Erin McCallum, thank you for making the last two years truly enjoyable. Your happiness and humour are contagious, and you always seem to know how to lift my spirits, especially with Starbucks. Thank you for finding the time to read over anything I sent your way and for the late night chats about science, stats, food, and life. To Dr. Joelle Thorpe, thank you for being such a great friend. I always know I can count on you to be there when I need you most, and even though our regular coffee dates usually centered on venting, panic, and stress, it was always comforting to know I was not alone in all of this. To Dr. Julie Marentette, thank you for your endless support and for always being a person I can turn to when in need. As my older academic sibling (from both the Corkum and Balshine labs), I generally look to you for guidance and without fail, you are always able to provide me with both words of comfort and *rational* ideas. And finally, to the best lab manager anyone could ever ask for, Susan Marsh-Rollo. I don't even know where to begin except for with a huge THANK YOU. Thank you for all the support and advice you have given me over the years in both academics and life. You always seemed to know when I needed to talk, and always found the time in your busy day for coffee or lunch dates. To this day, it still amazes me how you manage to do all that you do.

In addition to all the support I've received within academia, I would have likely crumbled under the pressure if it were not for the support I received from outside as well. To my parents and sisters, thank you for your continued love and support during my decade-long adventure in post-secondary education, even without fully understanding what I do or why I do it. Thank you to all the lovely ladies at the barn. I always look forward to our weekend rides, as it is (usually) the most relaxing time of my week, barring no accidents. To one of my most cherished friends, Allison Mistakidis, thank you for always being there for me. I could not have asked for a better field assistant in 2011, where we drove from Vancouver to California (3200 km round trip), not once but TWICE. We've come a long way from ignoring each other on the city bus on our way to the SAME undergrad class, and I thank you from the bottom of my heart for being the first to break the silence while we sat next to each other on the plane to Costa Rica. I could not imagine my life without you in it, and I hope I never have to.

Last but most certainly not least, I have a very special thank you to my husband, Clayton Hiltz. Thank you for being so patient and understanding of all the highs and lows of grad school, and for all of your love and support over the years. You have been my rock through all of this and have always tried to keep me calm and relaxed, even from afar. Not only have you put up with me leaving you for months on end while I embarked on field work adventures all along the beautiful west coast, but for all the weeks I spent traveling to and from London for research, leaving you to fend for yourself. And although your talk about “real jobs” and the “real world” always annoyed me, thank you for not only reminding me but also for keeping me connected to life outside of academia. I look forward to wherever life takes me next, because I know I’ll have you by my side.

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DECLARATION OF ACADEMIC ACHIEVEMENT

This dissertation is organized in a "sandwich" thesis format, as approved by McMaster University. It consists of six chapters in total. **Chapter 1** provides a general introduction for the motivation of my thesis, the study species used for my research, and the specific goals of my thesis. **Chapter 2** has been published. **Chapter 3** is currently under review. **Chapter 4** has been accepted pending revisions. **Chapter 5** is a manuscript in preparation for submission. **Chapter 6** provides a general discussion of the research findings in Chapters 2-5 and provides suggestions for future research.

Chapter 1 – General Introduction

Author: Karen M. Cogliati

Chapter 2 – High degree of paternity loss in a species with alternative reproductive tactics

Authors: Karen M. Cogliati, Bryan D. Neff, Sigal Balshine

Publication: Behavioral Ecology and Sociobiology (2013) 67:399-408.

Comments: This manuscript was conceived by KMC, BDN, and SB. KMC and SB collected the samples from the field. KMC conducted the genetic work, analysed the data, and wrote the manuscript under the supervision of BDN and SB. Reprinted with permission.

Chapter 3 - Comparing population level sexual selection in a species with alternative reproductive tactics

Authors: Karen M. Cogliati, Allison F. Mistakidis, Julie R. Marentette, Adrienne Lau, Bryan D. Neff, Sigal Balshine

Publication: Behavioral Ecology (Submitted Oct. 20, 2013)

Comments: This manuscript was conceived by KMC, BDN, and SB. KMC, AFM, JRM, and SB collected field samples. KMC and AL conducted genetic analyses. KMC analysed the data and wrote the manuscript under the supervision of SB and BDN.

Chapter 4 – Competition and cuckoldry: estimating fitness of alternative reproductive tactics in plainfin midshipman

Authors: Karen M. Cogliati, Sigal Balshine, Bryan D. Neff

Publication: Behaviour (Accepted pending revisions, Dec. 3, 2013)

Comments: This manuscript was conceived by KMC, SB, and BDN. KMC and SB collected field samples. KMC analysed the data and wrote the manuscript under the supervision of SB and BDN.

Chapter 5 – Diet and cannibalism in plainfin midshipman (*Porichthys notatus*)

Authors: Claire Danukarjanto*, Karen M. Cogliati*, Amanda C. Pereira, Malcolm J. Lau, Ahdia Hassan, Allison F. Mistakidis, Benjamin M. Bolker, Bryan D. Neff, Sigal Balshine
* Authors contributed equally to this manuscript

Publication: In prep

Comments: This manuscript was conceived by KMC and SB. KMC, AH, and AFM collected samples from the field. CD, ACP and MJL analysed samples. CD and KMC analysed the data under the supervision of BMB. KMC and CD wrote the manuscript under the supervision of BMB, BDN and SB.

Chapter 6 – General Discussion

Author: Karen M. Cogliati

Chapter 1

GENERAL INTRODUCTION

1.1 MOTIVATION FOR THESIS

Environmental conditions and the degree to which resources or mates can be monopolized often dictate the type of mating system and mating patterns observed (Emlen and Oring 1977; Ims 1988; Shuster and Wade 2003). However, ecological factors can vary both spatially and temporally and such variation across a species' distribution, and over the reproductive season, can lead to differences in the degree of within-sex competition. This variation is often ignored and the mating patterns of a species are commonly based instead on a single exemplar population sampled at one time point in the breeding season, or averaged across the breeding season (e.g. Jones et al. 1999; Jones and Avise 2001; McCoy et al. 2001). Ignoring variation over time and space could potentially lead to an incomplete or misleading characterization of the mating patterns of a species. Another means by which researchers have been able to more accurately assess the mating system and mating patterns of a species has been through the application of modern molecular techniques (e.g. reviewed in DeWoody and Avise 2001; Avise et al. 2002; Griffith et al. 2002; Coleman and Jones 2011). The widespread application of molecular methods in behavioural ecology has revolutionized and revised our understanding of mating systems and subsequently, our understanding of variation in individual reproductive success.

To date, there are a small number of studies that have used genetic techniques to investigate how mating patterns vary both spatially and temporally (e.g. Neff and Clare 2008; Reichard et al. 2008; Sefc et al. 2009; including studies detailed below). These studies are based on comparing distinct populations or time points that differ in some environmental factors such as population density or predation intensity and exploring how this influences the mating patterns determined through molecular analyses. Some of these studies have found considerable intraspecific variation in mating patterns across populations (Trexler et al. 1997; Kelly et al. 1999; Griffith et al. 1999; Durrant and Hughes 2005; Bessert et al. 2007; Mobley and Jones 2007). For example, there was a higher frequency of nest takeovers in a nest-limited population of fathead minnow (*Pimephales promelas*) where nesting males were also larger and acquired more mates in this population (Bessert et al. 2007). However, other studies have found essentially no variation in mating patterns between populations despite significant environmental differences (Jones et al. 2001; Neff et al. 2008). For example, Jones et al. (2001) found similar rates of cuckoldry between two sand goby (*Pomatoschistus minutus*) populations that differed significantly in nest density. More data are clearly needed to address how ecological variation specifically influences reproductive success and mating patterns. In species with male alternative reproductive tactics (ARTs; see below), ecological variation may lead to differences in the competitive environment experienced by males and may have important implications for reproductive success and the frequency of alternative tactics. Indeed, to date only a handful of studies have applied modern molecular techniques to explore variation in reproductive success across time and space in a species with ARTs (Jones et al. 2001; Neff and Clare 2008; Sefc et al. 2009; Alonzo and Heckman 2010; see also Coleman and Jones 2011).

The overall aims of my thesis were to apply molecular methods to first investigate the reproductive ecology of the plainfin midshipman (*Porichthys notatus*), a species with fixed male ARTs, and second to explore these mating patterns across time and space. In this general introduction, I provide a brief review of the theories on which my thesis is based, including sexual selection, parental investment, and the evolution of ARTs. I then introduce my study species and study area, and end this general introduction with an outline describing the structure of my thesis.

1.2 THEORETICAL BACKGROUND

1.2.1 Sexual selection

Darwin (1859) first proposed his theory of sexual selection to explain the evolution of elaborate traits that appear to impede rather than enhance survival, an observation that was contradictory to his theory of natural selection. Rather than enhancing survival, sexual selection acts to increase the reproductive success of an individual. In typical mating systems, males are often the more competitive and ornamented sex, while females tend to be the more choosy and drabber sex. Darwin (1871) first realized that particular showy traits evolved because they helped males gain access to members of the opposite sex, either by competing directly with other males (intrasexual selection) or by advertising their quality to the more choosy females (intersexual selection). Later Bateman (1948) and Trivers (1972) recognized that sexual selection would ultimately lead to a greater variance in reproductive success for the more competitive sex. That is, some males may end up mating with many females and other males may mate with none at all. In contrast, all females will commonly be able to mate with at least one male. Thus, systems in which there is greater variance among males in competitive traits and reproductive success should be experiencing more intense sexual selection pressure than systems with minimal variance among males.

1.2.2 Parental investment and the strength of sexual selection

While a number of factors influence the strength of sexual selection and variance in reproductive success, such as resource availability and population density (Emlen and Oring 1977), another important modulating factor is parental investment (Trivers 1972; Parker and Simmons 1996). Parental investment refers to any investment in an offspring by a parent that both increases the offspring's survival and reduces the parent's ability to invest in other offspring (Trivers 1972; Clutton-Brock 1991; Smiseth et al. 2012). The level of parental investment given by each sex influences how quickly each sex can produce offspring (also known as the reproductive rate; Clutton-Brock and Vincent 1991; Clutton-Brock and Parker 1992) and hence the degree of bias in the operational sex ratio (OSR: the number of reproductively capable males to females; Emlen and Oring 1977; Kvarnemo and Ahnesjo 1996). Parental investment can cause a larger skew in the OSR, as the investing parent may be out of the mating pool while providing care (reviewed in Reynolds 1996; Kokko and Jennions 2012). Importantly, it is not always the case that the investing sex is limiting in the population. In many fish species, for example, males continue to court additional females while providing parental care (Blumer 1979; Clutton-Brock 1991; Mank et al. 2005). For this reason, many care-giving male fishes may still have faster reproductive rates than females, leading to an overall male-biased OSR. This would lead to a greater degree of competition among males when it comes to access to females, and result in greater variance in competitive characteristics and reproductive success, and ultimately, more intense sexual selection among males.

1.2.3 Alternative reproductive tactics and paternity

Large variance in reproductive success, and hence strong sexual selection in the form of mating competition, has likely led to the evolution of alternative reproductive tactics (Gross 1996; Taborsky et al. 2008). Alternative reproductive tactics refer to a type of mating system characterized by the presence of two or more discontinuous phenotypes of the same sex that compete for reproductive success using different tactics or strategies (Taborsky et al. 2008). Alternative reproductive tactics are expected to evolve whenever there are opportunities to obtain reproductive fitness through alternative means (Taborsky et al. 2008). Commonly observed among males, one of the tactics (often called guarder, territorial, or parental) is typically associated with showy displays used to court females and deter rivals, and in some cases, also provide parental care. The alternative male tactic (often called cuckolder or sneaker) is associated with a lack of courtship and parental care, and instead gains reproductive success in sneaky or coercive ways. Alternative reproductive tactics may also evolve as a means for males to avoid costly parental responsibilities and still obtain fitness in species with parental care (Jones et al. 2001). Both of the selective pressures (strong sexual selection and avoiding care) may jointly contribute to the evolution of ARTs.

In fishes, both male parental care and ARTs are common (Reynolds et al. 2002; Taborsky 2008; Balshine 2012). Indeed, in species with uniparental care (found in 23% of fish families), male care predominates (50-84% of those with uniparental care; Balshine 2012), and ARTs have been documented behaviourally or morphologically in 170 different fish species across 32 families (Taborsky 2008). Alternative reproductive tactics are also highly variable in fishes such that the behaviours performed by each tactic type and the number of tactics present in a species vary widely (Taborsky 2008). For example, male guppies (*Poecilia reticulata*) have flexible ARTs where any given male can display one of two behaviours for mating (Magurran and Seghers 1994). A male guppy may court a female with showy displays or he may choose to force copulate, and this decision may be based on the context or situation. Male guppies in areas of high predation are more likely to use forced copulations while those in areas of low predation use showy courtship tactics (Magurran and Seghers 1994). Each male in the population can adopt either of these mating tactics in a flexible manner. Conversely, male bluegill sunfish (*Lepomis macrochirus*) have fixed ARTs, where males follow one of two irreversible developmental trajectories termed parental or cuckolder (Gross and Charnov 1980). Parental males build and defend nests and provide care for developing embryos. Cuckolder males first develop into small sneaker males that use speed and stealth to fertilize eggs by darting through the nest of a parental male. These sneaker males may further develop into larger satellite males that use female mimicry to gain access to fertilizations (Gross 1982; Dominey 1981; Fu et al. 2001). As evidenced by the bluegill system, there are sometimes more than two alternative ways to achieve reproductive success. Indeed, there are also three observed tactics in the ocellated wrasse (*Symphodus ocellatus*), including territory owners, satellites, and sneakers (Taborsky et al. 1987). Unlike the bluegill system, ocellated wrasse satellite males share in some of the effort of nest defence.

In systems with ARTs, multiple paternity or reduced paternity for the territorial or care-giving tactic is widespread (for reviews, see Avise et al. 2002; Coleman and Jones 2011). However, although ARTS have been observed in 170 species (Taborsky 2008), the genetic reproductive success of the alternative male tactics has been characterized using molecular techniques in relatively few species (~20%; see Avise et al. 2002; Taborsky 2008; Coleman and Jones 2011). Among the studies that have employed molecular techniques to assign paternity, researchers have found an average paternity for guarding nest owner males as high as 99% (DeWoody et al. 2000) or as low as 72% (Alonzo and Heckman 2010; see also Avise et al. 2002; Coleman and Jones 2011). Given the variability in average paternity across species and the overall variability of ARTs in fishes, additional paternity studies are warranted, especially if we want to ascertain how ecological variation influences paternity loss to the alternative form. Such analyses will also provide new insights into how ecological factors and sexual selection influence the evolution of ARTs. For example, mate monopolization, which can be influenced by ecological factors such as nest density, is often a precursor to the evolution of sneaking behaviours. Moreover, sexually selected traits in the guarder male type is strongly correlated with the occurrence of male ARTs (Mank and Avise 2006).

1.3 STUDY SPECIES: PLAINFIN MIDSHIPMAN (*PORICHTHYS NOTATUS*)

In this thesis, I have used the plainfin midshipman (*Porichthys notatus*) to investigate mating patterns and genetic success of alternative tactics across their distribution and over the breeding season. This is a nocturnally active marine fish distributed along the Pacific coast of North America, from Sitka, Alaska, to Magdalena Bay, Mexico (Hubbs 1920; Arora 1948; Miller and Lea 1972). Its distribution, however, appears to show a discontinuity along the Oregon coast (Warner and Case 1980; Thompson and Tsuji 1989; Harper and Case 1999; **Figure 1.1a**). Typically found at depths up to 300m during the non-breeding season,

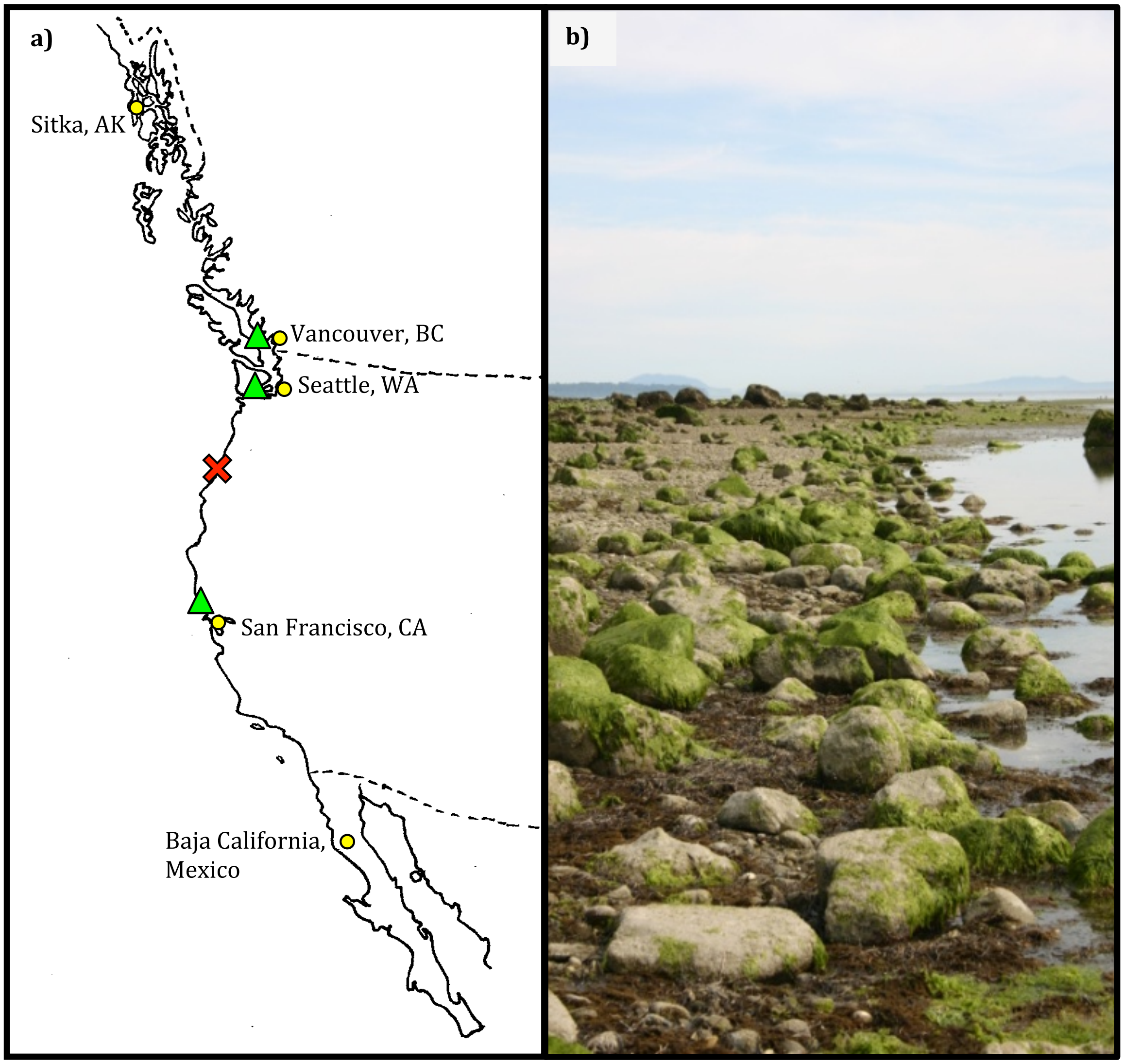

Figure 1.1 a) Distribution map of plainfin midshipman (*Porichthys notatus*), from Sitka, Alaska to Baja California, Mexico. Map shows the reported discontinuity in their distribution in Oregon, USA (red 'X'), as well as the three main locations used in the studies described in my thesis (BC, WA, CA; marked with green triangles). Well known cities are labeled for reference (yellow circles). b) An example of a rocky intertidal zone at low tide, an area where plainfin midshipman nests occur. Nests of guarding males are found underneath rocks.

reproductively mature adults will undergo a vertical migration to the intertidal zone during the breeding season in spring and summer (Miller and Lea 1972). Plainfin midshipman nest under rocks in sheltered, rocky shores, and can be entirely cut off from ocean water during low tide for up to 8 hrs (KC pers. obs.; **Figure 1.1b**). Their diet consists of crustaceans and small fishes (Eigenmann 1892; Hubbs 1920; Arora 1948; Hart 1973). However, no study to date has conducted a comprehensive analysis of their diet, though reports suggest that fish sampled from the intertidal zone had largely empty digestive tracts (Hubbs 1920; Arora 1948; Sisneros et al. 2009) and instances of cannibalism have been noted in this species (Mensinger and Case 1991; Sisneros et al. 2009).

In this species, there are two distinct male reproductive tactics called guarder "type I" and sneaker "type II" males (Brantley and Bass 1994; **Figure 1.2**). Guarder males aggressively compete for and defend nest sites and use acoustic signals to court females. In contrast, sneaker males do not guard nests or court females, but instead, steal fertilizations from the guarder males by either sneaking into the nest when a female is present, or by fanning sperm into the nest from the periphery (Brantley and Bass 1994; **Figure 1.3**). Interestingly, type I males are behaviourally flexible, and may adopt cuckolding behaviours when smaller than average or when nests are limited (Lee and Bass 2004). Type I and type II males appear to be mutually exclusive endpoints and not simply two parts of a single ontogenetic sequence. This observation is based on substantial differentiation in neuronal structures and sonic muscle growth prior to differentiation, and on

Figure 1.2 Male alternative reproductive tactics in plainfin midshipman. Guarder type I male above and sneaker type II male below. Photo courtesy of Dr. John Fitzpatrick.

Figure 1.3 Example of plainfin midshipman nest. Rock (on left; artificial tile) has been overturned to open the nest cavity. The fish located near the top of the photo is a sneaker male. The largest centrally located fish of the four below is the guarder male and he was surrounded by three females. The bottom female is positioned with her abdomen upwards in the classic spawning position.

overlapping ages of males developing into type I and type II with divergent growth trajectories (Bass et al. 1996). This substantial investment in growth for type I males may involve a cost that not all males can sustain leading to some males following the type II trajectory. Females are attracted to the courtship signals of the guarder males (Brantley and Bass 1994), and will each produce a single clutch of embryos released into a single nest (DeMartini 1990). Females tend to produce around 200 eggs per year (DeMartini 1990), but some females have up to 300 eggs (KC pers. obs.). Females and type II males are often found in the nests of type I males during low tides, as spawning takes several hours to complete (Brantley and Bass 1994). Indeed, only juvenile plainfin midshipman are found in offshore eelgrass beds, suggesting that reproductive adults are likely only found in nests (Bass 1992). However, guarding type I males have been seen outside of nests during high tide suggesting there is also some movement (K.C. pers. obs.). Larger guarder males typically win larger nest sites, attract more females, and acquire more eggs (DeMartini 1988). Guarder males will remain in their nest for up to four months, both providing parental care for developing embryos, which take up to 60 days to leave the nest, and continuing to court and spawn with additional females while caring for previous clutches (Arora 1948; DeMartini 1988; Brantley and Bass 1994).

The plainfin midshipman is an ideal study species to address my proposed research questions for multiple reasons. First, ARTs have been well characterized in this species through a number of behavioural, morphological, and neurobiological studies that distinguish reproductively mature guarding type I from sneaker type II males (Bass 1990, 1992, 1993; Brantley and Bass 1994). Indeed, plainfin midshipman are often cited as a classic ART system based on the many differences between tactics (Bass and Forlano 2008; Dugatkin 2013). However, the use of molecular techniques to quantify the genetic success of the alternative tactics in plainfin midshipman had never been attempted before. Second, plainfin midshipman and their embryos are easily sampled in their nests at low tides, and they have a wide distribution (from Alaska to Mexico) and a long breeding season (from April to August). These features make them ideal to investigate how ecological factors that differ over time and across different environments, influence individual reproductive success, mating patterns, and the frequency of alternative tactics.

1.4 STUDY AREA: PACIFIC NORTHWEST

My research on the plainfin midshipman has included study sites in British Columbia, Canada, and both California and Washington in the United States of America (**Figure 1.1a**). In British Columbia, I had several sampling locations including three different sites in Ladysmith Inlet (49°01'N, 123°83'W), one site at Mill Bay (48°63'N, 123°53'W), and one site at Two Cove Inlet in Stuart Channel (49°04´N, 123°75´W) on the eastern coast of Vancouver Island (**Figure 1.4a**). Additionally, we sampled one site on the mainland at Crescent Beach (49°04'N, 122°88'W) located in South Surrey, BC (**Figure 1.4b**). To investigate cross-population differences, I added multiple sampling sites in the United States to

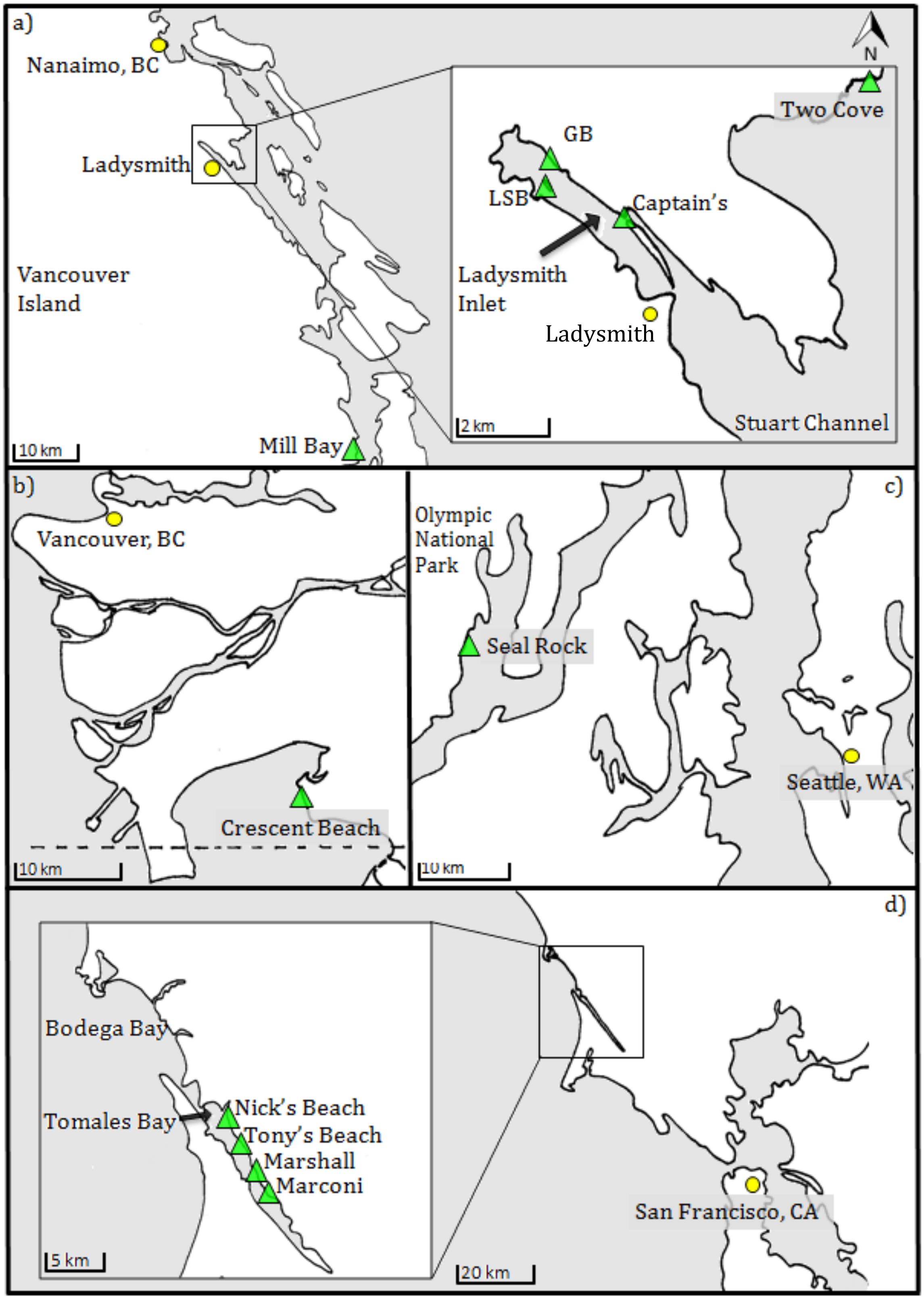
a)
Nanaimo, BC
Ladysmith
Vancouver Island
10 km
Mill Bay
N
Two Cove
GB
LSB
Captain's
Ladysmith Inlet
Ladysmith
2 km
Stuart Channel
b)
Vancouver, BC
Crescent Beach
10 km
c)
Olympic National Park
Seal Rock
Seattle, WA
10 km
d)
Bodega Bay
Tomales Bay
Nick's Beach
Tony's Beach
Marshall
Marconi
5 km
San Francisco, CA
20 km

Figure 1.4 Location of my study sites along the Pacific coast of North America. a) Shows the five study sites on the East coast of Vancouver Island, south of Nanaimo, BC. The inset shows three sites within Ladysmith Inlet and one site in Stuart Channel. b) Shows the study site at Crescent Beach on the West coast of mainland BC, south of Vancouver. c) Shows the study site in Puget Sound, south of Strait of Juan de Fuca. This study site is located east of Olympic National Park and west of Seattle, WA. d) Shows the four study sites on the West coast of California, north of San Francisco. The inset shows Bodega Bay and Tomales Bay, with all four study sites found in Tomales Bay. Scales shown at bottom left are specific to each location. Water shaded in grey.

include sites both north and south of the supposed discontinuity in the species range (Warner and Case 1980; Thompson and Tsuji 1989; Harper and Case 1999). Specifically, I included one site in Seal Rock, WA (47°71´N, 122°89´W; **Figure 1.4c**) and four sites in Tomales Bay, CA (38°15´N, 122°90´W; **Figure 1.4d**). I chose these sites because plainfin midshipman have been previously sampled and studied there, and I was able to collaborate with researchers from Cornell University (Dr. Andrew Bass) in California and from University of Washington (Dr. Joseph Sisneros) in Washington. The sites differed in ecological conditions including rock density, duration that nests were out of water during low tide, sediment type, and number of aerial predators.

