REPRODUCTIVE CONSEQUENCES OF NESTING SITE DECISIONS IN A MARINE TOADFISH (*PORICHTHYS NOTATUS*)

REPRODUCTIVE CONSEQUENCES OF NESTING SITE DECISIONS IN A MARINE TOADFISH (*PORICHTHYS NOTATUS*)

By

NICHOLAS A.W. BROWN, B.Sc.

A Thesis

Submitted to the School of Graduate Studies

In Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

MASTER OF SCIENCE (2019)

(Psychology, Neuroscience & Behaviour)

McMaster University

Hamilton, Ontario

TITLE: Reproductive consequences of nesting site decisions in a marine toadfish (*Porichthys notatus*)

AUTHOR: Nicholas A.W. Brown, B.Sc. (Memorial University of Newfoundland)

SUPERVISORS: Drs. Sigal Balshine and Francis Juanes

NUMBER OF PAGES: i-xiv, 133

ABSTRACT

Animals should favour breeding locations that maximize their lifetime reproductive output. Parents ought to rear young in sites that positively affect offspring but sometimes these same sites inflict costs on parents. How parents balance their own needs against those of their offspring when selecting a site for reproduction remains unclear, particularly in animals that provide extended parental care at one location. Further, few studies have linked variation in life history traits to reproductive site choices within a single species. In this thesis, I addressed both knowledge gaps by studying the plainfin midshipman (*Porichthys notatus*), a marine toadfish where fathers provide sole parental care to broods of young in intertidal and shallow subtidal nests for up to two months. In Chapter 2, I measured the costs incurred by caring males and the benefits conferred to offspring in nest sites along a tidal gradient. Males suffered similar rates of body condition deterioration in all nests across the intertidal gradient. Young developed more quickly in intertidal nests compared to subtidal nests, but broods in the highest intertidal nests suffered the highest mortality rates, despite receiving more parental care from the males at these nest sites. We found the most competitive males in lower intertidal nests, a trend that agrees with life history theory—in species with relatively slow offspring development, parents should accrue greater reproductive benefits from nest sites where offspring benefits are highest. In Chapter 3, I describe a laboratory experiment designed to examine how warm water and air exposure (two abiotic conditions that vary dramatically within the intertidal) affect development and survival of plainfin midshipman young, and how these effects vary with maternal traits. Exposure to warm water enhanced embryonic and larval development rates; this effect was attenuated by air exposure. Juveniles raised in warm water also exhibited superior swimming performance, while air-exposed young suffered higher mortality rates. Although larger juveniles emerged from larger eggs, development rates were similar across egg sizes. Offspring survival increased with maternal body condition in cold water but decreased with maternal body condition in warm water. Juvenile body sizes increased with maternal condition in cold water without emersion, and in warm water with emersion—the two ecologically relevant rearing environments. Thus, low condition mothers might accrue greater benefits by depositing their eggs in nests at higher tidal elevations—where development is more rapid—further supporting the idea that among-individual variation in the expression of life history traits might influence nest site preferences in these fish. In sum, my research (**Chapters 2 & 3**) elucidates the link between life history traits and the spatial component of animal reproductive strategies.

ACKNOWLEDGEMENTS

Thank you, Sigal and Francis, for your tireless encouragement, enthusiasm, and patience while guiding me through this process, from conception to realization. You collaborate in impeccable harmony. I'm incredibly grateful to have learned from your unique skill sets, collective expertise, and to have been welcomed into both the ABEL and Juanes lab communities. Ben, my quantitative skills and scientific thinking have been deeply enriched through our discussions and collaboration on both study design and analyses; thank you for contributing countless insights.

Noah, thank you for sticking out a long summer in British Columbia and sharing your experience gained during the previous field season. You put incredible thought and effort into the work we carried out together and contributed greatly to its success. Thanks also to Hailey Shafer, whose alloparental care during our absence ensured the health and survival of many young fish, and for taking leadership on the behavioural assays. Thank you, Dr. Aneesh Bose and Jess Miller, for your thoughtful mentorship, and for elucidating the secret ways of the plainfin midshipman fish. To my colleagues in the ABEL and Juanes labs—Hossein Medhi, Brett Culbert, Nicole Yee, Katrina Nikolich, Mackenzie Woods, Dr. William Halliday, Kieran Cox, and Garth Coverton—thank you for contributing wisdom, helpful suggestions, and for productive collaborations. I'm grateful to all members, past and present, of the ABEL lab, Juanes lab, and Dr. Julia Baum's lab for welcoming me into such happy ecosystems; it was a joy to work in your fine company. Several volunteers and research assistants poured immense time and effort into data collection for my research: Simi Dhanota, Maha Siddiqui, Avani Pathak, Samuel Matthew, Anna-Lisa Nguyen, Jennifer Curtis, Hailey Davies, Katie Innes, and Lucas Agagnier;

thank you all so much. In addition, Matt, Ken, Raymond, Lucy, Michelle, and all other animal technicians at the OAU, you have my sincerest gratitude for ensuring the best possible animal housing conditions and experimental resources were available for my work in the facility.

Finally, my thanks and love to my parents, my sister, and my partner Megan, whose nurturing, encouragement, and unconditional support have afforded me the privilege to pursue my academic development and apply unwavering focus to my work, when needed. You gave me assurance during every challenge I encountered along this journey

TABLE OF CONTENTS

Appendix

LIST OF FIGURES

LIST OF TABLES

THESIS ORGANIZATION AND FORMAT

This thesis is organized into four chapters. **Chapter 1** provides the theoretical background and motivation for the thesis, and then introduces the research aims and study species. **Chapters 2 & 3** are manuscripts in preparation for their first submission to two ecological journals. **Chapter 4** provides a synthetic discussion of the results of **Chapters 2 & 3** and includes suggestions for future experiments that would nicely expand on the work presented in this thesis.

CHAPTER 1: General introduction

*Author***:** Nicholas A. W. Brown

CHAPTER 2:

*Authors***:** Nicholas A.W. Brown, Nicole Yee, Noah Houpt, Francis Juanes, and Sigal Balshine

*Publication***:** In preparation for submission to *Journal of Animal Ecology*

*Comments***:** NB, BB, FJ, and SB conceived and designed the experiments. NB, NH, SB, and NY collected the data, NB, BB, and NY conducted the analyses, and NB wrote the first draft of the manuscript under the supervision of FJ and SB.

CHAPTER 3:

*Authors***:** Nicholas A. W. Brown, Hailey Shafer, Francis Juanes, and Sigal Balshine

*Publication***:** In preparation for submission to an as-yet undetermined peer-reviewed journal

*Comments***:** NB, FJ, and SB conceived and designed the experiments. NB and HS collected the data and conducted the analyses. NB wrote the first draft of the manuscript under the supervision of FJ and SB.

CHAPTER 4: General discussion

Author: Nicholas A. W. Brown

Chapter 1: General introduction

1.1 Introduction

Understanding the ecological and evolutionary forces that shape animals' reproductive strategies is essential if we wish to predict how populations will respond to disturbance (Partridge & Harvey, 1988). Many components of reproductive strategies, such as the time to reach sexual maturity and the optimal number/size of offspring to produce, have been studied intensively (Pianka, 1976; Stearns, 1992). However, *where* animals choose to deposit their eggs and rear their young has not been studied nearly as well (Resetarits, 1996). This is odd given the clear potential for environmental perturbations and global change to disrupt this component of reproduction (Refsnider & Janzen, 2010).

With my Masters research, I endeavoured to address this gap in our understanding of the spatial components of reproductive strategies by studying nest site selection. Oviparous animals seldom deposit their eggs randomly; rather, they usually select sites according to particular habitat qualities—a phenomenon known as *oviposition site selection*, which encompasses nest and spawning site selection (Bernardo, 1996; Refsnider & Janzen, 2010). Mounting evidence shows that oviposition site selection can profoundly impact the lifetime reproductive success of both parents and offspring (Madsen & Shine, 1999; Van De Pol et al., 2006; Mitchell, Warner, et al., 2013). However, despite this awareness, our capacity to predict where animals will attain high reproductive success is still limited by an incomplete understanding of the factors that drive oviposition decisions. This is especially true in animals that provide extended parental care at a single site because they are subjected to the same environmental conditions as their developing young. These parents that provision care on the nest must therefore address both their own needs and those of their offspring when choosing where to nest, potentially resulting in parent–offspring conflicts (Spencer, 2002; Amat & Masero, 2004; Stahlschmidt & Adamo, 2013).

My thesis links the reproductive payoffs of oviposition sites to local environmental conditions and to parental phenotypes. In this introduction, I outline the benefits that oviposition sites can confer to parents and their offspring and review the ecological and evolutionary processes that are known to influence oviposition decisions. I also introduce the questions I addressed in my two data chapters (**Chapters 2 & 3**), introduce the study species that I used to tackle these questions, and outline the structure of my thesis.

1.2 The benefits of being choosy

Oviposition strategies are diverse. Some animals merely deposit their eggs in certain locations, while others will extensively prepare, alter, and then maintain the physical rearing environment to improve its protective value (Balshine, 2012). Among animals that construct and deposit their eggs in nests, the phenomenon is known as *nest site selection* and among externally fertilizing animals as *spawning site selection* (Refsnider & Janzen, 2010). Oviposition, nesting, or spawning sites can serve numerous functions. Sometimes certain sites are chosen solely for the benefits conferred to parents. Ensuring their gametes are fertilized is the primary function of an oviposition site for some species with external fertilization (Refsnider & Janzen, 2010; Wootton & Smith, 2015). For example, the fertilization success of numerous marine organisms, such as the seaweed *Fucus vesiculosus*, increases considerably in calm waters (Serrao et al., 1996). Accordingly, some pelagic marine fishes spawn only in relatively calm waters, where fertilization success is highest (Petersen et al., 1992; Sponaugle & Cowen, 1997). Oviposition sites can also be selected to facilitate mating. Nests can even be viewed as extended phenotypes of their builders in species such as the satin bowerbird (*Ptilonorhynchus violaceus*) that build elaborate nests whose primary function is to attract the interest of potential mates (Soler et al., 1998; Coleman et al., 2004; Schaedelin & Taborsky, 2009; Kawase et al., 2013). Some nest features are also modified to signal resource holding potential to conspecifics (Schaedelin & Taborsky, 2009). For example, black kites (*Milvus migrans*) in good body condition and of prime reproductive age will decorate their nests extensively with high-visibility objects that convey the nest owner's quality and thereby reduce conspecific attack rates (Fabrizio et al., 2011).

While many species select sites to reproduce that increase their chances of mating or fertilization, oviposition sites are most commonly selected or constructed to shelter offspring and enhance the conditions they experience during development (Balshine, 2012). These two purposes are not mutually exclusive—larger, more elaborate nests are usually honest indicators of mate quality and can also reliably predict beneficial rearing conditions under the care of a good partner (Grubbauer & Hoi, 1996; Quader, 2006). The main benefit offspring receive from a good oviposition site is enhanced survival (Refsnider & Janzen, 2010). For example, sawflies (*Euura lasiolepis*) exhibit hierarchical site preferences according to host plant traits that most improve larval survival (Craig et al., 1989). Similarly, male marsh wrens (*Cistothorus palustris*) diminish egg predation by building several decoy nests in addition to the nest in which their eggs are eventually incubated (Leonard & Picman, 1987). Some oviposition sites can also enhance juvenile survival and even future reproductive success if they result in fitter young and/or are positioned close to suitable habitats (Refsnider & Janzen, 2010). Female toad-headed agamas (*Phrynocephalus przewalskii*), for example, select nest sites that contain near-optimal temperature and moisture conditions for offspring; all offspring that emerge from these nests grow faster, and any female offspring from these nests will reach sexual maturity earlier (Li et al., 2018). Oystercatchers (*Haematopus ostralegus*) that nest closer to foraging patches can escort their young to food, which facilitates higher fledgling consumption rates and thereby enhances offspring growth and future reproductive success (Ens et al., 1992; Van De Pol et al., 2006). Thus, a good oviposition or nesting site can provide offspring with numerous advantages that help them well into the future.

1.3 Ecological factors that influence oviposition site selection

Oviposition sites are chosen for their specific microclimates, but which environmental factors influence oviposition decisions and why? Both abiotic and biotic features affect the choices made by ovipositing mothers. Across taxa, rearing site temperature is perhaps the most important determinant of oviposition site selection due to its profound effects on offspring development rates and growth (Gillooly et al., 2002; Noble et al., 2018). Many studies have shown that animals preferentially oviposit in warmer over cooler sites that enhance offspring development rates and/or in sites with thermal properties that positively affect offspring traits (Jeanne & Morgan, 1992; With & Webb, 1993; Angilletta et al., 2009; Dayananda et al., 2017; Li et al., 2018). In birds, for example, sub-optimal incubation temperatures can result in abnormal tissue development, whereas near-optimal temperatures enhance offspring metabolism, food-conversion efficiency, and growth (Durant et al., 2013). In many reptiles and some teleost fishes, offspring sexes are controlled by incubation temperature (Bachtrog et al., 2014), which allows parents to alter the sex ratios of their broods through selection of warmer or cooler sites (Roosenburg, 1996; Conover, 2002; Warner & Shine, 2005, 2008; Doody et al., 2006; Mitchell et al., 2013; Schwanz, 2016). Oxygen availability is another important abiotic factor in oviposition site decisions, particularly in amphibians and fishes. For example, female bitterlings (*Rhodeus amarus*) that spawn in the gills of living mussels exhibit preferences for mussels that emit high levels of dissolved oxygen (Smith et al., 2001; Phillips et al., 2017). Other abiotic factors with known effects on oviposition site selection include moisture content in the rearing environment (Wood & Bjorndal, 2000; Warner & Andrews, 2002; Brown & Shine, 2004; Stahlschmidt et al., 2011), wind exposure (With & Webb, 1993; D'Alba et al., 2009), and sun exposure (Calder, 1973; Walsberg, 1981; With & Webb, 1993).

Biotic factors can exert equal or even greater effects. Predation risk to both parents and offspring profoundly affects the choice of oviposition sites in numerous taxa (Resetarits & Wilbur, 1989; Candolin & Voigt, 1998; Spencer & Thompson, 2003; Peluc et al., 2008; Ghalambor et al., 2013; Touchon & Worley, 2015). Predation risk supersedes beneficial abiotic conditions in female field crickets (*Gryllus texensis*) by driving them to sheltered sites with sub-optimal incubation temperatures (Stahlschmidt & Adamo, 2013). Likewise, Kentish plovers (*Charadrius alexandrinus*) reduce their own risk of predation during incubation by building nests in exposed areas, where visibility is higher and predators are more easily spotted, but thermal conditions are sub-optimal for their eggs (Amat & Masero, 2004). Food availability is another important biotic factor in the oviposition decisions of some parents. For example, the mosquito *Anopheles punctipennis* avoids depositing its eggs in pools with cues of high interspecific competition (Petranka & Fakhoury, 1991); another mosquito, *Wyeomyia smithii*, preferentially deposits its eggs in pitchers with higher prey availability (Heard, 1994). Risk of parasitic infection (Kiesecker & Skelly, 2000; Amano et al., 2008) and degree of foliage concealment (which affect how easily predators can find a nest, and be detected by guarding parents)

(Götmark et al., 1995; Remeš, 2005; Peluc et al., 2008), are other biotic factors known to influence oviposition site decisions.

1.4 Selective forces shaping oviposition site selection

Oviposition site selection is one of several non-genetic mechanisms through which parents influence the phenotypes of their offspring (Bernardo, 1996; Mousseau & Fox, 1998). Such parental effects are adaptive when parents adjust offspring phenotypes to maximize their own fitness, which sometimes increases offspring fitness as well (Marshall & Uller, 2007; Badyaev & Uller, 2009). For example, in animals with temperature-dependent sex determination, developing under incubation temperatures that bias sex ratios toward a certain sex can increase reproductive success in offspring of that sex by matching their seasonal hatching time to its optimal time to reach sexual maturity (Warner & Shine, 2005, 2008). Thus, oviposition site selection that enhances maternal fitness also enhances offspring fitness in these animals.

However adaptive parental effects (including adaptive oviposition site choice) do not always enhance offspring fitness. Although parental and offspring interests commonly align, some ‘selfish’ animals exert neutral or even negative effects on offspring fitness to maximize their own (Einum & Fleming, 2000; Marshall & Uller, 2007). Accordingly, oviposition sites are not always chosen to provide the best possible conditions for offspring. For example, females of the herbivorous fly *Chromatomyia nigra* selectively feed and oviposit on grasses that enhance their own fecundity, but not the fitness of their offspring (Scheirs et al., 2000). These flies maximize their lifetime reproductive success through producing the most offspring possible rather than ovipositing where their offspring will have the highest survivorship. Ultimately,

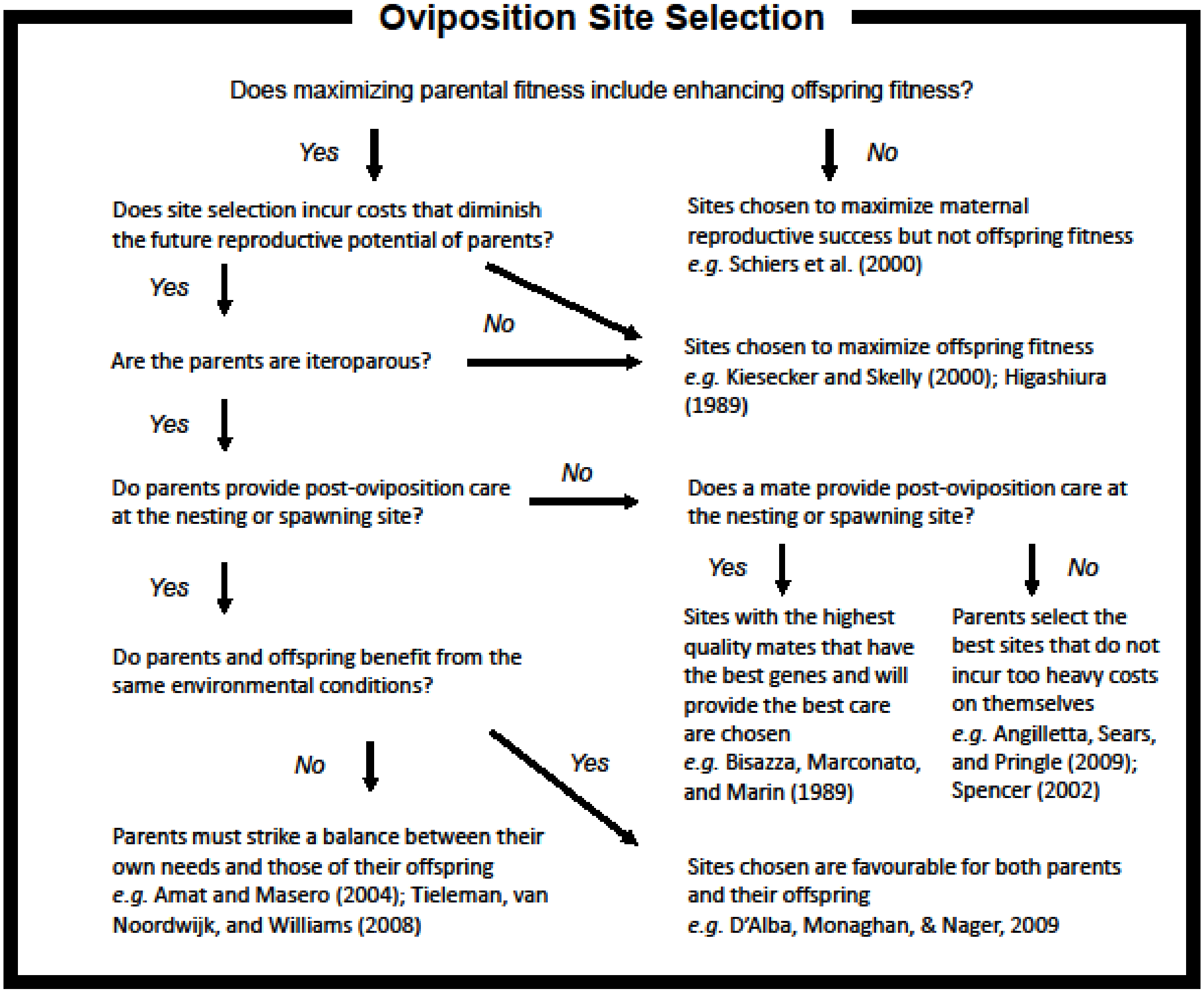


Figure 1.1 Decision tree outlining important life history variables and their influence on optimal oviposition site selection. Credit: Nicholas AW Brown

selection favours choosing oviposition sites that will enhance offspring fitness only when this also enhances parental fitness (Fig. 1.1.; Marshall & Uller, 2007; Refsnider & Janzen, 2010).