1.5 STRUCTURE OF THESIS

Throughout this thesis, I use molecular techniques to calculate genetic reproductive success of each of the two male alternative reproductive tactics and attempt to link this to ecological variation in time and space. In **Chapter 2** I determine the genetic reproductive success of guarder males using six microsatellite markers developed from plainfin midshipman (Suk et al. 2009) and explore how paternity changes over the course of the breeding season. In **Chapter 3** I use molecular and ecological data to investigate how environmental differences across two geographically distinct populations influence reproductive success, mating patterns, tactic frequency, and the strength of sexual selection. In **Chapter 4** I apply a life history model and estimate the frequency of cuckoldry based on the genetic reproductive success of each tactic to further understand the evolution of alternative reproductive tactics in plainfin midshipman. And in **Chapter 5** I explore differences in diet between males and females and between guarder type I and sneaker type II males in relation to time spent in the nest. Finally, in **Chapter 6** I summarize the results of my thesis while highlighting significant contributions to the field and given these results, discuss possible avenues for future research.

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Chapter 2

HIGH DEGREE OF PATERNITY LOSS IN A SPECIES WITH ALTERNATIVE REPRODUCTIVE TACTICS

Behavioral Ecology and Sociobiology (2013) 67:399-408

2.1 ABSTRACT

In many mating systems, males adopt alternative reproductive tactics (ARTs) to maximize reproductive success. In fishes, guarding males often invest more energy into courtship, defense, and paternal care, whereas cuckolding males forego such costs and steal fertilizations by releasing their sperm in the nest of a guarding male. These two tactics have been documented in the plainfin midshipman fish (*Porichthys notatus*), yet the relative reproductive success of the guarding and cuckolding male tactics remains unknown. In this study, we used microsatellite markers to determine the level of paternity of the guarding type I males. We explored how paternity varied with male phenotype and across the breeding season. Our results revealed the lowest documented levels of paternity in a species with obligate paternal care. Although paternity remained consistently low, it did increase as the breeding season progressed. Male body size did not significantly

predict paternity. The low paternity in this species may be explained, in part, by aspects of their reproductive ecology including the duration of parental care period, limited nest availability and competition for nests, as well as the occurrence of nest takeovers. Overall, our findings contribute to the understanding of the ultimate factors underlying ARTs in this species, and highlight the importance of investigating reproductive success across the entire breeding season.

2.2 INTRODUCTION

Alternative reproductive tactics (ARTs) occur when there are two or more discontinuous reproductive phenotypes within a population, each attempting to maximize reproductive success through alternative means (Taborksy et al. 2008). One male morph, sometimes called guarding, bourgeois, parental, territorial, or type I, is typically associated with showy displays used to court females and deter rivals. The alternative male morph instead exploits the guarding male's investment in courtship by gaining reproductive success in sneaky or coercive ways. Such alternative male morphs are often called parasitic, sneakers, cuckolders, or type II males. Although many studies have described the existence of ARTs across a wide variety of taxa, relatively few studies have used molecular techniques to quantify the reproductive success of the male morphs.

In the last few decades, the application of molecular techniques to behavioral research has revolutionized our understanding of mating systems and the fitness consequences of mating behavior. These techniques have been applied to quantify individual reproductive success, and the reproductive success associated with the alternative male tactics (e.g. Shuster 1989; Brockmann et al. 2000; Neff 2001; Lank et al. 2002). Among fishes with obligate male care, the mean paternity of guarding males is typically greater than 80% (for reviews see Avise et al. 2002; Coleman and Jones 2011). Møller and Thornhill (1998) predicted that the degree of male parental care should be negatively correlated with extra-pair paternity, such that low paternity is only expected in species where male care is not required or important for offspring survival (also see Møller 2000). This hypothesis was developed based on avian mating systems, where paternal care is typically a depreciable resource; that is, there are greater costs imposed on the parent for each additional offspring in the nest (Altmann et al. 1977; Clutton-Brock 1991). Although paternal care in fishes is often thought to be nondepreciable, parental investment can increase in relation to brood size (Gross 1980; Coleman et al. 1985; Coleman and Fischer 1991). Thus, the Møller and Thornhill (1998) hypothesis should still hold for many fish mating systems, yet several fish species with obligate male care have low paternity (e.g. Bessert et al. 2007; Neff and Clare 2008; Alonzo and Heckman 2010). For example, Alonzo and Heckman (2010) found that paternity in the ocellated wrasse (*Symphodus ocellatus*) was around 72% and they argued that their data did not lend support for the Møller and Thornhill (1998) hypothesis. Instead, other aspects of an animal's mating system and ecology might also be important in governing parental care patterns, including the opportunity for remating, the temporal distribution and density of females, and resource availability (Emlen and Oring 1977; Westneat and Sherman 1992; Avise et al. 2002).

Among the studies that have investigated the paternity of male ARTs, only a handful have incorporated temporal variation in reproductive success (Sefc et al. 2009; Alonzo and Heckman 2010; see also Coleman and Jones 2011). Temporal variation in reproductive success has long been recognized as an important factor influencing mating system dynamics (e.g. Schultz 1993; Verhulst et al. 1995; Oliveira et al. 1999) as well as an important factor to consider when calculating the relative reproductive success of the ARTs (e.g. Neff and Lister 2006). Importantly, measures of cuckoldry rates calculated at one time point during the reproductive season may not accurately reflect the relative reproductive success of each tactic overall (Neff and Clare 2008; Sefc et al. 2009). As proposed by Neff and Clare (2008), temporal variation in paternity could arise from: (1) variation in number of spawning males and females across the season; (2) variation in male body condition and the ability to effectively defend against cuckolders; and (3) variation in the number of reproductively active cuckolders across the season. In this study, we determined the reproductive success of the "type I" male tactic, and how this varied across a protracted breeding season, in a species with ARTs, the plainfin midshipman (*Porichthys notatus*).

The plainfin midshipman is a nocturnally active deep-water marine fish distributed along the Pacific coast of North America (Hubbs 1920; Arora 1948; Miller and Lea 1972). It is characterized by two distinct reproductive tactics known as guarding “type I” and cuckolder “type II” males (Brantley and Bass 1994). At the onset of the breeding season in early spring, reproductive adults undergo a large scale vertical migration from deep waters (> 200 m) to the spawning grounds in calm rocky shores in the intertidal zone (Arora 1948; Miller and Lea 1972). Type I males aggressively compete for limited nest sites, and the largest males typically win the largest nest sites (DeMartini 1988). Larger males and larger nests attract more females (DeMartini 1988) and also attract more type II males (Lee and Bass 2004). Type I males acoustically court females (Ibara et al. 1983; Bass 1992; Brantley et al. 1993), who produce only a single clutch of fewer than 200 eggs (each 5-7 mm in diameter) per year, that are released into a single nest (DeMartini 1990). Type I males will remain in the nest and continue to court and spawn with additional females until the nest is filled with multiple “age cohorts” that typically are at different developmental phases (Arora 1948; DeMartini 1988; Brantley and Bass 1994). Midshipman have direct development, where embryos transition into juveniles with no major morphological remodeling or a distinct larval phase (Balon 1999). The offspring developmental phases include embryos (pre-hatching), free embryos (post-hatching), and juveniles (first oral feeding, but may still be absorbing their yolk sacs, Crane 1981; Balon 1999). Offspring “ages” can be inferred by current developmental status. It takes about 60 days for embryos to develop into free-swimming juveniles, and because there are multiple cohorts acquired across the breeding season, type I males may be caring for offspring for up to four months (Arora 1948; K. Cogliati pers. obs.). Paternal care involves fanning and brushing the eggs and hatched embryos, to help keep them clean and deliver fresh water and oxygen (Arora 1948). Because larger broods typically require more fanning behavior (Coleman and Fischer 1991), this aspect of parental care in midshipman is likely depreciable. Type II males, on the other hand, do not guard nests or court females. Instead, they steal fertilizations from type I males by either sneaking into the nest when a female is present, or fanning sperm into the nest from the periphery. Interestingly, type I males are behaviorally plastic, and may adopt cuckolding behaviors, particularly when nesting sites are severely limited (Lee and Bass 2004).

Although alternative reproductive tactics have been well described in plainfin midshipman (Brantley and Bass 1994), the genetic reproductive success of each tactic has never before been examined. The aim of this study was to determine the level of paternity assigned to the nest guarding type I males and in turn, the relative rate of cuckoldry in this species. In addition, given the lengthy breeding and parental care period, and the presence of multiple age cohorts within a nest, we explored how male phenotype and the timing in the breeding season influenced paternity. Specifically, we postulated that if male condition deteriorated as the breeding season progressed, then paternity should decrease due to a decrease in males' abilities to effectively defend their nest against cuckoldry.

2.3 METHODS

2.3.1 Field collections

Between May 5th and June 5th, 2008, May 18th and May 27th, 2009, and May 10th and July 14th, 2010, we located and sampled plainfin midshipman nests during low tide in the intertidal zone on three rocky beaches in British Columbia (Ladysmith Inlet: 49°01´N, 123°83´W and Mill Bay: 48°63´N, 123°53´W on the eastern coast of Vancouver Island, and Crescent Beach: 49°04´N, 122°88´W in South Surrey). At each nest, we measured total length (TL in cm), standard length (SL in cm) and body mass (in grams) of each fish present, and sexed each fish (based on the shape of the urogenital papilla and overall body coloration). Using mass and length values, we calculated a body condition index using the residuals from the regression of log (mass) against log (length) (Fechhelm et al. 1995; Blackwell et al. 2000). We digitally photographed each nest so that we could later quantify the offspring in terms of number, size and developmental phase (embryo, free embryo, juvenile). We collected a small amount of fin tissue from each adult for genetic analyses and preserved the tissue in 95% ethanol. In addition, for a subset of nests, we collected and preserved 40-50 offspring from each cohort present within the nest that had suitable embryonic development for genetic analyses (2008: N=10; 2009: N=13; 2010: N=24). Here, we define a “cohort” as a group of offspring laid by one or more females in a nest in relative synchrony such that they are developing together, but distinct from other cohorts in the same nest based on age and the timing of egg laying. Thus, cohorts in the nests may be from multiple offspring developmental phases. Embryos were suitable for genetic analyses once they passed the blastodermic cap stage, when the neural tube was visible through the egg envelope. After sampling the adults and the offspring, we returned the adults to the nest and carefully replaced all rocks to their original position.

In 2010, we also estimated the duration of male parental care and nest tenure by labeling 131 nests with a marked plastic tent peg positioned by the nest and returned to these nests in subsequent low tides. In 61 of these 131 nests, we tagged the type I male in the dorsal fin tissue between fin rays with a unique 4-mark code of non-toxic injectable Elastomer (Northwest Marine Technology, Inc., Washington, USA). On return to the labeled nests, we took morphological measurements from all fish present, collected fin clips from all non-tagged fish, re-photographed the nests, and collected a sample of any embryos (new cohort) that were not previously present in these nests. Additionally, we determined if the original type I male was still the nest owner or not based on the unique code. The majority of the nests included in our genetic analyses in 2010 were marked and monitored over the season (17 of 24 nests); however, seven were located for the first time late in the breeding season and therefore only sampled once.

2.3.2 Embryonic development classification

Because we collected across the breeding season, cohorts were at different development phases. We therefore used a back-calculation to determine a Julian date of egg laying for all cohorts in each nest. We developed this back-calculation based on the cohort's current development state and the Julian date of collection. During both sampling and DNA extractions, we observed key embryonic development characteristics (Balon 1999). From these characteristics, we developed a classification scheme that placed each sample into one of nine distinct categories based on the degree of embryonic development. We tested the reliability of our classification assignment (inter-observer reliability was 87%). Four of 30 samples were inconsistently classified among observers but in those cases, the discrepancy involved one (of three) observers classifying the sample either one category before or after the classification designated by the other observers. In addition, we used photographs of broods from nests that were sampled multiple times during the breeding season to ground-truth our technique and to determine the numbers of days between development categories represented in the nests. For each cohort in our analyses, we determined the developmental category and then subtracted the back-calculated number of days for that developmental category from the sample date to arrive at the date of egg laying.

2.3.3 Genetic analyses

In total, we extracted DNA from 222 adults and 1713 offspring (approximately 25 offspring per phase of development per nest) and genotyped these individuals using six microsatellite loci developed from plainfin midshipman (Pon22, Pon23, Pon25, Pon30, Pon32, and Pon47; see Suk et al. 2009 for primer sequences). We carried out PCR amplifications on T1 Thermocycler (Whatman-Biometra) using fluorescent dyed forward primers (Sigma-Genosys, Woodland, Texas) following the protocol outlined in Suk et al. (2009), and conducted fragment analyses on a capillary sequencer (CEQ 8000, Beckman Coulter; Fullerton, California). Microsatellite alleles for each locus were scored for each individual based on characteristics peaks.

Using adult male and female genotypes, we investigated whether individuals from our three different sampling sites were from distinct genetic populations using *structure* (v2.3) software (Pritchard et al. 2000). *Structure* indicated a single panmictic population (data not shown; consistent with results described in Suk et al. 2009). Therefore, we calculated population level allele frequencies for each locus using all adult genotypes.

2.3.4 Paternity analyses

We calculated the paternity estimate for each type I male using the genotypes of the male and offspring (offspring were required to show at least 3 representative loci for inclusion in the analysis), the allele frequencies of the breeding population, and the *two-sex paternity* model developed in Neff et al. (2000a,b) and Neff (2001). The *two-sex paternity* model calculates the proportion of the offspring that are compatible with the putative father (ng_{dad}), the expected proportion of offspring that were compatible with the putative father by chance (NG_{dad}), an estimated paternity measure expressed as a proportion, and a 95% confidence interval (CI). Males with multiple cohorts present in their nest had an additional model constructed to obtain individual paternity estimates for each cohort.

In addition to calculating paternity, we used COLONY (v2.0) software to calculate the number of females that were genetically represented in the sample of offspring that also belonged to the type I guarding male found in the nest (Wang 2004; Jones and Wang 2010). We had very low paternity estimates in some cases (<10%), which we considered to be a possible result of nest takeovers. For those cohorts, we used COLONY to infer the likely male genotype of the previous nest owner, as well as the number of possible females represented, based on best family grouping assigned by the program (Wang 2004; Jones and Wang 2010). COLONY is unable to differentiate male from female genotypes. Using the distribution of maternity values within a cohort, paternity values were typically equal to or greater than the maternity values (in 48 of 62 cohorts). In addition, there were 3 out of 62 cohorts where only one female was represented (100% maternity), while there were 11 cohorts out of 62 where the male was the sole father for that cohort (100% paternity). Therefore, we chose the most common genotype represented in COLONY as that of the previous type I male nest owner. The inferred genotype of the previous nest owner was then used in the *two-sex paternity* model to obtain a new paternity estimate with confidence intervals. We followed Jones et al. (2010) and used the *two-sex paternity* model rather than COLONY to calculate the parentage in all cases so that we could use the calculated confidence intervals in further analyses. Also, the *two-sex paternity* model is especially useful in species with nest-holding males and suspected cuckoldry (Jones et al. 2010).

2.3.5 Statistical analyses

For all statistical analyses, we arcsine square root transformed the paternity estimates. Paternity estimates calculated in the *two-sex paternity* model have individual 95% CI indicating varying levels of confidence for each estimate. Therefore, we weighted all paternity data by their confidence using log (1/CI +1). We checked data for normality and then ran one-way ANOVAs to test for effects of location (sampling beach) and collection year (2008-2010) and controlled for these effects in further analyses when these factors were significant.

To investigate how paternity varied on a temporal scale, we constructed a linear mixed effects model with random slopes fit by restricted maximum likelihood (REML) using transformed paternity as our response variable and the back-calculated Julian date of egg laying as our predictor variable. We included nest ID as a random factor (to account for males with multiple paternity estimates) and the random interaction between Julian date and nest ID was also incorporated into the model. Similarly, we constructed additional linear mixed effect models fit by REML using male phenotypic characteristics as predictor variables in place of Julian date of egg laying, keeping nest ID as a random factor.

2.4 RESULTS

2.4.1 Paternity analyses

In total, we calculated paternity for 74 different cohorts within 47 separate nests. Some nests (N=30) had only one cohort and therefore only one associated paternity estimate, while other nests (N=17) had up to 4 cohorts and paternity estimates (two cohorts: N=8; three cohorts: N=8; four cohorts: N=1) (**Table 2.1**). Using all 74 paternity estimates calculated from all cohorts, the average paternity for type I males was 52% ± 0.04 (mean ± SE; N=74, range 0-100%). There were no differences in paternity based on sampling location (ANOVA: $F_{2,71}=0.34$, p=0.71) or year of collection (ANOVA: $F_{2,71}=0.86$, p=0.43), so neither factor was included in further analyses. On average and within a cohort, males mated with 3.3 ± 0.2 females (mean ± SE; N=65, range 1-5). In general, we observed a decrease in paternity as the number of females that mated with the male increased ($F_{4,60}=2.74$, p=0.04; **Fig. 2.1**); however, post-hoc comparisons revealed no significant difference in average paternity between males that mated with 1, 2, 3, 4 or 5 females (Tukey HSD: all p>0.12). The average NG_{dad} value (1-exclusion probability) calculated from our samples was 0.14 ± 0.009 (mean ± SE; range 0.01-0.33), indicating that on average and within this population, 86% (1-0.14) of all individuals could be excluded as a potential father by chance alone. There were no significant differences in the paternity values estimated using the *two-sex paternity* model versus those generated by COLONY (paired t-test: $t_{86}=1.13$, p=0.26), and, as expected, the paternity values from each of these programs were highly correlated ($R^2=0.72$, $p<0.0001$).

Table 2.1 Summary of paternity analyses from nests with multiple cohorts in 2010 in plainfin midshipman (*Porichthys notatus*)

Nest	Cohort[a]	Date[b]	$N_{offspring}$	NG_{dad}[c]	ng_{dad}[d]	Pat (%)	95% CI	Females[e]
1	1	May 31	28	0.30	0.36 (10)	8	1-45	1
1	2	June 14	25	0.30	0.84 (21)	77	29-91	2
1	3	June 28	24	0.31	0.33 (8)	3	0-44	1
2	1	June 5	24	0.11	0.79 (19)	77	48-90	3
2	2	June 12	24	0.11	0.63 (15)	58	25-77	4
2	3	July 3	25	0.11	0.92 (23)	91	69-97	1
3	1	May 10	25	0.13	0.00 (0)	0	0-13	0
3	2	May 18	24	0.14	0.75 (18)	71	41-86	5
3	3	June 10	24	0.15	0.88 (21)	85	60-95	3
4	1	May 18	24	0.14	0.75 (18)	71	39-86	5
4	2	May 25	25	0.14	0.60 (15)	54	20-74	3
4	3	June 10	24	0.15	0.79 (19)	76	45-89	2
5	1	May 9	26	0.17	0.85 (22)	81	54-92	2
5	2	May 17	24	0.17	0.58 (14)	50	15-71	3
5	3	June 24	26	0.17	0.38 (10)	26	3-51	2
6	1	May 23	25	0.21	0.56 (14)	44	8-67	5
6	2	May 30	30	0.21	0.50 (15)	36	5-60	5
6	3	June 21	29	0.21	0.90 (26)	87	60-95	3
7	1	May 9	23	0.12	0.83 (19)	80	54-92	4
7	2	May 17	24	0.15	0.42 (10)	31	5-57	5
8	1	May 12	25	0.12	0.00 (0)	0	0-13	0
8	2	June 3	25	0.12	0.84 (21)	82	56-93	2
8	3	June 10	24	0.12	0.75 (18)	72	41-86	3
8	4	June 24	24	0.12	0.92 (22)	90	68-97	4
9	1	May 29	25	0.05	0.76 (19)	75	52-88	4
9	2	June 18	25	0.05	0.48 (12)	46	23-65	5
10	1	May 11	48	0.06	0.65 (31)	62	44-75	5
10	2	May 29	25	0.07	0.80 (20)	79	57-91	4
11	1	May 21	25	0.11	0.68 (17)	64	37-81	4
11	2	July 2	25	0.15	0.48 (12)	39	10-62	5
12	1	May 23	25	0.05	0.20 (5)	15	2-37	4
12	2	July 6	25	0.05	0.44 (11)	41	19-61	2
13	1	June 3	23	0.03	0.00 (0)	0	0-14	0
13	2	June 24	25	0.03	0.80 (20)	79	59-91	3
14	1	May 7	25	0.11	0.00 (0)	0	0-13	0
14	2	June 3	22	0.11	0.95 (21)	95	74-99	2
14	3	June 10	25	0.12	0.92 (23)	91	69-97	3
15	1	May 14	24	0.25	0.42 (10)	22	2-53	2
15	2	June 5	25	0.23	0.64 (16)	53	12-75	4
15	3	June 18	25	0.22	0.40 (10)	23	2-52	3
16	1	June 7	25	0.08	0.48 (12)	43	17-64	3
16	2	June 14	24	0.08	0.38 (9)	32	8-55	1

17	1	June 20	25	0.11	0.04 (1)	0	0-18	0
17	2	June 28	25	0.12	0.92 (23)	91	70-97	4

[a] The different age cohorts collected for each nest, shown in chronological order from oldest to youngest offspring

[b] The back-calculated date on which the eggs were likely laid in the nest (see text for details), all in year 2010

[c] The expected proportion of offspring compatible with the father by chance alone; analogue of the exclusion probability, calculated according to Neff et al. (2000a,b)

[d] The proportion of offspring (and number) that were compatible with the putative father calculated according to Neff et al. (2000a,b)

[e] The number of females that sired the compatible offspring of the putative father, determined using COLONY

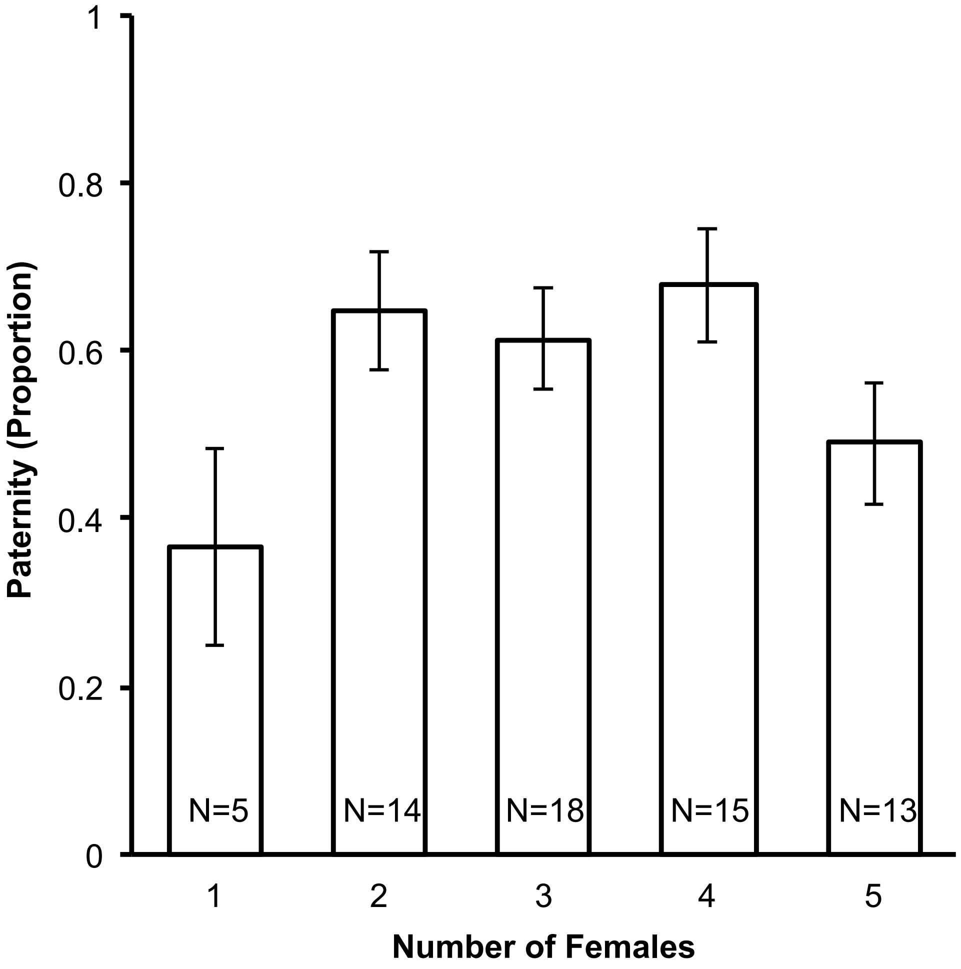

Fig. 2.1 Paternity in plainfin midshipman (*Porichthys notatus*) as a function of the number of females that mated with the nest owner per cohort. Statistics were performed on weighted transformed data, but for clarity we present the raw data here. Bars represent mean values ± 1 SE

2.4.2 Temporal and phenotypic patterns of paternity

Paternity increased across the breeding season (Julian date $F_{1,29.39}$=5.59, p= 0.02). To determine if this seasonal variation in paternity may have arisen from variation in total brood size, we included brood size as a predictor variable in our original model and found no effect ($F_{1,39.08}$=0.48, p=0.49). Likewise, male size, mass, and body condition based on the residuals of body mass and body length, were not significant predictors of paternity when these were included in the linear mixed effects model (standard length: $F_{1,32.04}$=0.02, p=0.90; body mass: $F_{1,19.82}$=0.91, p=0.35; mass by length residuals: $F_{1,50.71}$=0.71, p=0.40). Taken together, these results indicate that paternity increased later in the season regardless of brood size, male size, and male body condition.

2.4.3 Nest takeovers and loss of paternity

Of the 47 nests sampled in our study, 11 had paternity estimates of less than 10% for at least one cohort (**Table 2.2**). When we compared paternity based on multiple cohorts sampled and controlling for the random effect of nest, these low values of paternity were found significantly more often in the oldest or most developed offspring ($F_{3,13}$=39.98 p<0.0001; **Fig. 2.2**). Similarly, when we controlled for multiple cohorts collected from some of the nests using the linear mixed effects model, we found that the low paternity values occurred significantly more often earlier in the breeding season ($F_{1,20.73}$=11.82, p=0.003). Previous changes in male resident status may have contributed to these low values of paternity. Looking at

Table 2.2 Summary of paternity analyses from nests with suspected takeovers in plainfin midshipman (*Porichthys notatus*)

Nest	Cohort[a]	Male[b]	Date[c]	$N_{offspring}$	NG_{dad}[d]	ng_{dad}[e]	Pat (%)	95% CI	Females[f]
1	1	1	May 28 '08	12	0.08	0.08 (1)	1	0-34	0
1	1	Inferred 1	May 28 '08	12	0.01	0.58 (7)	58	31-80	2
2	1	2	May 19 '08	24	0.16	0.08 (2)	0	0-22	0
2	1	Inferred 2	May 19 '08	24	0.35	0.88 (21)	81	28-93	2
3	1	3	May 20 '09	12	0.09	0.17 (2)	8	1-43	2
3	1	Inferred 3	May 20 '09	12	0.03	0.75 (9)	74	44-91	4
4	1	4	May 20 '09	19	0.09	0.00 (0)	0	0-17	0
4	1	Inferred 4	May 20 '09	19	0.02	0.47 (9)	46	25-68	3
5	1	5	May 20 '09	15	0.19	0.13 (2)	0	0-34	2
5	1	Inferred 5	May 20 '09	15	0.09	0.47 (7)	42	14-68	2
6	1	6	June 3 '10	23	0.03	0.00 (0)	0	0-14	0
6	1	Inferred 6	June 3 '10	23	0.06	0.96 (22)	95	77-99	2
6	2	6	June 24 '10	25	0.03	0.80 (20)	79	59-91	3
7	1	7	May 12 '10	25	0.12	0.00 (0)	0	0-13	0
7	1	Inferred 7	May 12 '10	23	0.02	0.64 (16)	63	43-79	2
7	2	7	June 3 '10	25	0.12	0.84 (21)	82	56-93	2
7	3	7	June 10 '10	24	0.12	0.75 (18)	72	41-86	3
7	4	7	June 24 '10	24	0.12	0.92 (22)	90	68-97	4
8	1	8	May 31 '10	28	0.30	0.36 (10)	8	1-45	1
8	1	Inferred 8a	May 31 '10	28	0.06	0.39 (11)	36	14-56	3
8	2	8	June 14 '10	25	0.30	0.84 (21)	77	29-91	2
8	3	8	June 28 '10	24	0.31	0.33 (8)	3	0-44	1
8	3	Inferred 8b	June 28 '10	24	0.09	0.58 (14)	54	24-74	1
9	1	9	May 7 '10	25	0.11	0.00 (0)	0	0-13	0
9	1	Inferred 9	May 7 '10	25	0.03	0.80 (20)	79	69-97	4
9	2	9	June 3 '10	22	0.11	0.95 (21)	95	74-99	2
9	3	9	June 10 '10	25	0.12	0.92 (23)	91	69-97	3
10	1	10	May 10 '10	25	0.13	0.00 (0)	0	0-13	0
10	1	Inferred 10	May 10 '10	25	0.11	1.00(25)	100	85-10	2
10	2	10	May 18 '10	24	0.14	0.75 (18)	71	41-86	5
10	3	10	June 10 '10	24	0.15	0.88 (21)	85	60-95	3
11	1	11	June 20 '10	25	0.11	0.04 (1)	0	0-18	0
11	1	Inferred 11	June 20 '10	25	0.07	0.68 (17)	65	43-82	3
11	2	11	June 28 '10	25	0.12	0.92 (23)	91	70-97	4

[a] The different age cohorts collected for each nest, shown in chronological order from oldest to youngest offspring

[b] Using genotype data from the Type I male found on the nest (number only) or using inferred genotypes obtained from COLONY (inferred #)

[c] The back-calculated date on which the eggs were likely laid in the nest (see text for details)

[d] The expected proportion of offspring compatible with the father by chance alone; analogue of the exclusion probability, calculated according to Neff et al. (2000a,b)

[e] The proportion of offspring (and number) that were compatible with the putative father calculated according to Neff et al. (2000a,b)

[f] The number of females that sired the compatible offspring of the putative father, determined using COLONY

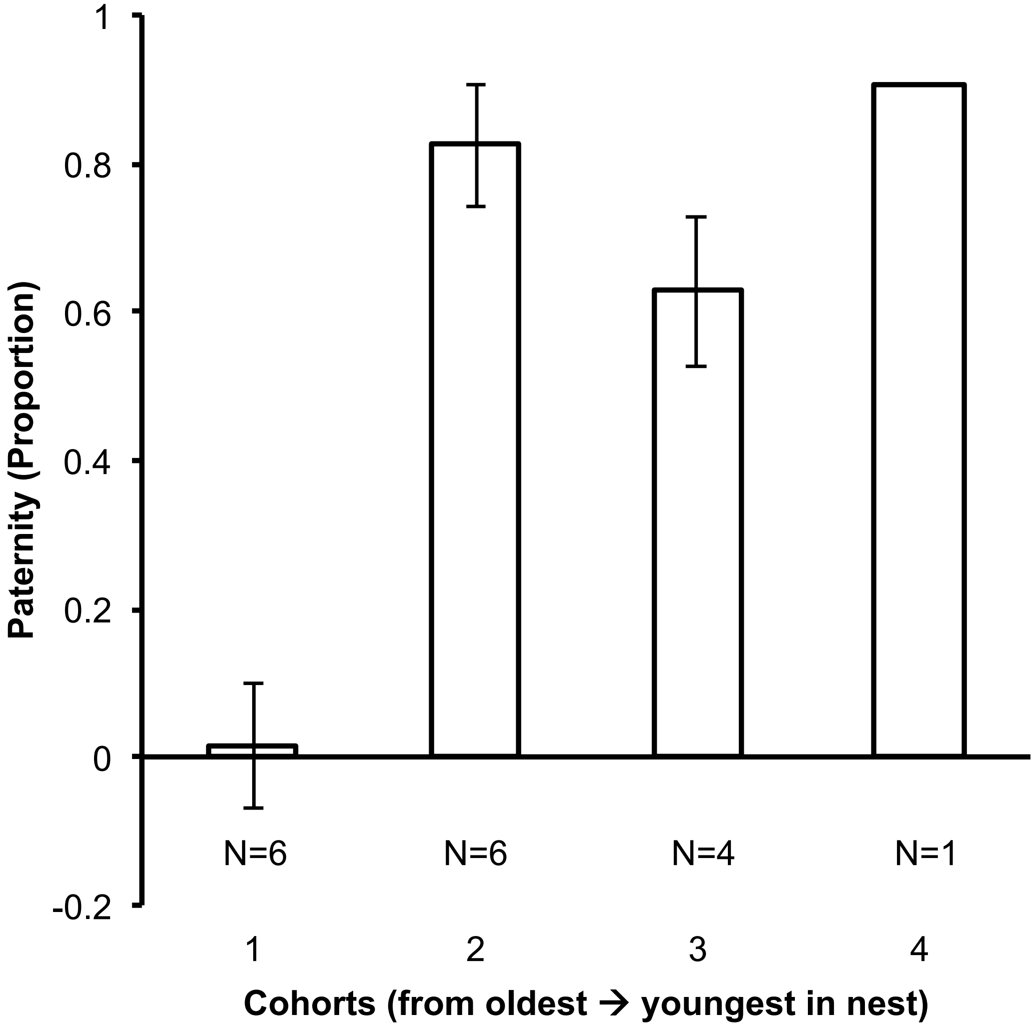

Fig. 2.2 Proportion of paternity based on cohort in plainfin midshipman (*Porichthys notatus*). Data included nests that had a possible takeover event and multiple cohorts sampled (less than 10% paternity; N=6), from oldest (1, laid earlier in season) to youngest (4, laid later in season) offspring. Statistics were performed on weighted transformed data controlling for nest, although the raw data are presented. Bars represent mean values ± 1 SE

the 131 nests that we monitored in 2010, we found that 45% were no longer in use or were abandoned after our initial sampling, 34% of the re-sampled nests were occupied by new type I males, while the original type I male remained in only 21% of the resampled nests. Amongst the 34% of nests where a new male had been observed, the new takeover males were not significantly larger (matched pairs – length: $t_{1,31}$=1.45, p=0.16; mass: $t_{1,23}$=1.52, p=0.14) or in better condition (residuals matched pairs: $t_{1,23}$=0.33, p=0.74) when compared to previous, now missing, nest owners. Furthermore, males that remained on the nest throughout the season were not larger or in better condition than either males who lost or abandoned their nests or males that took over nests (all body measures taken: $F_{2,79}$<1.65, p>0.20).

2.4.4. Average paternity excluding nest takeovers

Considering cohorts with a likely takeover, average paternity for the previous type I male calculated using COLONY and the *two-sex paternity* model was 66% ± 0.06 (mean ± SE; N=12, range 35-100%). When we excluded potential takeover events (where paternity estimates were less than 10%) and included the estimates of the previous putative type I male, average paternity for type I males rose from 52% to 63% ± 0.03 (mean ± SE; N=74, range 15-100%). Also, egg laying date no longer significantly predicted paternity when we included these paternity estimates of the previous nest owners in place of the very low paternity values in our original linear mixed effects model ($F_{1,22.20}$=1.32, p=0.26). That is, our initial pattern for seasonal variation in paternity appeared to be driven by these very low paternity estimates and likely takeovers occurring early in the season.

2.5 DISCUSSION

Our study documents the lowest reported levels of paternity in a vertebrate with male-only care; on average only 52% of the young in plainfin midshipman nests could be assigned to the adult type I male guarding them. Additionally, paternity remained consistently low even after accounting for likely nest takeovers by type I males. In two reviews of paternity in fishes, Avise et al. (2002) and Coleman and Jones (2011) showed that in species with alternative male tactics, the typical paternity for the guarding male tactic was generally around 80% or higher. A number of authors have argued that paternity cannot be any lower in species where male care is required and important for offspring survival (Møller and Thornhill 1998; Møller 2000). This argument, however, does not hold for plainfin midshipman where paternal care is obligate and at least to some degree, depreciable.

There are several important ecological and reproductive differences between plainfin midshipman and other species with published paternity estimates that may explain why our observed levels of paternity are relatively low. First, the costs of missed foraging opportunities associated with parental care vary across species. Occelated wrasse (72% mean paternity to guarding males), bluegill sunfish (*Lepomis macrochirus*, 77% paternity), and pumpkinseed sunfish (*Lepomis gibbosus*, 62% paternity) all breed repeatedly throughout the season, but males in each of these three species have time between broods to replenish energy stores before they rebuild new nests in subsequent reproductive bouts. The broods that are cared for also develop quickly (3-10 days) and all young undergo development in synchrony (e.g. Neff and Clare 2008; Alonzo and Heckman 2010). Hence, the time between broods provides foraging opportunities that plainfin midshipman male parents do not have. Plainfin midshipman also have a protracted breeding season, with a long embryo development time (50-65 days), as well they have staggered offspring development (Arora 1948; Sisneros et al. 2009; K.Cogliati pers. obs.). Thus, the high cost to care associated with missed foraging opportunities and duration of care in plainfin midshipman may give rise to a greater degree of cuckoldry as a means to avoid the investment in parental care.

Second, nest site availability appears to be more limited in plainfin midshipman compared to these other species with ARTs (DeMartini 1988, 1991). The high male-male competition for nesting sites has probably led to the evolution of the cuckolding tactic (type II males), as well as to the behaviorally plastic cuckoldry behaviors seen by smaller type I males (Lee and Bass 2004; K. Cogliati pers. obs.), and possibly to males tolerating a high degree of paternity loss. This pattern is mirrored by a study of fathead minnows (*Pimephales promelas*), which estimated 95% paternity to the guarding male when nesting substrate was not limited, but only 79% when nesting substrate was limited (Bessert et al. 2007). Our two hypotheses (costs associated with missed foraging opportunities and limited nest availability) are not mutually exclusive and it is possible that the low observed paternity in plainfin midshipman is driven by both factors (Emlen and Oring 1977; Clutton-Brock et al. 1997; Coltman et al. 1999; Bessert et al. 2007).

In addition to the partial paternity loss arising from sneaker males, some guarding males experienced a complete paternity loss in some cohorts within their nest. In fact, 17% of nests exhibited a complete loss of paternity for at least one cohort in the nest, and another 6% of nests exhibited nearly complete loss of paternity (less than 10% paternity) for at least one cohort in the nest. Three scenarios could explain these very low paternity levels in plainfin midshipman: (1) it may be a result of some sterile type I males in our population, (2) it may be a result of highly successful cuckoldry, or (3) it could arise from nest takeovers. A nest takeover is an extreme form of male-male competition where males continue to compete for nest sites even after a male has "won" a nest and previously spawned with one or more females in the nest (see Coleman and Jones 2011). Our examples of very low paternity occurred significantly more often in the oldest offspring in nests when multiple cohorts were present (5 of 6 nests; 6 of 7 cohorts). This pattern lends support for the occurrence of nest takeovers in plainfin midshipman, rather than sterile males or highly successful cuckoldry, both of which would not show temporal variation. Our findings are similar to those of Bessert et al. (2007) where the low paternity values in fathead minnows were always in the oldest cohort, a finding they also used to infer takeovers. Also when we sampled across the breeding season, we observed a new untagged male in 34% of our sampled nests. This value is higher than what was generated by our paternity analyses and it likely included both cases of takeover and nest abandonment by type I males. Taken together, these data indicate that nest takeovers occurred in at least 23% of nests in plainfin midshipman.

Why would new takeover males guard the eggs of a previous competitor? First, takeovers may be necessary when nests are a limited resource such as in plainfin midshipman (DeMartini 1988; DeWoody et al. 2000; Coleman and Jones 2011). Takeover rates differed between substrate limited (29%) and substrate unlimited (5%) sampling sites in the fathead minnow (Bessert et al. 2007). We found that nest takeovers occurred more often early in the season (83% of observed takeovers occurred in the first half of the breeding season), likely while males were still competing for these limited nest sites on a particular stretch of beach. As the season progressed, the occurrences of takeovers became less frequent, possibly because the males were more established in their nests and were less likely to desert or to move to a new nest when challenged (sensu Coleman and Gross 1991). Second, takeovers may be particularly beneficial if females have a strong preference for nests with eggs (Rohwer 1978; Ridley and Rechten 1981; Porter et al. 2002). Females may find the presence of eggs attractive because it increases the overall chances that her eggs will survive, largely through the dilution effect (Rohwer 1978; Foster and Treherne 1981). In threespine sticklebacks (*Gasterosteus aculeatus*; Ridley and Rechten 1981) and the sand goby (*Pomatoschistus minutus*; Forsgren et al. 1996), for example, females prefer to lay their eggs in nests that already contain eggs (Dugatkin 1992). Whether or not there is a female preference for eggs in the nest in plainfin midshipman is not yet known.

We found that paternity in plainfin midshipman varied across the breeding season with cuckoldry being more common early in the breeding season. We explored the cause of this variation using the three hypotheses proposed by Neff and Clare (2008). First, as predicted, we found more spawning activity earlier in the breeding season with more eggs being laid early compared to later in the breeding season (a mean of 761 eggs/nest early in the season vs. 482 eggs/nest late in the season; unpublished data). Second, while it is possible that males in poor condition are unable to effectively defend their nest against cuckolders, a hypothesis supported by the study in bluegill (Neff and Clare 2008; Cargnelli and Neff 2006), we found no evidence for such relationship in plainfin midshipman: type I male body condition did not vary across the breeding season, nor did it correlate with paternity. Through our population monitoring, we observed type II males at equal frequencies across the breeding season (data not shown), refuting the third hypothesis that temporal variation in paternity could be driven by seasonal changes in sneaker number. However, some caution is warranted here because the frequency of type II males observed was low and we did not sample nests outside of low tide events. Finally, an additional possibility that arose from our study to explain seasonal variation in paternity was the occurrence of nest takeover events early in the breeding season. Altogether, our data highlight the importance of investigating reproductive success across the breeding season as opposed to one single time point to ensure accurate population-level paternity estimates.

The results of this study demonstrate low paternity levels in plainfin midshipman that are likely mediated by key aspects of their reproductive ecology, including patterns of parental care and limited resource availability. The comparatively low levels of paternity in plainfin midshipman also demonstrates the need for additional studies that investigate the genetic reproductive success in species with alternative reproductive tactics to further explore the variation in paternity among species and the underlying ecological driving forces. In addition, we have shown that nest takeovers occur, and that they contribute to the overall low paternity in this species. Takeovers are likely a result of male-male competition among type I males for limited nest sites. These results collectively demonstrate that direct competition among males can play an important role in determining paternity in a species with alternative reproductive tactics.

2.6 ACKNOWLEDGEMENTS

We thank Celia Chui, John Fitzpatrick, Ahdia Hassan, Julie Marentette, Jen Reynolds, Natalie Sopinka, and Jonathan Taves for their assistance with data collection in British Columbia. We thank Michael Roubakha, Shawn Garner, Cayli Hunt, and Amanda Pereira for their assistance with DNA extractions and sample analyses. We thank Cayli Hunt, Erin McCallum, Ryan Caldwell, and Celeste Bouchard for assistance with developing the classification scheme and verifying its validity. We also thank Mr. & Mrs. Cogswell, Mr. & Mrs. Weatherell, and especially Mr. & Mrs. Flader for providing access to midshipman habitats and lodgings for field crew. This work was funded by the Natural Sciences and Engineering Research Council of Canada (grants to S.B. and B.D.N.).

2.7 ETHICAL STANDARDS

This work conformed to the guidelines outlined by the Canadian Council on Animal Care, and permission to undertake this work was granted by the Department of Fisheries and Oceans, and the First Nations Bands in which sampling occurred.

2.8 CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Chapter 3

COMPARING POPULATION LEVEL SEXUAL SELECTION IN A SPECIES WITH ALTERNATIVE REPRODUCTIVE TACTICS

Submitted to: Behavioral Ecology (October 2013)

3.1 ABSTRACT

The description of a species' mating patterns is often based on observations from a single exemplar population; however, environmental variation can lead to variation in mating patterns and to differences in the strength of sexual selection among populations. In this study, we explored how resource distribution and ecological variation across a species' range affects competition and the strength of sexual selection in two populations of plainfin midshipman (*Porichthys notatus*), a species with two male reproductive tactics. Male plainfin midshipman can be guarders that compete for nest sites and court females, or sneakers that attempt to steal fertilizations from the guarder males during spawning. When we compared across populations, we found that there were fewer unoccupied nests, more variance in nest size, and higher nest density in the north compared to the south, all suggesting greater male-male competition in the north. Males from the north were also larger, but the relative variance in body size was similar across the two populations. We found no significant population differences in egg numbers per nest (north: mean 554 eggs/nest; south: mean 434 eggs/nest), paternity of the guarding male (north: 52%; south: 58%), or tactic frequencies (north: 93% guarders; south: 92% guarders). Finally, despite significant differences in nest availability, the strength of sexual selection did not differ across populations. Thus, significant environmental variation between these populations did not translate into differences in mating patterns or sexual selection. We discuss our findings in relation to sexual selection theory and the evolution of alternative reproductive tactics.

3.2 INTRODUCTION

The strength of sexual selection is shaped by both social and environmental factors, or 'ecological constraints' (Emlen and Oring 1977; Andersson 1994; Shuster and Wade 2003). Studies that look at the strength of selection and mating behaviors often do so in a single exemplar population and extrapolate the results for the species in general. Yet, it is known that temporal and spatial variation in ecological factors exist across populations and this can lead to variation in mating behavior and in the strength of sexual selection (e.g. Endler and Houde 1995; Reichard et al. 2008; Candolin and Vlieger 2013). For example, a comprehensive study looking at five low and five high predation populations of guppy (*Poecilia reticulata*) found a significantly higher frequency of multiply sired broods in the higher predation populations, likely a result of more coercive copulations by males (Kelly et al. 1999; also see Neff et al. 2008). Additionally, Mobley and Jones (2007) compared two populations of the sex-role reversed dusky pipefish (*Syngnathus floridae*) and found that males from the larger population had higher rates of multiple mating and greater reproductive success. The authors argued that these differences were driven by variation in population densities. Certainly, more cross-population studies of mating patterns are needed to better understand the ecological factors that influence mating systems.

Precisely how to measure sexual selection remains an ongoing debate (e.g. Mills et al. 2007; Klug et al. 2010a; Krakauer et al. 2011; Fritzsche and Booksmythe 2013). Several metrics have been proposed and are thought to capture the potential or the actual strength of sexual selection. An early popular choice for assessing the potential strength of sexual selection was the operational sex ratio (OSR; Emlen and Oring 1977). The OSR is based on calculating the ratio of reproductively active males to females in the population at a given time; individuals in highly skewed populations are expected to experience high mating competition and thus stronger sexual selection (Emlen and Oring 1977; Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjo 1996). Additional metrics developed to measure the maximum strength of selection are based on Bateman’s three principles (Bateman 1948; Trivers 1972; Arnold 1986; Arnold and Duvall 1994). The sex experiencing stronger sexual selection is expected to have (i) greater variance in reproductive success (# of offspring), (ii) greater variance in mating success (# of mates), and (iii) a steeper regression slope relating reproductive success to mating success (Wade 1979; Wade and Arnold 1980; Arnold 1986; Arnold and Duvall 1994). From the first two principles, the opportunity for selection (I) and the opportunity for sexual selection (I_S) metrics can be calculated, respectively, and these metrics capture the maximum potential of selection (Crow 1958; Wade 1979; Wade and Arnold 1980; Shuster and Wade 2003; Jones 2009). Bateman's third principle, the linear relationship between reproductive success and mating success, is now often called Bateman's gradient (β_{SS}; Arnold and Duvall 1994; Andersson and Iwasa 1996). A steeper Bateman gradient indicates a greater potential for sexual selection to occur (Jones 2009). An alternative measure of sexual selection is known as the selection gradient (β'), a trait based measure examining the degree of sexual selection on a particular phenotypic characteristic (Lande and Arnold 1983). The selection gradient metric of sexual selection measures the actual strength of selection on a phenotypic trait, and will always have a lower value than the maximum value of the opportunity for sexual selection (I_S; Arnold and Wade 1984a,b; Krakauer et al. 2011). Although debate continues about which metric to use, the emerging consensus is that the best metric of sexual selection will depend on the research question (e.g. actual selection on a trait versus potential selection across sexes or populations), and that multiple measures should be employed whenever possible (see Fritzsche and Booksmythe 2013).

In this study, we investigated population differences in the strength of sexual selection and its impact on mating patterns in a species with alternative reproductive tactics (ARTs), the plainfin midshipman (*Porichthys notatus*). Alternative reproductive tactics refer to the occurrence of two or more discontinuous reproductive phenotypes, with each phenotype attempting to maximize reproductive fitness through alternative means (Gross 1996; Taborsky et al. 2008). Variation in the frequency of alternative tactics can influence the strength of sexual selection. To date, only a small subset of studies have investigated the strength of sexual selection and how it influences mating patterns in species with alternative reproductive tactics (e.g. Fleming and Gross 1994; Mills and Reynolds 2003; Pomfret and Knell 2008; Simmons et al. 2004). Even fewer studies have compared the strength of sexual selection in species with ARTs across ecologically distinct environments, and results show a significant change in mating patterns across time and space (Mobley and Jones 2007; Reichard et al. 2008; Candolin and Vlieger 2013).

In plainfin midshipman, there are two distinct male reproductive tactics called guarder "type I" and sneaker "type II" males (Brantley and Bass 1994). In early spring, all plainfin midshipman reproductive adults undergo a large-scale vertical migration from deep waters (> 300 m) to the spawning grounds in sheltered, rocky shores in the intertidal zone (Arora 1948; Miller and Lea 1972). Type I guarder males arrive first on the breeding grounds and aggressively compete for limited nest sites, which are excavated areas under rocks. The largest type I males typically win the largest rock/nest sites and, by acoustically courting spawning females, will also gain the most eggs (DeMartini 1988; Ibara et al. 1983; Bass 1992; Brantley et al. 1993; Fitzpatrick et al. submitted). In contrast, type II sneaker males do not guard nests or court females, but instead, steal fertilizations from type I guarder males by either sneaking into the nest when a female is present, or by fanning sperm into the nest from the nest periphery (Brantley and Bass 1994). Females, attracted to the courtship songs of type I males, each produce a single clutch of up to 300 eggs per year (each 5-7 mm in diameter; KC and SB pers. obs.), which are laid into a single male's nest usually around the lowest tides of the month (DeMartini 1990). Females and type II males are often found in the nests of type I males during low tides, as spawning takes several hours to complete (Brantley and Bass 1994). Additionally, type I males are behaviorally plastic, and some will adopt a cuckolding behavior and move to a nest of another guarding male (Lee and Bass 2004). This behavior occurs particularly when nesting sites are severely limited and these cuckolding type I males can fertilize between 11% and 24% of the offspring within a nest (Cogliati et al. submitted).

Plainfin midshipman are distributed along the Pacific coast of North America, from Sitka, Alaska to Baja California, Mexico (Hubbs 1920; Arora 1948; Miller and Lea 1972). However, the distribution of this species appears to be discontinuous along the Oregon coast, with a northern population stretching from Oregon to Alaska and a southern population stretching from Oregon to Mexico (Warner and Case 1980; Thompson and Tsuji 1989; Harper and Case 1999). Guarding males from the south (California) are much smaller (Brantley and Bass 1994) than guarding males sampled in the north (Washington; Lee and Bass 2004; Sisneros et al. 2009). Because large male body size is an important trait in competitive interactions and strongly influences reproductive success in this species (DeMartini 1988), we propose that there will be more competition and stronger sexual selection in the north than in the south population. The overall aim of our study was to explore how differences in ecological factors influence the degree of male competition in a species with alternative reproductive tactics.