The degree to which offspring and parental fitness are linked or are in opposition depends on species-specific life history traits. Oviposition site selection must ensure that physiologically constrained life history traits (*e.g.* offspring development times) are matched to the environments where they have the highest adaptive value (Stearns, 1992; Resetarits, 1996; Ricklefs & Wikelski, 2002). However, studies relating variation in life history traits to oviposition site choice are limited. Madsen and Shine (1999) describe two linked oviposition and care provisioning strategies in water pythons (*Liasis fuscus*): females that oviposit in relatively cool nests remain to provide post-hatching parental care whereas females that oviposit in relatively warm nests abandon their broods shortly after oviposition. Neither strategy appeared to confer a fitness advantage, nor did fecundity, clutch size, or body size vary notably between females that adopted either strategy; thus, life history variation among water pythons does not appear to affect their oviposition site choices (Madsen & Shine, 1999). This finding is surprising considering numerous studies have linked patterns of other parental behaviours to variation in life history traits (Klug et al., 2012). South American birds that produce smaller clutches and survive in higher proportions than do North American birds will more readily sacrifice offspring provisioning to reduce their own risk of predation (Ghalambor & Martin, 2001). Life history traits also affect patterns of parental care in fish. Consistent with a theoretical model of parental care evolution (Klug & Bonsall, 2010), prolonged parental care is far more common in fishes with shorter lifespans (lower future reproductive output) and longer offspring development periods (more benefits from guarding and cleaning behaviours) than in those with longer lifespans and shorter offspring development (Winemiller & Rose, 1992). As a form of parental care, oviposition site selection should be similarly linked to life history traits. Oviposition sites should be chosen according to life history traits that affect the benefits offspring receive from different sites (*e.g.* offspring size affecting thermal benefits) and the costs parents incur during site selection (*e.g.* diminished energy for future reproductive efforts is more costly for iteroparous parents; Resetarits, 1996; Marshall & Uller, 2007; Klug & Bonsall, 2010).

1.5 Thesis aims and structure

Despite growing empirical interest, we still lack a thorough understanding of how oviposition site selection is affected by life history traits and, in turn, how these traits affect the optimal balance between costs incurred by parents and benefits received by offspring from certain nesting locations. Tension between parents and offspring manifested in the benefit–cost ratio of oviposition site selection should be highest when parents and offspring do not benefit from the same rearing conditions (Fig. 1.1). Parents that provide extended post-hatching parental care on the nest are more likely to suffer higher costs in more challenging nest sites than parents that abandon their eggs following oviposition; thus, such individuals and species present a compelling opportunity to examine how oviposition site decisions are affected by life history traits. The aims of my MSc research were to measure the reproductive costs and benefits incurred by care-giving parents nesting in different microhabitats and to determine whether their oviposition site decisions match what is predicted from life history theory. More specifically, my thesis addresses the following two questions: 1) how do parents balance the benefits and costs of care when selecting a nest site? and 2) how do the benefits conferred to offspring vary according to abiotic conditions and in relation to maternal traits? To answer these questions I carried out field (**Chapter 2**) and laboratory (**Chapter 3**) experiments on a marine toadfish to determine how the survival, development rates, and physical performance of their young are affected by their nesting location (and its microclimate) in the intertidal zone, and how parental reproductive success is affected as a result of particular nest choices.

1.6 Study species: the plainfin midshipman fish (*Porichthys notatus*)

The plainfin midshipman (*Porichthys notatus*) is a marine toadfish (Batrachoididae) that provides extended parental care in one of the Earth's most dynamic ecosystems, the intertidal zone. The toadfish are the only family of marine teleosts known to provide extended post-hatching parental care for young in the intertidal (Horn et al., 1999). Every year in the late spring, plainfin midshipman migrate from the deep ocean (> 150 m; Sisneros et al., 2004) to beaches along the Pacific coast of North America to spawn (Arora, 1948). Here, plainfin midshipman are subjected to extreme fluctuations in temperature, desiccation, osmotic stress, predation from both terrestrial and marine species, and intense hydrodynamic forces daily (Truchot & Duhamel-Jouve, 1980; Denny, 1988; Lloyd & Martin, 2004). Male plainfin midshipman adopt one of two alternative reproductive tactics: larger 'guarder' males clear out nesting cavities underneath rocks and produce an advertisement 'hum' to attract females (Fig. 1.2); smaller 'sneaker' males enter the nest during spawning and attempt to cuckold guarder males (Brantley & Bass, 1994). Once they have acquired a brood of eggs, guarder males remain in the nest until all eggs have hatched, a care period that frequently exceeds 60 days (Cogliati et al., 2013). Successful guarder males will spawn with several females in each season (DeMartini, 1988; Cogliati et al., 2013), but their body condition declines steadily over time while tending to eggs in this harsh environment (Bose et al., 2016a). Although guarder males are tolerant to aquatic hypoxia (LeMoine et al., 2014; Craig et al., 2014), many perish in the harsh environment, leading to the loss of their eggs as well (Arora, 1948; DeMartini, 1988; Bose et al., 2016b).

Figure 1.2. A plainfin midshipman guarder male tending a brood of eggs in a submerged intertidal nest. Photo credit: Nicholas A. W. Brown

Presumably, plainfin midshipman would not migrate from relatively stable conditions in the deep ocean to breed in the rapidly changing and challenging intertidal zone if such an environment was not somehow beneficial to their offspring (Klug & Bonsall, 2010). The intertidal zone is a highly productive ecosystem driven by high temperature, sunlight, and oxygen levels, but is also an area of ever fluctuating abiotic conditions (Leigh et al., 1987; Denny & Wethey, 2001). Many species of fish and amphibians spawn on beaches or lay their eggs above the water's surface because exposure to air enhances oxygen availability, and/or higher temperatures speed development (Martin et al., 2004). Shallow tidal pools reach far warmer temperatures than open seawater, possibly allowing midshipman to rear more or larger eggs (higher quality offspring) than they could in colder conditions over the same time period (Martin et al., 2004). Some males nest high in the intertidal, others nest low in the intertidal, and some males even nest in the subtidal. Guarding males nesting higher up in the intertidal zone experience longer periods of air exposure, and warmer temperatures (Bose et al., 2019). Because midshipman mobilize glycogen reserves to tolerate hypoxia (Craig et al., 2014), guarding males in air exposed, warmer nests might deplete energy reserves quicker than males nested at lower elevations. Eggs laid higher in the intertidal likely develop faster under these same extreme conditions (Martin et al., 2004), but might also suffer increased mortality (Bose et al., 2019). The variation in where these fish will nest imposes a varying degree of challenge and makes the plainfin midshipman an ideal system to examine how animals select nest sites according to their relative benefit–cost ratio.

1.7 References

Amano, H., Hayashi, K., & Kasuya, E. (2008). Avoidance of egg parasitism through submerged oviposition by tandem pairs in the water strider, *Aquarius paludum insularis* (Heteroptera: Gerridae)– Ecol. Entomol. 33: 560–563.

Amat, J.A. & Masero, J.A. (2004). Predation risk on incubating adults constrains the choice of thermally favourable nest sites in a plover– Anim. Behav. 67: 293–300.

Angilletta, M.J., Sears, M.W., & Pringle, R.M. (2009). Spatial dynamics of nesting behavior: Lizards shift microhabitats to construct nests with beneficial thermal properties– Ecology 90: 2933–2939.

Arora, H.L. (1948). Observations on the habits and early life history of the Batrachoid fish, *Porichthys notatus* Girard– Copeia 1948: 89–93.

Bachtrog, D., Mank, J.E., Peichel, C.L., Kirkpatrick, M., Otto, S.P., Ashman, T.L., Hahn, M.W., et al. (2014). Sex determination: why so many ways of doing it?– PLoS Biol. 12: 1–13.

Badyaev, A. V. & Uller, T. (2009). Parental effects in ecology and evolution: Mechanisms, processes and implications– Philos. Trans. R. Soc. B Biol. Sci. 364: 1169–1177.

Balshine, S. (2012). Patterns of parental care in vertebrates– In: Evol. Parent. Care (Royle, N.J., Smiseth, P.T., & Kölliker, M., eds). Oxford University Press, Oxford, p. 356.

Bernardo, J. (1996). Maternal effects in animal ecology– Am. Zool. 2: 83–105.

Bisazza, A., Marconato, A., & Marin, G. (1989). Male competition and female choice in *Padogobius martensi* (Pisces, Gobiidae)– Anim. Behav. 38: 406–413.

Bose, A.P.H., Borowiec, B., Scott, G., & Balshine, S. (2019). Nesting on high: reproductive and physiological consequences of breeding in the intertidal zone– Evol. Ecol. 33: 21–36.

Bose, A.P.H., McClelland, G.B., & Balshine, S. (2016a). Cannibalism, competition, and costly care in the plainfin midshipman fish, *Porichthys notatus*– Behav. Ecol. 27: 628–636.

Bose, A.P.H., Kou, H.H., & Balshine, S. (2016b). Impacts of direct and indirect paternity cues on paternal care in a singing toadfish– Behav. Ecol. 27: 1507–1514.

Brantley, R.K. & Bass, A.H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae)– Ethology 96: 213–232.

Brown, G.P. & Shine, R. (2004). Maternal nest-site choice and offspring fitness in a tropical snake (*Tropidonophis mairii*, Colubridae)– Ecology 85: 1627–1634.

Calder, W.A. (1973). Microhabitat selection during nesting of hummingbirds in the Rocky Mountains– Ecology 54: 127–134.

Candolin, U. & Voigt, H.R. (1998). Predator-induced nest site preference: Safe nests allow courtship in sticklebacks– Anim. Behav. 56: 1205–1211.

Cogliati, K.M., Neff, B.D., & Balshine, S. (2013). High degree of paternity loss in a species with alternative reproductive tactics– Behav. Ecol. Sociobiol. 67: 399–408.

Coleman, S.W., Patricelli, G.L., & Borgia, G. (2004). Variable female preferences drive complex male displays– Nature 428: 742–745.

Conover, D.O. (2002). Adaptive significance of temperature-dependent sex determination in a fish– Am. Nat. 123: 297–313.

Craig, P.M., Fitzpatrick, J.L., Walsh, P.J., Wood, C.M., & McClelland, G.B. (2014). Coping with aquatic hypoxia: How the plainfin midshipman (*Porichthys notatus*) tolerates the intertidal zone– Environ. Biol. Fishes 97: 163–172.

Craig, T.P., Itami, J.K., & Price, P.W. (1989). A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly– Ecology 70: 1691–1699.

D'Alba, L., Monaghan, P., & Nager, R.G. (2009). Thermal benefits of nest shelter for incubating female eiders– J. Therm. Biol. 34: 93–99.

Dayananda, B., Murray, B.R., & Webb, J.K. (2017). Hotter nests produce hatchling lizards with lower thermal tolerance– J. Exp. Biol. 220: 2159–2165.

DeMartini, E.E. (1988). Spawning success of the male plainfin midshipman. I. Influences of male body size and area of spawning site– J. Exp. Mar. Bio. Ecol. 121: 177–192.

Denny, M.W. (1988). Biology and the mechanics of the wave-swept environment. Princeton University Press, Princeton.

Denny, M.W. & Wethey, D. (2001). Physical processes that generate patterns in marine communities.– In: Mar. Community Ecol. Sinauer Associates, Sunderland, p. 3–37.

Doody, J.S., Guarino, E., Georges, A., Corey, B., Murray, G., & Ewert, M. (2006). Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination– Evol. Ecol. 20: 307–330.

Durant, S.E., Hopkins, W.A., Hepp, G.R., & Walters, J.R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds– Biol. Rev. 88: 499–509.

Einum, S. & Fleming, I.A. (2000). Highly fecund mothers sacrifice offspring survival to maximize fitness– Nature 405: 565–567.

Ens, B.J., Kersten, M., Brenninkmeijer, A., & Hulscher, J.B. (1992). Territory quality, parental effort and reproductive success of oystercatchers (*Haematopus ostralegus*)– J. Anim. Ecol. 61: 703–715.

Fabrizio, S., Blas, J., Blanco, G., Tanferna, A., López, L., Lemus, J.A., & Hiraldo, F. (2011). Raptor nest decorations are a reliable threat against conspecifics– Science 331: 327–330.

Ghalambor, C.K. & Martin, T.E. (2001). Fecundity-survival trade-offs and parental risk-taking in birds– Science 292: 494–497.

Ghalambor, C.K., Peluc, S.I., & Martin, T.E. (2013). Plasticity of parental care under the risk of predation: how much should parents reduce care?– Biol. Lett. 9: 20130154.

Gillooly, J.F., Charnov, E.L., West, G.B., Savage, V.M., & Brown, J.H. (2002). Effects of size and temperature on developmental time– Nature 417: 70–73.

Götmark, F., Blomqvist, D., Johansson, O.C., & Bergkvist, J. (1995). Nest site selection: a trade-off between concealment and view of the surroundings?– J. Avian Biol. 26: 305–312.

Grubbauer, P. & Hoi, H. (1996). Female penduline tits (*Remiz pendulinus*) choosing high quality nests benefit by decreased incubation effort and increased hatching success– Écoscience 3: 274–279.

Heard, S.B. (1994). Imperfect oviposition decisions by the pitcher plant mosquito (*Wyeomyia smithii*)– Evol. Ecol. 8: 493–502.

Higashiura, Y. (1989). Survival of eggs in the gypsy moth *Lymantria dispar*. II . Oviposition site selection in changing environments– J. Anim. Ecol. 58: 413–426.

Horn, M.H., Martin, K.L.M., & Chotkowski, M.A., eds (1999). Intertidal Fishes: Life in Two Worlds– Encycl. Ocean Sci. Academic Press, San Diego, CA.

Jeanne, R.L. & Morgan, R.C. (1992). The influence of temperature on nest site choice and reproductive strategy in a temperate zone *Polistes* wasp– Ecol. Entomol. 17: 135–141.

Kawase, H., Okata, Y., & Ito, K. (2013). Role of huge geometric circular structures in the reproduction of a Marine pufferfish– Sci. Rep. 3: 4–8.

Kiesecker, J.M. & Skelly, D.K. (2000). Choice of oviposition site by gray treefrogs: The role of potential parasitic infection– Ecology 81: 2939–2943.

Kinne, O. & Kinne, E.M. (1962). Rates of development in embryos of a cyprinodont fish exposed to different temperature-salinity-oxygen combinations– Can. J. Zool. 40: 231–253.

Klug, H., Alonzo, S.H., & Bonsall, M.B. (2012). Theoretical foundations of parental care– In: Evol. Parent. Care (Royle, N.J., Smiseth, P.T., & Kölliker, M., eds). Oxford University Press, Oxford, p. 356.

Klug, H. & Bonsall, M.B. (2010). Life history and the evolution of parental care– Evolution 64: 823–835.

Leigh, E.G., Paine, R.T., Quinn, J.F., & Suchanek, T.H. (1987). Wave energy and intertidal productivity– Proc. Natl. Acad. Sci. 84: 1314–1318.

LeMoine, C.M.R., Bucking, C., Craig, P.M., & Walsh, P.J. (2014). Divergent hypoxia tolerance in adult males and females of the plainfin midshipman (*Porichthys notatus*)– Physiol. Biochem. Zool. 87: 325–333.

Leonard, M.L. & Picman, J. (1987). The adaptive significance of multiple nest building by male marsh wrens– Anim. Behav. 35: 170-271–277.

Li, S.R., Hao, X., Wang, Y., Sun, B.J., Bi, J.H., Zhang, Y.P., Janzen, F.J., & Du, W.G. (2018). Female lizards choose warm, moist nests that improve embryonic survivorship and offspring fitness– Funct. Ecol. 32: 416–423.

Lloyd, J.D. & Martin, T.E. (2004). Nest-site preference and maternal effects on offspring growth– Behav. Ecol. 15: 816–823.

Madsen, T. & Shine, R. (1999). Life history consequences of nest-site variation in tropical pythons (*Liasis fuscus*)– Ecology 80: 989–997.

Marshall, D.J. & Uller, T. (2007). When is a maternal effect adaptive?– Oikos 116: 1957–1963.

Martin, K.L.M., Van Winkle, R.C., Drais, J.E., & Lakisic, H. (2004). Beach-spawning fishes, terrestrial eggs, and air breathing– Physiol. Biochem. Zool. Ecol. Evol. Approaches 77: 341–356.

Mitchell, T.S., Maciel, J.A., & Janzen, F.J. (2013). Does sex-ratio selection influence nest-site choice in a reptile with temperature dependent sex determination?– Proc. R. Soc. B Biol. Sci. 280.

Mitchell, T.S., Warner, D.A., & Janzen, F.J. (2013). Phenotypic and fitness consequences of maternal nest-site choice across multiple older life stages.– Ecology 94: 336–345.

Mousseau, T.A. & Fox, C.W. (1998). The adaptive significance of maternal effects– Trends Ecol. Evol. 13: 403–407.

Noble, D.W.A., Stenhouse, V., & Schwanz, L.E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis– Biol. Rev. 93: 72–97.

Partridge, L. & Harvey, P.H. (1988). The ecological context of life history evolution– Science 241: 1449–1455.

Peluc, S.I., Sillett, T.S., Rotenberry, J.T., & Ghalambor, C.K. (2008). Adaptive phenotypic plasticity in an island songbird exposed to a novel predation risk– Behav. Ecol. 19: 830–835.

Petersen, C.W., Warner, R.R., Cohen, S., Hess, H.C., & Sewell, A.T. (1992). Variable pelagic fertilization success: implications for mate choice and spatial patterns of mating– Ecology 73: 391–401.

Petranka, J.W. & Fakhoury, K. (1991). Evidence of a chemically-mediated avoidance response of ovipositing insects to blue-gills and green frog tadpoles– Copeia 1991: 234–239.

Phillips, A., Reichard, M., & Smith, C. (2017). Sex differences in the responses to oviposition site cues by a fish revealed by tests with an artificial host– Anim. Behav. 126: 187–194.

Pianka, E.R. (1976). Natural selection of optimal reproductive tactics– Am. Zool. 784: 775–784.

Pörtner, H.O. & Farrell, A.P. (2008). Physiology and climate change– Science 322: 690–692.

Quader, S. (2006). What makes a good nest? Benefits of nest choice to female baya weavers (*Ploceus philippinus*)– Auk 123: 475.

Refsnider, J.M. & Janzen, F.J. (2010). Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice– Annu. Rev. Ecol. Evol. Syst. 41: 39–57.

Remeš, V. (2005). Nest concealment and parental behaviour interact in affecting nest survival in the blackcap (*Sylvia atricapilla*): An experimental evaluation of the parental compensation hypothesis– Behav. Ecol. Sociobiol. 58: 326–332.

Resetarits, W.J.J. (1996). Oviposition site choice and life history evolution– Am. Zool. 36: 205–215.

Resetarits, W.J.J. & Wilbur, H.M. (1989). Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors– Ecology 70: 220–228.

Ricklefs, R. & Wikelski, M. (2002). The physiology/life-history nexus– Trends Ecol. Evol. 17: 462–468.

Roosenburg, W.M. (1996). Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination?– Am. Zool. 36: 157–168.

Schaedelin, F.C. & Taborsky, M. (2009). Extended phenotypes as signals– Biol. Rev. 84: 293–313.

Scheirs, J., De Bruyn, L., & Verhagen, R. (2000). Optimization of adult performance determines host choice in a grass miner– Proc. R. Soc. B Biol. Sci. 267: 2065–2069.

Schwanz, L.E. (2016). Parental thermal environment alters offspring sex ratio and fitness in an oviparous lizard– J. Exp. Biol. 219: 2349–2357.

Serrao, E.A., Pearson, G., Kautsky, L., & Brawley, S.H. (1996). Successful external fertilization in turbulent environments.– Proc. Natl. Acad. Sci. 93: 5286–5290.

Sisneros, J.A., Forlano, P.M., Knapp, R., & Bass, A.H. (2004). Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman– Gen. Comp. Endocrinol. 136: 101–116.

Smith, C., Rippon, K., Douglas, A., & Jurajda, P. (2001). A proximate cue for oviposition site choice in the bitterling (*Rhodeus sericeus*)– Freshw. Biol. 46: 903–911.

Soler, J.J., Møller, A.P., & Soler, M. (1998). Nest building, sexual selection and parental investment– Evol. Ecol. 12: 427–441.

Spencer, R.J. (2002). Experimentally testing nest site selection: Fitness trade-offs and predation risk in turtles– Ecology 83: 2136–2144.

Spencer, R.J. & Thompson, M.B. (2003). The significance of predation in nest site selection of turtles: an experimental consideration of macro-and microhabitat preferences– Oikos 102: 592–600.

Sponaugle, S. & Cowen, R. (1997). Early life history traits and recruitment patterns of Caribbean wrasses (Labridae)– Ecol. Monogr. 67: 177–202.

Stahlschmidt, Z.R. & Adamo, S.A. (2013). Warm and cozy: Temperature and predation risk interactively affect oviposition site selection– Anim. Behav. 86: 553–558.

Stahlschmidt, Z.R., Brashears, J., & DeNardo, D.F. (2011). The role of temperature and humidity in python nest site selection– Anim. Behav. 81: 1077–1081.

Stearns, S.C. (1992). The evolution of life histories. Oxford University Press, Oxford, UK.

Tieleman, B.I., van Noordwijk, H.J., & Williams, J.B. (2008). Nest site selection in a hot desert:

trade-off between microclimate and predation risk?– Condor 110: 116–124.

Touchon, J.C. & Worley, J.L. (2015). Oviposition site choice under conflicting risks demonstrates that aquatic predators drive terrestrial egg-laying– Proc. R. Soc. B Biol. Sci. 282: 20150376–20150376.

Truchot, J.P. & Duhamel-Jouve, A. (1980). Oxygen and carbon dioxide in the marine intertidal environment: Diurnal and tidal changes in rockpools– Respir. Physiol. 39: 241–254.

Van De Pol, M., Bruinzeel, L.W., Heg, D., Van Der Jeugd, H.P., & Verhulst, S. (2006). A silver spoon for a golden future: Long-term effects of natal origin on fitness prospects of oystercatchers (*Haematopus ostralegus*)– J. Anim. Ecol. 75: 616–626.

Walsberg, G.E. (1981). Nest-site selection and the radiative environment of the warbling vireo– Condor 83: 86–88.

Warner, D.A. & Andrews, R. (2002). Nest-site selection in relation to temperature and moisture by the lizard *Sceloporus undulatus*– Herpetologica 58: 399–407.

Warner, D.A. & Shine, R. (2005). The adaptive significance of temperature-dependent sex determination: experimental tests with a short-lived lizard– Evolution 59: 2209.

Warner, D.A. & Shine, R. (2008). The adaptive significance of temperature-dependent sex determination in a reptile– Nature 451: 566–568.

Winemiller, K.O. & Rose, K.A. (1992). Patterns of life-history diversification in North American fishes: implications for population regulation– Can. J. Fish. Aquat. Sci. 49: 2196–2218.

With, K.A. & Webb, D.R. (1993). Microclimate of ground nests: the relative importance of radiative cover and wind breaks for three grassland species– Condor 95: 401–413.

Wood, D.W. & Bjorndal, K.A. (2000). Relation of temperature, moisture, salinity, and slope to nest site selection in loggerhead sea turtles– Copeia 1: 119–128.

Wootton, R.J. & Smith, C. (2015). Reproductive Biology of Teleost Fishes. John Wiley & Sons, Ltd., West Sussex, UK.

Chapter 2: Nest site selection in a toadfish is linked to increased offspring survival and reduced parental care

Nicholas A. W. Brown, Nicole Yee, Noah Houpt, Francis Juanes, & Sigal Balshine

In preparation for submission to *Journal of Animal Ecology*

2.1 Abstract

Parents attain higher reproductive success by increasing the benefits or lowering the costs of parental care. Choosing a well-located nesting or rearing environment allows parents to improve the benefit–cost ratio associated with care. However, parents must sometimes choose between locations that are most favourable for their offspring and those that are most favourable for themselves. Surprisingly, we know little about how parents balance the costs to themselves against the benefits to offspring when selecting a nesting site. In this study, we used the plainfin midshipman (*Porichthys notatus*), a marine toadfish that nests along a marine tidal elevation gradient, to measure the costs and benefits conferred to caring parents and their offspring at different nest sites. We constructed artificial nests along a tidal elevation gradient and found that all caring males showed body condition deterioration, but the magnitude of this deterioration did not increase with intertidal elevation. However, nests higher up in the intertidal zone experienced higher brood mortality rates. Additionally, parental care activity increased with intertidal elevation—males in the highest nests were the most active caregivers. Nesting in the middle-to-low intertidal rather than high in the intertidal enhances offspring survival rates and reduces the demand for parental care. By competing for nests in these more favourable sites, parental males attain higher reproductive success with lower parental effort.

2.2 Introduction

Improved offspring fitness is the principal evolutionary driver of parental care (Clutton-Brock, 1991; Alonso-Alvarez & Velando, 2012; Klug & Bonsall, 2010). Parents can directly enhance offspring fitness by providing defence and food for their offspring (Komdeur & Kats, 1999; Buckley et al., 2010). Parents can also indirectly enhance offspring fitness by improving their rearing environment (Diesel, 1992; Martin, 1998). *Nest site selection* is one such form of parental care that occurs before young are born or laid. However, the choice of nesting or rearing microhabitats can also affect parents themselves by altering their risk of predation while selecting a site, or by altering the costs of providing care or spawning success at a site (Refsnider & Janzen, 2010). If conditions that favour offspring and parental success differ, selection of nest sites can represent a trade-off for parents between locations that are most favourable for offspring and locations that are most favourable for parents (Bonsall & Klug, 2011; Badyaev & Ghalambor, 2013). To date, few studies have examined how parents balance potential trade-offs imposed by their selection of a nesting environment (Amat & Masero, 2004; Spencer, 2002; Tieleman et al., 2008). The aim of our study was to determine which sites allow parents to maximize their reproductive success when both the costs and benefits of providing care vary according to environmental conditions at different nesting locations.

Provisioning young incurs costs for parents, including decreased time and energy for feeding themselves or for mating and increased mortality risk, all of which can diminish future reproductive output (Svensson, 1988; Buzatto et al., 2007; Burris, 2011). Life history traits dictate the precise costs of care and influence the trade-off in investment between current versus future offspring (Klug & Bonsall, 2010). For example, Southern Hemisphere birds, which have higher remating potential and smaller clutches than Northern Hemisphere birds, tend to prioritize their own safety over that of their offspring (Ghalambor & Martin, 2001). Similarly, male burying beetles (*Nicrophorus vespilloides*) have higher remating potential than females and are accordingly more likely to abandon experimentally reduced broods (Ward et al., 2009). Animals might reduce their parental effort, and abandon or even cannibalize their offspring when costs begin to outweigh the benefits of continuing to provide care (DeMartini, 1987; Ghalambor et al., 2013). Such plasticity in the ability to terminate care allows parents to maximize the benefits and/or minimize the costs of care, and thus optimize their lifetime reproductive success (Alonso-Alvarez & Velando, 2012).

Nest site selection can also allow parents to alter the costs and benefits of care. Certain nesting or rearing sites provide offspring with optimal conditions for development (Muth, 1980; Wilson, 1998), refuge from predation (Rieger et al., 2004), and/or close proximity to favourable juvenile habitat (*e.g.* with higher food availability, Baden, 2018; fewer parasites, Kiesecker & Skelly, 2000). Likewise, selection of nests and the microhabitats around them can benefit parents by reducing their exposure to predation (Spencer, 2002; Amat & Masero, 2004), or reducing the physiological costs of providing care (Madsen & Shine, 1999). Parents and offspring sometimes benefit from the same environmental conditions. For example, female common eiders (*Somateria mollissima*), which nest in the arctic, lose less body mass and maintain higher and more constant incubation temperatures in sheltered nests compared to those in more wind-exposed locations (D'Alba et al., 2009). However, in other species, parents face a trade-off when selecting a nest site between their needs and the needs of their offspring. Both hoopoe larks (*Alaemon alaudipes*) and Kentish plovers (*Charadrius alexandrines*) reduce their own risk of predation by building nests in exposed areas where predators are more easily spotted, but eggs are more likely to overheat (Amat & Masero, 2004; Tieleman et al., 2008). Yet, we still know little about how variable costs and benefits can shape nest choice and favour certain microhabitats over others. To investigate the extent to which the costs and benefits of care differ across nesting sites, we studied a marine toadfish, the plainfin midshipman (*Porichthys notatus*), that nests along an intertidal gradient.

Each spring, plainfin midshipman migrate from deep waters (> 150 m) to intertidal shores along the Pacific coast of North America to breed (Arora, 1948; Sisneros et al., 2004). Large parental males called *guarder males* excavate nest cavities under rocks and produce a vocal advertisement signal that attracts females (Brantley & Bass, 1994). Males typically spawn with several females in a season and then remain alone in the nest for 60 or more days tending and guarding a brood of eggs until they develop into free-swimming juveniles (DeMartini, 1988; Cogliati et al., 2013). Males in intertidal nests are periodically emersed (air exposed) for several hours per day during low tides (Arora, 1948; Bose et al., 2019a). Intertidal animals tend to be distributed with respect to tidal elevation according to their physiological tolerance of extreme abiotic conditions (Somero, 2002; Tomanek & Helmuth, 2002); thus, costs and benefits of providing care in the intertidal zone likely vary with tidal elevation as plainfin midshipman guarder males approach their physiological limits (Bose et al., 2019a). Males that nest high in the intertidal must endure longer periods in small pools of increasingly warming and hypoxic water, as well as longer emersion periods compared to males nesting in the lower intertidal zone (Bose et al., 2019a). To withstand these extreme events, males mobilize energy reserves and depress their metabolism, so energy and aerobic scope are progressively depleted with increasing cumulative emersion time (Craig et al., 2014; Bose et al., 2019a; Houpt et al., *in review*). Body condition of parental males also declines as they starve during the care period; they often perish as a result, leaving their broods vulnerable to predation, disease, and complete mortality (DeMartini, 1988; Sisneros et al., 2009; Bose et al., 2016a; Bose et al., 2016b). Thus, nesting high in the intertidal conceivably incurs heavier physiological and survival costs to parents than nesting lower in the intertidal or subtidal. However, offspring could benefit from conditions in higher nests—emersed eggs are exposed to higher temperatures and, potentially, increased oxygen availability, which are associated with faster development in other species (Martin et al., 2004; Seymour & Bradford, 1995; Strathmann & Hess, 1999). Males nesting higher in the intertidal might receive reproductive benefits if these conditions translate to rearing young more quickly or successfully compared to in lower intertidal or subtidal nests, and these benefits are not outweighed by the challenges of tolerating harsher conditions.

To investigate these ideas, we tested two linked hypotheses: 1) the rate of body condition deterioration in caring males increases with intertidal elevation, and 2) egg development rates increase with tidal elevation. The proposed balance between costs and benefits of nesting along the tidal elevation gradient suggested three further, interrelated predictions: 3) egg survival will be highest in the middle intertidal where the benefit–cost ratio of care is highest, 4) the largest, most competitive males will be found in these preferred middle intertidal nests, and 5) males in higher nests will provide less care because they will be constrained by the physiological demands of inhabiting a harsher environment.

2.3 Methods

2.3.1 Study site, nest construction, and water quality measures

We studied plainfin midshipman fish nesting along the tidal gradient in Dabob Bay, Washington, USA (47°76′N, 122°86′W) from May to August 2018. Substrate composition at the site was a mixture of oyster reef (dominated by *Crassostrea gigas*) and beach rock, which descended into a subtidal macroalgae bed with occasional beach rock. Initially, the macroalgae bed was mainly *Sargassum muticum* but with increasing depth *Zostera marina* becomes more dominant. On 14–16 May we constructed 110 artificial nests on this beach by deploying 30 square concrete tiles (929 cm^2) along 'high', 'middle', and 'low' intertidal contours. Each nest was separated from the next by ≈ 1 m and the contour lines were demarcated by the waterline at +0.0, −1.1, and −2.0 ft, relative to the tidal chart. In addition, 20 tiles were placed at a fourth 'subtidal' contour along a distinct *S. muticum*–*Z. marina* border (at approximately −4.0 ft, see Figure 2.1C). These contours reflect the natural population distribution of nesting plainfin midshipman at the study site. We surveyed all the artificial nests along these contours during low spring tides on 16–19 May, 13–18 and 28–29 June, and 11–13 and 28–29 July 2018.

Digital temperature loggers (Onset ± 0.1 ºC HOBO Pendants and Thermochron ± 0.5 ºC iButtons set to record a temperature measurement every hour) were deployed in 10 nests along each intertidal contour and in three subtidal nests. Water quality tests were also carried out during the lowest tide of each spring cycle at three nests per intertidal contour—one at each end of the contour, and one approximately in the middle—and, as a comparison point, we also recorded the water quality 1 m offshore from low the water line ('subtidal'). We recorded pH (± 0.01 unit; Oakton PCSTestr 35), salinity (± 1 ppt; handheld Thermofisher refractometer), and dissolved oxygen (± 0.01 mg L^{-1}; Oakton DO 450 dissolved oxygen meter and probe). If the focal nest had completely dried during testing, we recorded only air temperature and salinity (when possible, using a pipette to collect water if a small amount of remained in the nest).

2.3.2 Experimental Procedure

Twenty of the 30 tiles at each intertidal contour were designated *experimental nests* and were covered with plastic mesh once a male excavated a cavity underneath and received eggs. Mesh prevented nesting males from leaving and other individuals from entering, which allowed us to track the condition of males and development of eggs without the possibility of nest-takeovers, cuckoldry by other males, or avian predation, all of which are common in this species (Bose et al., 2019b; Cogliati et al., 2014; Elliot et al., 2003). It also prevented new females from entering nests, allowing us to track brood survival from a known spawning date. The remaining 10 tiles at each intertidal contour and 20 tiles in the subtidal, were designated *natural nests* and left uncovered. Fish and eggs were measured and photographed at these natural, unmeshed nests (see below); we used these nests to assess the impact of mesh covering on spawning success (number of eggs acquired).

Nests were checked during ebbing tides, just after the water line receded past a contour. If no fish were present, the tile was replaced and checked again the following day. If a guarder male was discovered under a tile without eggs, we gently laid the tile back down, and checked it again the following day. Once a guarder male was discovered with a brood of eggs, we photographed the eggs and measured the male’s body mass (± 0.01 g) and standard length (SL; ± 1 mm). If one or more females or any peripheral cuckolder males were present in the nest, their mass and length were also recorded, and then they were released into the sea. Following measurements, guarder males were returned to their nests and the tile was covered with mesh (for experimental nests only). Eighty-seven plainfin midshipman guarder males (initial mass: 56.4–335.0 g; initial SL: 153–287 mm) took up residence in experimental and natural nests, combined.