One of the most important resources for a male midshipman is the nest (DeMartini 1988). Nest availability, density, and nest size variation should all strongly influence the degree of mating competition experienced by males. Greater variation in nest availability, density and nest size should select for more exaggerated male competitive traits and a higher frequency of male alternative tactics. Because males are larger in the north, we predicted that competition among guarding males over nest availability would be greater in the north than in the south population. We further predicted that we would observe more variance among guarding males in body size and in reproductive success in the north population. Another way that male midshipman can compete with one another is via nest takeovers and cuckoldry, by both type I and type II males (Lee and Bass 2004). Therefore, we also predicted that we would see more of these competitive behaviors resulting in an overall lower paternity for guarding type I males in the north population. Finally, we predicted that, as a result of more competition among males, all measures of sexual selection would be higher in the north compared to the south including the OSR, opportunity for selection (I), opportunity for sexual selection (I_S), Bateman gradient (β_{SS}), and trait based selection gradient for male size (β').

3.3 METHODS

3.3.1 Field sampling

From May 10th to July 14th, 2010, and May 29th to July 4th, 2011, we located and sampled a total of 374 plainfin midshipman nests during low tide in the intertidal zone on six rocky beaches in British Columbia, CA, one beach in Washington, USA, and four beaches in California, USA (3 sites in Ladysmith Inlet, BC: 49°01´N, 123°83´W, 1 site in Mill Bay, BC: 48°63´N, 123°53´W, and 1 site in Stuart Channel, BC: 49°04´N, 123°75´W on the eastern coast of Vancouver Island, and 1 site in Crescent Beach, BC: 49°04´N, 122º88´W in South Surrey; 1 site in Seal Rock, WA: 47°71’N, 122°89’W; 4 sites in Tomales Bay, CA: 38°15´N, 122°90´W). Based on the population divergence described in Warner and Case (1980), we designated nests on British Columbia and Washington beaches as our north population (n=310), and the nests on California beaches (n=64) as our south population.

At each of the 11 different beaches used, we sampled in an identical manner by laying down a 50 m transect and sampling all nests in a 2.5 × 2.5 m grid, every 10 m along the transect. In total, we sampled 163 quadrants over two years in the north population and 21 quadrants in one year in the south population. Within each quadrant, we checked all rocks that were suitable as a nest (i.e. rocks with a relatively flat underside that were greater in size than the mean male length in each population), and classified rocks as either active nests (occupied), potential nests (unoccupied but suitable), or unsuitable as a nest (embedded or too small). We marked quadrants and each nest in the quadrants with plastic pegs, and also marked the type I guarding males with a unique elastomer injection (see Cogliati et al. 2013). We then mapped out each quadrant and measured the distances between all suitable rocks within the quadrant to the nearest cm. For all nests, we measured the length and width and calculated a nest surface area. We returned to the same quadrants in subsequent low tides over the course of the breeding season. When we re-sampled a quadrant, we recorded whether or not a nest was still in use and recorded whenever a previously unused rock (a potential nest) became an active nest. We always checked whether existing nests had a new resident male or the same resident male as the previous sampling date and marked any new resident males that we found in the nest.

At each nest, we sexed all fish based on the shape of the urogenital papilla and overall body coloration, and identified males as type I guarding or type II sneaker based on their body size (Brantley and Bass 1994). Sometimes more than one type I male was found in a nest and we further distinguished between guarding and cuckolder type I males based on their position in the nest; guarding males are frequently the largest and most centrally placed fish in the nest (Lee and Bass 2004; KC pers. obs.) whereas cuckolders are typically smaller and remain on the periphery. Such field-based classifications to identify sex and reproductive type have been verified in extensive studies and further confirmed through dissections (Bass and Marchaterre 1989; Bass and Anderson 1991; Brantley and Bass 1994; **Chapter 5**).

For each fish in each nest, we measured standard length (SL in cm) and body mass (g) and collected a small caudal fin clip for genetic analyses. Fish length and mass were highly correlated across all fish sampled ($R^2=0.96$, $N=708$, $p<0.0001$). Due to a few instances of technical difficulties with the balance in the field, we opted to use SL over body mass in all analyses as our measure of body size. To estimate reproductive success, we digitally photographed the embryos in each nest and, because midshipman eggs are laid in a monolayer, we later enumerated the eggs from the photos using ImageJ software (Schneider et al. 2012). We used egg number as one measure of reproductive success and used paternity as an additional measure. From a subset of nests (N = 47 in BC and N = 21 in CA), we collected and preserved 40-50 offspring from each cohort per nest (i.e. a distinct group of offspring at the same developmental stage within the nest) to calculate paternity (see Cogliati et al. 2013). After sampling, we returned all adults to their nest and repositioned all rocks to their original location.

3.3.2 Genetic analyses

Microsatellite data for our north population were collected using 222 adults and 1,713 offspring and are described in detail in Cogliati et al. (2013). Following the same protocols, we obtained here microsatellite data from 92 adults and 531 offspring from our south population. Using the adult male and female microsatellite data only, we first investigated whether individuals from the north and south populations were actually from distinct genetic populations using *structure* (v2.3) software (Pritchard et al. 2000). *Structure* assigns individuals to populations and infers the structure of populations using a clustering method (Pritchard et al. 2000). We performed five independent simulations for each value of K (number of populations assumed) from 1-6, for a total of 30 simulations. The parameters of each simulation included a "burn length" of 50,000 followed by 100,000 Markov chain Monte Carlo (MCMC) repetitions after burning, an admixture ancestry model without using prior sampling location, and independent allele frequencies. To identify the number of possible populations in our study, we inputted the output files into *Structure Harvester* for analyses (Dent and vonHoldt 2012). In addition, we calculated a measure of genetic differentiation (F_{ST}) between the north and south adult samples using GenAlEx (v6.5b3; Peakall and Smouse 2006, 2012).

We used the paternity estimates for guarder type I males produced in Cogliati et al. (2013) for our north population and, following the same protocols, calculated a paternity estimate for the guarder type I males in our south population. In some cases, we had low paternity estimates (<10% paternity), which we considered to be a result of nest takeovers (see Cogliati et al. 2013). For those cohorts, we used COLONY (v2.0) software to infer the likely male genotype of the previous nest owner (Wang 2004; Jones and Wang 2010) and calculated a new paternity estimate for the previous nest owner (see Cogliati et al. 2013). Additionally, we used COLONY to determine the number of cuckolders that sired offspring in each cohort, and the number of offspring that each cuckolder sired.

3.3.3 Selection metrics

As suggested in the recent review by Fritzsche and Booksmythe (2013), we used multiple measures to compare the strength of sexual selection between the north and south populations. First, we determined the total population level OSR based on the total number of reproductive nesting males and females observed in each population. Although this does not directly measure the strength of selection, we include it here to describe the potential level of competition among individuals in each population. Second, similar to Klug et al. (2010b), we calculated the opportunities for selection (I) based on nesting success (I_{nest}: whether nesting males received eggs or not in their nests as 1 or 0), fertility of mates (I_{eggs}: # of eggs received), and paternity (I_{pat}). The variables measuring the opportunity for selection are calculated by dividing the variance in reproductive success by the mean reproductive success squared, where reproductive success refers to the three variables listed above. Third, we calculated the opportunity for sexual selection (I_s) using the variance in mating success (# of genetic mates; Barreto and Avise 2010) divided by the mean mating success squared. Fourth, we calculated the Bateman gradient (β_{ss}) for the north and south populations based on the least squares regression slope of reproductive success (with both paternity, β_{ss_pat}, and egg number, β_{ss_egg}) on mating success. Both mating success and reproductive success measures were divided by their means to produce measures of relative fitness with transformed means equal to 1 (see Jones et al. 2004). Finally, because male size is an important trait for reproductive success in plainfin midshipman (DeMartini 1988), we calculated the selection gradient (β') as the slope of the regression of reproductive success (using egg number) on SL. We first log transformed SL and standardized this variable within population to have a mean of 0 and a variance of 1 (Jones et al. 2004).

3.3.4 Statistical analyses

We used three measures of nest occupancy per quadrant sampled: the total number of potential nests, the total number of occupied nests, and the proportion of potential nests that were occupied. We ran three linear mixed effects (LME) models using the three measures of nest occupancy as our dependent variable. In each LME, we included population and year as fixed effects, and quadrant ID as a random effect to account for the repeated sampling.

To test for differences in nest size (rock surface area), type I male body size (SL), and reproductive success (egg number) between the north and the south populations, we used one-tailed Wilcoxon tests measured by site within each population. For each of the variables, we also tested whether the absolute variance (Var) and the relative variance (measured using the coefficent of variation; CV) differed between populations, controlling for site. We tested for possible year effects in our north population and found no significant effect and so we excluded year from analyses. To check for trait differences in sexual and tactic dimorphism, we ran two linear models. The log of the SL was the dependent variable, and population, sex or tactic, and the interaction between population and sex or tactic were fixed effects. The interaction term indicates degree of dimorphism between populations.

Paternity estimates were obtained from all four sites in the south population and from five of seven sites in the north population. We arcsine square root transformed the paternity proportions and tested for difference between populations with t-tests. Based on data from COLONY, we determined the number of actual sires and calculated the number of effective sires for each cohort following Neff et al. (2008). The number of effective sires is a measure of reproductive skew among males, and the number of effective sires will equal the number of actual sires when all males contribute equally in terms of offspring number. Briefly, we calculated the number of effective sires as $1/\sum(rs_i/brood\ size)^2$, where rs_i is the number of offspring assigned to sire *i*, and *brood size* is the total number of offspring analyzed in the cohort. We compared the actual number of sires and the effective number of sires between populations using two linear mixed effects (LME) models. In each LME, we included population as a fixed effect, and nestID as a random effect to account for multiple cohorts from the same nest.

To check for differences in tactic frequency between the north and south populations, we ran two generalized linear mixed models (see Bolker et al. 2008). In the first test, we identified males based on their morphological tactic (type I vs. type II) and used this as the dependent variable, while in the second test we identified males based on their behavior (guarding vs. cuckolding). We included this second test because cuckolding behavior is not limited to type II sneaker males. Type I males are behaviorally plastic and readily cuckold (Lee and Bass 2004; Cogliati et al. submitted), thus the intensity of selection may affect the degree of cuckoldry behavior observed by type I males. In both tests, the north and south population variable was the fixed effect, and we included site (within each population) as a random effect.

We compared the OSR across the north and south populations using a Fisher's exact test. To compare the opportunities for selection (*I*) and sexual selection (*I*) between populations, we used Levene's homogeneity of variance tests. Finally, we compared the Bateman gradient and selection gradient slopes between the north and south populations with ANCOVAs. We performed analyses using JMP (10.0) and R (2.15.2). Unless otherwise indicated, we provided results as mean ± SE.

3.4 RESULTS

3.4.1 Ecological differences

A greater proportion of potential nests were occupied in the north than the south ($F_{1,124.2}=13.59$, $p=0.0003$; least square mean proportion for north: 0.44 ± 0.03; south: 0.18 ± 0.06). Additionally, there were significantly more potential and occupied nests per quadrant in the north (LME, potential nests: $F_{1,181}=21.81$, $p<0.0001$, least square mean for north: 7.56 ± 0.30, for south: 4.42 ± 0.58; occupied nests: $F_{1,94.53}=38.02$, $p<0.0001$, least square mean for north: 3.55 ± 0.23, for south: 0.18 ± 0.47). The average nearest neighbor distance was closer in the north compared to in the south population (Wilcoxon test: $W=5946.5$, $p<0.0001$; north: 71

± 3 cm; south: 94 ± 7 cm^2). These data suggest that the nest resources were more limited and dense in the north, which could result in a more competitive environment.

Rocks used for nests were significantly larger in the north compared to the south (Wilcoxon test: W=10963.5, p<0.0001; north: 1913 ± 58 cm^2; south: 1410 ± 87 cm^2). Rock size tended to be more variable in the north compared to the south (Var; W=22, p=0.08); however, the coefficient of variation for rock size was not different between populations (CV; W=14, p=0.54).

3.4.2 Variance in body size

As expected based on previous studies, type I guarding males were significantly larger in the north compared to the south (Wilcoxon test: W=30114.5, p<0.0001; **Table 3.1**, **Figure 3.1a**). Males from the north population also showed more variance in body size compared to the males from the south population (W=24, p=0.04; **Figure 3.1b**); however, the coefficient of variation was not different between populations (W=13, p=0.61; **Figure 3.1c**). Thus, although there was greater variation among males in the north in terms of absolute body size, the relative difference among males in body size did not differ across populations. Females and type II males were also significantly larger in the north compared to the south (Wilcoxon tests: females: W=1806.5, p<0.0001; type II males: W=415, p=0.0002; **Table 3.1**). We found no significant difference in the degree of sexual

Table 3.1 Standard lengths (mean ± SE and range) for plainfin midshipman (*Porichthys notatus*) by sex and population.

	North			South		
	Mean (cm) ± SE	*N*	*Range (cm)*	*Mean (cm) ± SE*	*N*	*Range (cm)*
Type I males	20.5 ± 0.15	500	10.4 – 29.5	15.6 ± 0.29	68	10.7 – 20.9
Type II males	12.3 ± 0.32	34	9.2 – 16.6	10.1 ±0.29	14*	7.4 – 11.4
Females	16.6 ± 0.24	126	10.0 – 21.9	12.5 ± 0.22	16	11.3 – 13.9

*eight type II males were added for body size measurements only and not included in analyses because they were collected outside of our quadrants.

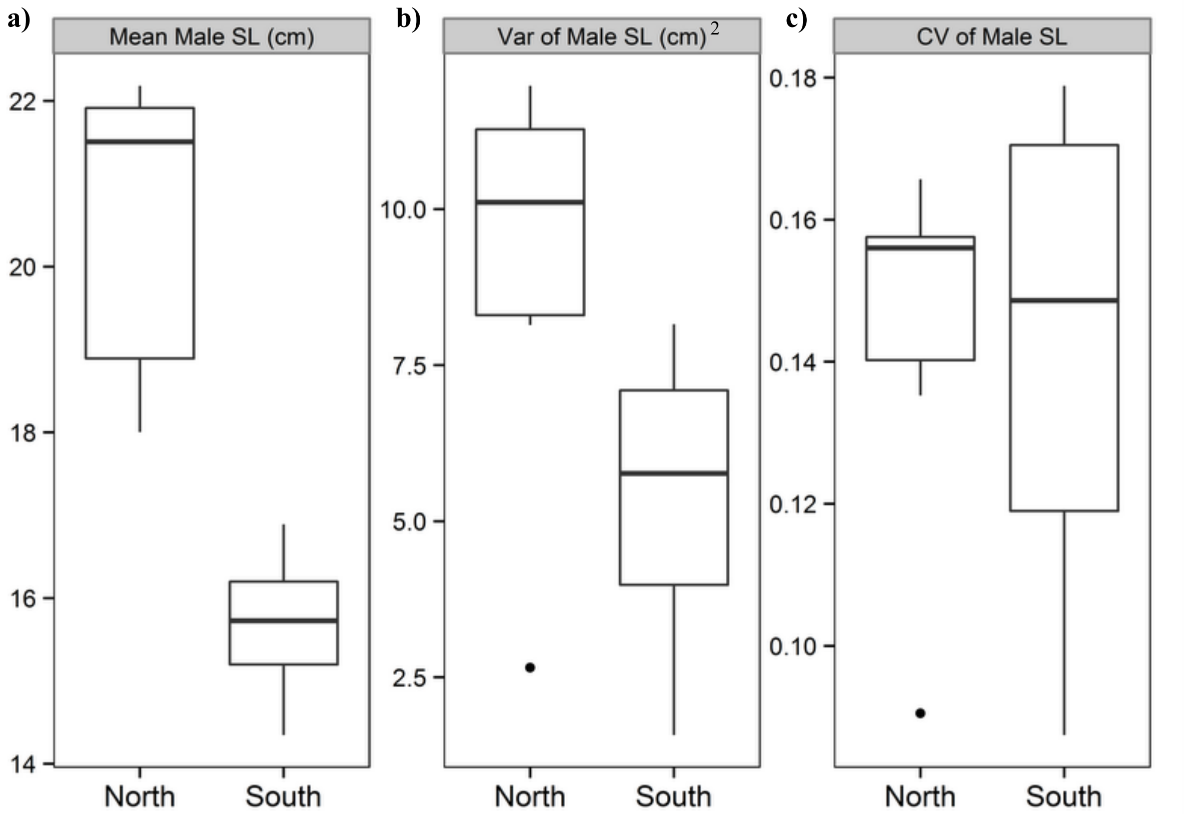

Figure 3.1 Standard length differences between north and south populations of plainfin midshipman (*Porichthys notatus*) as measured by a) mean (cm), b) variance (cm)^2, and c) coefficient of variation. Box plots show median with first and third quartiles. Whiskers represent 1.5*IQR (inter-quartile range) and dots represent outliers.

dimorphism (whole model: R^2=0.35, $F_{3,706}$=127.49, p<0.0001, interaction term: t=0.08, p=0.94) or in the degree of size dimorphism b+etween male tactics (whole model: R^2=0.51, $F_{3,613}$=211.94, p<0.0001, interaction term: t =1.27, p=0.21) between north and south populations.

3.4.3 Variance in reproductive success

The average number of eggs per nest in the north was 554 ± 23 (N=464; range 0 – 3193) while in the south, nests tended to have fewer eggs with only 434 ± 51 eggs per nest (N=64; range 0 – 2032; W=17041, p=0.09, **Figure 3.2a**). Of the nests sampled in the north, 13% of guarding males did not have embryos (eggs or hatched young) in their nests, while in the south, 16% of guarding males did not have any embryos. The variance in reproductive success among males did not differ between populations (W=14, p=0.54, **Figure 3.2b**), nor did the coefficient of variation for reproductive success (W=13, p=0.61, **Figure 3.2c**).

3.4.4 Genetic divergence and variance in paternity

Structure analysis indicated that there were two distinct genetic populations, with 93% and 96% of individuals aligning with their predefined north and south collection regions, respectively. The two populations showed a moderate level of genetic differentiation (F_{ST} = 0.07 ± 0.02), with an estimated 8.7 ± 5.5 effective migrants, or individuals, moving between populations (based on 222 adults in BC and 92 adults in CA). Therefore, for the paternity analyses, we calculated separate

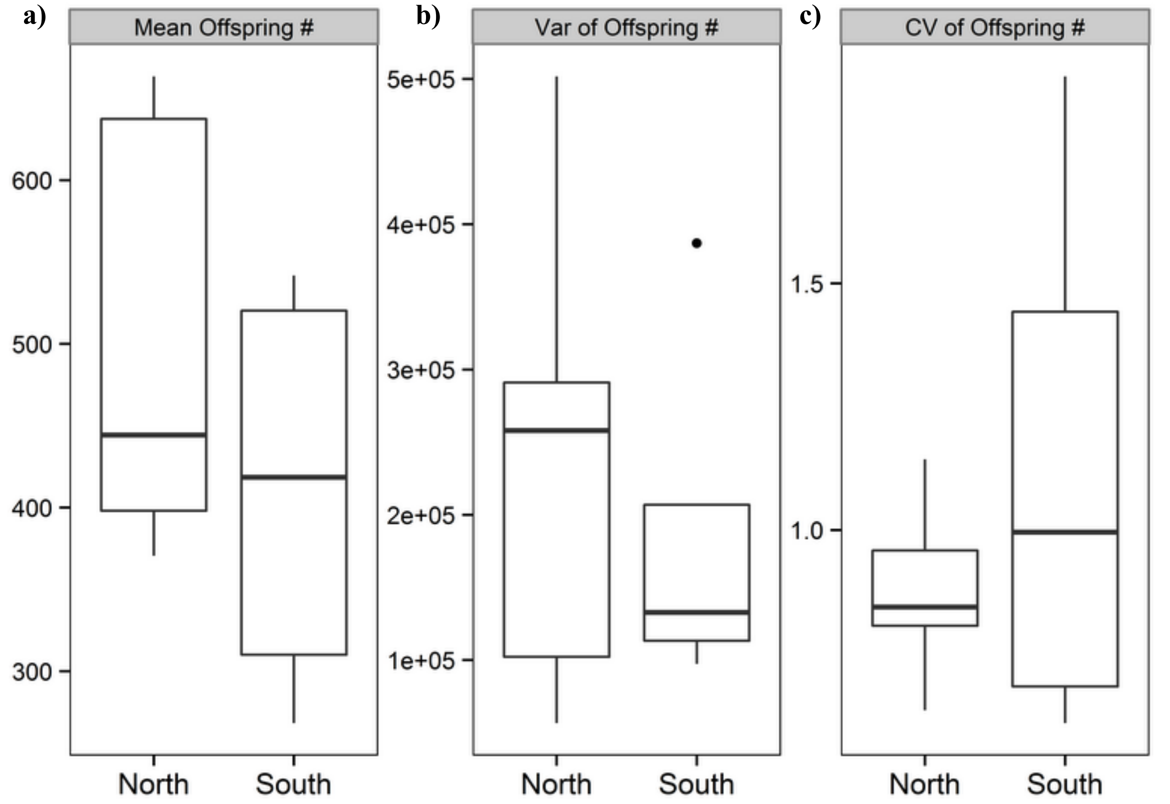

Figure 3.2 Differences in male reproductive success between north and south populations of plainfin midshipman (*Porichthys notatus*) as measured by a) mean number of offspring, b) variance in offspring number, and b) the coefficient of variation for offspring number. Box plots show median with first and third quartiles. Whiskers represent 1.5*IQR (inter-quartile range) and dots represent outliers.

population level allele frequencies for our microsatellite loci using the adult genotypes.

On average, nest guarding males in the north sired 52% ± 0.04 young/nest (N=74; range 0 – 100%, Cogliati et al. 2013) and 58% ± 0.07 of the young/nest (N=27; range 0 – 100%) in the south. We classified very low paternity values (<10%) for the guarding male as likely caused by nest takeover events. We observed takeovers in 23% of nests in the north, and in 24% of nests in the south (**Figure 3.3a**). There was no significant difference in the frequency of takeovers between populations (Fisher's exact test: p=0.64). When we account for nest takeovers by including the previous nest owners' paternity in cases of probable takeovers, the average paternity for nest-guarding type I males was 63% ± 0.03 (range 15-100%) in the north and 69% ± 0.05 (range 17-100%) in the south. In either scenario, overall paternity levels did not differ between guarding males from the north and south populations (t-test, without takeovers: t_{99}=1.23, p=0.22; with takeovers: t_{99}=1.28, p=0.20; **Figure 3.3b**). Also, paternity was not correlated with total offspring number in either population (north: R^2=0.005, p=0.65, N=43; south: R^2=0.10, p=0.94, N=21).

Known cuckoldry occurred in 78% of cohorts in the north and 67% of cohorts in the south. In the north, we detected 2.5 ± 0.13 actual sires (N=74; range 1 – 5) and 1.76 ± 0.09 effective sires (range 1 – 4.0) per cohort. In the south, we detected 2.22 ± 0.23 actual sires (N=27; range 1 – 5) and 1.70 ± 0.16 effective sires (range 1 – 4.3) per cohort. There were no significant differences between

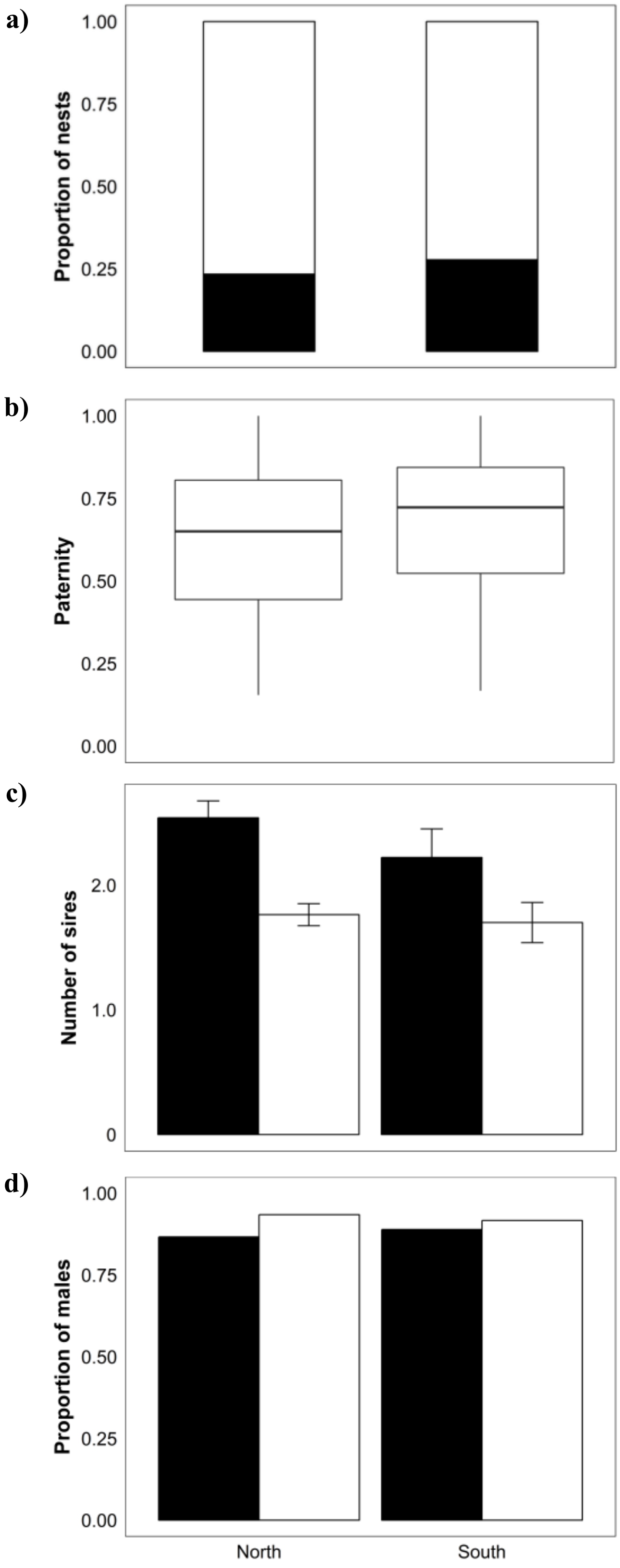

Figure 3.3 Cross-population comparison of mating patterns in plainfin midshipman (*Porichthys notatus*). a) Proportion of nests with takeovers (black portion: inferred takeover based on paternity analyses; white portion depicts no takeovers); b) Paternity accounting for takeover values from previous nest guarders. Box plots show median with first and third quartiles. Whiskers represent 1.5*IQR (inter-quartile range); c) Number of actual sires (black bars) and effective sires (white bars) per cohort (mean ± SE). The number of effective sires is a measure of reproductive skew; d) Proportion of males displaying the guarding behavior (vs. cuckolding behavior; black bars) and the proportion of males displaying the type I tactic (vs. type II tactic; white bars).

populations for either the number of actual sires (LME: $F_{1,99}$=1.47, p=0.23) or number of effective sires ($F_{1,62.77}$=0.10, p=0.75; **Figure 3.3c**).