Using GoPro cameras (model: Hero 5 Black), we collected 48 videos of 36 caring males with broods of eggs: 13 videos (of 12 males) in the high elevation, 16 videos (of 14 males) in the middle, and 19 videos (of 10 males) in the low. Hence of the 36 males recorded, some were repeatedly recorded but most were recorded once only (*N* measured once = 28; *N* measured twice = 2; *N* measured thrice = 6). We analyzed all videos that were recorded from the same male but accounted for this pseudoreplication in our statistical model (see below). Recording always began during the flood stage of the daily tide cycle, as soon as the ocean returned to the focal nest. Videos were recorded until camera batteries died and they lasted, on average, 90 minutes (range: 45–134 min).

2.3.3 Statistical Analyses

All analyses were conducted in R (version 3.2.3; R Core Team, 2016). We used the ‘lme4’ (Bates et al., 2015), ‘mgcv’ (Wood, 2017), and ‘glmmTMB’ (Brooks et al., 2017) packages to fit linear, additive, and negative-binomial mixed effects models, respectively. We used the ‘car’ package (Fox & Weisberg, 2011) to carry out Wald chi-squared tests of main effects and interactions in mixed effects models, and the ‘emmeans’ (Lenth, 2019) package to carry out planned and *post-hoc* comparisons. Because many of our predictions were predicated on the assumption that abiotic conditions increase in severity with tidal elevation, we applied successive differences contrast coding to the elevation groups in some models and note this where applicable in the results. Continuous covariates in all models were centered at 0 and scaled by their standard deviations (Schielzeth, 2010).

To test our assumption that thermal conditions increased in severity with tidal elevation, we calculated daily mean temperatures, daily temperature standard deviations, and daily maximum temperatures from measurements recorded at each logger. These data fluctuated notably with the tidal cycle (Fig. 2.2), so we used generalized additive mixed models (GAMMs) to fit penalized smoothing splines to the data. We fit three GAMMs: one each for average daily temperature, daily standard deviation, and daily maximum temperature. Intertidal elevation was the only fixed effect in both models. We used cubic regression splines to fit a different smoothing function for each elevation along the number of days since its first measurement (Wood, 2017). Temperature logger identity was included as a random effect to account for repeated measurements. We included an auto-regressive term (auto-correlation of order 1) to account for temporal auto-correlation between days and temperature loggers (Zuur et al., 2009).

To determine if nests in the middle elevation were preferred by males, we used a one-way ANOVA with a custom contrast (middle > low & high) for elevation to test whether larger males (larger SL) took up these presumed best sites. We evaluated whether males in nests at certain elevations were more likely to attract females by fitting a binomial generalized linear model (GLM) to the proportion of nests that received spawnings within 96 h of their construction. Intertidal elevation was included as the only fixed effect. We also fit a GLM with a Conway-Maxwell Poisson distribution (Brooks et al., 2019) to evaluate the extent to which the number of eggs received in nests differed between elevations. Intertidal elevation and nest manipulation (*i.e.* experimental or natural) were included as fixed effects, and male body size (SL) was included as a covariate.

Residuals from a regression of each male's $\log_{10}$-mass and $\log_{10}$-SL were used as a body condition index (Bose et al., 2016b). We inferred that males suffered heavier physiological costs when their body condition deteriorated faster. To determine if body condition deteriorated more quickly at higher elevations, we fit condition index values with a linear mixed effects model (LMM). Fixed effects were elevation, observation day, and their interaction. Subject identity was included as a random intercept to account for repeated measurements.

A rater (N.Y.), blind to elevation, used Behavioural Observation Research Interactive Software (BORIS; version 7.0.12; Friard & Gamba, 2016) to score parental behaviours from the first hour of each video recording (5 videos that recorded for less than 60 min were excluded). We defined parental care as the total counts for all egg-directed behaviours (nosing eggs, egg squirting, and egg fanning) and nest maintenance behaviors. Because some behaviours were rare and zero occurrences were common, we used a negative-binomial GLMM to test whether parental care varied with elevation. Elevation was included as a fixed effect, with male SL and brood size (see below) as covariates; we also included the interactions: elevation × male SL and elevation × brood size to assess whether these relationships varied with elevation. Male identity was included as a random intercept to account for repeated measurements.

A second rater, also kept blind to nest elevation, used ImageJ (Schneider et al., 2012) to count the number of eggs present (brood size) in each nest photograph and score developmental stages. Each stage was assigned a numeric score corresponding to the proportion of total development time it represented, ranging from 0 (newly laid egg) to 1 (nearly free-swimming juvenile with no visible yolk sac; see **Chapter 3**). To assess whether development rates varied by elevation in experimental and natural nests, we fit a binomial generalized linear mixed effects model (GLMM) to the development data. The response variable was the most advanced developmental stage present in a nest on a given date; observations were weighted by the number of living eggs of the corresponding developmental cohort in the brood. Fixed effects were intertidal elevation, observation day, and their interaction; the number of days ($\log_{10}$ transformed and then scaled) between the previous and focal observation was entered as a covariate. Brood identity was included as a random intercept to account for repeated measurements.

To determine if egg mortality increased with intertidal elevation, we used a binomial GLMM to carry out a survival analysis on counts of living eggs from the experimental nests (where, because they were covered with mesh, we could be certain new spawnings hadn't occurred between our observations). The response was the proportion of eggs in each brood that had perished since the previous observation. Fixed effects were intertidal elevation, observation day, and their interaction; the number of days ($\log_{10}$ transformed and then scaled) between the previous and focal observation was entered as a covariate. Brood identity was included as a random intercept.

2.4 Results

2.4.1 Abiotic conditions were more severe in nests at higher intertidal elevations

During our 88-day study, the high elevation nests were emersed more frequently (51 days), compared to nests at the middle elevation (31 days), or to nests at the low elevation (15 days; Table 2.1). Subtidal nests were never emersed. Temperature loggers recorded the highest daily average temperatures at nests along the high and middle contours (GAMM, successive differences contrast: high−middle, estimate [est.] ± standard error [SE] = 0.23 ± 0.20 °C, $z = 1.13$, $p = 0.58$; middle−low, est. ± SE = 0.31 ± 0.11 °C, $z = 2.86$, $p = 0.013$; low−subtidal, est. ± SE = 0.96 ± 0.29 °C, $z = 50.72$, $p < 0.001$; Fig. 2.2A). Surprisingly, we did not detect an equivalent pattern in daily temperature fluctuations (standard deviation). The subtidal contour was less variable overall than the intertidal contours, but the intertidal contours were not clearly different (GAMM, successive differences contrast: high–middle, est. ± SE = 0.045 ± 0.18 °C, $z = 0.25$, $p = 0.997$; middle–low, est. ± SE = 0.023 ± 0.062 °C, $z = 0.37$, $p = 0.97$; low–subtidal, est. ± SE = 0.79 ± 0.16 °C, $z = 4.93$, $p < 0.001$; Fig. 2.2B). Daily *maximum* temperatures followed the same pattern as average temperatures notably with elevation. Maximum temperatures were highest in high and middle elevation nests, and much higher in low than subtidal nests (GAMM, successive differences contrast: high–middle, est. ± SE = 0.30 ± 0.68 °C, $z = 0.44$, $p = 0.96$; middle–low, est. ± SE = 0.71 ± 0.23 °C, $z = 3.12$, $p = 0.0054$; low–subtidal, est. ± SE = 1.63 ± 0.60 °C, $z = 25.99$, $p < 0.001$; Fig. 2.2C).

2.4.2 There was more competition for nests in the low intertidal but spawnings did not clearly differ across the elevation gradient

Guarder males readily took up experimental nests along *all* intertidal contours; each of the 60 experimental tile nests laid out initially (20 at each intertidal contour) were occupied by a guarding male within 96 hours of deployment. However, the largest males were not found in middle elevation nests (one-way ANOVA, main effect of elevation: $F_{2,84} = 5.17$, $p = 0.0077$; custom contrast—middle > low & high: $F_{1,84} = 1.04$, $p = 0.31$). Instead, the largest males were found in nests at the low contour (mean ± SD SL: 238 ± 30 mm). Somewhat smaller males occupied nests along the middle (217 ± 34 mm) and high contours (210 ± 29 mm; Tukey's HSD: middle–low, $p_{\text{adj}} = 0.04$; high–low, $p_{\text{adj}} = 0.007$; high–middle, $p_{\text{adj}} = 0.67$; Fig. 2.3A).

However, only some males managed to not only acquire a nest but also attract a female and acquire eggs within this 96-h period. While 95% of males in the middle elevation nests received eggs within the first four days following deployment (19 of the 20 nests), and 75% of the males in high elevation nests received eggs (15 of 20 nests), only 45% of the males in low elevation nests (9 of 20 nests) and 40% (4 of 10 nests) of subtidal nests received eggs in this time frame (GLM, type II analysis of deviance test: elevation, $\chi^2(3) = 17.2$, $p < 0.001$; Fig. 2.3B). Examining data collected from throughout our study duration, experimental nests at the high elevation received an average (median [interquartile range: IQR]) of 99 eggs (79–159), nests in the middle elevation received 87 eggs (59–125) and nests in the low elevation received 128 eggs (61–221). Males in natural nests received many more eggs than those in the experimental nests at every elevation. Natural nests in the high contour received a median (IQR) of 651 eggs (494–1217), nests in the middle received 776 eggs (117–1673), and nests in the low elevation received 1010 eggs (834–1890; GLM, type II Wald chi-squared tests: nest manipulation, $\chi^2(1) = 111.80$, *p*

< 0.001). However, brood size did not differ clearly between elevations once male body size was accounted for (larger males received more eggs; GLM, type II Wald chi-squared tests: elevation, $\chi^2(1) = 1.88$, $p = 0.39$; male SL: $\chi^2(1) = 13.10$, $p < 0.001$; Fig. 2.3D).

2.4.3 Costs of care were similar across the intertidal gradient

Not only were the males nesting lower in the intertidal larger, they also tended to be in better condition compared to males nesting in the middle and high contours (LMM, type III Wald chi-squared test: effect of elevation: $\chi^2(2) = 6.3$, $p = 0.044$; est. ± SE differences in average body condition index scores: high−middle = –0.035 ± 0.033; middle−low = −0.054 ± 0.035). Males persisted in middle and low nests, on average, two weeks longer than males in high nests (median [IQR] number of days on the nest: high = 29 [28–30], middle = 43 [42–45], low = 42 [35–54]; one-way ANCOVA, main effect of elevation: $F_{2,26} = 8.75$, $p = 0.0012$; main effect of initial male mass: $F_{1,26} = 0.23$, $p = 0.63$). Body condition of all guarder males, regardless of intertidal elevation, deteriorated by 4.31 ± 0.51 % (est. ± SE) per day ($N = 30$ males; LMM, type III Wald chi-squared test: effect of time: $\chi^2(1) = 71.6$, $p < 0.001$; Fig. 2.4A). However, we did not detect a clear difference in body condition deterioration rates across intertidal elevations (likelihood ratio test, elevation × time: $\chi^2(2) = 2.21$, $p = 0.33$; est. ± SE differences in body condition deterioration rates: high = −4.49 ± 1.00 %; middle = −4.04 ± 0.77 %; low = −4.39 ± 1.01 %; Fig. 2.4A).

2.4.4 Parental care was most intensive in high elevation nests

Care behaviours were most frequent among males in middle and high intertidal nests (GLMM, successive differences contrasts, low–middle: est. ± SE = −24 ± 10, $t_{36} = –2.47$, p = 0.018; middle–high: est. ± SE = –21 ± 16, $t_{36} = –1.33$, $p = 0.19$). The median (IQR) frequencies of male care behaviours recorded in the 1-h videos were 56 (21–92), 38 (5–46), and 21 (0–35) in the high, middle, and low contour nests, respectively (Fig. 2.4B). Parental care frequency also increased with the number of eggs found in a male's nest and with the size of the parental male (GLMM, type III Wald chi-squared tests, egg number: $\chi^2 (1) = 7.46$, $p = 0.006$; male size: $\chi^2 (1) = 5.24$, $p = 0.022$; Table 2.2B). These relationships between the amount of care males provided and their body and brood sizes were not clearly affected by the intertidal elevation of their nests (GLMM, number of eggs × elevation: $\chi^2 (1) = 2.26$, $p = 0.32$; male size × elevation: $\chi^2 (1) = 2.99$, $p = 0.22$; Table 2.2B).

2.4.5 Egg development was slowest in the subtidal and brood survival was highest in low intertidal nests

Development rates did not vary notably between any of the intertidal contours, but eggs in subtidal nests developed slower than those developing in intertidal nests. Broods in low intertidal nests developed 1.45 ± 0.44 % (est. ± SE) faster per day than broods in subtidal nests, whereas development of broods in nests across the different intertidal contours differed by no more than (est. ± SE) 0.68 ± 0.42 % per day (N = 66 experimental and natural nests; GLMM, successive differences contrasts, high–middle: $z = 0.67$, $p = 0.51$; middle–low: $z = –1.61$, p = 0.11; low–subtidal: $z = 3.29$, $p = 0.001$; Table 2B, Fig. 2.5A). Broods that developed in middle nests suffered a 3.0 ± 0.6 % (est. ± SE) higher likelihood of mortality per day (called the *hazard*) compared to broods in low nests; broods in high nests were exposed to mortality risk similar to those in middle nests, with a 1.7 ± 2.5 % (est. ± SE) difference in the hazard ($N = 55$ experimental nests; successive differences contrasts, high–middle: $z = 0.68$, $p = 0.49$; middle–low: $z = 5.09$, $p < 0.001$; Table 2.2B, Fig. 2.5B).

2.5 Discussion

We detected clear differences in the body sizes and condition of plainfin midshipman guarder males occupying nests at different tidal elevations. Males in the low contour were larger and maintained better body condition compared to males in the high and middle intertidal throughout the study. Males also remained in middle and low nests longer before disappearing than males in high nests (males that disappeared could have either perished or abandoned their nest). Bose et al. (2019a) also observed that the highest intertidal nests were occupied by smaller guarder males. Smaller males are likely forced up by larger males and move into these higher, less profitable nests as a result of competition (Briffa & Sneddon, 2007; Bose et al., 2019a). Nesting sites are a limited resource for these fish (DeMartini, 1991), so guarder males compete intensely for adequate sites, particularly in the early breeding season when nest take-overs and fighting injuries are most common (DeMartini, 1988; Cogliati et al., 2013; Bose et al., 2014). The size-assortative pattern we detected among intertidal elevations strongly suggests that better competitors defend and retain lower nests.

Our primary goal was to investigate why nests at certain intertidal elevations might be more favourable or costly for caring males, and thus experience higher competition. Our first hypothesis was that more extreme environmental conditions higher in the intertidal zone (*i.e.* higher temperatures and longer periods of emersion) would inflict a heavier cost on males nesting there. Daily average temperatures were similar in the high and middle contours, even though the total number of emersion days increased as we expected with intertidal elevation. All intertidal nests experienced similar temperature variability, but subtidal nests had overall lower and more stable temperatures throughout our study. Despite these seemingly harsher abiotic conditions higher in the intertidal, our first hypothesis was not supported—body condition deterioration rates were similar across elevations. This result is surprising for two reasons: 1) larger plainfin midshipman (the fish we found in the low contour) have higher glycolytic capacity in their gills and skeletal muscles and a lower mass-specific metabolic rate, presumably affording increased hypoxia tolerance (LeMoine et al., 2014; Nilsson & Östlund-Nilsson, 2008); and 2) guarder males switch to energetically expensive anaerobic metabolism during emersion, and the levels of anaerobic metabolites increase with emersion time (Bose et al., 2019a; Houpt et al., *in review*). Thus, we expected smaller males higher in the intertidal to suffer higher costs compared to larger males nesting lower in the intertidal. Our timeseries of condition measurements might have been too short to detect a difference in deterioration rates. Plainfin midshipman guarder males are remarkably resilient and physiologically adapted for challenging and variable intertidal conditions (Bose et al., 2019a; Craig et al., 2014; Houpt et al., *in review*), and nesting males will often provide care for 60 or more days (Cogliati et al., 2013). In this study, males in high elevation nests were commonly measured only twice and for a period of less than 30 days, which might not have been long enough to resolve different deterioration rates between elevations. Further, we were unable to capture or retain males in subtidal nests, yet these nests experienced more stable temperatures and were never emersed. Future work is needed to determine whether the lower, more stable water temperatures and lack of emersion translate to lower body condition deterioration rates among males nesting in the subtidal.

It was also surprising that egg development rates, while higher in the intertidal than in the subtidal, varied little between intertidal nests. Higher temperatures generally enhance development of fish eggs and are thought to be among the advantages of intertidal spawning (Martin et al., 2004). Daily *average* and *maximum* temperatures were highest at the high and middle contours; in some high and middle nests, recorded temperatures exceeded 30 and 32 °C, respectively. Thermal conditions in the higher intertidal nests might have occasionally exceeded the thermal optima of plainfin midshipman eggs (Kinne & Kinne, 1962). During extreme temperature events, development of eggs in higher nests could temporarily arrest—a phenomenon called *thermal pejus* (Pörtner & Farrell, 2008), which has been measured across life stages in numerous fishes (Neuheimer et al., 2011; Anderson & Podrabsky, 2014; Di Santo, 2015), but its exact values are presently unknown in plainfin midshipman embryos. Temperatures in high and middle intertidal nests might have occasionally reached pejus, negating any increases in development rates relative to low nests, where temperatures infrequently or never reached pejus.

High elevation nests also had drastically low brood survival—69% (11/16) of broods in these nests perished entirely in less than 34 days. We expected, as per our third hypothesis, that survival would be highest in middle contour nests where the benefit–cost ratio of care would be highest. Yet, brood mortality rates were highest in middle and high nests. Males in these nests were smaller on average compared to males in low nests. Smaller males sometimes provide lower quality or less-frequent parental care (*e.g.* smallmouth bass, *Micropterus dolomieu;* Suski & Ridgway, 2007). However, videos we recorded of parental care behaviours *in situ* provide evidence to the contrary. Males in high and middle nests exhibited a higher number of parental care behaviours over the one-hour observation periods compared to males in low nests. Our results suggest parental care was not energetically constrained within the timeframe of our study. Instead, males driven by competition to a harsher nesting environment seemingly adjust care to meet increased offspring needs as a strategy to maximize their fitness (Bonsall & Klug, 2011; Delia et al., 2013). When the tide returns, suspended sediment is added to the nest, which likely necessitates cleaning to prevent eggs from suffocating (as seen in bromeliad crabs; Diesel, 1992).

Egg mortality in higher nests is caused by the harsher environment rather than by a lower quality of parental care. Plainfin midshipman eggs frequently become infected with an exogenous fungus (Arora, 1948; Alderks & Sisneros, 2013). If fungal growth is enhanced under higher temperatures (Lorenz & Molitoris, 1992) or more frequent and extended emersion, eggs could perish faster in higher nests where males are more frequently emersed and cannot clean them as often. Accordingly, parental care should increase with brood size—more eggs require more cleaning—exactly as we observed.

Taken together, these results indicate that nest site selection affects the benefit–cost ratio of parental care for plainfin midshipman guarder males. Nesting in the low intertidal affords males high offspring survival with relatively low levels of parental care (as seen in longfinned gobies, *Valenciennea longipinnis*; Takegaki, 2002), and these males also maintained better body condition throughout our study. We were unable to accurately measure survival of subtidal broods due to the challenges of meshing submerged nests. However, we determined that broods in subtidal nests had the slowest development rates of all and that these subtidal nests, similar to low intertidal nests, did not receive spawnings as readily as nests higher up in the intertidal. Seasonal shifts in abiotic conditions and associated benefits and costs might explain the discrepancy we observed between the distribution of spawnings in May and the success of nests throughout the season (Edgerly et al., 1998; Shine, 2004). Seasonal variation in nesting site value allows female frogs (*Edalorhina perezi*) to begin depositing their eggs in pools that are initially subject to higher predation risk later in the breeding season when predator density has declined by up to 50% (Murphy, 2003a, 2003b). Similarly, as the season progresses, air and water temperatures increase in the intertidal, emersion lengthens during spring tides, and higher nests become increasingly exposed to terrestrial predation, extreme temperature events, and severe desiccation (Somero, 2002; Tomanek & Helmuth, 2002). In late-July, daily average temperatures recorded in subtidal nests in our study nearly matched those in high intertidal nests two months prior. Thus, the relative value of higher intertidal nesting sites likely decreases as the breeding season progresses, whereas low intertidal and subtidal nests become increasingly favourable.

A seasonal shift toward harsher conditions in the high intertidal could drive condition-dependent nesting strategies among guarder males. Nesting in harsher, higher intertidal sites might preclude males from breeding several times but provide a narrow window of prime breeding opportunity early in the season (Schultz et al., 1991). In the bluegill sunfish (*Lepomis macrochirus*), larger males possess more robust energy stores and can thus endure costly parental care for longer during the breeding season. These males nest relatively early and continue to spawn and rear broods through the entire season to produce as many offspring as possible. In contrast, smaller males cannot endure such a long period of parental care and therefore nest only once at the peak of the breeding season when spawning opportunities are highest (Cargnelli & Neff, 2006). Smaller plainfin midshipman guarder males might exhibit a similar strategy where nesting once in higher nests increases their chances of spawning and rearing a brood early in the season, whereas larger males take up lower intertidal and possibly subtidal nests where they persist for longer and successfully rear more young across the entire season (Rowe et al., 1994; Dickerson et al., 2005; Hennin et al., 2016).

Our study adds to a rich literature on animal nesting behaviour by demonstrating how parents can maximize the benefit–cost ratio of parental care through competing for nesting sites with the most favourable environmental conditions for their offspring. We propose that, by successfully competing for sites lower in the intertidal zone, plainfin midshipman males rear broods with lower mortality but similar offspring development rates compared to males in higher intertidal nests. In these more benign conditions, males can persist longer on the nest, and thereby enjoy higher reproductive success through rearing more offspring to independence. While males in higher intertidal nests were more active care-givers than males in lower nests in our study, this active care can only persist until parental energy reserves are exhausted, at which point a male will either perish, abandon the nest, or cannibalize his young (Clutton-Brock, 1991). Our measurements of males in high elevation nests might have been too short to uncover the expected higher energetic costs of care given this species' arsenal of intertidal adaptations. We suspect that nesting higher in the intertidal does incur higher physiological costs, but further research and longer individual timeseries are needed to confirm this prediction. In sum, male plainfin midshipman fish that nest high in the intertidal suffer increased brood mortality without accruing developmental benefits for their offspring. Nest sites closer to the ocean edge, with less-frequent emersion and less-extreme high temperatures, are retained by the most competitive males and have the highest offspring survival.

2.6 Acknowledgements

We extend our sincerest gratitude to Eileen Carr and family as well as Michael, Dvori and Shelley Balshine for facilitating access to the field site, storing field supplies and the field vehicle. We also thank Aneesh Bose and Jessica Miller for sage advice on the study design and fieldwork logistics, and Hailey Shafer, Simi Dhanota, and Avani Pathak for assistance with field work and laboratory analyses. This work was funded by Natural Sciences and Engineering Research Council of Canada (NSERC) grants to SB and FJ, a Liber Ero Foundation grant to FJ, Animal Behaviour Society, American Museum of Natural History, and Sigma Xi Foundation grants to NB, and an NSERC Undergraduate Student Research Award to NH. Additional funding was provided to NB from the Department of Psychology, Neuroscience, and Behaviour at McMaster University. Our research procedures were approved by the McMaster University Research Ethics Committee (AUP: 18-01-02), and by the University of Victoria Animal Care Committee (AUP: Juanes-2017-003). All protocols employed comply with guidelines set forth by the Canadian Council on Animal Care (Olfert, Cross, & McWilliam, 1993).

2.7 References

Alderks, P.W. & Sisneros, J.A. (2013). Development of the acoustically evoked behavioral response in larval plainfin midshipman fish, *Porichthys notatus*– PLoS One 8: 1–11.

Alonso-Alvarez, C. & Velando, A. (2012). Benefits and costs of parental care– In: Evol. Parent. Care (Royle, N.J., Smiseth, P.T., & Kölliker, M., eds). Oxford University Press, Oxford, p. 356.

Amat, J.A. & Masero, J.A. (2004). Predation risk on incubating adults constrains the choice of thermally favourable nest sites in a plover– Anim. Behav. 67: 293–300.

Anderson, S.N. & Podrabsky, J.E. (2014). The effects of hypoxia and temperature on metabolic aspects of embryonic development in the annual killifish *Austrofundulus limnaeus*– J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 184: 355–370.

Arora, H.L. (1948). Observations on the habits and early life history of the Batrachoid fish, *Porichthys notatus* Girard– Copeia 1948: 89–93.

Baden, A.L. (2018). A description of nesting behaviors, including factors impacting nest site selection, in black-and-white ruffed lemurs (*Varecia variegata*)– Ecol. Evol. 1–19.

Badyaev, A. V. & Ghalambor, C.K. (2013). Evolution of life histories along elevational gradients: trade-off between parental care and fecundity– Ecology 82: 2948–2960.

Bates, D., Mächler, M., Bolker, B.M., & Walker, S. (2015). Fitting linear mixed-effects models using lme4– J. Stat. Softw. 67: 1–48.

Bonsall, M.B. & Klug, H. (2011). The evolution of parental care in stochastic environments– J. Evol. Biol. 24: 645–655.

Bose, A.P.H., Borowiec, B., Scott, G., & Balshine, S. (2019a). Nesting on high: reproductive and physiological consequences of breeding in the intertidal zone– Evol. Ecol. 33: 21–36.

Bose, A.P.H., Lau, M.J., Cogliati, K.M., Neff, B., & Balshine, S. (2019b). Cannibalism of young is related to low paternity and nest take-overs in an intertidal fish– Anim. Behav. 153: 41–48.

Bose, A.P.H., Cogliati, K.M., Howe, H.S., & Balshine, S. (2014). Factors influencing cannibalism in the plainfin midshipman fish– Anim. Behav. 96: 159–166.

Bose, A.P.H., Kou, H.H., & Balshine, S. (2016a). Impacts of direct and indirect paternity cues on paternal care in a singing toadfish– Behav. Ecol. 27: 1507–1514.

Bose, A.P.H., McClelland, G.B., & Balshine, S. (2016b). Cannibalism, competition, and costly care in the plainfin midshipman fish, *Porichthys notatus*– Behav. Ecol. 27: 628–636.

Brantley, R.K. & Bass, A.H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae)– Ethology 96: 213–232.

Briffa, M. & Sneddon, L.U. (2007). Physiological constraints on contest behaviour– Funct. Ecol. 21: 627–637.

Brooks, M.E., Kristensen, K., Darrigo, M.R., Rubim, P., Uriarte, M., Bruna, E., & Bolker, B.M. (2019). Statistical modeling of patterns in annual reproductive rates– Ecology 0: e02706.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., & Bolker, B.M. (2017). Modeling Zero-Inflated Count Data With glmmTMB– bioRxiv 132753.

Buckley, J., Maunder, R.J., Foey, A., Pearce, J., Val, A.L., & Sloman, K.A. (2010). Biparental mucus feeding: a unique example of parental care in an Amazonian cichlid– J. Exp. Biol. 213: 3787–3795.

Burris, Z.P. (2011). Costs of exclusive male parental care in the sea spider *Achelia simplissima*

(Arthropoda: Pycnogonida)– Mar. Biol. 158: 381–390.

Buzatto, B.A., Requena, G.S., Martins, E.G., & Machado, G. (2007). Effects of maternal care on the lifetime reproductive success of females in a neotropical harvestman– J. Anim. Ecol. 76: 937–945.

Cargnelli, L.M. & Neff, B.D. (2006). Condition-dependent nesting in bluegill sunfish *Lepomis macrochirus*– J. Anim. Ecol. 75: 627–633.

Clutton-Brock, T.H. (1991). The Evolution of Parental Care. Princeton University Press, Princeton, New Jersey.

Cogliati, K.M., Balshine, S., & Neff, B.D. (2014). Competition and cuckoldry: estimating fitness of alternative reproductive tactics in plainfin midshipman– Behaviour 151: 1209–1227.

Cogliati, K.M., Neff, B.D., & Balshine, S. (2013). High degree of paternity loss in a species with alternative reproductive tactics– Behav. Ecol. Sociobiol. 67: 399–408.

Craig, P.M., Fitzpatrick, J.L., Walsh, P.J., Wood, C.M., & McClelland, G.B. (2014). Coping with aquatic hypoxia: How the plainfin midshipman (*Porichthys notatus*) tolerates the intertidal zone– Environ. Biol. Fishes 97: 163–172.

D’Alba, L., Monaghan, P., & Nager, R.G. (2009). Thermal benefits of nest shelter for incubating female eiders– J. Therm. Biol. 34: 93–99.

Delia, J.R.J., Ramírez-Bautista, A., & Summers, K. (2013). Parents adjust care in response to weather conditions and egg dehydration in a Neotropical glassfrog– Behav. Ecol. Sociobiol. 67: 557–569.

DeMartini, E.E. (1987). Paternal defence, cannibalism and polygamy: factors influencing the reproductive success of painted greenling (Pices, Hexagrammidae)– Anim. Behav. 35: 1145–1158.

DeMartini, E.E. (1988). Spawning success of the male plainfin midshipman. I. Influences of male body size and area of spawning site– J. Exp. Mar. Bio. Ecol. 121: 177–192.

DeMartini, E.E. (1991). Spawning success of the male plainfin midshipman. II. Substratum as a limiting spatial resource– J. Exp. Mar. Bio. Ecol. 146: 235–251.

Di Santo, V. (2015). Ocean acidification exacerbates the impacts of global warming on embryonic little skate, *Leucoraja erinacea* (Mitchill)– J. Exp. Mar. Bio. Ecol. 463: 72–78.

Dickerson, B.R., Brinck, K.W., Willson, M.F., Bentzen, P., & Quinn, T.P. (2005). Relative importance of salmon body size and arrival time at breeding grounds to reproductive success– Ecology 86: 347–352.

Diesel, R. (1992). Managing the offspring environment: brood care in the bromeliad crab, *Metopaulias depressus*– Behav. Ecol. Sociobiol. 30: 125–134.

Edgerly, J.S., McFarland, M., Morgan, P., & Livdahl, T. (1998). A seasonal shift in egg-laying behaviour in response to cues of future competition in a treehole mosquito– J. Anim. Ecol. 67: 805–818.

Elliott, K.H., Struik, C.L., & Elliott, J.E. (2003). Bald eagles, *Haliaeetus leucocephalus*, feeding on spawning plainfin midshipman, *Porichthys notatus*, at Crescent Beach, British Columbia– Can. Field-Naturalist 117: 601–604.

Fox, J. & Weisberg, S. (2011). An {R} Companion to Applied Regression, Second Edition. Sage, Thousand Oaks, CA.

Friard, O. & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations.– Methods Ecol. Evol. 11: 1325–1330.

Ghalambor, C.K. & Martin, T.E. (2001). Fecundity-survival trade-offs and parental risk-taking in birds– Science 292: 494–497.

Ghalambor, C.K., Peluc, S.I., & Martin, T.E. (2013). Plasticity of parental care under the risk of predation: how much should parents reduce care?– Biol. Lett. 9: 20130154.

Hennin, H.L., Bêty, J., Legagneux, P., Gilchrist, H.G., Williams, T.D., & Love, O.P. (2016). Energetic physiology mediates individual optimization of breeding phenology in a migratory Arctic seabird– Am. Nat. 188: 434–445.

Kiesecker, J.M. & Skelly, D.K. (2000). Choice of oviposition site by gray treefrogs: The role of potential parasitic infection– Ecology 81: 2939–2943.

Kinne, O. & Kinne, E.M. (1962). Rates of development in embryos of a cyprinodont fish exposed to different temperature-salinity-oxygen combinations– Can. J. Zool. 40: 231–253.

Klug, H. & Bonsall, M.B. (2010). Life history and the evolution of parental care– Evolution 64: 823–835.

Komdeur, J. & Kats, R.K.H. (1999). Predation risk affects trade-off between nest guarding and foraging in Seychelles warblers– Behav. Ecol. 10: 648–658.

LeMoine, C.M.R., Bucking, C., Craig, P.M., & Walsh, P.J. (2014). Divergent hypoxia tolerance in adult males and females of the plainfin midshipman (*Porichthys notatus*)– Physiol. Biochem. Zool. 87: 325–333.

Lenth, R. (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package.

Lorenz, R. & Molitoris, H.P. (1992). Combined influence of salinity and temperature (Phoma-pattern) on growth of marine fungi– Can. J. Bot. 70: 2111–2115.

Madsen, T. & Shine, R. (1999). Life history consequences of nest-site variation in tropical pythons (*Liasis fuscus*)– Ecology 80: 989–997.

Martin, K.L.M., Van Winkle, R.C., Drais, J.E., & Lakisic, H. (2004). Beach-spawning fishes, terrestrial eggs, and air breathing– Physiol. Biochem. Zool. Ecol. Evol. Approaches 77:

341–356.

Martin, T.E. (1998). Are microhabitat preferences of coexisting species under selection and adaptive?– Ecology 79: 656–670.

Murphy, P.J. (2003a). Context-dependent reproductive site choice in a Neotropical frog– Behav. Ecol. 14: 626–633.

Murphy, P.J. (2003b). Does reproductive site choice in a neotropical frog mirror variable risks facing offspring ?– Ecol. Monogr. 73: 1–24.

Muth, A. (1980). Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations– Ecology 61: 1335–1343.

Neuheimer, A.B., Thresher, R.E., Lyle, J.M., & Semmens, J.M. (2011). Tolerance limit for fish growth exceeded by warming waters– Nat. Clim. Chang. 1: 110–113.

Nilsson, G.E. & Östlund-Nilsson, S. (2008). Does size matter for hypoxia tolerance in fish?– Biol. Rev. 83: 173–189.

Pörtner, H.O. & Farrell, A.P. (2008). Physiology and climate change– Science 322: 690–692.

R Core Team (2016). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Refsnider, J.M. & Janzen, F.J. (2010). Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice– Annu. Rev. Ecol. Evol. Syst. 41: 39–57.

Rieger, J.F., Binckley, C.A., & Resetarits, W.J.J. (2004). Larval performance and oviposition site preference along a predation gradient– Ecology 85: 2094–2099.

Rowe, L., Ludwig, D., & Schluter, D. (1994). Time, condition, and the seasonal decline of avian clutch size– Am. Nat. 143: 698–722.

Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients–

Methods Ecol. Evol. 1: 103–113.

Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis– Nat. Methods 9: 671–675.

Schultz, E.T., Clifton, L.M., & Warner, R.R. (1991). Energetic constraints and size-based tactics: the adaptive significance of breeding-schedule variation in a marine fish (Embiotocidae: *Micrometrus minimus*)– Am. Nat. 138: 1408–1430.

Seymour, R.S. & Bradford, D.F. (1995). Respiration of amphibian eggs– In: Physiol. Zool., Vol. 68, p. 203–232.

Shine, R. (2004). Seasonal shifts in nest temperature can modify the phenotypes of hatchling lizards, regardless of overall mean incubation temperature– Funct. Ecol. 18: 43–49.

Sisneros, J.A., Alderks, P.W., Leon, K., & Sniffen, B. (2009). Morphometric changes associated with the reproductive cycle and behaviour of the intertidal-nesting, male plainfin midshipman *Porichthys notatus*– J. Fish Biol. 74: 18–36.

Sisneros, J.A., Forlano, P.M., Knapp, R., & Bass, A.H. (2004). Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman– Gen. Comp. Endocrinol. 136: 101–116.

Somero, G.N. (2002). Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living– Integr. Comp. Biol. 42: 780–789.

Spencer, R.J. (2002). Experimentally testing nest site selection: Fitness trade-offs and predation risk in turtles– Ecology 83: 2136–2144.

Strathmann, R.R. & Hess, H.C. (1999). Two designs of marine egg masses and their divergent consequences for oxygen supply and desiccation in air– Am. Zool. 39: 253–260.

Suski, C.D. & Ridgway, M.S. (2007). Climate and body size influence nest survival in a fish with parental care– J. Anim. Ecol. 76: 730–739.

Svensson, I. (1988). Reproductive costs in two sex-role reversed pipefish species (Syngnathidae)– J. Anim. Ecol. 57: 929.

Takegaki, T. (2001). Environmental factors affecting the spawning burrow selection by the gobiid *Valenciennea longipinnis*– J. Fish Biol. 58: 222–229.

Tieleman, B.I., van Noordwijk, H.J., & Williams, J.B. (2008). Nest site selection in a hot desert: trade-off between microclimate and predation risk?– Condor 110: 116–124.

Tomanek, L. & Helmuth, B. (2002). Physiological ecology of rocky intertidal organisms: a synergy of concepts– Integr. Comp. Biol. 42: 771–775.

Ward, R.J.S., Cotter, S.C., & Kilner, R.M. (2009). Current brood size and residual reproductive value predict offspring desertion in the burying beetle *Nicrophorus vespilloides*– Behav. Ecol. 20: 1274–1281.

Wilson, D.S. (1998). Nest-site selection: Microhabitat variation and its effects on the survival of turtle embryos– Ecology 79: 1884–1892.

Wood, S.N. (2017). Generalized additive models. An introduction with R. Chapman and Hall/CRC, New York, NY, 2nd ed.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., & Smith, G.M. (2009). Mixed effects models and extensions in ecology and evolution with R. Springer-Verlag, New York, NY, 1st ed.