3.4.5 Tactic frequencies

The proportion of type I males sampled was 0.93 in the north population and was 0.92 in the south population (**Figure 3.3d**). We found no significant difference in the proportion of each tactic type (type I vs. type II) between populations (GLMM: Z=0.34, p=0.74). When we looked strictly at the behavioral types (guarding vs. cuckolding), the proportion of guarding males sampled was 0.87 in our north population and 0.89 in our south population and again was not significantly different between populations (Z=0.49, p=0.63, **Figure 3.3d**).

3.4.6 Sexual selection across populations

Selection metrics are summarized in **Table 3.2**. The observed operational sex ratios were strongly male biased and were similar across the range (Fisher's exact test: p=0.58); on average the OSR was 3.7 males per female in the north and 4.1 males per female in south. We found that 92% of type I males sampled in the north and 97% of type I males sampled in the south had acquired a nest site, resulting in a higher opportunity for selection (I_{nest}) in the north than in the south (Levene's homogeneity of variances test: $F_{1,570}$=8.56, p=0.004). In the north, 87% of the nesting males had received eggs, while in the south 84% of nesting males received eggs. Therefore, there was no significant difference in the opportunity for selection

Table 3.2 Population comparison of sexual selection metrics calculated for plainfin midshipman (*Porichthys notatus*).

	North	**South**
# of all males	538	74
# of nesting type I males	466	66
# of nesting type I males with young	408	54
# of cuckolding type I males	38	2
# of sneaker type II males	34	6
# of females	126	16
Sex Ratio M:F	1:0.23	1:0.22
Operational Sex Ratio (OSR) M:F	1:0.27	1:0.24
Mean nesting success[a]	0.925	0.971
Variance in nesting success	0.070	0.029
I_{nest}	0.082	0.031
Mean egg number/nest[b]	554	434
Variance in egg number/nest	251409	165193
I_{egg}	0.819	0.878
Mean paternity	0.631	0.686
Variance in paternity	0.055	0.057
I_{pat}	0.137	0.127
Mean mating success[c]	3.23	3.12
Variance in mating success	1.33	0.99
I_s	0.127	0.102
Bateman Gradient ($\beta_{\text{ss\_pat}}$)[d]	-0.23	0.13
Bateman Gradient ($\beta_{\text{ss\_egg}}$)[e]	0.94	0.53
Selection Gradient (β')[f]	0.29	0.27

[a] Based on whether a type I male obtained a nest (scored as a 1) or did not acquire his own nest when sampled (scored as a 0).

[b] Based on the number of eggs a male received, including males that received zero eggs in their nests.

[c] Mating success refers to the number of genetic mates per cohort, determined by COLONY.

[d] Slope of the regression using paternity as the measure of reproductive success on mating success.

[e] Slope of the regression using egg number as the measure of reproductive success on mating success.

[f] Slope of the regression between egg number and guarding male standard length.

among nesting males based on our egg count measure of reproductive success ($F_{1,526}$=2.03, p=0.16), nor was there a difference based on the paternity assigned to the guarding males in the north versus the south population ($F_{1,99}$=0.004, p=0.95). The opportunity for sexual selection (I_s) based on mating success was not significantly different between populations ($F_{1,98}$=2.13, p=0.15). The standardized Bateman gradients (based on relative fitness using male paternity on relative mating success) for the north and south populations were not significantly different (ANCOVA: t=-1.39, p=0.17); there was a nearly significant negative regression slope in the north (y = -0.23x + 1.23, r=0.218, N=74, p=0.06) but the regression slope for the south population was not significantly different from zero (y = 0.13x + 0.85, r=0.119, N=26, p=0.56). This suggests that paternity, as a measure of fitness, was not strongly tied to mating success. When we instead considered relative fitness using the total number of offspring in each cohort (as opposed to paternity), the standardized Bateman gradients for the north and south populations were again not significantly different (ANCOVA: t=0.64, p=0.52). However, in the north, the regression now was significantly positive (y = 0.94x + 0.06, r=0.36, N=70, p=0.002), whereas the regression slope for the males from south population was not significantly different from zero (y = 0.53x + 0.49, r=0.18, N=26, p=0.38). The selection gradients (based on the regression of relative reproductive success on male body size) were not significantly different between populations (ANCOVA: t=0.18, p=0.85) and both slopes were significantly different from zero (north: y = 0.29x + 1.0, r=0.32, N=463, p<0.0001; south: y = 0.27x + 1.0, r=0.289, N=64, p=0.02).

3.5 DISCUSSION

Our results confirm previous claims that there are two genetically distinct plainfin midshipman populations along the Pacific Northwest, with a moderate level of genetic differentiation (Warner and Case 1980; Thompson and Tsuji 1989; Harper and Case 1999). When we investigated the ecology of the two populations, we found that they differed; the north population had a higher density of nests, larger nests overall, and more variation in nest size than the south population. Furthermore, there were fewer available (unoccupied) nests in the north population. We confirmed that male body size was larger in the north, and the associated absolute variance (Var) in body size among males was also greater. However, the relative variance of body size (CV) among type I guarder males did not differ between populations. When we subsequently investigated differences in mating patterns between populations, we found no significant effect for any of our measures: there were no significant population differences in male reproductive success (as measured by the # of eggs per nest or by paternity), in nest takeover frequency, in patterns of cuckoldry, or in terms of observed tactic frequencies when we considered either the developmental tactics (type I and type II) or behavioral tactics (guarding and cuckolding). Finally, despite finding significant ecological differences, most measures of sexual selection, aside from the opportunity for selection based on nesting success (I_{nest}), did not reveal any significant difference between populations.

Although ecological differences can influence mating patterns, the differences we detected did not appear to be sufficient to influence the maximum strength of sexual selection, tactic frequency, or reproductive success. Our results were consistent with other studies based on a variety of taxa, which also show that populations experiencing different ecological factors may not always experience differences in measures of sexual selection and reproductive success (e.g. Jones et al. 2001; Griffith et al. 2002; Singer et al. 2006; Lindstedt et al. 2007). Because male size is an important trait for competition and reproductive success in plainfin midshipman (DeMartini 1988), as well as in other species (e.g. Andersson 1994), the similar values of *relative* variance of male size between populations, as measured by the coefficient of variation, may be the key component needed to explain the apparent similarity in sexual selection. Indeed, the outcomes of competitive interactions are often based on relative differences in fighting characteristics (Parker 1974). For example, in round goby (*Neogobius melanostomus*) small differences in body size (as low as 3%) between competitors was sufficient to predict conflict outcomes (Stammler and Corkum 2005; Groen et al. 2012). We also found a similar frequency of alternative tactics across populations, which was consistent with a similar level of sexual selection in the two populations. Thus, variation in body size, as measured by the coefficient of variation, may be an appropriate metric for assessing differences in competition among populations and may be a key determinant of the strength of sexual selection, at least in plainfin midshipman.

Why did the significant difference in nest density and availability between the two populations not translate into significant differences in the degree of sexual selection? Resource availability is one of the main factors influencing intra-sexual competition and therefore the strength of sexual selection (Emlen and Oring 1977; Andersson 1994). For example, in the sand goby (*Pomatoschistus minutus*), there was more intense male-male competition in a population with fewer available nesting sites compared to a population in which nesting sites were abundant (Forsgren et al. 1996). In contrast, Debuse et al. (2003) investigated breeding shelter density and male and female interactions in European lobster (*Homarus gammarus*) and found little support for the relationship between resource availability and sexual selection. Those authors argued that with scarce resource availability, overt competition between males may be too costly and inefficient and therefore the strength of sexual selection was reduced instead of enhanced, as theory predicts. A similar situation may have occurred in the north population of plainfin midshipman, where there were fewer available nests. If overt male-male competition is costly in light of the scarcity of nests, this could ultimately dampen or constrain the overt competition and cuckoldry in the north population. An alternative hypothesis for why differences in resource availability did not lead to differences in sexual selection is that the nests may not actually be limiting in either population in plainfin midshipman. Future studies investigating the degree of competitive behaviors of guarding males between populations are needed to elucidate the relationship between nest availability and sexual selection.

Although the results of our study suggest that there were minimal differences in reproductive success and sexual selection across the populations we studied, guarder males from the north population were still larger than guarder males from the south population. Moreover, sneaker males and females were also larger in the north. Hence, differences in body size across the populations may be a result of a physiological adaptation due to natural selection and not sexual selection. There are longer low tide events in the north population compared to those in the south (Mobile Geographics LLC 2005; KC pers. obs.). That is, fish nesting and mating in the north population remain out of water and cut off from the ocean for longer durations than fish in the south population. Therefore, fish in the north must endure a greater degree of possible desiccation, hypoxia, and a buildup of waste material in their nest (Craig et al. 2013). Fish respond to hypoxia in a number of ways, including both enhancing the uptake of oxygen present, or limiting metabolic use (e.g. Nikinmaa and Rees 2005; Nilsson 1990; Richards et al. 2009; Richards 2011). Fish in the north may grow to a larger size in order to increase their gill structure (Sollid et al. 2003, 2005) or to reduce their metabolic rate to reduce oxygen demand (Martinez et al. 2006; Richards et al. 2008; Richards 2009). Alternative explanations for the difference in fish body size includes predation pressures, where fish in the south may be experiencing greater predation intensity resulting in more individuals with a smaller body size at sexual maturity (e.g. Reznick et al. 2001) or differences in mate preferences, where females in the north may have a stronger preference selecting for larger male body size. Further investigation into physiological differences in response to different hypoxic conditions, other natural selection pressures, and differences in female mate choice between the north and south populations is warranted, as we now know that this increase in body size in the northern population is not likely a result of differences in the intensity of sexual selection.

This study found significant differences in nest site availability but this did not translate into differences in mating patterns and sexual selection between two populations. There are two important conclusions we can draw from these results. First, our data suggest, at least for plainfin midshipman, that one population can capture many aspects of the mating dynamics and is sufficient to describe the overall mating patterns of the species. Second, our results indicate that nest site availability is not a limiting factor driving the intensity of sexual selection in the plainfin midshipman. It is possible that other aspects of the environment, such as food availability or predation pressure (not measured in this study), may play a more critical role in shaping life history and mating patterns of this species. These other aspects of the environment that may play a more important role in shaping mating patterns may not have varied between the two populations. Future studies examining variation in other ecological factors across populations would be useful, and provide a more comprehensive understanding of the role of the environment in shaping mating dynamics. We recommend that cross-population studies continue to be employed whenever possible.

3.6 ACKNOWLEDGEMENTS

We thank Andrew Bass, Midge Marchaterre, and Joe Sisneros who assisted with permits and provided access to the sites in California and Washington. We also thank Celia Chui, Ahdia Hassan, and Jennifer Reynolds for their help with data collection. We thank Amanda Pereira and Holly Howe for counting embryos, Celeste Bouchard and Cayli Hunt for help with DNA extractions from California samples, and Jonah Marcovitch for help with microsatellite analyses from California samples. Mr. and Mrs. Cogswell, Mr. and Mrs. Weatherell, Mr. and Mrs. Balshine, and especially Mr. and Mrs. Flader for providing us access to midshipman sampling sites and lodgings for field crew in BC. This work was supported by Discovery grants from the Natural Sciences and Engineering Research Council of Canada to BDN and SB.

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Chapter 4

COMPETITION AND CUCKOLDRY: ESTIMATING FITNESS OF ALTERNATIVE REPRODUCTIVE TACTICS IN PLAINFIN MIDSHIPMAN

Submitted to: Behaviour, accepted pending revisions

4.1 ABSTRACT

There has been much debate about how male alternative reproductive tactics (ARTs) evolve. In particular, researchers question whether ARTs have evolved as a conditional, ‘best of a bad job’ strategy where one tactic has higher fitness than the other, or whether they have evolved as a result of a genetic polymorphism where both tactics have equal fitness. Despite the large number of species known to have ARTs, tests of equal fitness between tactics have only been conducted in a handful of species. We tested the prediction of equal fitness using the plainfin midshipman (*Porichthys notatus*), a species with two well characterized male ARTs: guarding type I males and cuckolding type II males. We collected data across three years and three sampling locations to determine the proportion of each reproductive tactic, as well as the proportion of offspring sired by each male type using microsatellite markers. Our analysis suggests that males adopting the conventional guarding tactic

likely have greater fitness compared to males adopting the cuckolder type II tactic. Also, we show that the guarding male tactic is able to gain paternity through cuckoldry, and that these males, who sometimes guard and sometimes cuckold, are responsible for the majority of paternity lost within nests. Indeed, the classic cuckolding type II males were responsible for only a small fraction of the paternity lost. These results highlight the degree of plasticity in male behaviour even within one male tactic. Taken together, our results provide the first exploration of the evolution of male ARTs in plainfin midshipman and, given the tractability of midshipman system, a valuable next step will be to look for gene-by-environment interactions on tactic development and expression.

4.2 INTRODUCTION

Alternative reproductive tactics (ARTs) occur when there are two or more reproductive phenotypes within a population, each attempting to maximize reproductive success using different behaviours (Taborksy et al., 2008). Most commonly reported in males, one morph, sometimes called guarding, parental, territorial, bourgeois, or type I, is typically associated with delayed sexual maturation, showy displays used to court females and deter rivals, and in some cases paternal care. The alternative male morph, called cuckolder, sneaker, parasitic, or type II, is more often associated with precocial maturation, and does not invest in courtship or paternal care, instead gaining reproductive success in sneaky or coercive ways. Intense male-male competition and large variation in male reproductive success are thought to select for the evolution of ARTs (Gross, 1996; Taborsky et al., 2008). These conditions promote the evolution of ARTs by providing some males a means to gain reproductive success by pursuing an alternative tactic.

Two mechanisms have been put forth to explain the evolution of alternative reproductive tactics. The first hypothesis argues that a genetic polymorphism is responsible for the expression of two or more tactics, and that different alleles at a single gene determine which tactic is expressed. Game theory shows that negative frequency dependent selection maintains the evolutionary stability of the alternative tactics, with each tactic having equal fitness (Maynard Smith, 1982; reviewed in Gross, 1996). The frequency dependence ensures stability because when one of the alternative tactics is rarer, it has greater fitness leading to an increase in frequency of the underlying allele in the population. A genetic polymorphism has been implicated as the mechanism responsible for ARTs in species that include the ruff (*Philomachus pugnax*; Lank et al., 1995), a marine isopod (*Paracerceis sculpta*; Shuster, 1989; Shuster & Wade, 1991), and a swordtail (*Xiphophorus nigrensis*; Ryan et al., 1992). The second hypothesis proposes that male tactics depend on individual differences in condition or status and hence on environmental and social influences (reviewed in Gross, 1996; Oliveira et al., 2008). In a conditional strategy, tactics are expressed based on a single gene or genes that code for a 'decision rule', where a male 'chooses' the reproductive tactic that maximizes his fitness based on his condition or status at some critical point during development (Gross, 1996; Piche et al., 2008; Tomkins & Moczek, 2009; Roff, 2011).

An individual's status can be influenced by environmental effects, including population density, the size of the individual, and other biotic and abiotic conditions (Emlen, 2008). Thus, individuals may develop into different tactics if they experience divergent environmental or growing conditions. Because each male utilizes the best tactic for his own situation, in a conditional strategy, the reproductive tactics can have unequal fitness and negative frequency-dependent selection is not required (Gross, 1996). A conditional strategy has been implicated as the mechanism responsible for ARTs in species that include the scarab dung beetle (genus *Onthophagus*; Eberhard, 1982; Emlen, 1994) and scorpionfly (*Panorpa* sp.; Thornhill, 1981).

Gross and Charnov (1980) developed a life history model to calculate the relative fitness of each tactic and thus to differentiate between ARTs caused by genetic polymorphism or a conditional strategy. In their model, the proportion of males that develop into each male type in the population as well as the proportion of all offspring that are fertilized by each male type are calculated. If the proportion of males adopting each tactic is equal to the proportion of offspring sired by males using that tactic, then the tactics have equal fitness and a genetic polymorphism is inferred. If the tactics have unequal fitness, then a conditional strategy is inferred. Based on their initial application of the model, Gross and Charnov (1980) determined that parental and cuckolder males in bluegill sunfish (*Lepomis macrochirus*) had equal fitness. However, with improved paternity data and controlled breeding experiments, Neff and Lister (2007) calculated that cuckolder males had higher fitness relative to parental males, suggesting instead a conditional strategy. Because of the challenges associated with measuring reproductive success and the frequency of each tactic in wild populations, models to test equal fitness have only been applied to a small number of species (Gross & Charnov, 1980; Shuster & Wade, 1991; Ryan et al., 1992; Sinervo & Lively, 1996; Neff & Lister, 2007; Rios-Cardenas & Webster, 2008).

Armed with paternity data and multi-year population sampling, we applied the Gross and Charnov (1980) life history model to another species with well characterized alternative reproductive tactics, the plainfin midshipman (*Porichthys notatus*). This species is a nocturnally active deep-water marine fish distributed along the Pacific coast of North America (Hubbs, 1920; Arora, 1948; Miller & Lea, 1972). There are two distinct male reproductive tactics in this species known as guarding 'type I' and sneaker 'type II' males (Brantley & Bass, 1994). At the onset of the breeding season in early spring, all reproductive adults undergo a large-scale vertical migration from deep waters (> 200 m) to the spawning grounds in calm, rocky shores in the intertidal zone (Arora, 1948; Miller & Lea, 1972). Type I males aggressively compete for limited nest sites, and the largest males typically win the largest nest sites (DeMartini, 1988). Guarding type I males use their sonic muscles, which are attached to their swim bladder, to acoustically court females (Ibara et al., 1983; Bass, 1992; Brantley et al., 1993). Females are attracted to these courtship songs and will produce a single clutch of up to 300 eggs (each 5-7 mm in diameter) per year, which are released into a single male's nest (DeMartini, 1990; authors unpublished data). Females and type II males are often sampled in the nests of guarding type I males, because spawning takes several hours to complete (Brantley & Bass, 1994). Type I males will remain in the nest and continue to court and spawn with additional females until their nest is filled with multiple 'age cohorts' that are typically at different developmental phases (Arora, 1948; DeMartini, 1988; Brantley & Bass, 1994; Cogliati et al., 2013). In contrast, type II males do not guard nests or court females, and have limited sonic muscle development. Instead, they steal fertilizations from type I males by either sneaking into the nest when a female is present, and also by fanning sperm into the nest from the periphery. Interestingly, type I males are behaviourally flexible, and can also facultatively adopt a cuckolding behaviour, particularly when nesting sites are severely limited or when they have no young in their nests (Lee & Bass, 2004; K.C., personal observation).

There are a number of features that distinguish reproductively mature type I from type II males, including a series of behavioural, morphological, and neurobiological traits (Bass, 1990, 1992, 1993; Brantley & Bass, 1994). Bass et al. (1996) have argued, based on differences in neuronal structures and in otolith growth, that type I and type II males are mutually exclusive endpoints and not simply two parts of a single ontogenetic sequence. Tactic divergence occurs at an early age of development (approximately 200 days), with type I males reaching sexual maturity three to four months after type II males (Bass et al., 1996; Bass, 1996). Based on the early differentiation in brain and muscle structures between the two tactics, Bass (1996) has argued that the tactics must be genetically determined. However, this conclusion was challenged by Foran (1998) who showed that male density during development affected morph outcomes. When male plainfin midshipman were reared in tanks under high density, a greater proportion of males developed into type II males as compared to tanks in which fish were reared in low density conditions, suggesting that the two morphs come about as a result of a conditional strategy (Foran, 1998). Thus, it remains unclear if the two reproductive tactics in midshipman represent alternative strategies based on a genetic polymorphism with equal fitness, as argued by Bass (1996), or a single conditional strategy based on a genetic monomorphism with unequal fitness, as suggested by the data in Foran (1998).

In this study, we examined the fitness of the two male reproductive tactics in plainfin midshipman. We measured the frequency of both male tactics from a population in British Columbia over a number of years, and combined these estimates with paternity data for nest-guarding males from this same population, previously published in Cogliati et al. (2013). Additionally, for a subset of the nests, we also conducted extensive population-level sampling so that we could estimate the extent of cuckoldry by type I males. Finally, we used these data to apply the Gross and Charnov (1980) life history model to determine if alternative reproductive tactics in plainfin midshipman have evolved as a genetic polymorphism or as a conditional strategy.

4.3 MATERIAL AND METHODS

4.3.1 Field collections

In 2008, 2009, and 2010, between May and July, we monitored 473 plainfin midshipman nests during low tide in the intertidal zone on three rocky beaches in British Columbia (Ladysmith Inlet: 49°01´N, 123°83´W; Mill Bay: 48°63´N, 123°53´W; and Crescent Beach: 49°04´N, 122°88´W). This population has been extensively monitored since 2007 and shows stable numbers of reproductively mature adults and a stable ratio of male types (**Table 4.1**). At each nest, we sexed all fish based on the shape of the urogenital papilla and overall body coloration and identified males as type I or type II based on their body size and dissection of supposed type II males (Brantley & Bass, 1994). We further distinguished between guarding and cuckolder type I males based on their position in the nest, because cuckolder males are most often found on the periphery facing outwards and their urogenital papilla pointing inwards (Lee & Bass, 2004; K.C., personal observation). These characteristics used to identify sex and reproductive type have been verified in extensive studies and field observations have been confirmed through dissections (Bass & Marchaterre, 1989; Bass & Anderson, 1991; Brantley & Bass, 1994). We collected a small amount of fin tissue from each adult and preserved the tissue in 95% ethanol. In addition, from a subset of nests (N = 47), we collected and preserved 40-50 offspring from each cohort found in each nest. A cohort was defined as a distinct group of offspring laid by one or more females in relative

Table 4.1 An estimate of the proportion of type I males in a population of plainfin midshipman (*Porichthys notatus*) from British Columbia.

Year	**Number of males**			
	Type I	**Type II**	**Total**	***q****
2008	140	10	150	0.93
2009	114	22	136	0.84
2010	254	15	269	0.94
Total	508	47	555	0.92

***q** is the proportion of type I males in our population sample synchrony such that they were at the same developmental stage (see Cogliati et al., 2013 for the unique characteristics used to classify developmental stage in midshipman young). Plainfin midshipman nests typically have between 1 and 4 cohorts. After sampling, we returned the adults (expect for type II males) to the nest and then repositioned all rocks to their original position.

4.3.2 Genetic analyses

We genotyped adults and offspring collected across the three years and three beaches using six microsatellite loci developed from plainfin midshipman (loci: Pon22, Pon23, Pon25, Pon30, Pon32, and Pon47; see Suk et al., 2009 for primer sequences). Across the 47 nests from which offspring were collected, we genotyped 1,713 offspring (approximately 25 offspring per cohort per nest) using the six microsatellite loci (see Cogliati et al., 2013). Additionally, to determine the degree of type I male cuckoldry, here, we focused our genotyping on one well sampled beach in one year (Ladysmith Inlet in 2010; henceforth called LSI2010) and genotyped all adults sampled from this site, for a total of 102 adults (20 females, 78 type I males, and 4 type II males). We conducted PCR amplifications following the protocol described in Suk et al. (2009), and conducted fragment analyses on a CEQ 8000 sequencer (Beckman Coulter, Fullerton, California).

4.3.3. Paternity analyses

We have previously published the paternity results for midshipman males for the 47 genotyped nests, including those from LSI2010, using the *two-sex paternity* model (Neff et al., 2000a,b; Neff, 2001) and COLONY (v2.0; Wang, 2004; Jones & Wang, 2010; see Cogliati et al., 2013). Across nests, we did not find a difference in paternity between the two models. We used the COLONY assignments in this study because this program generates putative parental genotypes that we could use to identify parent offspring relationships (as opposed to the overall proportional assignments generated by the *two-sex paternity* model). Unique to this study, we were able to use COLONY to assign cuckolded offspring to individual type I and type II males collected during our intense sampling at the LSI2010 site.

4.3.4 Applying the Gross and Charnov life history model

We used the Gross and Charnov (1980) life history model to calculate the relative fitness of type I and type II males. This model uses the proportion of males in the population that develop into type I (q) and the proportion of eggs fertilized in a reproductive season by all type I males (h) to calculate the relative fitness of the two male tactics:

$$\frac{\omega_I}{\omega_{II}} = \frac{(1-q) \times h}{q \times (1-h)} \tag{1}$$

where ω denotes the fitness of type I males (I) or type II males (II). Thus, the two life histories have equal fitness ($\omega_I = \omega_{II}$) when the proportion of type I males in the population equals the proportion of all eggs fertilized by type I males (i.e., $q = h$; see derivation in Gross & Charnov, 1980).

Using the data collected from our population monitoring, we calculated q as the proportion of type I males over the total number of all males sampled in the population across the three breeding seasons. During the breeding season, type II males are commonly found in nests whereas only juveniles are found in nearby offshore areas (Bass, 1992; Brantley & Bass, 1994), so it is unlikely that our sampling method strongly biases against type II males. Furthermore, although we sampled reproductive adults that already diverged into each tactic as opposed to the males at the point of divergence, we are assuming minimal differential survivorship between tactics. Because h denotes the proportion of *all* eggs fertilized by type I males in the population, we needed to incorporate the proportion of eggs sired by type I male cuckolders. To do this, we first defined $h_{guarding}$ as the proportion of offspring that were assigned by COLONY to nest-guarding males (of a possible 1,713 genotyped offspring). We then defined $h_{cuckolder}$ as the proportion of cuckolded eggs that were also sired by type I males. We estimated this latter value based on the 512 offspring sampled from LSI2010, of which 132 were cuckolded eggs. COLONY was unable to assign all cuckolded offspring to known males, indicating that we did not sample all possible sires (67 of the 132 cuckolded offspring were unassigned in the LSI2010 samples). Thus, we considered three scenarios. First, we assumed that all

67 offspring of unknown paternity were sired by type II males and $h_{cuckolder}$ would then equal only the proportion of cuckolder offspring assigned to known, non-nest guarding, type I males in our sample (55 of 132; $h_{cuckolder\_low}$). Second, we assumed that all 67 offspring of unknown paternity were sired by type I males and $h_{cuckolder}$ would then equal the proportion of cuckolder offspring assigned to known, non-nest guarding, type I males in addition to all of the offspring assigned to the unknown males (55 + 67 = 122 of 132; $h_{cuckolder\_high}$). Given these two scenarios, h is bounded by:

$$h_{low} = h_{guarding} + \left(1 - h_{guarding}\right) \times h_{cuckolder\_low}\text{; and} \qquad (2)$$

$$h_{high} = h_{guarding} + \left(1 - h_{guarding}\right) \times h_{cuckolder\_high} \qquad (3)$$

Third, we assumed that the proportion of cuckoldry performed by type I and type II males in the unassigned offspring was equivalent to the proportion of each male's cuckoldry calculated from the offspring that could be assigned (we called this our 'proportional' scenario):

$$h_{prop} = h_{guarding} + \left(1 - h_{guarding}\right) \times h_{cuckolder\_prop} \qquad (4)$$

4.3.5 Statistical analyses

Using equation (1), we calculated the relative fitness (ω_I/ω_{II}) of type I males versus type II males for h_{low} and h_{high} scenarios, as well as the proportional scenario. To obtain confidence intervals for the three relative fitness estimates, we performed Monte Carlo analyses that re-sampled with replacement the original data at the level of the individual offspring or adult for each variable (q, $h_{guarding}$, $h_{cuckolder\_low}$, $h_{cuckolder\_high}$, $h_{cuckolder\_prop}$). Specifically, confidence parameters for q were estimated by re-sampling the adult males, $h_{guarding}$ were estimated by re-sampling the 1,713 offspring, and $h_{cuckolder\_low}$, $h_{cuckolder\_high}$ or $h_{cuckolder\_prop}$ were estimated by re-sampling the 132 cuckolder offspring given the three scenarios for assigning offspring with unknown sires. Each resampled estimate was also used to recalculate the relative fitness (ω_I/ω_{II}). We performed this procedure 10,000 times and used the resulting values to obtain 95% confidence intervals for the parameters and for the relative fitness estimate.