Table 2.1 Summary of water quality measures and emersion events in subtidal and intertidal nests between 15 May–27 July 2018. We calculated the number of days each contour was emersed by fitting tide chart data with an arccosine function.

	High		**Middle**		**Low**		**Subtidal**	
	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max
Dissolved oxygen (mg L^{-1})	0.95 (1)	–	1.80 ± 1.64 (15)	0.93–3.18	1.47 ± 0.94 (5)	0.91–3.14	9.05 ± 1.21 (4)	7.39–10.26
pH	7.56 (1)	–	7.73 ± 0.13 (15)	7.46–7.88	7.76 ± 0.15 (5)	7.55–7.90	8.23 ± 0.14 (4)	8.07–8.36
Salinity (ppt)	25 (1)	–	25 ± 2 (16)	20–29	24 ± 1 (5)	22–26	28 ± 2 (4)	26–30
Number of days emersed	57		31		15		0	

Figure captions

Figure 2.1 (A) An experimental nest (foreground) and several others (background) along the low contour emersed during a spring tide in June 2018. (B) A plainfin midshipman guarder male (center) with two spawning (belly-up) females in an overturned natural nest photographed at the middle contour in June 2018. (C) Diagram of the field site. Coloured squares show every *fifth* tile along the high, middle, low, and subtidal contours. High nests were wrapped around the oyster mound corresponding to the contour at +0.0 ft on tidal charts.

Figure 2.2 (A) Daily averages of temperature data collected from digital loggers (see Methods) between 4 May and 27 July 2018. (B) Daily standard deviations of temperature measurements. (C) Maximum daily temperatures. Each dot in all panels represents one daily value from one logger. Lines are fitted to predicted values generated by GAMM smoothing functions; 90% CIs are plotted in light grey around each smooth (see Methods).

Figure 2.3 (A) Initial body size (mm) of males that took up natural or experimental intertidal nests ($N = 89$). (B) Proportion of nests that received eggs at the field site between 15–19 May 2018. Error bars show the 95% binomial CIs. Panel (C) shows the relationships between elevation, male body size, and the number of eggs photographed in experimental and natural nests, respectively. Egg number did not vary with elevation in either nest type once male size was accounted for. Subtidal nests were not included in these analyses because we were unable to capture subtidal males.

Figure 2.4 (A) Change in body condition of males in experimental nests. The solid lines are fitted to LMM predictions; shaded areas around each line correspond to the 90% CI of predictions. We note one outlier in the low elevation (body condition index = 0.54); however, there was no biological reason to exclude this individual, so we kept it in the analysis. (B) Total counts of parental care behaviours exhibited by parental males for 60 minutes immediately following nest immersion during a rising tide.

Figure 2.5 (A) Development rates and (B) brood mortality rates in nests at different tidal elevations. Plotted lines in both panels are fitted to predicted values generated by GLMM's; shaded areas around each line correspond to the 90% CI's. We could not accurately measure brood survival in subtidal nests.

Figure 2.1

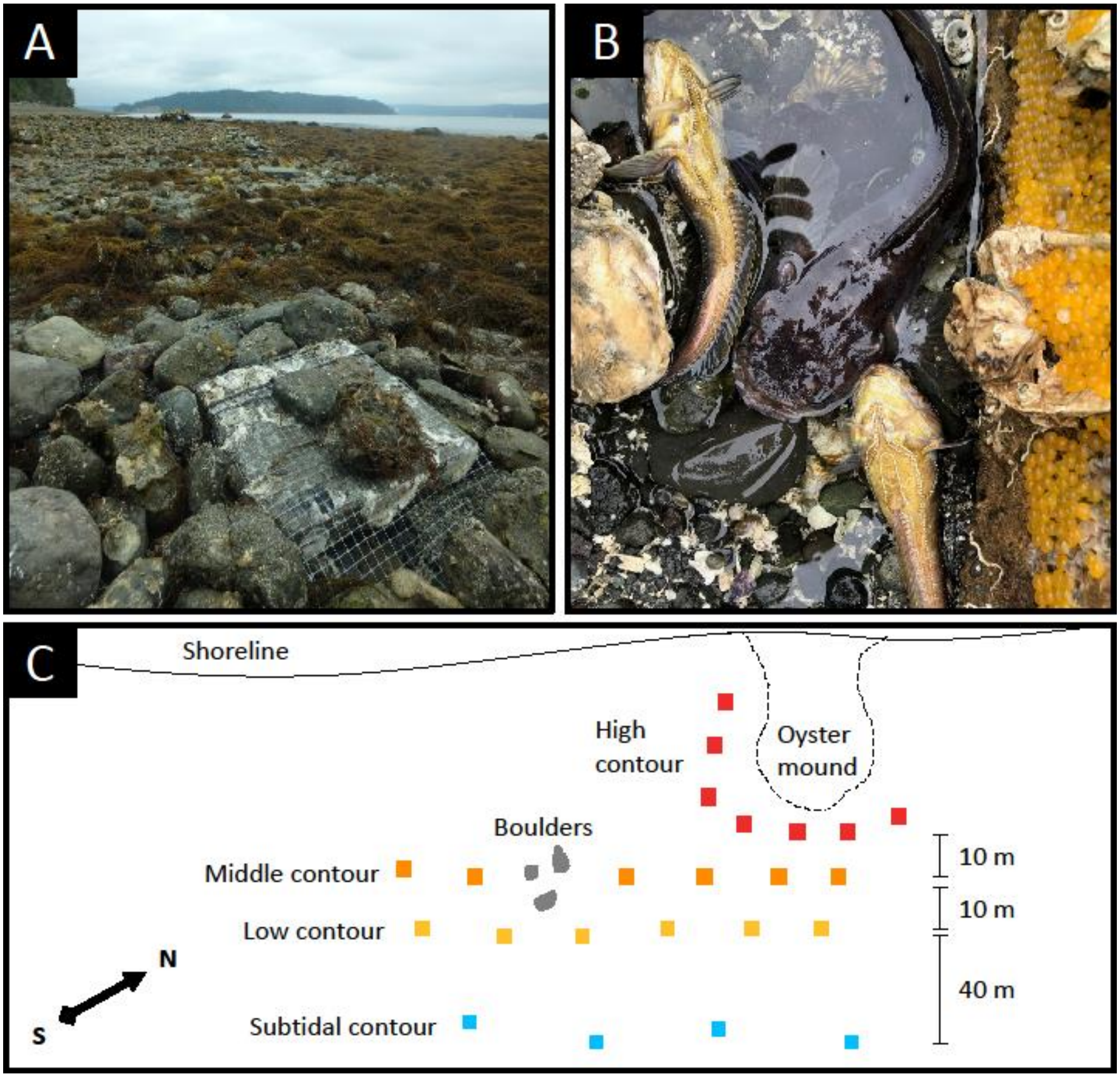

A
B
C
Shoreline
High contour
Oyster mound
Boulders
Middle contour
Low contour
Subtidal contour
N
S
10 m
10 m
40 m

Figure 2.2

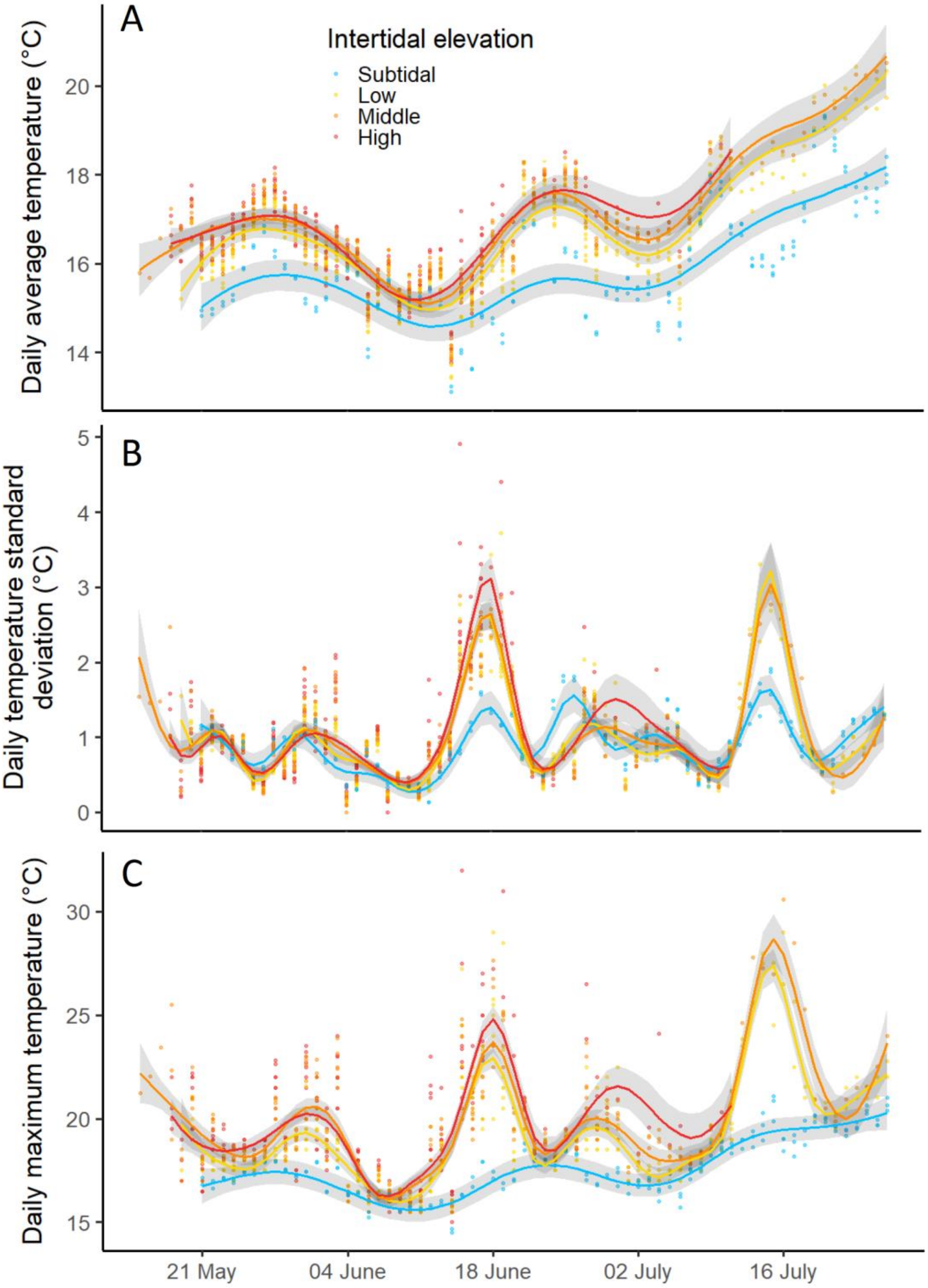

A
Intertidal elevation
Subtidal
Low
Middle
High
Daily average temperature (°C)
14
16
18
20
B
Daily temperature standard deviation (°C)
0
1
2
3
4
5
C
Daily maximum temperature (°C)
15
20
25
30
21 May
04 June
18 June
02 July
16 July

Figure 2.3

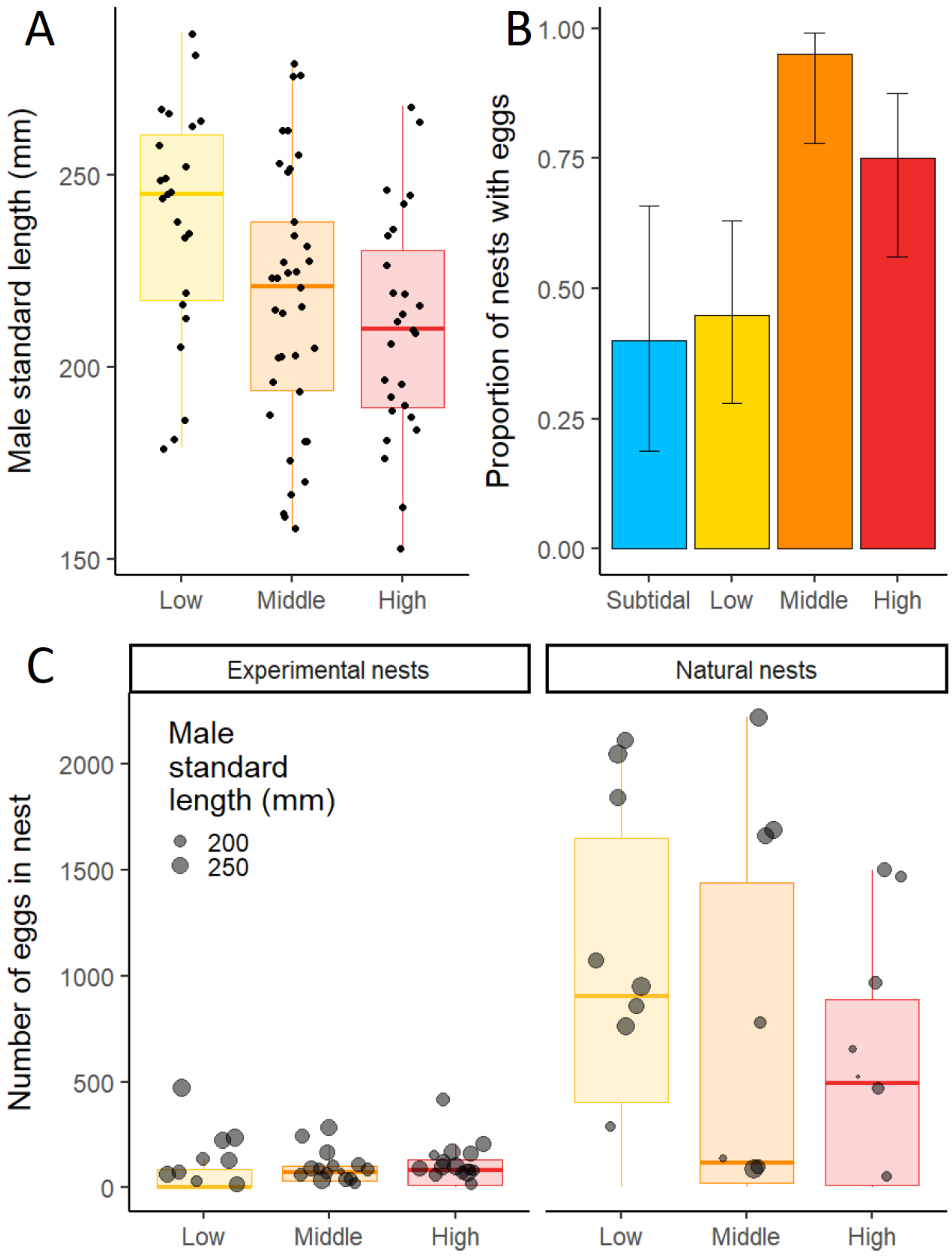

A
Male standard length (mm)
150
200
250
Low
Middle
High
B
Proportion of nests with eggs
0.00
0.25
0.50
0.75
1.00
Subtidal
Low
Middle
High
C
Experimental nests
Natural nests
Number of eggs in nest
0
500
1000
1500
2000
Male standard length (mm)
200
250
Low
Middle
High
Low
Middle
High

Figure 2.4

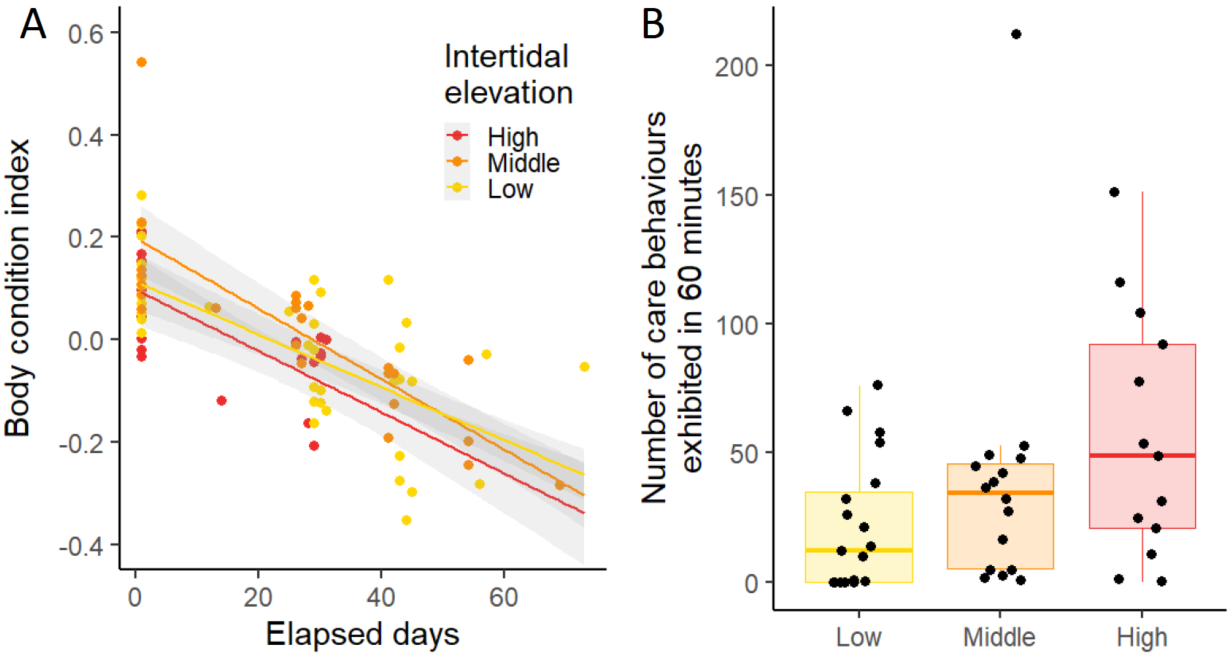
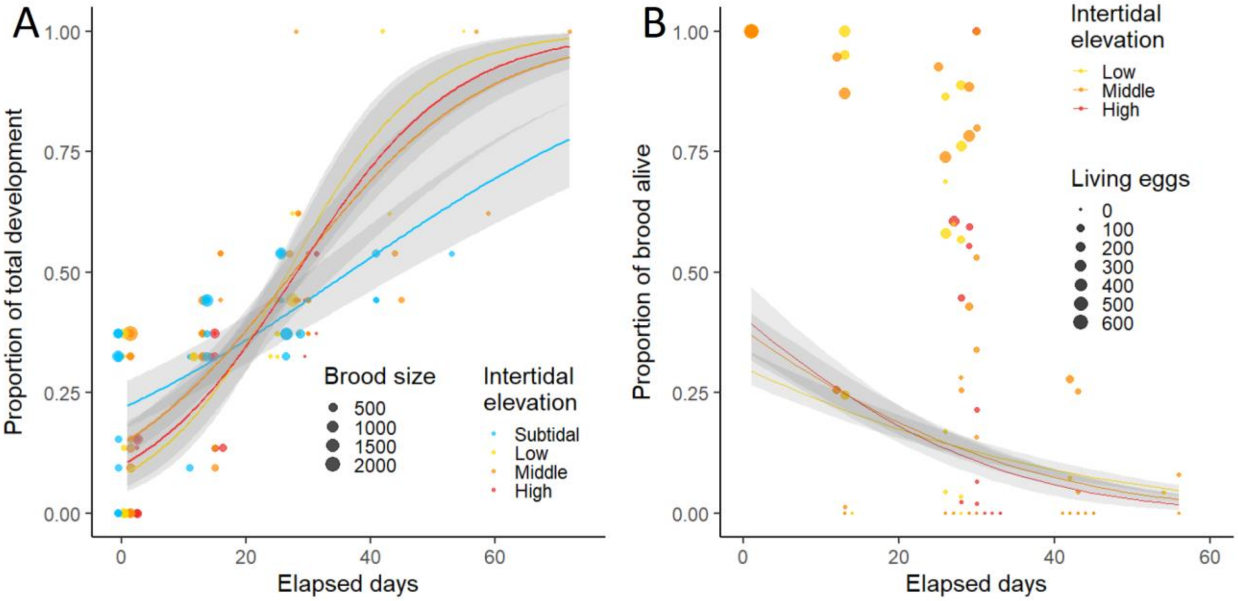

A
Body condition index
0.6
0.4
0.2
0.0
-0.2
-0.4
0
20
40
60
Elapsed days
Intertidal elevation
High
Middle
Low
B
Number of care behaviours exhibited in 60 minutes
200
150
100
50
0
Low
Middle
High

Figure 2.5


A
Proportion of total development
1.00
0.75
0.50
0.25
0.00
Elapsed days
0
20
40
60
Brood size
500
1000
1500
2000
Intertidal elevation
Subtidal
Low
Middle
High
B
Proportion of brood alive
1.00
0.75
0.50
0.25
0.00
Elapsed days
0
20
40
60
Intertidal elevation
Low
Middle
High
Living eggs
0
100
200
300
400
500
600

Chapter 3: Abiotic conditions and maternal traits enhance offspring development, survival, and behaviour in a toadfish

Nicholas A. W. Brown, Hailey Shafer, Francis Juanes, Sigal Balshine

In preparation for submission to an as-yet undetermined peer-reviewed journal

3.1 Abstract

Experiencing good conditions early in life can have long term fitness benefits for offspring. In many species, parents have evolved physiological and behavioural strategies that improve the conditions their offspring experience. However, few studies have investigated whether the payoffs of different behavioural strategies depend on the physical state (body condition) of the parent. Here, we examined whether female plainfin midshipman fish (*Porichthys notatus*) can enhance their reproductive success by ovipositing in locations with warmer temperatures and periodic emersion or allocating resources to egg size over egg number, and whether these strategies depend on maternal body condition. Specifically, we examined how offspring survival, development, and behaviour are affected by water temperature and emersion, two abiotic conditions that vary notably across the intertidal zone where these fish spawn and nest in the wild. We further investigated whether the benefits of different water temperatures and emersion durations depend on maternal condition. Young incubated in warmer water developed faster (especially those laid by mothers in good condition) and exhibited faster swimming escape speeds. Experiencing a 4 h daily emersion attenuated temperature's effect on development times and increased brood mortality rates. Young laid by mothers in relatively poor body condition survived in much higher proportions when incubated in warm rather than cold water. These results suggest that ovipositing in nests higher in the intertidal zone, where incubation temperatures are warmer and emersion is longer and more frequent, could allow mothers in poor body condition to enhance the survival and development rates of their young.

3.2 Introduction

A good start in life can go a long way. Being born in strong physical condition and/or developing in a favourable environment with many resources (*i.e.* receiving the "silver spoon") increase an individual's likelihood of survival and future reproductive success (Lindström, 1999; Ferrière et al., 2004; Monaghan, 2008). Parents, especially mothers, can control the degree to which these advantages are available to their offspring (Bernardo, 1996; Mousseau & Fox, 1998; Badyaev & Uller, 2009). Environmental conditions, including resource abundance/depletion, competition, or even a mother's own body condition can induce mothers to alter their relative investments in offspring number, size, or degree of provisioning (Bernardo, 1996; Mousseau & Fox, 1998). In some cases, mothers will predispose their offspring to better match an anticipated future environment (Marshall & Uller, 2007; Monaghan, 2008; Burgess & Marshall, 2014). For example, red squirrel mothers (*Tamiasciurus hudsonicus*) that experience cues of high population density exhibit elevated glucocorticoid levels, which stimulate enhanced offspring growth rates (Dantzer et al., 2013). However, it is not always in a mother's best interest to maximize offspring fitness; selection acts first on mothers and thereby maximizes maternal reproductive success over offspring fitness (Marshall & Uller, 2007). Selection can thereby favour mothers that produce more but smaller (less fit) offspring (Einum & Fleming, 2000), and induces a trade-off for most mothers between investment in their current versus future progeny (Stearns, 1992; Wootton & Smith, 2015). When environmental conditions weaken maternal condition and/or physiology, mothers might diminish their investment in the current progeny—resulting in offspring with suboptimal phenotypes—to save energy for future reproduction (Hanssen et al., 2005; Marshall & Uller, 2007; McCormick, 2006).

Mothers can also employ behavioural strategies that enhance phenotypes and survivorship of their young (Bernardo, 1996). For example, viviparous female skinks (*Niveoscincus ocellatus*) can spend more time basking to produce larger offspring and more females (Wapstra, 2000; Wapstra et al., 2004). A more prevalent behavioural strategy is for mothers to deposit their eggs in certain locations—a phenomenon known as *oviposition site selection* that ensures beneficial environmental conditions are present during development (Resetarits, 1996; Refsnider & Janzen, 2010). Favourable sites might offer optimal temperatures or oxygen-rich conditions, fewer parasites, or close proximity to suitable juvenile habitat, which can profoundly affect offspring fitness (Refsnider & Janzen, 2010). However, despite numerous studies that demonstrate remarkable individual maternal effects, few have investigated how mothers might integrate behavioural strategies with their physical state to alter offspring phenotypes and maximize their fitness. This is surprising because mothers often exhibit several mechanisms that, combined, maximize the number of their offspring that survive to reproduce (Ratikainen & Kokko, 2010). The aim of this study was to determine how offspring phenotypes and survival vary between rearing environments and with maternal allocation between offspring number and quality.

Marine fishes have proven useful study organisms to explore maternal allocation because of their notable interspecific and interindividual variation in egg and clutch sizes (*e.g.* Einum & Fleming, 2000, 2002; McCormick, 2006). Further, oviposition site selection appears to be an important determinant of offspring survival across fishes (Wootton & Smith, 2015). Therefore, we studied the plainfin midshipman (*Porichthys notatus*), a marine toadfish with considerable intraspecific egg and clutch size variation (DeMartini, 1990) that lays eggs and provides parental care in nesting sites spanning the shallow subtidal to the high intertidal zone.

Male plainfin midshipman provide sole parental care to broods of young in their nests for up to 3 months (Arora, 1948; Brantley & Bass, 1994; Cogliati et al., 2013). Females control resource allocation when producing broods of 200+ eggs per year, and decide where to lay these eggs, typically favouring larger nests with larger males (DeMartini, 1988, 1990, 1991; Bose et al., 2018). Thus, early in life, offspring survival in the plainfin midshipman is strongly affected by both fathers (that care for eggs) and mothers (that produce and chose where to lay their eggs). Males and their nests (oviposition sites) in the rocky intertidal zone span a pronounced environmental gradient where temperatures and emersion (air exposure) increase in severity with elevation above the low water line (Doty, 1946; Somero, 2002). Nests at extremes of the intertidal zone can differ by up to 11 °C and 5 h of emersion during spring tidal cycles in the summer breeding season (**Chapter 2**; Bose et al., 2019). Eggs develop faster in intertidal nests than subtidal nests, but broods in the highest nests experience higher mortality rates and temperatures in the highest nests might occasionally exceed the thermal optima of eggs (**Chapter 2**). However, whether females exhibit oviposition preferences with respect to intertidal elevation remains unknown.

Depending on their intrinsic quality and feeding conditions experienced at sea leading up to the breeding season, gravid females reach the intertidal zone in a range of body condition, and as a result vary in resource allocation among their eggs (DeMartini, 1990). Females that make higher per-offspring investments typically produce larger young that enjoy higher survival, but offspring survival can also depend on abiotic conditions like temperature. In Ambon damselfish (*Pomacentrus amboinensis*) eggs with greater yolk sac reserves enjoy higher survival in high (31 °C) but not ambient (29 °C) or low (25 °C) water temperatures (Gagliano et al., 2007a). If the benefits of emerging from a relatively well or poorly provisioned egg vary between rearing environments in plainfin midshipman, mothers might gain reproductive benefits by targeting oviposition sites that best suit the condition of their offspring. Females could accomplish this goal by targeting nests at certain intertidal elevations.

Our aim was to investigate how survival, development, and behaviour of plainfin midshipman young are affected by their rearing environment (specifically, water temperature and emersion). We also investigated maternal allocation between egg size and number, and whether the benefits of different rearing environments could vary according to maternal body condition (Bonduriansky & Crean, 2018). We subjected eggs laid by females of varying body condition to different combinations of water temperatures and emersion presence or absence in the laboratory to simulate features of their natural nesting microhabitats. Our study investigated three questions: 1) how do water temperature and emersion affect offspring development rates, survival, and physical performance? 2) how do development rates and survival vary between brood or egg sizes, or with maternal body condition? and 3) how do the benefits of water temperature or emersion on offspring development and survival vary according to maternal body condition?

3.3 Methods

3.3.1 Animal collection, housing, and mating conditions

Nest-guarding males and ripe females for this study were collected by overturning large rocks during low spring tides between 2 May and 2 June 2018 at four sites in southern British Columbia, Canada: Ladysmith Inlet (49°01′N, 123°83′W), Lantzville (49°15'N, 124°04'W), Bowser Tidal Lagoons (49°27'N, 124°41'W), and Crescent Beach (49°04'N, 122°88'W). Fish were transported in sex-specific bins filled with aerated seawater to the University of Victoria's Outdoor Aquatic Unit where they were transferred to sex-specific 400-L cylindrical outdoor holding tanks (maximum of 8 fish per tank) supplied with free-flowing, ambient temperature (*c.* 13 ºC) seawater and shelters.

Males from holding tanks were then placed individually in aerated 175-L glass 'spawning' aquaria lined with coarse gravel and supplied with free-flowing, ambient temperature seawater. Each spawning tank contained an artificial nest made of bricks (see Fig. 3.1A). Males were given three days to discover and excavate a nest; if a male failed to take up a nest within three days, he was swapped for a new male from the holding tanks. Once a male had taken up a nest, a ripe female from the holding tanks was weighed (± 0.01 g), measured (standard length [SL] ± 1 mm), and then added to his spawning tank. Like males, females were given up to three days to spawn with a partner; if no spawning occurred, the female was moved into another male's tank. Females were given the opportunity to spawn with up to three different males before being permanently excluded from the study. Each tank was checked daily for spawning. When eggs were detected, both the male and female were immediately removed and measured again. All the eggs were used in Experiment 1 while free-swimming juveniles were used in Experiments 2–4 (see below)

3.3.2 Ethical note

All research practices complied with guidelines set forth by the Canadian Council on Animal Care and the Animal Behaviour Society (Olfert et al., 1993; Buchanan et al., 2012). Animals were collected in accordance with the Fisheries and Oceans Canada guidelines (Scientific license: XR 48 2018). These experiments were conducted under McMaster University animal utilization protocol (AUP): 18-01-02, and under the University of Victoria AUP: Juanes-2018-003.

3.3.3 Experiment 1: How do rearing conditions affect egg and larval development rates and survival??

3.3.3.1 Methods

Once a spawning occurred, bricks constituting the roof of the nesting cavity were immediately separated such that bricks with eggs of the same brood were placed into different rearing treatments. Each brick with eggs was photographed alongside a ruler and then randomly assigned to one of four rearing environments: 1) cold seawater (ambient temperature—13.5 ± 0.5 ºC) without any prolonged daily emersion; 2) cold seawater (13.5 ± 0.5 ºC) with a 4-h daily emersion; 3) warm seawater (18.0 ± 2.0 ºC) without any prolonged daily emersion and 4) warm seawater (18.5 ± 1.5 ºC) with a 4-h daily emersion. Each rearing environment was replicated in two continuously aerated tanks (tank dimensions were identical to spawning tanks) supplied with free-flowing seawater and were heated by two 250-W aquarium heaters (EHEIM GmbH & Co. KG) in the warm seawater treatment tanks only. Bricks containing young in the emersion treatments were removed and kept out of water for a 4 h daily period (mean ± SD air temperature = 18.4 ± 1.3 °C; range: 15.4–22.7 °C) so that the young would experience oxygenic conditions like those encountered during a low tide. During emersion, bricks were covered (to avoid light damage) and gently misted with seawater every 1 h (to prevent desiccation). All eggs attached to the bricks were inspected daily so we could record the day when each brood reached any of 10 standardized developmental stages (see Table 3.1). Dead eggs were counted and then removed carefully with tweezers. Bricks were photographed every three days until the day the young reached the free-swimming juvenile stage and detached from the bricks, at which point the free-swimming young were used for Experiments 2–4 (see below). An enumerator, blind to treatment group, counted the number of eggs and attached larvae in each photo. The enumerator also measured diameters (± 1 mm) of 20 randomly selected eggs from their initial (day 1) photo. A second enumerator measured SL (± 1 mm) of up to 10 juveniles on each brick as each cohort reached the free-swimming developmental stage (Table 3.1).

3.3.3.2 *Statistical analyses*

All statistical analyses in the present and subsequent experiments were carried out in R (version 3.6.0; R Core Team, 2016) using the ‘lme4’ package (Bates et al., 2015). To standardize effect sizes, continuous covariates in all models were scaled by their standard deviations (Schielzeth, 2010). Rearing tank identity was included as a random intercept in all models to account for pseudo-replication. Female body condition has well-known effects on fecundity and the offspring size–number trade-off (Kamler, 2005; Wootton & Smith, 2015; Bonduriansky & Crean, 2018). For each female, we calculated her fecundity (number of eggs laid), average egg diameter (mm; averaged across all measured eggs that were laid by the same female), and relative somatic condition (RSC; see calculation in DeMartini, 1990). First, we investigated the influence of these factors on development rates in young by fitting a linear mixed effects model

(LMM) to total development time (days until young became free-swimming; square-root transformed). Fixed factors were water temperature, emersion, female RSC and their interaction; average egg diameter and fecundity were included as covariates. Observations were weighted by the initial number of eggs on each brick, and female identity was entered as a random intercept.

Second, we investigated how rearing conditions and maternal body condition influenced survival to the independent free-swimming stage of life by fitting a binomial GLMM to the count data (converted to proportion of initial egg number alive) obtained from photos taken 1–2 days before juveniles on each brick first became free-swimming. Fixed factors were water temperature, emersion regime, female RSC, and their interaction; initial egg diameter and fecundity were included as covariates. Female identity and rearing tank were entered as random intercepts.

To investigate how rearing conditions and maternal body condition affected the body sizes (SL) of juveniles (the day they reached the free-swimming stage) we fit an LMM to the body size data. Fixed factors were water temperature, emersion regime, female RSC, and their interaction; initial egg diameter and fecundity were included as covariates. Female and replicate identity were entered as random intercepts.

3.3.3.3 *Results*

Young exposed to warm seawater (*c.* 18°C) and brief emersion developed fastest, with an average (median) full development time of 37 days (inter-quartile range [IQR]: 35–38 days). A somewhat slower average full development time of 40 days (IQR: 40–42 days) was observed among broods exposed to warm seawater with a 4-h daily emersion. Young in cold seawater (*c.* 13°C) developed much more slowly—those that experienced a 4-h daily emersion, on average, reached the end of development by 105 days (IQR: 102–107 days) while young developing without emersion took 108 days (IQR: 106–112 days; Fig 3.2.; LMM, type III Wald chi-squared tests; water temperature: $\chi^2(1) = 7916.30$, $P < 0.001$; emersion: $\chi^2(1) = 4.76$, $P = 0.03$; water temperature × emersion: $\chi^2(1) = 17.90$, $P < 0.001$). This cold water development time is considerably longer, and the warm water development time is considerably shorter, than the estimated development times and care duration in field studies (approximately 60 days; Cogliati et al., 2013; **Chapter 2**). The impacts of rearing conditions on brood survival to free-swimming and juvenile body size depended on maternal body condition (see below).

3.3.3.4 *Effects of maternal body condition and allocation*

Initial diameters of eggs in our experiment ranged widely from 5.4–9.9 mm, and brood sizes ranged from 19–230 eggs. Larger females produced larger eggs but not significantly more eggs. For every (estimate [est.] ± SE) 0.6 ± 1.3 mm increase in female standard length we saw a 20-egg increase in fecundity; whereas for every (est. ± SE) 16 ± 3 mm increase in female standard length we saw a 1-mm egg size increase (OLS regression on female SL; average egg diameter: $R^2_{\text{adj}} = 0.43$ $P < 0.001$; fecundity: $R^2_{\text{adj}} = 0.003$, $P = 0.67$). Females in better body condition produced more eggs but there was not a clear effect of body condition on egg size. For every (est. ± SE) 1.6 ± 0.6 % increase in female somatic condition we saw a 20-egg increase in fecundity; whereas for every (est. ± SE) 2.5 ± 1.6 % increase in female somatic condition we saw a 1-mm egg size increase (OLS regression on female RSC average egg diameter: $R^2_{\text{adj}} = 0.06$, $P = 0.11$; fecundity: $R^2_{\text{adj}} = 0.18$, $P = 0.006$).

Mothers that laid more eggs produced offspring that developed slower, but, surprisingly, egg size did not clearly affect total development times (LMM, type III Wald chi-squared tests;

increase in development time with 20-egg increase in fecundity: est. ± SE = 0.9 ± 0.3 days; $\chi^2(1) = 9.54$, $P = 0.002$; increase in development time with 1-mm increase in initial egg diameter: est. ± SE = 0.5 ± 0.6, $\chi^2(1) = 0.95$, $P = 0.33$). Mothers in better body condition produced young that developed faster, but this effect was only present for young reared in warm water; when offspring developed in cold water, maternal condition did not clearly affect development (effect of 1-SD increase in female RSC in cold water: est. ± SE = −0.0 ± 0.6 days; in warm water: est. ± SE = −1.3 ± 0.6 days; LMM, type III Wald chi-squared tests: water temperature × female RSC, $\chi^2(1) = 5.29$, $P = 0.021$).