We also conducted a sensitivity analysis to determine where in the bounds fitness changed from type I males having higher fitness to equal fitness to type II males having higher fitness. This analysis was done by resampling the 132 cuckolder offspring in 67 subsequent Monte Carlo analyses, to titrate through the 67 offspring, assigning a different number to type I males in each analysis. We started the Monte Carlo analyses with 0 of the 67 offspring assigned to type I males and ended with 67 of 67 offspring assigned to type I males. For each assignment, we calculated the relative fitness and confidence interval. As an example, assignments for which the subsequent confidence interval included 1.0 defined a bin where the two male types have equal fitness.

4.4 RESULTS

Across the three years, we sampled 555 adult male midshipman, of which 508 were type I males (**Table 4.1**). The Monte Carlo simulation revealed that the mean proportion of type I males in the population (q) was 0.92 with a 95% confidence interval of 0.89 – 0.94. In the 47 nests that we examined parentage, 1,193 of the 1,713 genotyped offspring were assigned by COLONY to a guarding type I male. Thus, the proportion of eggs fertilized by guarding type I males, or $h_{guarding}$, is equal to 0.70 with a 95% confidence interval of 0.67 – 0.72. This assignment accounts for the occurrence of takeovers and assigns paternity to any previous nest holding type I male (see Cogliati et al., 2013). By examining the 512 offspring genotyped from our well sampled LSI2010 site which consisted of 21 cohorts in 8 nests from across the breeding season, we were able to determine the degree of type I male cuckoldry (**Table 4.2**). In this sample, 380 offspring were assigned to a guarding type I male, leaving 132 cuckolded offspring. Of the 132 cuckolded offspring, COLONY assigned 55 to known type I males, 10 to known type II males, and 67 to unknown males (i.e. males not sampled; **Table 4.2**). Altogether, we were able to assign 445 of the 512 offspring (87%) to males sampled in our population from our well sampled LSI2010 site.

If we assume that all 67 offspring of unknown paternity were sired by type II males, we generate an $h_{cuckolder\_low}$ that equals 0.42 (95% CI = 0.33 – 0.50). The proportion of offspring fertilized by all type I males (h_{low}) would then equal 0.82

Table 4.2 Summary of paternity assignments for plainfin midshipman (*Porichthys notatus*) collected at one beach in Ladysmith Inlet during 2010 using COLONY. Paternity represents the number of offspring assigned to each male type.

			Paternity			
					Cuckoldry	
Nest	Cohort	Offspring	Guarding	Type I	Type II	Unknown
1	1	25	21	0	3	1
	2	23	23	0	0	0
2	1	24	6	3	0	15
	2	26	21	1	2	2
	3	26	0	12	0	14
3	1	25	10	0	0	15
4	1	23	23	0	0	0
	2	24	9	9	0	6
5	1	24	19	1	3	1
	2	24	20	4	0	0
	3	25	23	0	0	2
	4	25	23	0	0	2
6	1	25	23	0	2	0
	2	22	22	0	0	0
	3	25	24	1	0	0
7	1	24	21	0	0	3
	2	25	25	0	0	0
	3	24	0	24	0	0
8	1	24	24	0	0	0
	2	24	18	0	0	6
	3	25	25	0	0	0
Totals		**512**	**380**	**55**	**10**	**67**

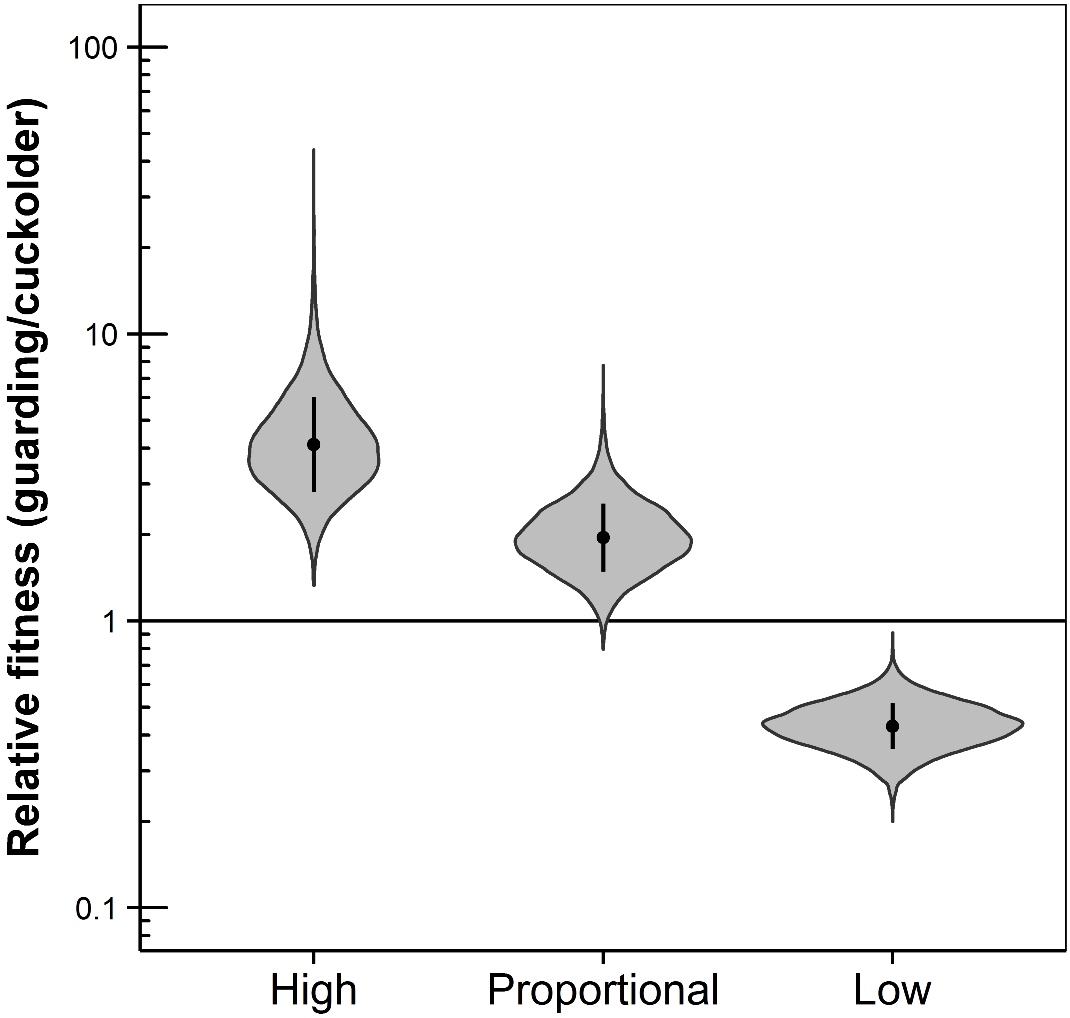

Figure 4.1 Estimates of the relative fitness of ARTs in plainfin midshipman (*Porichthys notatus*). Shown are the mean relative fitness (± 1SD, dot and vertical line) for type I males given high (all unknown sires as type I), proportional (85% of unknown sires as type I), and low (all unknown sires as type II) scenarios (see text for details). The violin plots show the vertical frequency histograms of the distribution of data from the Monte Carlo analyses. The solid horizontal line depicts equal fitness (= 1) between type I and type II males. The y-axis is a log scale to better depict the ratio data.

(95% CI = 0.79 – 0.85), and the relative fitness of the two male tactics $\omega_{I}/\omega_{II\ low}$ would be 0.44, with a 95% confidence interval of 0.29 – 0.61 (**Figure 4.1**). If instead, all 67 offspring of unknown paternity were sired by type I males, we generate an $h_{cuckolder\_high}$ that equals 0.92 (95% CI = 0.88 – 0.97) and the proportion of offspring fertilized by type I males (h_{high}) would equal 0.98 (95% CI = 0.96 – 0.99). The relative fitness $\omega_{I}/\omega_{II\ high}$ would be 4.5 with a 95% confidence interval of 2.1 – 9.4 (**Figure 4.1**). Finally, if we assume the proportional ratio, that 85% or 57 of the 67 offspring of unknown paternity were sired by type I males (based on 55 type I offspring out of 65 known cuckolder offspring), then $h_{cuckolder\_prop}$ equals 0.85 (95% CI = 0.79 – 0.91). The proportion of offspring fertilized by type I males (h_{prop}) would then equal 0.95 (95% CI = 0.93 – 0.97), and the relative fitness $\omega_{I}/\omega_{II\ prop}$ would be 2.0 with a 95% confidence interval of 1.2 – 3.4 (**Figure 4.1**). Thus, if the unknown paternity is attributed entirely to type I males, or if 85% is attributed to them (our proportional scenario), then the fitness of type I males is significantly higher than that of type II males. Conversely, of our three scenarios, it is only when all of the unknown paternity is attributed entirely to type II males is the fitness of type I males significantly lower than that of type II males.

We then used our sensitivity analysis to titrate through the number of offspring that were assigned to unknown males, to determine the critical points where relative fitness between the two tactics would be equal. We found that when 27 or fewer of the possible 67 offspring were sired by type I males, the relative fitness of type I males was significantly less than 1. At the other extreme, when 52 or more of the possible 67 offspring were sired by type I males, the relative fitness of type I males was significantly greater than 1. No significant difference in the relative fitness of type I and type II males was observed when 28 to 51 of the offspring sired by unknown males were assigned to type I males.

4.5 DISCUSSION

In this study, we conducted intense field sampling and used genetic markers to explore patterns of paternity to estimate the degree of cuckoldry in the plainfin midshipman, a species with well-described ARTs. Our analyses built on those presented in Cogliati et al. (2013), who first estimated cuckoldry rates in this system and documented nest takeover behaviour by type I males. Here, we used extensive sampling at the Ladysmith Inlet site (LSI2010) to partition the observed paternity loss between specialized cuckolder males (type II) and opportunistic cuckoldry performed by type I males. Surprisingly, based on these samples, we found that nearly 42% of all documented cuckoldry was done by type I males (fathering 11% of all offspring at this site), whereas only 7% was done by known type II males (2% of all offspring at this site). The remaining 51% of the cuckolded offspring could not be assigned to any of the males that we sampled (13% of all offspring at this site). Our results thereby corroborate observations of cuckoldry behaviour by type I males reported in Lee and Bass (2004), and provide the first estimate of the frequency of cuckoldry by type I males in plainfin midshipman. Examples of such behavioural plasticity within a male tactic are restricted to species where male alternative tactics are distinct. Such within tactic variation cannot be determined in species where males display flexible tactics and do not have distinct morphs (e.g. guppies; Magurran & Seghers, 1990, 1994). We know of only one other study that has reported estimates of paternity loss from competition within a tactic type, not including takeovers or abandonment. In bluegill, Neff (2001) documented paternity loss of 1.8% to other parental males who were cuckolding. This high occurrence of plastic cuckoldry in plainfin midshipman may reflect greater nest site limitations and the protracted parental care period (3-4 months), which may considerably increase the benefit of type I cuckoldry (Cogliati et al., 2013). Thus, our genetic analyses have documented several reproductive behaviours used by type I males, comprising guarding, nest takeovers, and cuckoldry.

We were also able to use our genetic data to estimate the relative fitness of the ARTs in plainfin midshipman. The life history model developed by Gross and Charnov (1980) provides a useful method for calculating the relative fitness of ARTs. Male ARTs can arise from a genetic polymorphism or from a conditional strategy, with the former mechanism predicting equal fitness between the tactics and the latter predicting that one tactic will have higher fitness (see Gross, 1996). Applying the life history model to our multi-year data indicated that the relative fitness of type I males had an upper estimate of 4.5 and a lower estimate of 0.44, where a relative fitness of 1 indicates equal fitness for the two male tactics. Our sensitivity analyses revealed that type I males had lower fitness than type II males when fewer than 62% of all cuckolded offspring were sired by type I males, equal fitness when 63% to 80% of cuckolded offspring were sired by type I males, and greater fitness when more than 81% of the offspring were sired by type I males. At first pass, our analysis is equivocal, encompassing higher, equal, and lower fitness for type I males.

However, based on two lines of evidence, we argue that type I males are most likely to have higher or equal fitness compared to type II males. First, type I males sired more than 2 times as many of the cuckolded offspring that could be assigned to known males than did type II males. Of the offspring that were assigned to cuckolders, type I males sired an average of 5.5 offspring per cohort (range 1-24), compared to type II males who sired an average of 2.5 offspring per cohort (range 2-3). Given this distribution of paternity assignment, with type II males fathering a smaller number of offspring per cohort, we can reasonably assume that any male that sired 6 or more offspring in a cohort was likely to be a type I male. Of the unassigned offspring, the reconstructed genotypes provided by COLONY found 22 distinct sires of which four had sired 6 or more offspring within a cohort (overall range 1-12; data not shown) for a total of 35 offspring. If we consider these four unknown males who sired a relatively large number of young to be type I males, then type I males would account for at least 35 of the 67 unknown offspring and type I males would have equal or higher fitness compared to type II males. Second, it is likely that type I males sired more than 77% (52 of 67) of the unassigned offspring. For example, based on the 65 assigned cuckolded offspring, type I males sired 85% (=55/65). Assuming the same proportion (0.85) in the unassigned offspring would give 57 of the 67 unassigned offspring to type I males (our proportional scenario), and supports the notion that type I males have higher fitness than type II males. Indeed, the Gross and Charnov (1980) model assumes that q is based on the number of males at the point of tactic divergence, and while we were unable to sample males at this point, q would have to equal 0.98 or greater to shift our prediction such that type II males have higher fitness. Thus, although we cannot conclusively differentiate between alternative strategies and a conditional strategy, our data do suggest that type I males have equal or higher fitness and it is type II males that might be making the best of a bad situation (sensu Dawkins, 1980; Eberhard, 1982).

Understanding the evolution of ARTs has been an area of great interest for decades, yet testing the prediction of equal fitness has only been done in a handful of systems (Gross & Charnov, 1980; Shuster & Wade, 1991; Ryan et al., 1992; Sinervo & Lively, 1996; Neff & Lister, 2007; Rios-Cardenas & Webster, 2008). In comparison to the hundreds of species with documented ARTs, the relative paucity of tests for the prediction of equal or unequal fitness is likely due, in part, to the challenges of measuring reproductive success for each male tactic, particularly when reproductive success fluctuates in both space and time (e.g. Neff & Clare, 2008; Cogliati et al., 2013). In the extensively studied bluegill sunfish, Neff and Lister (2007) observed that the cuckolder morph had slightly higher fitness than the parental morph, suggesting a conditional strategy. Their result differed from the initial conclusions by Gross and Charnov (1980), who calculated equal fitness between the two morphs in the same population. This discrepancy was largely driven by the inclusion of differential survivorship of offspring sired by parental versus cuckolder males in the Neff and Lister (2007) study, where cuckolder offspring had significantly higher survivorship than parental offspring prior to differentiation into the two life histories. The Gross and Charnov (1980) model assumes equal survivorship of offspring prior to differentiation into the two tactics. Indeed, in our study we were unable to include survivorship data in the relative fitness calculation. However, because the tactics in plainfin midshipman differentiate at approximately 200 days of age and type I males are known to mature just a few months after type II males based on otolith growth (Bass et al., 1996), we doubt there will be a major effect of differential survivorship of the offspring on the calculation of fitness of the two tactics. Bluegill tactics, in comparison, diverge at 2 years of age when sneakers first mature (Gross, 1982). Therefore, the impacts of differential survivorship are likely considerably less severe in plainfin midshipman. Nevertheless, our assumption of similar survivorship to tactic divergence remains to be empirically tested in plainfin midshipman.

We investigated whether the plainfin midshipman male reproductive tactics evolved via one of two mutually exclusive possibilities: genetic or conditional strategies (Gross, 1996). In a recent review, Neff and Svensson (2013) proposed a new model termed ‘conditional alternative strategies’, which treats the two approaches (genetic and conditional) as two ends of a single continuum and thus combines both genetic and environmental effects on tactic expression. Given their approach, it is possible to observe additive genetic variance in condition dependent 'thresholds' (Piche et al., 2008; Tomkins & Moczek, 2009) or 'switch points' (Roff, 2011). The plainfin midshipman would be an ideal species to test Neff and Svensson (2013) model. For example, those authors suggest the use of natural breeding experiments and multiple rearing environments to investigate gene-by-environment interactions (i.e., interactions between sire life history and the environment, such as food availability, predation risk, or competition). These experiments can be executed with relative ease in plainfin midshipman, because they can be sampled readily at low tide and have an extended breeding season. Furthermore, sexual maturity and tactic differentiation occurs at a relatively young age for midshipman (Bass et al., 1996) and therefore monitoring or manipulating aspects of the environment before tactic differentiation occurs could be performed and then evaluated. This approach could get at a gene-by-environment interaction, which is a key predictor of the Neff and Svensson (2013) model.

In conclusion, the results of our study delineate the possible upper and lower bounds of relative fitness between the two male tactics seen in plainfin midshipman, encompassing both traditional mechanisms for the evolution of ARTs: genetically determined alternative strategies or a single conditional strategy. Based on how rare and unsuccessful type II males were at our extensively sampled site, we argue that type I males are likely to have greater fitness to type II males. In addition, we show that type I males cuckold at a relatively high frequency, adding to the repertoire of reproductive behaviours used by these males. Given the tractability of the midshipman system, a valuable next step will be to look for gene-by-environment interactions on tactic development and expression as predicted by the conditional alternative strategies model of Neff and Svensson (2013).

4.6 ACKNOWLEDGEMENTS

We thank Shawn Garner and John Fitzpatrick for valuable comments on the manuscript. We thank Celia Chui, John Fitzpatrick, Ahdia Hassan, Julie Marentette, Jennifer Reynolds, Natalie Sopinka, and Jonathan Taves for their assistance with data collection in British Columbia. Michael Roubakha, Shawn Garner, Cayli Hunt, Celeste Bouchard, and Amanda Pereira assisted with DNA extractions and sample analyses. Ben Bolker provided advice on the data analysis and Shawn Garner wrote the Excel macros to conduct the Monte Carlo analyses. We also thank Mr. and Mrs. Cogswell, Mr. and Mrs. Weatherell, and especially Mr. and Mrs. Flader for providing access to midshipman habitats and lodgings for field crew. This work was funded by the Natural Sciences and Engineering Research Council of Canada (grants to S.B. and B.D.N.).

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Chapter 5

DIET AND CANNIBALISM IN PLAINFIN MIDSHIPMAN (*PORICHTHYS NOTATUS*)

Manuscript in prep

5.1 ABSTRACT

Reduced opportunity to feed has long been recognized as a cost of providing parental care. However, few studies have quantified food availability for parents and compared this to the actual diet. We quantified the macroscopic and microscopic diversity of potential food items in the nests of plainfin midshipman (*Porichthys notatus*) and investigated which items were found in the digestive tracts of the care-giving males. In this species, males occur as one of two possible reproductive morphs: guarder males that care for young, and sneaker males that parasitize the courtship and care of guarder males. Although we predicted that guarder males would have fewer feeding opportunities due to their confinement to the nest, they in fact had more food items in their guts than did sneaker males and females. Date in the breeding season (a proxy of care duration) and body condition were not correlated with the amount of food consumed by guarder males. The main type of food consumed was midshipman embryos; 69% of all guarder males sampled had cannibalised offspring. By comparing the diet of both sexes and tactics, our results

shed light on some of the strategies designed to cope with the costs of providing parental care.

5.2 INTRODUCTION

Of the more than 514 families of fishes, 20% show some form of parental care for offspring (Gross and Sargent 1985; Mank et al. 2005; Nelson 2006). Parental care in fishes includes activities such as hiding or guarding embryos, chasing-off predators, fanning to ventilate embryos, and removing debris or microorganisms from the embryos (Blumer 1982). Such caring behaviours increase rates of offspring development and survivorship, but also carry costs for the caring parent including reductions in survival, mating opportunities, and fecundity (Trivers 1972; Gross and Sargent 1985; Smith and Wootton 1995).

Many of the costs associated with care are a result of decreased opportunities to feed. There can be significant physical deterioration and a reduction of energy stores during care (e.g., Marconato and Bisazza 1988; Smith 1993; Cooke et al. 2002; Cargnelli and Neff 2006; Ito et al. 2010). For example, in the stream goby (*Rhinogobius* sp), body condition and liver size, two measures of energy stores, both decreased in caring males as the breeding season progressed (Ito et al. 2010). Indeed, 68% of guarding stream goby males did not eat during the four-week period between nest building and egg hatching (Ito and Yanagisawa 2000; Ito et al. 2010). Decreased body condition can have effects on growth and survival; for example, individuals in poor condition are more likely to be captured by predators

(Fitzgibbon and Fanshawe 1989; Mesa et al. 1994). Reduced growth can in turn influence future mating opportunities (Andersson 1994) and fecundity (Balshine-Earn 1995). One possible response to the decreased opportunities to feed during care is cannibalism of embryos (Rohwer 1978). Theory suggests that cannibalism can be adaptive even when the parents are related to the consumed offspring, because cannibalism can enable parents to recoup the necessary energy to continue caring for the remaining offspring (Rohwer 1978; Sargent 1992; Klug and Bonsall 2007).

The high costs of parental care are thought to select for the evolution of alternative reproductive tactics (ARTs), where some individuals parasitize the care and courtship of other conspecifics (Gross 1996; Taborsky et al. 2008). ARTs, which are discontinuous variations in behaviours and other traits that represent alternative ways to achieve and maximize fitness (Gross 1984; Taborsky 1997), are commonly found in fishes, especially among males, and typically result in two male reproductive morphs broadly classified as guarder and cuckolder males (Taborsky et al. 2008). Guarder males actively compete for access to resources or females, defend breeding territories, and commonly provide parental care (Caro and Bateson 1986). In contrast, cuckolder males exploit the investment of guarder males by satellite or sneak-spawning using stealth, speed, or female mimicry to gain fertilizations (Taborsky et al. 2008). Cuckolder males do not provide care and invest instead in gonad size and sperm quality (Taborsky 1998). Using a fish species with well-characterized ARTs, the plainfin midshipman (*Porichthys notatus*), we investigated the potential cost of lost feeding opportunities associated with parental care of guarder males and compared the costs across tactics and sexes.

The mating system and caring behaviour of the plainfin midshipman make it an excellent candidate for investigating the costs of parental care associated with lost feeding opportunities. This fish is a nocturnally active, deep-water species found along the Pacific coast of North America from Sitka, Alaska, U.S.A., to Magdelena Bay, Mexico (Arora 1948; Walker and Rosenblatt 1988). Plainfin midshipman males occur as one of two morphs called either guarder (type I) or sneaker (type II) males; the morphs are anatomically, physiologically, and behaviourally distinct (Brantley and Bass 1994). Guarder males are eight times larger in body mass than sneaker males. They build and defend nests under rocks in the intertidal zone during the spawning period and use acoustic signals to court females (Brantley and Bass 1994). Females lay all of their eggs in a nest and will leave once spawning is completed, relinquishing all parental duties to the guarder male (Brantley and Bass 1994). The guarder males will spend up to 4 months caring for the embryos by guarding them from predators, brushing the embryos with their bodies or fins to remove debris, and fanning to oxygenate them until they are free swimming (Arora 1948; Brantley and Bass 1994). While providing parental care for their offspring, guarder males are confined to their nest cavity and do not appear to leave (Arora 1948; Brantley and Bass 1994). In contrast, sneaker males forego all courting, territorial and egg tending behaviour and instead simply steal fertilizations from guarder males when females are present (Brantley and Bass

1994). Sneaker males invest heavily in gonadal tissue and sperm competitive traits, and have testes, which even after controlling for body size are seven times larger than the testes of guarder males (Brantley and Bass 1994; Fitzpatrick et al. submitted). Spawning takes several hours to complete, after which females and sneaker males leave the nest (Brantley and Bass, 1994). Although previous studies of plainfin midshipman have anecdotally described aspects of their diet (see Arora 1948, Hubbs 1920; Sisneros et al. 2009), no detailed analyses of diet content and of differences in diet between male tactics and between the sexes have been conducted.

In this study, we explored the availability of food items and the diet of reproductive plainfin midshipman. We completed a macroscopic and microscopic survey of abundance and biodiversity of organisms in plainfin midshipman nests. We compared the organisms found in the nests, which would be immediately available as food to a guarder male during the parental care period, to the organisms found in the digestive tract of guarder males. We also explored how food abundance and gut mass correlated with fish body condition and time in the breeding season. Additionally, we quantified the organisms found in the digestive tracts of reproductive sneaker males and females (collected opportunistically from within the nests), which allows us to make some comparisons between sexes and tactics. We confirmed that midshipman cannibalise young (also described by Mensinger and Case 1991, and Sisneros et al. 2009), and, for the first time, we quantified the extent of this behaviour in the plainfin midshipman, and how cannibalism is influenced by paternity. Specifically for the guarder males, we predicted that (1) the body condition would decrease over the course of the breeding season (as proxy for the duration of care), and (2) as body condition deteriorates, food intake and cannibalism rates would increase. Based on all fish sampled from nests, we also predicted that (3) guarder males, who are confined to their nests for a long duration, would have less food intake than sneaker males and females who are only in the nests for several hours and would therefore have more opportunities to feed.

5.3 MATERIALS AND METHODS

5.3.1 Field collections

From May 10 to July 15, 2010, we sampled plainfin midshipman nests from rocky beaches during low tide in the intertidal zone in British Columbia (Ladysmith Inlet: 49°01'N, 123°83'W and Mill Bay: 48°63'N, 123°53'W on the eastern coast of Vancouver Island, and Crescent Beach: 49°04'N, 122°88' W in South Surrey). Additionally, from May 31 to July 17, 2011, we sampled nests from Ladysmith Inlet and Crescent Beach in British Columbia, and nests from Washington (Seal Rock: 47°71' N, 122°88' W), and California (Tomales Bay: 38°15' N, 122°90' W).

5.3.2 Macroscopic biodiversity in nests

To assess possible food resources immediately available to guarder males during the reproductive season, we surveyed the biodiversity in 105 nests across all sites in British Columbia in June 2010. Here, we performed a visual count of all macroscopic organisms found within the nest and identified species to the lowest possible taxonomic rank. We completed these visual counts immediately following the opening of the nest cavity to ensure we did not miss mobile organisms.

5.3.3 Microscopic biodiversity on broods

To assess the diversity of microscopic food items directly on the embryos across the reproductive season, we collected organisms from the broods of 25 nests in British Columbia in 2011. We sampled 15 nests early in the breeding season (June) and 10 nests later in the breeding season (July). To collect the organisms on the broods, we positioned a flexible funnel below the embryos, washed the embryos using 200 mL of filtered sea water (filtered through a 40 micron cell strainer), and collected everything on the broods into a small container. We then poured these washings through a square of 65 micron plankton net and transferred the mesh plankton net and its contents to a 50 mL tube and filled with 95% ethanol for storage. Once the washings were collected, we removed and euthanized the guarder male from each nest so that the contents of his digestive tract could be compared to organisms from the washings at the nest. The developmental stage of all offspring found in the nests was also recorded (i.e. we noted whether the nest contained eggs only, both eggs and hatched embryos, or hatched embryos only); this order of development generally follows the progression of the breeding season (Cogliati et al. 2013).

5.3.4 Gut analysis

Across both sampling years and all sampling sites, we collected 151 fish from nests and measured their body mass (g), total length (TL in cm), and standard length (SL in cm). We recorded the sex of each fish based on the shape of their urogenital papilla, overall colouration, and gonads once dissected. We sampled fish by overturning rocks to locate nests and the guarder males, and opportunistically collected females and sneaker males from the nests. We determined morph type using criteria that have been well developed for this species, including body size and the gonadosomatic index (GSI: gonad mass/body mass × 100; Brantley and Bass 1994; Bass 1996). In total we sampled 123 guarder males, 10 sneaker males, and 18 females. We used a solution of Benzocaine (Sigma-Aldrich, St. Louis, Missouri), ethanol and sea-water, followed by cervical severance to euthanize fish. We then dissected the digestive tract of each fish, henceforth referred to as the gut, by making an incision from the gill to the anus to remove the whole gut and intestine (G_F), which we then weighed (g). We rolled out the contents of the gut from the anterior to posterior end using a probe and preserved the contents in 95% ethanol. We then reweighed the empty gut (G_E) and determined the mass of the gut contents alone (G_C), where $G_C = G_F - G_E$.

5.3.5 Microscope analysis

We quantified the microscopic organisms for the 25 embryo washings and in the guts of 101 of the 151 plainfin midshipman collected from the nests (73 guarder males, 10 sneaker males, and 18 females). In this subset of 101 gut samples, all females and sneaker males were included, and the 73 guarder male samples were haphazardly chosen. The researchers who quantified organisms in the washings and in the gut were blind to the identity of the nest and reproductive type. We removed all organisms caught in the plankton net by soaking for 48 hours and rinsing with 95% ethanol. Afterwards, we passed the net under a Nikon SMZ 1500 stereomicroscope fitted with a 1× WD54 lens (Nikon Instruments Incorporated, Melville, New York) to detect any remaining organisms not removed by the rinsing, and added any organisms still on the net to the sample. We removed excess ethanol in the sample by decantation with a pipette, leaving each sample in approximately 20mL of ethanol. We transferred this remaining solution to a gridded 90 mm square petri dish with 13 mm × 13 mm squares and illuminated using a Fiber-Lite MI-150 High Intensity Illuminator (Dolan-Jenner Industries, Dayton, Ohio) under the microscope. We identified organisms to the lowest feasible taxonomic rank, counted each type of organism, and extracted all organisms for preservation in 95% ethanol. We examined preserved gut samples using the same protocols, except that the samples were transferred immediately to the petri dish without a 48 hour soaking period. Following previous studies, we classified invertebrates such as crustaceans, gastropods, and zooplankton, as well as plainfin midshipman offspring as "food items" (Hubbs 1920; Arora 1948; Sisneros et al. 2009). We classified abiotic items and plant matter as "non-food items".

The remaining 50 fish (of the original 151 fish collected) were all guarder males and their guts were analyzed for instances of cannibalism only. To analyze rates of cannibalism we combined the data from these 50 guarder males with the 73 guarder males scrutinized for whole-gut contents.

5.3.6 Cannibalism analysis

When instances of cannibalism were detected during examination of the guts, we removed any intact embryos from the samples for genetic analyses. Following the methods outlined in Cogliati et al. (2013), we extracted DNA from a subset of these embryos and from the associated fin clips that we collected from these males during field sampling. In total, we genotyped 67 embryos found in the guts of 11 males using 6 microsatellite loci developed for plainfin midshipman (Suk et al. 2009; Cogliati et al. 2013). We conducted fragment analyses on a capillary sequencer (CEQ 8000, Beckman Coulter; Fullerton, California) and scored alleles from each locus based on characteristic peaks. We used the *two-sex paternity* model (Neff et al. 2000a,b; Neff 2001) to calculate a paternity value for each male based on the embryos found in their guts.

5.3.7 Statistical analyses

For each nest, brood, and gut examined we calculated a *biodiversity* measure as the number of different organism classifications found per sample, an *abundance* measure as the total number of organisms found per sample, and a *prevalence* measure as the total number of samples in which a specific organism occurred. Specifically for the guts examined, we also used *gut mass* as a measure of overall fullness. For this measure, we controlled for differences in body mass by dividing the mass of the gut contents (G_c) with the eviscerated body mass. Eviscerated body mass was calculated as the total mass of the fish (M_{total}) minus the mass of the gut contents ($M_{evis} = M_{total} - G_c$). Finally, we used the Julian date of collection as a proxy for the duration of parental care provided by guarder males, and assumed that samples collected later in the season were from males who had been providing care for longer than for the samples collected earlier in the breeding season.

We characterized body condition by plotting the log of the eviscerated body mass against the log of the SL of the fish and obtaining the residuals from a linear regression (Jakob et al. 1996). A positive value signified an individual in better condition compared to the average and a negative value represented individuals in poorer condition. Following well established protocols, we calculated the hepatosomatic index of each fish using the formula: HSI = (liver mass / eviscerated body mass) × 100 (Htun-Han 1978). We compared these two measures of body condition in relation to what was found in the guts and across the breeding season. The gut mass for one guarder male and the body condition for two females were not collected due to technical issues with our balance in the field.

We performed all statistical analyses using JMP 10.0 (SAS Institute Inc.), with the exception of the generalized linear models, which we conducted in R v2.15.2 (The R Foundation for Statistical Computing). Unless otherwise indicated, we present data as mean ± SE. We transformed data to reach normality wherever possible and analysed data using Pearson regressions, ANOVAs and post-hoc Tukey's HSD tests. We analysed all count data (*abundance* measure) using negative binomial generalized linear models to accommodate the over-dispersed Poisson distributed data. All other data that did not conform to normality or that violated the assumption of homogeneity of variance were analysed using non-parametric statistics (Krukal-Wallis and Wilcoxon tests). Specifically for the gut analyses, there was no effect of region of collection (British Columbia, Washington, and California), other than on parasite load, and so we did not include region of collection in these other statistical models.

5.3.8 Ethics

All research conformed to the guidelines outlined by the Canadian Council on Animal Care and was conducted in accordance with the protocols approved by the McMaster University Animal Research Ethics Board (AUP #10-11-70) and the UC Davis Institutional Animal Care and Use Committee (AUP #15870). We were also granted permission to undertake this work by the Department of Fisheries and Oceans, Canada, the California Department of Fish and Game, and the Washington Department of Fish and Wildlife, and received the approval for collection from the Fisheries officer of the Stz'uminus and Malahat First Nations bands on Vancouver Island.

5.4 RESULTS

5.4.1 Biodiversity, abundance, and prevalence

In the 105 nests sampled macroscopically, we identified 43 different organisms, with an average of six different types of organisms per nest (range 0-11 types of organisms). Many of the organisms found in nests were also abundant with an average of 91 organisms per nest (range 0-1089 organisms). Shore crabs (present in 88% of nests), hermit crabs (73% of nests) and barnacles (64% of nests) were by far the most prevalent organisms found inside midshipman nests. The distribution of the 10 most prevalent organisms in plainfin midshipman nests is plotted in **Fig. 5.1a**.

On the 25 broods examined, we identified 20 different microscopic organisms. In terms of biodiversity, there were on average seven different organisms per brood (range 2-12 organisms). The average abundance of organisms found on the broods was 480 per brood (range 53-2054). Copepods were the most common organisms found on the broods and were present in every nest sampled. The distribution of the 10 most prevalent organisms on broods is plotted in **Fig. 5.1b**.

Across the 101 fish examined in detail for gut content, we found a total of 50 different items in the guts of which 18 could be classified as food. Five items were various species of parasites (one species of Acanthocephala, one species of Trematoda, one species of Cestoda, and two unknown species) and the remaining 27 items were objects such as shell fragments and various classes of sediment and

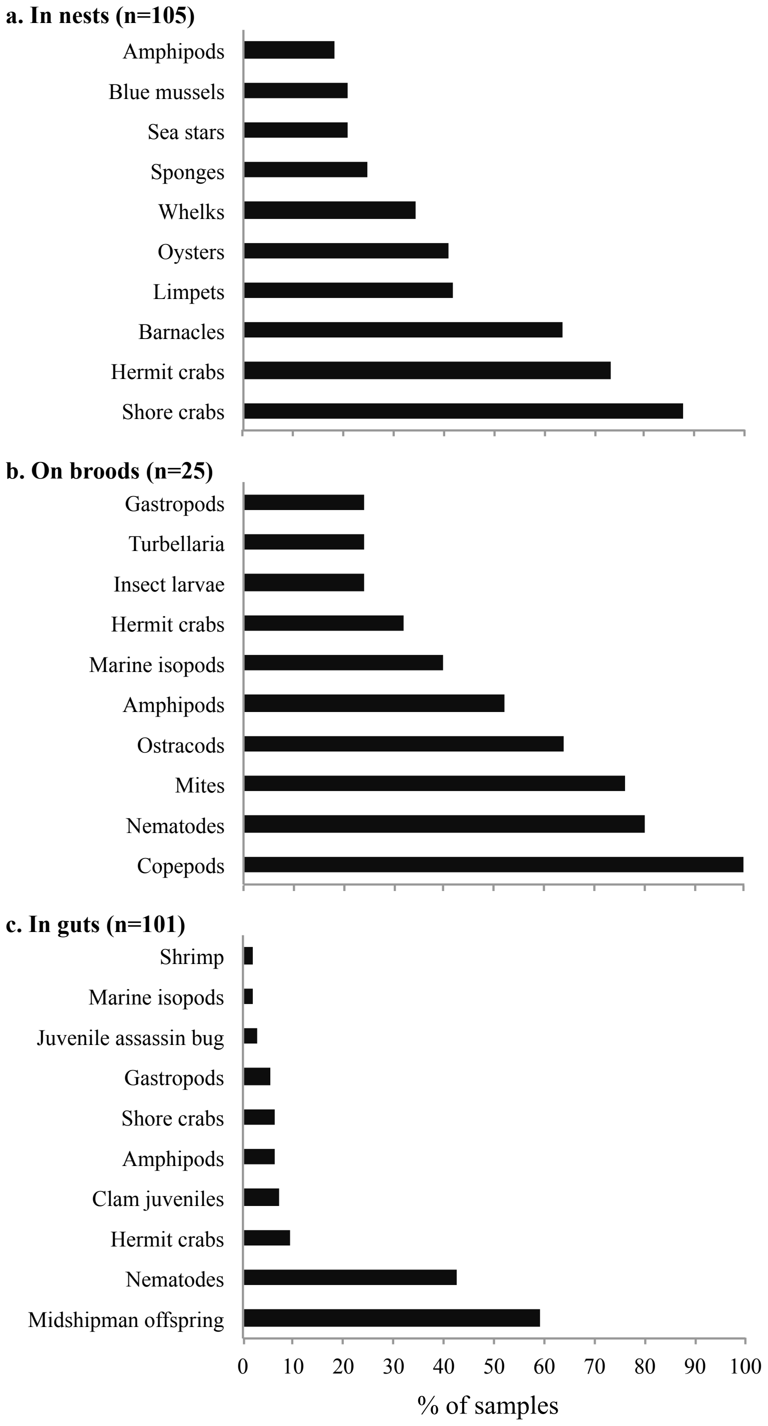

Fig. 5.1 Prevalence of the ten most commonly found organisms (a) in the nests, (b) on the broods, and (c) in the guts of reproductively mature plainfin midshipman (*Porichthys notatus*). Gut samples include those from guarder males, sneaker males, and females.

plant matter and so were classified as non-food. Only food items were considered in the subsequent analyses. Biodiversity in the gut was low with an average of two different organisms found (range 0-5); abundance was also low with an average of nine items per gut (range from 0-67). The most prevalent organism found in the guts across all the fish sampled was plainfin midshipman offspring either in the form of eggs, egg casings or hatched embryos, which were found in 58% of all fish examined. The distribution of the ten most prevalent organisms in the guts is plotted in **Fig. 5.1c**. Nematodes also appeared frequently (43% of all fish examined) and were prevalent in both the guts and on the broods (**Fig. 5.2**). Eight organisms were found on both the broods and in the guts of guarder males (**Fig. 5.2**) and there was a strong positive correlation in the prevalence of these organisms when we compared the broods and the guts (R^2=0.71, n=8, p=0.009).

5.4.2 Food availability across the breeding season

There were significantly more organisms on the broods later in the breeding season (655 ± 115) than early in the breeding season (218 ± 141; t=test: t_{23}=3.1, p=0.005). When nests were further divided into those containing only eggs (more common in the early part of the breeding season) versus those with mainly hatched embryos, the broods with more developed young had significantly more organisms on them than broods with eggs only (ANOVA: $F_{2,22}$=8.41, p=0.002). When we examined only the nests from later in the breeding season, those with eggs-only had significantly fewer organisms (452 ± 163; n=9),

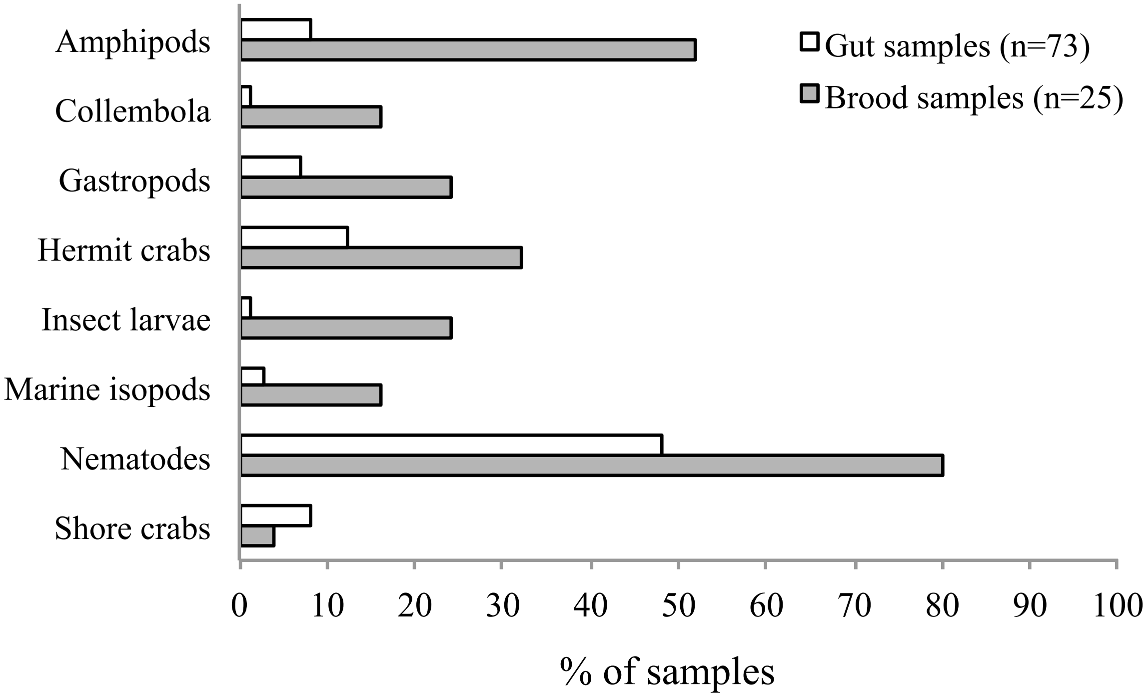

Fig. 5.2 Comparison of the prevalence of organisms that were identified both on the broods and in the guts of guarder male plainfin midshipman (*Porichthys notatus*).

while the nests with mainly hatched young had a mean ± SE of 959 ± 199 organisms (n=6; t_{13}=2.41, p=0.03).

5.4.3 Guarder male diet and condition across the breeding season

We had predicted that guarder males, as a result of their parental care, would eventually begin to consume food later in the breeding season. However, we saw no significant change in food abundance in their guts over time (increase in log food abundance per unit time: 0.002 ± 0.008 SE, χ^2=0.03, p=0.87). Guarder male gut mass also did not significantly change across the season (R^2=0.004, n=72, p=0.58).

There was no effect of Julian date on the body condition of guarder males (R^2=0.010, n=73, p=0.40) or on their hepatosomatic indices (HSI; R^2=0.016, n=73, p=0.29). We also found no significant relationship between body condition and food abundance (increase in log food abundance per unit of body condition: 3.5 ± 2.8 SE, χ^2=1.25, p=0.20) nor was there a relationship between body condition and gut mass (R^2=0.002, n=72, p=0.68). However, we found a significant positive relationship between HSI and food abundance (+0.54 log abundance/HSI ± 0.13 SE, χ^2=4.12, $p<0.0001$), but no significant relationship between HSI and gut mass (R^2=0.013, n=72, p=0.33).

5.4.4 Sex and tactic differences in diet and condition

We found no difference between females and sneaker males in terms of food abundance per gut (post hoc Wilcoxon: Z=0.05, p=0.96). Females had 3.6 ± 3.0 items on average in their guts while sneaker males had 1.0 ± 4.1 items on average. Guarder males had 11.6 ± 1.5 items on average, a significantly greater number of items than either females (Kruskal-Wallis: $\chi^2=31.52$, $p<0.0001$; post hoc Wilcoxon test: $Z=4.53$, $p<0.0001$) or sneaker males (post hoc Wilcoxon test: $Z=-3.94$, $p<0.0001$; **Fig. 5.3**). Guarder male gut mass tended to be two times greater than sneaker male gut mass (ANOVA: $F_{2,97}=2.71$, $p=0.07$; Tukey's HSD: $p=0.06$). All other comparisons were not significant (Tukey's HSD: all $p>0.35$).

5.4.5 Parasites

We identified five different intestinal parasitic worms in the guts of the 101 midshipman analysed in detail; 67% of the guarder males sampled had at least one of these parasites. The three most common were Acanthocephalan (spiny headed worms), Cestoda (tapeworms) and Trematoda (tissue flukes; **Table 5.1**). These parasites were often found in large numbers, ranging from 1 to 2991 individual parasites in a given guarder male. Only Acanthocephalan and Cestoda were found in females and sneakers males, with a prevalence of 50% and 40%, respectively. Guarder males sampled from California had significantly more parasites (600 ± 200) than the guarder males sampled from British Columbia (6 ± 4) and Washington (5 ± 1, Kruskal-Wallis: $\chi^2=15.57$, $p=0.0004$). Guarder males that cannibalised eggs (see below) were not more likely to have parasites in their guts (Chi-squared test: $\chi^2=2.4$, $p=0.12$). The total number of parasites sampled from

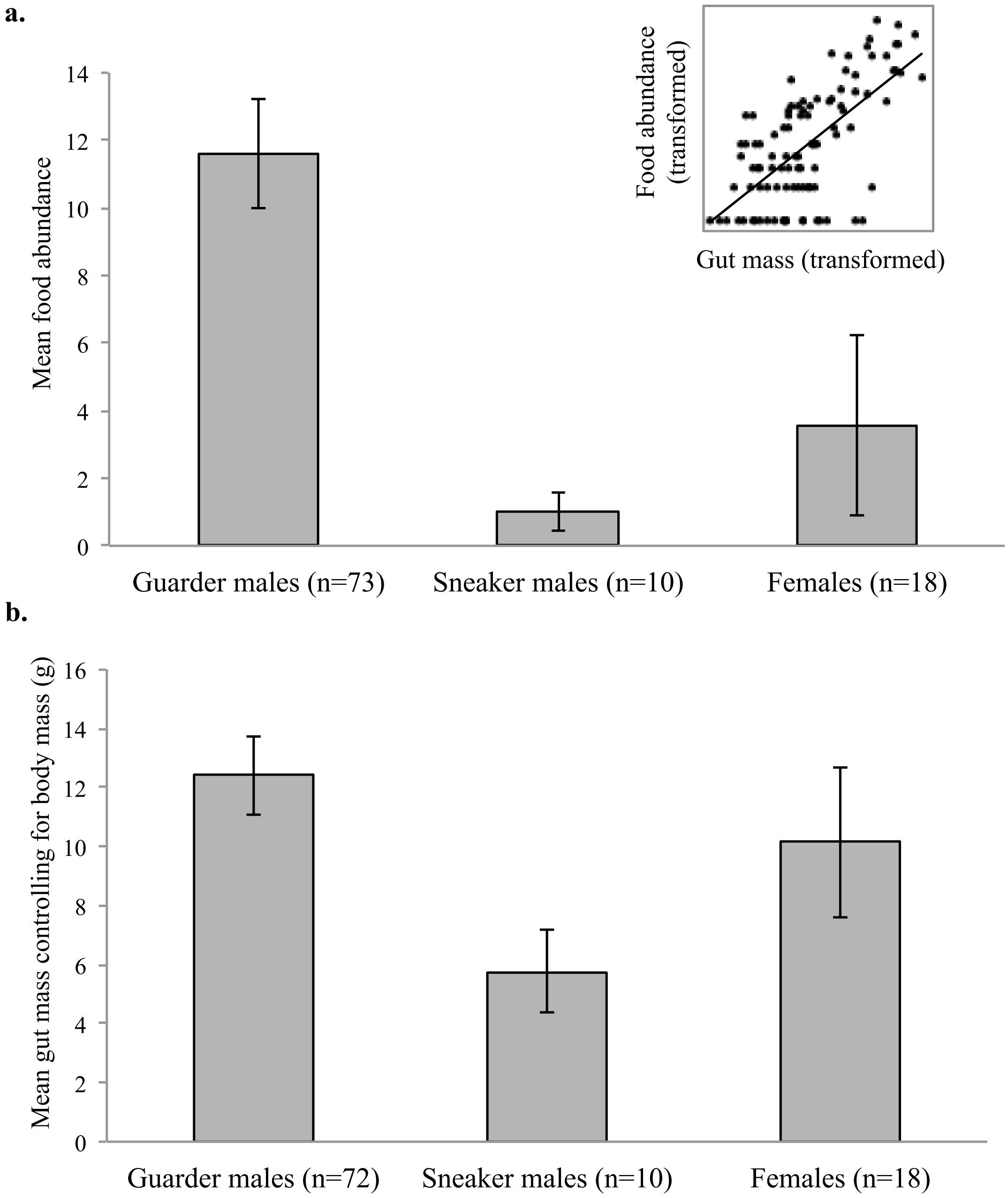

Fig. 5.3 Comparison among sex and male reproductive types of the (a) mean of total food abundance in the guts and (b) mean gut content mass controlling for eviscerated body mass (g X 1000) in the plainfin midshipman (*Porichthys notatus*). Inset shows the relationship between food abundance and gut mass controlling for body mass. For the inset, we used a log(1 + x) transformation for the zero-inflated total food abundance and we used a log transformation for gut content mass.

Table 5.1 The various classes of parasites identified in the guts of plainfin midshipman (*Porichthys notatus*). All of these parasites were identified by Dr. Brian Leander at the University of British Columbia.

Parasite Class	Prevalence[a]	Abundance[b]	Life cycle[c]	Impacts[d]
Acanthocephala (Spiny-headed worms)	46%	4.9 (range 1-32)	Complex IH: decapods or other crustaceans	• Weight loss • Seasonal mortality • Intestinal perforation
Cestoda (Tapeworms)	25%	510 (range 1-2991)	Complex IH: often snails	• Weight loss • Intestinal irritation • Low reproductive output
Trematoda (Tissue flukes)	11%	1	Complex IH: often molluscs	• Inflammatory response • Lowered hemoglobin and hematocrit level
Unknown 1	1%	1	-	-
Unknown 2	1%	1	-	-

[a] Percent of samples in which the parasite was found, out of 101 possible fish.

[b] Mean number of parasites found per sample

[c] Complex life cycle includes two hosts. Most common intermediate hosts (IH) are listed.

[d] Arai 1969; Möller and Anders 1986; Barber et al. 2000

guarder males decreased as the season progressed (increase in log parasite abundance per unit time: -0.041 ± 0.014 SE, χ^2=6.5, p=0.01). There was no significant interaction between sampling site and Julian date on the number of parasites found in the guts of guarder males (likelihood ratio test χ^2_3=7.4, p=0.06). Because all the parasites identified have complex life cycles with an intermediate host that is organism typically found in midshipman diets (e.g. snails or mollusks), it is likely that the parasites were acquired when the fish ingested an infected intermediate host (Barber et al. 2000).

5.4.6 Cannibalism

Of the 151 plainfin midshipman whose guts were examined for signs of cannibalism, 58% had consumed midshipman eggs or hatched embryos. The majority (96.6%) of the cannibals were guarder males, none were sneaker males and the remaining 3.4% of the cannibals were females. Cannibalistic guarder males consumed on average 7.7 ±1.3 offspring (range 1-106 offspring, median of 2). The three female cannibals consumed 1, 2 and 31 offspring.

Larger guarder males tended to eat more embryos than did smaller guarder males regardless of body condition (increase in log embryo number per unit of body length: +0.09 ± 0.05 SE, χ^2=1.68, p=0.09; increase in log embryo number per unit of body condition: +3.44 ± 3.49 SE, χ^2=0.98, p=0.33). For guarder males, time in the breeding season did not affect the number of offspring eaten (log embryo number per unit of time: +0.002 ± 0.01 SE χ^2=0.21, p=0.84), nor did the number of young cannibalised vary across stages of offspring developmental (Kruskal-Wallis: χ^2=1.8, p=0.40).

Among the 11 males from which we genotyped the consumed cannibalised embryos collected from their guts, eight males (72%) were unrelated to the cannibalised embryos. The remaining three males had paternity values of 9%, 26%, and 100%. Because the number of embryos consumed and successfully genotyped varied among males (**Table 5.2**), these results need to be treated with caution. For example, the male with 100% paternity had only one embryo in his gut. The overall mean paternity, between cannibals and the embryos from their guts was 12% (range = 0-100%, median = 0%; **Table 5.2**).

5.5 DISCUSSION

We found that there were food resources available for guarder males in their nests and on their broods. However, most of the guts examined were relatively empty (apart from embryos) and the most prevalent organism found in guarder males was plainfin midshipman embryos rather than any other available surrounding food item. Although we had initially predicted that guarder males, as a result of their caring duties and their long confinement to the nest area, would have fewer opportunities to feed and therefore would have less food in their guts, our results showed the opposite pattern. In general, guarder males ate more than females and sneaker males. This observed difference was mainly driven by cannibalism by

Table 5.2 Cannibalism paternity data from a set of 11 guarder plainfin midshipman males (*Porichthys notatus*).

Male ID	# of offspring consumed	# of offspring genotyped	Cannibalism paternity (%)[a]
1	1	1	0
2	1	1	0
3	1	1	0
4	1	1	0
5	1	1	100
6	10	9	0
7	18	7	0
8	21	1	0
9	22	20	9
10	38	5	26
11	53	17	0

[a] The proportion of offspring that was compatible with the cannibal father calculated according to Neff et al. (2000a, b).

guarder males. For guarder males, food abundance in the guts was not related to body condition, and both food abundance and gut content mass were not related to time in the breeding season. Our results suggest that all fish collected from nests during the breeding season regardless of sex and tactic were not actively feeding, but that guarder males with access to embryos ate them under some circumstances.

The diet of the plainfin midshipman has been briefly mentioned in a number of previous studies, reporting that midshipman eat crustaceans and small fishes (Eigenmann 1892; Hubbs 1920; Arora 1948; Hart 1973). Cannibalism has also been documented anecdotally (Mensinger and Case 1991; Sisneros et al. 2009). Here, we confirmed that reproductively mature plainfin midshipman do eat a number of different crustaceans and midshipman embryos, in addition to nematodes and mollusks. Though infrequent, we also found instances of terrestrial insects in the diet of plainfin midshipman. We found no evidence of other fish species in their guts, not even in the form of bony structures or scales. Midshipman embryos were the most prevalent and abundant food item found. However, many factors could influence abundance and prevalence of particular organisms found in gut samples, including differences in digestion and evacuation times, and the size of the meal (Bromley 1994). For example, 85% of eggs that were force fed to Pacific salmon (*Oncorhyncus tshawytscha*) were cleared from the digestive tract within 48 hrs (Garner et al. 2009); a similar digestion time of 48 hrs for embryos has been observed in plainfin midshipman (A. Bose, personal communication). Although we acknowledge that there are likely differences in digestion and evacuation times among prey items, because the majority of the diet of plainfin midshipman consists of hard structures (including egg casings) we are unlikely to be grossly under- or overestimating the prevalence and abundance of diet items in this species.

Three previous studies noted that the stomachs of plainfin midshipman fish located in intertidal zones were largely empty (Hubbs 1920; Arora 1948; Sisneros et al. 2009). Although our study mostly agrees with these previous findings, our results also indicate that guarder males are in fact eating a small number of organisms over the breeding season and these organisms are often found in the nest or on the brood. Similarly, a study of smallmouth bass (*Micropterus dolomieu*) found that nesting males had food in their stomachs and the authors argued that these sources of food are important to supplement the endogenous energy reserves during care (Mackereth et al. 1999). In contrast to the results of our study, Magnhagen (1986) found that nesting male common goby (*Pomatoschistus microps*) ate less than females, a result attributed to limited mobility of male gobies during the parental care period. In our study, contrary to our third prediction, sneaker males and females had even lower food intake than the nest guarding males, which may suggest that even minimal feeding by the guarding male during the care period is important in this species.

Parental care is a costly behaviour and can result in significant physical deterioration and a loss of energy reserves (e.g. Magnhagen 1986; Marconatto and Bisazza 1988; Lindström and Hellström 1993; Okuda and Yanagisawa 1996). As a result, we had initially predicted that guarder males would lose body condition over the reproductive season, and that food intake would increase accordingly. Instead, we found no evidence that condition or food intake changed across the season. By eating a small number of embryos and other prey items throughout the breeding season, guarder males may be able to retain condition during the parental care period. Cannibalism may be an adaptation to maintain the necessary energy required for performing care and in part, may explain the observed high prevalence of partial cannibalism (consuming only part of the brood; Rowher 1978; Sargent 1992; Sargent et al. 1995). Neff (2003) found that male bluegill sunfish (*Lepomis macrochirus*) in lower body condition were more likely to partially cannibalise their brood, suggesting that this behaviour is important for replenishing energy reserves. However, in contrast to Neff's (2003) results in bluegill, the energy replenishing hypothesis was not supported in our study as we found no significant relationship between body condition and the number of embryos cannibalised for guarder males (Rowher 1978; Sargent 1992; Sargent et al. 1995). Conversely, other studies have instead found a positive relationship between condition and number of cannibalised offspring in other species including the long-snout clingfish (*Diademichthys lineatus*; Gomagano and Kohda 2008) and three-spined sticklebacks (*Gasterosteus aculeatus*; Mehlis et al. 2009).

Why then did so many plainfin midshipman guarder males consume embryos? The reasons for partial cannibalism vary both within and among species (Smith and Reay 1991; Klug and Bonsall 2007). First, parents will often cannibalise in situations where nest size is limited, where offspring are of low quality and quantity, or in situations of low oxygen concentration, facilitating parental investment in remaining offspring (Rohwer 1978; Sargent 1992). For example, males of the beaugregory damselfish (*Stegastes leucostictus*) consume more embryos in areas of low oxygen, which the authors interpret as an adaptive strategy to ensure the success of remaining embryos (Payne et al. 2002). In plainfin midshipman, nest space is limited and guarder males must withstand cyclical environmental perturbations when they remain in the nests at low tide resulting in periods of hypoxia (Sisneros et al. 2009; Craig et al. 2013). These conditions could select for partial cannibalism, and would be adaptive if the remaining uneaten offspring have higher survival as a result of the parental behaviour. Second, parental care theory predicts that cannibalism should increase as the mean paternity to the group of offspring decreases (Trivers 1972; Whittingham et al. 1992; Westneat and Sherman 1993). Neff (2003) found that parental male bluegill that had more sneaker males present during spawning were more likely to consume part of their brood. Additionally, parental male bluegill may be selectively culling unrelated offspring after exposure to olfactory cues from the hatched embryos (Neff 2003). In plainfin midshipman, paternity is commonly lost to both sneaker and cuckolding guarder males, and a high degree of nest take-over has been documented for midshipman especially in the early part of the breeding season (Cogliati et al. 2013, submitted). Thus, partial cannibalism may be a response by guarder males to reduced paternity. Indeed, we found that guarder males were largely unrelated to the embryos they consumed. Although this result could provide preliminary evidence for selective cannibalism, guarder males were also largely unrelated to the embryos in their nest (unpublished data), suggesting instead that partial cannibalism in plainfin midshipman may be in response to taking over a nest.

This is the first comprehensive diet analysis study in a toadfish that determined both food availability and food consumed in reproductively mature individuals. Often, studies that examine diet fail to make comparisons between the sexes or between males adopting different behavioural tactics or to look at diet in relation to food availability. These were important facets of our study and accordingly, we found significant differences in diet between male alternative tactics and sexes. The diet choices were studied in the context of what food was available for guarder males and what items they could possibly acquire based on their presumably limited mobility outside of the nest. Guarder males had many available food items in their nests, but these items were largely untouched. Instead, they fed primarily on plainfin midshipman embryos. These cannibal males were also mainly unrelated to the offspring consumed, and are likely to have cannibalised as a result of a nest takeover. Altogether, by comparing the diet of both sexes and tactics in a species with a lengthy and costly parental care period, our results shed light on some of the adaptations and strategies designed to cope with the costs of providing parental care.

5.6 ACKNOWLEDGEMENTS

We thank Andy Bass, Midge Marchaterre, and Joe Sisneros who assisted with sample permits and site locations in California and Washington. We also thank Celia Chui, Julie Marentette, and Jennifer Reynolds for their help with data collection in British Columbia. Mr. and Mrs. Cogswell, Mr. and Mrs. Weatherell, Mr. and Mrs. Balshine, and especially Mr. and Mrs. Flader for providing access to midshipman sampling sites and lodgings for field crew. Susan Marsh-Rollo was invaluable for her microscope and analyses training provided for CD and ACP. Susan Marsh-Rollo, David Rollo, and Brian Leander helped with identification of organisms. Constance O'Connor and Cody Dey helped with statistical analyses. This work was funded by the Natural Sciences and Engineering Research Council of Canada (grants to BDN and SB).

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Chapter 6

GENERAL DISCUSSION

6.1 THESIS AIMS REVISITED

The overall aims of my thesis were twofold: 1) to use molecular methods to explore the reproductive ecology of a fish species with ARTs, and 2) to do so over time and space. Collectively, I sought to investigate how ecological factors influence mating patterns and the frequency of ARTs.

6.2 THESIS SUMMARY

While ARTs have been previously documented in plainfin midshipman (Brantley and Bass 1994), in **Chapter 2** I provided the first estimate of paternity for guarding males, and in **Chapter 4**, I provided the first conclusive evidence that type II sneaker males were successful at obtaining some genetic reproductive success. Overall, I showed that type I guarding males had relatively low estimates of paternity (52%) when compared to other species with ARTs where paternity has been quantified (reviewed in Avise et al. 2002; Coleman and Jones 2011). However, I revealed that most of this reduced paternity was attributed to nest takeovers by other type I guarding males and to cuckoldry by reproductively plastic type I males (**Chapters 2** and **4**). Only a small proportion of the paternity loss was attributed to type II sneaker males (**Chapter 4**). Importantly, I found that paternity for the guarding males increased as the breeding season progressed (**Chapter 2**). This pattern was a result of a higher frequency of nest takeovers and more male competition for nests earlier in the breeding season resulting in lower paternity, rather than higher rates of cuckoldry observed during this time period. In **Chapter 3**, I explored how variation in nest availability and nest density influenced mating patterns and the strength of sexual selection. In this chapter, I first confirmed that there were indeed two distinct genetic populations of plainfin midshipman. Second, I showed that these populations differed in both nest availability and density. However, despite finding significant environmental differences and significant differences in fish size between the two populations, I found no significant differences in paternity, cuckoldry, nest takeovers, or the strength of sexual selection. These results suggest that the degree of mating competition did not map on to differences in ecological conditions, as classic mating systems theory would predict (sensu Emlen and Oring 1977). From my results, I also inferred that variation in nest availability may not be a limiting factor influencing the intensity of sexual selection in this species.

In addition to estimating the genetic success of each tactic and ecological influences on mating patterns, in **Chapter 4** I applied a life history model to test whether or not the two tactics had equal fitness. Unfortunately, the results were inconclusive; the model supported scenarios in which the tactics had equal fitness and in which type I males had either significantly higher or lower fitness than type II males. However, in this chapter I argued, based on a number of factors, that it is likely that type I guarding males have greater fitness than type II sneaker males. Finally, I further explored differences in diet between the tactics and the sexes in **Chapter 5**, to elucidate some potential costs of providing parental care. I explored the diet of plainfin midshipman in relation to timing in the breeding season and to food availability in the nest. Guarding males ate more food than both sneaker males and females and, despite providing parental care for long periods and having limited foraging opportunities, their diet and body condition did not significantly change across the breeding season. In this chapter, I also provided the first quantitative assessment of cannibalism in this species, and showed that guarding males were mostly unrelated to the offspring cannibalised.

In the next sections, I address the two aims of my thesis by placing my results in the context of previous research conducted in the field and suggest avenues of future research that will increase our understanding of these topics.

6.3 CONTRIBUTIONS TO THE LITERATURE

6.3.1 The reproductive ecology of the plainfin midshipman

Alternative reproductive tactics are selected as a result of intense competition among males. Thus, when ARTs occur, it is thought to dampen the strength of overt competition among males (Gross 1996; Taborsky et al. 2008). For example, in a population with only one tactic, all males will compete in the same fashion to maximize reproductive success, leading to large variance in competitive traits and reproductive success among these males (Bateman 1948; Trivers 1972; Andersson

1994). As the intensity of sexual selection increases, some males may fair better by adopting an ART, thereby reducing the overall intensity of overt competition experienced by the remaining males employing the conventional tactic (Taborsky et al. 2008). Thus, in species with male ARTs, there can be competition both *within* a male tactic and *between* tactics. Overt competition occurs within a male tactic (among males employing the conventional guarding tactic) over access to nest sites and to spawning females, while indirect competition occurs between male tactics for fertilization success in the form of sperm competition (Taborsky et al. 2008; Fitzpatrick et al. submitted). Though ARTs have been identified in many species, there are relatively few studies that have applied molecular techniques to quantify the genetic reproductive success of tactics in fishes. For example, paternity has been estimated in ~20% of the 170 fish species with described ARTs (Avise et al. 2002; Taborsky 2008; Coleman and Jones 2011). Fortunately, the use of molecular techniques continues to be applied to species with ARTs and, as a result of my research, this body of work now includes the plainfin midshipman. As more studies are conducted, it is evident that there is a high variability in tactic fertilization success across species. For example, DeWoody et al. (2000) found that nest guarding male paternity in spotted sunfish (*Lepomis punctatus*) was 99%. Prior to my studies, Alonzo and Heckman (2010) documented the lowest paternity estimates at 72% in a species with obligate male care, the ocellated wrasse (*Symphodus ocellatus*). The results of **Chapter 2** showed that plainfin midshipman guarding males had even lower paternity estimates (52%); this is lower than any other previously investigated species. What is now needed is a better understanding of what factors contribute to this observed variability in paternity in species with ARTs. This will certainly be a prolific area of future research and is likely to foster a greater understanding of factors influencing the occurrence and success of ARTs.

Why is paternity for guarding plainfin midshipman males so low? In **Chapter 2** I argued that the low paternity observed in this species is most likely a result of 1) increased competition due to limited nest sites and 2) increased cuckoldry as a means to avoid high costs of parental care (due to a protracted breeding season and missed foraging opportunities). For plainfin midshipman, the nest is an important resource, as guarding males must acquire a nest site under a suitable rock in order to attract a female (Arora 1948). DeMartini (1988) demonstrated over a two-year study that nest sites were a limiting resource in this species. He based this conclusion on the proportion of occupied nests (greater than 90% of the natural nests were taken up) and on the occupancy rate of artificial nests (tiles) that were provided (99% of the tiles occupied over the two year study). This nest site limitation may be partially responsible for the high occurrence of nest takeovers and as a result, help explain part of the low paternity estimates of guarding males. Bessert et al. (2007) demonstrated that nest takeovers were more common in a nest-limited population of fathead minnow (*Pimephales promelas*) when compared to a non nest-limited population. However, in **Chapter 3**, I described how nest availability across populations did not map on to the degree of male-male competition as assessed by nest takeover rates. I observed nest takeovers in 23% of nests in the north and 24% of nests in the south, even though the constraint of nest availability was relaxed in the south.

Nest takeovers are a form of overt competition among guarding males and are only one way in which males can compete for nest sites. Another form of competition among guarding males is via cuckoldry and, in **Chapter 4,** I corroborated the behavioural observations of cuckoldry by guarding males (Lee and Bass 2004) by providing genetic evidence that males known to be of the guarder type were successful at fertilizing offspring in the nests of other males. To my knowledge, a quantification of the reproductive plasticity within a tactic in a species with distinct male types has only been previously reported in one other species, the bluegill sunfish (*Lepomis macrochirus*), where paternity lost to other nest-guarding males was only 1.8% (Neff 2001). Thus, the work of my thesis contributes to our understanding because paternity loss in plainfin midshipman is attributed to both competition *within* the guarding male tactic and to competition *between* male tactics.

In addition to intense competition among males, another factor that could contribute to the low observed paternity in plainfin midshipman is the costs associated with parental care. The high costs of parental care may lead to guarding males who are unable to effectively defend their nests against cuckoldry for the entire duration of breeding season as a result of reduced body condition (e.g. Komdeur 2001; Neff and Clare 2008; Järvi-Laturi et al. 2011). However, I found no support for a relationship between paternity and male body condition, and paternity was lowest early in the breeding season when males should have been in their best condition. The high costs of providing parental care may also select for increased cuckoldry as a means to avoid such costs. Theory predicts that missed foraging opportunities is a cost of providing parental care, and I investigated this potential cost in **Chapter 5**. Plainfin midshipman have a protracted breeding season (up to 4 months) with overlapping offspring development, resulting in guarding males being unable to leave the nest to forage for the duration of parental care. When I investigated the digestive tracts of guarding males (**Chapter 5**), I observed that the stomachs of guarding males were often empty or nearly so, suggesting a feeding cost associated with nest guarding. Magnhagen (1986) found that nesting male common goby (*Pomatoschistus microps*) ate less than females, a result attributed to limited mobility of male gobies during the parental care period. However, it is important to consider that reproductively mature females and sneaker males also had very little in their stomachs. Unlike guarding male plainfin midshipman, females and sneaker males were not confined to the nests for long periods and could still have foraged during high tide when they were not actively spawning. Thus, it is likely that missed foraging opportunities is a cost of parental care because guarding males are confined to their nests for longer durations than both females and sneaker males, but I was unable to provide direct evidence for this as a cost of care in plainfin midshipman.

Missed foraging opportunities may still explain why paternity in plainfin midshipman was lower on average when compared to other species with ARTs and male nest defence (e.g. Neff and Clare 2008; Alonzo and Heckman 2010). Because embryos take approximately 60 days to leave the nest and guarding males continue to acquire new eggs throughout the breeding season, parental care can last several months. In contrast, numerous other species have either a much shorter parental care period (weeks), or spawning bouts that provide males with an opportunity to forage (e.g. DeWoody et al. 2000; Jones et al. 2001a; Neff and Clare 2008; Alonzo and Heckman 2010). This suggests that missed foraging opportunities is a likely cost associated with care in plainfin midshipman and, as discussed in **Chapter 2**, may contribute to the comparatively low paternity in this species.

Diet analyses provide a transient picture of individual condition and only capture a short window of time during the breeding season. As a consequence, future studies should experimentally test whether missed foraging opportunities leads to a reduction in paternity. By setting up large outdoor tanks stocked with an exact number of similarly sized guarding males, sneaker males, and females, food availability could be varied during semi-natural spawning events. These tanks would be run in replicate, where some tanks receive no food and other tanks receive ample food. Then, paternity across treatment groups can be compared to determine if missed foraging opportunities, a likely cost of providing parental care, has a negative impact on paternity.

Why do plainfin midshipman males continue to care despite low paternity? The highly competitive environment experienced by guarding males may have selected for a higher tolerance of reduced paternity (Griffin et al. 2013). That is, with intense competition among guarding males, having some paternity in a nest may still be better than the options available to a male without a nest. Males without a nest are relegated to attempting to take over a nest or to cuckold another guarding male. An alternative hypothesis is that males continue to care because they do not know they have been cuckolded and are unable to recognize or discriminate related from unrelated embryos in the nest (Sherman et al. 1997; Neff and Sherman 2002). To shed light on why plainfin midshipman males have relatively low paternity and yet continue to care for unrelated offspring, additional studies are now needed that investigate whether guarding males can discriminate kin versus non-kin.

Another facet of the reproductive ecology of plainfin midshipman that I investigated was the frequency of cannibalism. In **Chapter 5**, I showed that when guarding males did consume food, the most prevalent food item was plainfin midshipman embryos. Indeed, I found evidence of cannibalism in the majority (69%) of guarding males analysed, with some individuals eating upwards of 100 embryos. There are several hypotheses designed to explain the evolution of cannibalism (e.g. see Manica 2002; Klug and Bonsall 2007). The hypothesis most widely accepted for the occurrence of cannibalism is that it is an adaptation to maintain a parent's necessary required energy for performing care (Rowher 1978; Sargent 1992; Kvarnemo et al. 1998; Manica 2004). Thus, guarding males may consume their own embryos to recoup energy lost during care, which may benefit either the current or future offspring. If males regularly cannibalise some of their embryos to maintain their energy and body condition, this may explain why I did not see a significant change in body condition across the breeding season (**Chapters 2** and **5**). However, not all studies have supported the prediction that cannibalism increases as food availability or parental body condition decreases; some studies found no relationship (e.g. Lindström and Sargent 1997; Payne et al. 2002) while other studies have found the opposite pattern that cannibalism increases with body condition (Klug and St. Mary 2005; Klug et al. 2006).

To help explain some of the variability in results across cannibalism studies, Klug and Bonsall (2007) developed an evolutionary model that investigated the occurrence of filial cannibalism (consumption of own offspring). Their model showed that multiple non-mutually exclusive hypotheses are possible, and thus multiple factors should be investigated when trying to understand the adaptive significance of cannibalism. For example, cannibalism may also occur as a response by guarding males to reduced paternity in the nest (Trivers 1972; Whittingham et al. 1992; Westneat and Sherman 1993). That is, guarding males may consume more offspring when paternity is low (e.g. Neff 2003). Although preliminary, my results in **Chapter 5** showed that guarding males do consume some related offspring, but were mostly unrelated to the consumed embryos. This is in contrast to the tessellated darter (*Etheostoma olmstedi*), where guarding males had both high paternity in the nest and were related to the embryos consumed (DeWoody et al. 2001). My results on the genetic relatedness of cannibalised embryos may have been due to nest takeovers rather than kin discrimination, but future experiments are needed to determine whether males can selectively cannibalise non-kin embryos. Additionally, cannibalism may be a response to the physical environment, such as low oxygen, as a means to reduce offspring demands and maximize the success of the current brood (e.g. Payne et al. 2002). A valuable next step for future research would be to investigate the relationship between energetic demands, paternity, environmental conditions, and cannibalism. I also documented three instances of cannibalism by females. This could be a behaviour that is selected as a result of nest space limitation, where a female prefers to spawn with a male whose nest is already full of embryos. Such females may choose to cannibalise to free up space for their embryos rather than find a new male. Because nest space itself appears to be limiting in this species (DeMartini 1991), males may also exert some choice in which females lay eggs in his nest, suggesting mutual mate choice in this species (e.g. Kraak and Bakker 1998; Baldauf et al. 2012). Therefore, in addition to the previous hypotheses used to explain cannibalism, I hypothesize that guarding males may also cannibalise to free up nest space for higher quality females.

6.3.2 Variation in mating patterns across time and space

Temporal variation has long been recognized as an important factor influencing mating system dynamics (e.g. Verhulst et al. 1995; Oliveira et al. 1999). As such, it is important to consider temporal variation when investigating mating patterns. In **Chapter 2**, I first investigated how genetic reproductive success for the guarding male tactic varied over the course of the lengthy breeding season. Temporal variation in genetic success has been previously shown in a mouthbrooding cichlid fish (*Ctenochromis horei*; Sefc et al. 2009), bluegill sunfish and pumpkinseed sunfish (*Lepomis gibbosus*; Neff and Clare 2008). For example, Sefc et al. (2009) found significantly more broods with multiple paternity in the mouthbrooding cichlid during the rainy season compared to the dry season, which was attributed to marked differences in the visual environment. In the sunfish species, Neff and Clare (2008) showed that paternity in the middle portion of the breeding season was significantly lowest in bluegill, while it was highest in pumpkinseed during this portion of the breeding season. These results, at least in bluegill, suggest that paternity was related to increased breeding opportunities, decreased body condition, or increased cuckoldry during the middle portion of the breeding season (Cargnelli and Neff 2006; Neff and Clare 2008). In plainfin midshipman, I showed that paternity for the guarding males increased across the breeding season. One hypothesis used to explain variation in paternity over time is that as the condition of the male changes over time, he may be unable to effectively defend his nest against cuckolders resulting in a positive correlation between body condition and paternity. This positive relationship between male body condition and paternity was shown in bluegill but not in pumpkinseed (Neff and Clare 2008). In two different studies (**Chapters 2** and **5)**, I showed that male body condition did *not* change across the breeding season in plainfin midshipman. Thus, a reduction in male body condition did not seem to explain the observed change in paternity over time. Instead, the results of **Chapter 2** indicated that the change in paternity was largely driven by a higher occurrence of nest takeover during the early portion of the breeding season.

My results suggest that competition, and therefore the intensity of sexual selection, may be higher among males early in the breeding season. Reichard et al. (2008), for example, found that the opportunity for sexual selection was lowest (i.e. less variance in reproductive success) at the start of the breeding season of the European bitterling (*Rhodeus amarus*), which was attributed to higher temporal clustering by spawning females during this time. Thus, a valuable next step would be to explore this change in the competitive environment and calculate the intensity of sexual selection over time for plainfin midshipman. I suggest that this could be done by combining the methods that I used in **Chapters 2** and **3**; at several time points over the course of the breeding season, quadrant sampling could be used to evaluate the intensity of the competitive environment and collect data needed to calculate sexual selection intensity over the season.

Just as temporal variation can influence the mating patterns of a given species, so too can spatial variation. Often, populations will differ in some ecological factors and several studies have investigated how such ecological factors influence the observed mating patterns (e.g. Mobley and Jones 1999; Jones et al. 2001b; Bessert et al. 2007; Neff et al. 2008). In **Chapter 3**, I sought to investigate whether there were population differences in mating patterns and the intensity of sexual selection as a result of measured differences in nesting resources. The distribution of plainfin midshipman shows a break along the Oregon coast (Warner and Case 1980; Thompson and Tsuji 1989; Harper and Case 1999), and I confirmed that there are in fact two distinct genetic populations found to the north and south of this break point. I compared nest availability between these populations, as this ecological factor has important implications for the intensity of sexual selection. I found significant population differences with fewer nests available in the north. Despite this, I did not observe any differences in mating patterns, including the frequency of male ARTs, and the intensity of sexual selection between populations. I argued that nest availability may not actually be limiting in plainfin midshipman or that other aspects of the environment have a more important role in shaping mating patterns. Indeed, I observed that less than 40% of potential nests were being used across both populations, and this is markedly lower than the 90%-99% of occupied nests observed by DeMartini (1988). Other studies have also documented significant ecological differences that resulted in similar mating patterns across populations, including the sand goby (*Pomatoschuistus minutus*), where populations differed in nest availability (Jones et al. 2001b) and the guppy (*Poecilia reticulata*), where populations differed in predation intensity (Neff et al. 2008). This suggests, at least for plainfin midshipman and a few other species, that studying one population can capture many aspects of the mating dynamics and is sufficient to describe the overall mating patterns of the species. Thus, some of the studies that have looked at only a single exemplar population to describe the mating patterns of the entire species may in fact be an accurate representation. However, without cross-population studies it is unclear how populations may vary (if at all), and our understanding of how ecological factors influence mating patterns will be limited.

6.4 GENERAL CONCLUSIONS

My thesis has improved our understanding of the reproductive ecology of plainfin midshipman, a classic model system of a species with ARTs (e.g. Shuster and Wade 2003; Bass and Forlano 2008; Dugatkin 2013). Through my research, I have been able to show that sneaking is indeed a viable alternative reproductive tactic in this species, as sneaker males did acquire some genetic reproductive success. However, the majority of paternity loss experienced by guarding males was a result of *within* tactic competition from plastic cuckoldry behaviours performed by other type I males. I have provided the first genetic evidence for a number of behavioural adaptations within the type I male tactic, including guarding, nest takeovers, and cuckoldry. My thesis has important implications for our understanding of the relationship between mating patterns and the environment. I showed that variation in the competitive environment across the breeding season had significant consequences on the paternity for guarding males, such that higher rates of nest takeovers observed earlier in the breeding season were correlated with lower paternity. However, my cross-population analyses did not reveal significant differences in mating patterns despite significant environmental differences such as those observed in nest availability and density. I have shown that nest resource availability and competition for nests may not be sufficient to influence sexual selection patterns and instead, other environmental factors acting in isolation or in combination may play a more critical role in shaping mating patterns.

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