The proportion of young that survived to the free-swimming stage was higher among broods laid by females with higher body condition scores but only when young were exposed to cold water; survival decreased with maternal body condition when young were exposed to warm water (increase in probability of mortality—called the *hazard*—per 1-SD increase in female RSC in cold water: est. ± SE = −98 ± 541 %; in warm water: est. ± SE = 517 ± 652 %; GLMM, type III Wald chi-squared test; water temperature × female RSC: $\chi^2(1) = 20.34$, $P < 0.001$; Fig. 3.3A). Survival was not clearly related to egg size nor to the number of eggs a mother laid, after accounting for maternal body condition (increase in hazard per 1-mm increase in initial egg size: est. ± SE = 4 ± 14 %; per 20-egg increase in fecundity: est. ± SE = −4 ± 7 %; GLMM, type III Wald chi-squared tests; initial egg diameter: $\chi^2(1) = 0.11$, $P = 0.75$; fecundity: $\chi^2(1) = 0.32$, $P = 0.57$)

Body sizes (SL) of free-swimming juveniles in our study ranged from 15.6–24.3 mm and increased (est. ± SE) 1.4 ± 0.3 mm, on average, with every 1 mm increase in their initial egg size; however, juvenile body size was not clearly related to maternal fecundity (est. ± SE = 0.1 ± 0.1 mm per 20-egg increase in fecundity; LMM, type III Wald chi-squared tests; initial egg diameter: $\chi^2(1) = 20.53$, $P < 0.001$; fecundity: $\chi^2(1) = 0.40$, $P = 0.53$). Body size of juveniles increased with maternal body condition under cold water and without prolonged emersion and in warm water with a 4-h daily emersion, but juvenile body size decreased with maternal body condition under cold water with a 4-h daily emersion and under warm water without prolonged emersion (increase in juvenile body length with 1-SD increase in female RSC—cold water without emersion: est. ± SE = 3.7 ± 7.6 mm; cold water with 4-h daily emersion: est. ± SE = −7.5 ± 5.9 mm; warm water without emersion: est. ± SE = −7.5 ± 7.6 mm; warm water with 4-h daily emersion: est. ± SE = 7.1 ± 5.0 mm; LMM, type III Wald chi-squared test; water temperature × emersion × female RSC: $\chi^2(1) = 5.10$, $P = 0.024$; Fig. 3.3B).

3.3.4 Experiment 2: How do rearing conditions affect juvenile survival, size and behaviour?

3.3.4.1 Methods

When juveniles finished detaching from bricks, we began daily counts of free-swimming juveniles and of any dead juveniles in each rearing tank. These numbers allowed us to compare mortality rates across rearing environments. Juveniles in all the rearing tanks were fed 2 mL of SELCO-enriched live adult brine shrimp daily on a per fish basis supplemented with marine amphipods (*Hyale sp.*) collected from nearby Cattle Point (48°26'17"N, 123°17'32"W).

To obtain direct behavioural performance measures in these juveniles (boldness, activity, and response to risk), we carried out behavioural assays on the juveniles as they reached three months of age. Unfortunately, too few juveniles remained at this stage from each emersion treatment to investigate the effect of air exposure on behaviour, so instead we compared performance of juveniles only between the two temperature treatments. Each subject was tested twice with three days between tests. Tests were carried out in a small (10-L) glass aquarium filled with seawater (6 cm depth) from the focal subject's source tank that also contained a 6-cm diameter cylindrical start chamber capped at both ends that opened into a 15-cm diameter circular test arena, which was divided into four equal quadrants (Fig. 3.1B).

To assess boldness, each subject was placed into the start chamber and allowed five minutes to acclimate. Then, a trap door to the start chamber opened and the subject was allowed eight minutes to emerge and swim out of the chamber. We defined boldness as the time it took for the subject's snout to clear the exit of the start chamber (Brown et al., 2005). If a fish did not emerge from the start chamber, it was given the maximum boldness score of eight minutes (Brown et al., 2005). Fish that did not emerge were then gently guided out by advancing the closed end of the start chamber forward through the tube (Fig. 3.1B). Following the boldness assay, each subject was given a 30-minute activity trial where it could move freely around the open arena. We recorded 1) the duration of time spent swimming in the first 3 minutes, and then again for 10 seconds at 10, 20, and 30 min mark into the trial; and 2) the total number quadrant line crosses by each fish (Fig. 3.1B). Finally, fish were tested for their fast-start swimming response. In this assay, one experimenter (H.S.) used a glass rod to tap the focal fish's tail, causing the fish to elicit a *fast-start* (Domenici & Blake, 1997). We obtained burst swimming velocities from this assay (only if fish performed a "C-start;" Domenici & Blake, 1997; Fig. 3.1C) by dividing the total distance (mm) covered by the fish during the first two axial bends of its tail (critical for avoiding predator attacks; Webb, 1976) by unit time (s) (Domenici & Blake, 1997). Assays were always conducted in the same order—each subject was tested for boldness first, activity second, and fast-start swimming performance third. After all behavioural assays were completed, each subject was measured for body mass (± 0.01 g) and SL (± 0.1 mm).

3.3.4.2 Statistical analyses

We used a binomial GLMM to investigate how mortality rates of juveniles varied across the four rearing environments. The response variable was the number of juveniles in each rearing tank that had died since the previous observation. Fixed effects were water temperature, emersion (only experienced while young were still attached to bricks), time (number of days since juveniles finished detaching in each rearing tank), and their interaction. The number of days between observations (scaled and $\log_{10}$-transformed) was included as a covariate to account for exposure times. Rearing tank was entered as a random intercept.

A rater, blind to treatment group, used the "Tracker" software (version 5.1; D. Brown & Cox, 2009) to quantify all behavioural measures from the videos. In all models for the behavioural assays, water temperature, and test number (1 or 2) were the only fixed factors; subject SL was entered as a covariate and subject identity as a random intercept. Duration swimming and boldness were both converted to proportions of total observation time and modeled with binomial GLMMs. Quadrant crosses were modeled with a negative binomial GLMM to account for frequent zero scores. Burst swimming velocities met normality assumptions and were thus modeled using an LMM.

3.3.4.3 Results

The hazard was 3.4 ± 1.3 % higher (est. ± SE) for juveniles in warm water compared to cold water, and 2.7 ± 1.3 % higher for juveniles that experienced a 4-h daily emersion during their development compared to those that were never emersed, regardless of water temperature (GLMM, type III Wald chi-squared tests; water temperature × time: $\chi^2(1) = 6.89$, $P = 0.0087$;

emersion × time: $\chi^2(1) = 4.12$, $P = 0.042$; water temperature × emersion × time: $\chi^2(1) = 0.0098$, $P = 0.92$).

In the physical assays, fish took, on average, 5 minutes to leave the start box and begin exploring. Boldness was not clearly influenced by any of the predictor variables; however, juveniles took (est. ± SE) 144 ± 65 seconds longer to exit the start box during their second test (GLMM, likelihood ratio tests [LRT]; water temperature: LRT = 2.83, *df* = 1, $P = 0.09$; body size: LRT = 1.66, *df* = 1, $P = 0.20$; test number: LRT = 4.46, *df* = 1, $P = 0.03$). Time spent swimming in the open arena increased (est. ± SE) 20 ± 16 seconds (14 ± 11 % of the total observation time) with each 1-mm increase in a fish's standard length (GLMM, LRT: body size: LRT = 8.20, *df* = 1, $P = 0.0053$). Fish reared in cold water spent a greater proportion of time swimming in the open arena (for their body sizes) than those that developed in warm water (warm water: est. ± SE = 24 ± 12 %; cold water: est. ± SE = 27 ± 10%; GLMM, LRTs; water temperature: LRT = 3.35, *df* = 1, $P = 0.045$; test number: LRT = 3.32, *df* = 1, $P = 0.070$). Our second activity measure, the number of quadrant line crosses, did not clearly differ between juveniles raised in either water temperature treatment, but fish exhibited (est. ± SE) 1.7 ± 0.3 fewer line crosses during their second trial, likely due to habituation (GLMM, LRTs; water temperature: LRT = 0.087, *df* = 1, $P = 0.89$; body size: LRT = 5.7, *df* = 1, $P = 0.042$; test number: LRT = 9.09, *df* = 1, $P = 0.0022$). In the fast-start swimming response assay, juveniles reared in warm water displayed slightly faster burst swimming speeds than those reared in cold water (warm water: est. ± SE = 290 ± 30 mm s^{-1}; cold water: est. ± SE = 200 ± 27 mm s^{-1}; LMM, water temperature: LRT = 9.21, *df* = 1, $P = 0.053$; body size: LRT = 1.63, *df* = 1, $P = 0.20$; test number: LRT = 0.29, *df* = 1, $P = 0.59$).

3.3.5 Experiment 3: How do rearing conditions influence juvenile habitat preferences (Part I)?

3.3.5.1 *Methods*

We tested habitat choices of free-swimming juveniles reared under different conditions (see Experiments 1 & 2, above) by dividing the rearing tanks into four quadrants, each with its own substrate/habitat type. Each tank had gravel (10–20 mm depth) on one side of the tank and fine sand (10–20 mm depth) on the other. These tank halves were then further subdivided lengthwise, such that each was half covered by artificial seagrass (made of green plastic tarpaulin cut into 470 × 15 mm [H × W] strips; Kenyon et al., 1999). Thus, tanks were partitioned into five possible habitats: sand with seagrass, gravel with seagrass, bare sand, bare gravel, and the water column. Each day (for a minimum of 25 days) we observed and counted all free-swimming juveniles and their location and substrate association in each tank. Any juvenile > 5 cm above the substrate or seagrass was included in the water column counts.

3.3.5.2 *Statistical Analysis*

We fit a Dirichlet regression model to the juvenile habitat counts (Hijazi & Jernigan, 2009), which were summed across the two tanks representing each rearing environment. The response variable was the proportion of juveniles that were counted in each of the five possible habitats on one day. Fixed factors were water temperature, emersion, days since reaching free-swimming (independence), and all interactions. Observations were weighted by the total number of juveniles that were counted.

3.3.5.3 *Results*

Habitat choices of juveniles depended on their rearing environment. Overall, juveniles aggregated more in habitats with seagrass than on bare substrates (mean ± SD proportion of counted juveniles in habitat: gravel or sand with seagrass = 49 ± 15 %, bare gravel or sand = 0.36 ± 14 %). Juveniles exposed to cold seawater tended to aggregate more in seagrass on gravel than those raised in warm water (mean ± SD proportion of counted juveniles in gravel with seagrass habitat: cold water development = 24 ± 16 % versus warm water development = 12 ± 7 %). Juveniles exposed to warm water more strongly preferred seagrass on sand versus seagrass on gravel (mean ± SD proportion of counted juveniles in sand with seagrass habitat: cold water development = 27 ± 9 % versus warm water development = 36 ± 16 %; Dirichlet regression, LRTs of highest order interaction term—water temperature × emersion × time—for each habitat type; gravel: LRT = 496.3, $df = 1$, $P < 0.001$; gravel and seagrass: LRT = 2106.5, $df = 1$, $P < 0.001$; sand: LRT = 740.2, $df = 1$, $P < 0.001$; sand and seagrass: LRT = 834.4, $df = 1$, $P < 0.001$; water column: LRT = 473.8, $df = 1$, $P < 0.001$). As time progressed, juveniles became increasingly pelagic, as represented by higher counts in the water column, and began to use all the habitats to the same extent regardless of their rearing environment (Fig. 3.4).

3.3.6 Experiment 4: How do rearing conditions influence juvenile habitat preferences (Part II)?

3.3.6.1 Methods

To further explore how rearing environment might influence habitat preferences in a more ecologically relevant setting, we conducted a second experiment between 9 August and 29

November 2018. For this experiment, we used three identical 10-L glass experimental tanks filled with rearing tank water and lined with sand. Each tank was then bisected widthwise, and either oysters or seagrass were added creating tanks where fish could choose from one of two possible habitats: 1) oyster shells (*Crassostrea gigas*) versus sand, 2) artificial seagrass versus sand, or 3) oyster shells versus seagrass. To minimize interference from external stimuli and standardize lighting conditions, an opaque blind was placed around the tanks, and two 300-lumen LED lights (Blackfire Waterproof LED Clamplight, BBM905) illuminated the tanks from within. Habitat choice trials were recorded by a small camera (GoPro, Hero5 Black) positioned above the tank. Fish were tested in *batches* of three. All fish in a batch went through all tests on the same day, concurrently, in an order determined by a random 3 × 3 balanced Latin square design. Each day, 6–12 individuals were tested. Fish were randomly selected and captured from rearing tanks in a hand-held dip net, and then transferred to 12 cm × 6 cm (H × D) cylindrical start chambers. Fish were transferred to experimental tanks in start chambers and allowed 10 minutes to acclimate and recover from handling. Immediately following the 10-min acclimation, fish were recorded moving around in the start chamber for 5-minutes as a baseline measure. We then began a 10-minute trial period by remotely opening the start box. Fish were then captured, returned to the start chamber, transferred to their next test tank, and the procedure was repeated. Each fish was tested three times; once in each of the three test tanks. All three tests occurred on the same day. After their final test, fish were measured for body mass (± 0.01 g) and standard length (± 0.1 mm). During the 5-minute baseline period, a rater blind to rearing conditions measured the time each fish spent active or still from the videos. During the 10-minute choice trial, the rater recorded latency to exit the start chamber, duration active or still in each habitat, initial habitat choice, initial habitat explored, and the number of centerline crosses.

3.3.6.2 Statistical Analyses

Because each test involved a choice between only two of the three possible habitats, we analyzed the data separately for each choice. Overall patterns of habitat preference were evaluated by arcsine-transforming the proportion of time each juvenile spent in both habitats, and then comparing the two proportions using a paired-samples *t*-test. To investigate whether rearing environments or subject body size influenced habitat preferences during the trial period, we fit a separate binomial generalized linear mixed model (GLM) for each choice, with the proportion of time spent in one of the two habitats as the response variable. Fixed factors were water temperature, emersion, and their interaction; subject SL was entered as a covariate.

3.3.6.3 Results

Fish did not show a clear preference between seagrass and oyster habitats (paired-samples *t*-test: $t_{27} = 2.63$, $P = 0.20$), nor did they show a clear preference between sand and oyster habitats (paired-samples *t*-test: $t_{38} = 0.085$, $P = 0.90$). However, fish did exhibit a preference for seagrass over a bare sand substrate (paired-samples *t*-test: $t_{38} = 0.085$, $P = 0.01$). Neither water temperature, emersion, their interaction, nor subject body size clearly affected the proportion of the trial period subjects spent in either habitat in any of the three forced choice tests (GLM, type III SS tests: all $P > 0.15$).

3.4 Discussion

3.4.1 Rearing environment affects offspring development, behaviour, and physical performance

Water temperature and emersion both affected plainfin midshipman throughout their embryonic, larval, early juvenile, and breeding adult stages. Here, we showed that young reared in warmer seawater (*c.* 18 °C) developed much faster compared to those reared in colder seawater (*c.* 13 °C). These young raised in warm water also enjoyed enhanced swimming performance in the face of danger (a measure that, in fish, correlates robustly with survival in the wild; McCormick et al., 2018) and showed stronger preferences for safer growing habitats (those with more cover). Emersion attenuated water temperature effects on development but increased mortality rates in free-swimming juveniles. In general, temperature had profound effects while emersion had more moderate effects on offspring phenotypes and behaviour.

Water temperature and emersion both vary naturally across nests along the tidal gradient; higher incubation temperatures and enhanced oxygen availability during emersion are considered principal benefits fishes receive from intertidal spawning (Horn et al., 1999; Martin et al., 2004). However, few studies to date have thoroughly examined emersion effects on intertidal fish eggs and young. In one notable study, Pacific herring eggs (*Clupea pallasi*) were subjected to twice daily emersion ranging from 2–8 h in 2-h increments (Jones, 1972). As we found in the present study, emersion decreased survival in pre-hatched eggs. Emersion was associated with faster development times, a pattern we observed only for eggs reared in cold water; further Jones (1972) found that 2-h emersion twice daily (4 h total) decreased incubation times by, on average, 6%. In this study we detected a 2% decrease in incubation times for plainfin midshipman young reared in cold water and an 8% increase for young reared in warm water. These differences were more likely driven by air temperatures than by oxygen availability. Average air temperatures during emersion in our study were similar to those experienced by eggs in the warm water treatment (*c.* 18.5 °C). During the 4-h emersion period, ambient air temperature likely increased metabolism in young reared in cold water but decreased metabolism in young reared in warm water, due to more efficient heat transfer in water than air.

The differences we observed in sheltering (habitat choice) behaviour between warm- and cold-water-reared juveniles could translate to survival differences in the wild if certain habitats are riskier. Eelgrass (*Zostera marina*) meadows are likely important nurseries for juvenile plainfin midshipman, sheltering them from predators (Robinson & Yakimishyn, 2013). Juveniles reared and housed in warm water were counted in higher numbers in the seagrass on sand habitat in Experiment 3. Although the sand may seem to offer fewer hiding places than gravel, its finer granularity allowed juveniles to partially or fully bury themselves and engage in ambush hunting, rather than actively swimming and chasing their crustacean prey (NB, personal observations). Juvenile fish reared under poor feeding conditions and/or high conspecific densities often achieve faster growth by foraging more actively and spending less time in shelters, especially if they are small, which increases their predation risk (Griffiths & Armstrong, 2002; Chapman et al., 2010; Walters & Juanes, 1993). In the wild, juveniles emerging from nests with lower incubation temperatures might hatch in poorer condition and compensate in this way. Although colder fish were not bolder in our study, they did have higher activity relative to their body size, which could draw increased attention from predators in less sheltered areas (Scharf et al., 2003; McCormick, 2012; McCormick et al., 2018).

3.4.2 Patterns of maternal allocation affect offspring development and body size

While water temperature had the largest and most pervasive effects on the phenotypic qualities investigated, we also uncovered some effects linked to maternal condition and allocation. Most notably, more fecund females produced young that developed slower, but development times did not depend clearly on egg size. Effects of egg size on development rates vary taxonomically and are not ubiquitous in fishes (Kamler, 2002); however, to our knowledge, ours is the first study to detect a connection between higher fecundity and slower development. Fecundity increases as a factor of body mass in teleost fishes (Wootton & Smith, 2015). Though we did not examine direct effects of female body mass on development, fecundity was not strongly related to body mass in our females. Instead, our data suggest that female plainfin midshipman diminish per-offspring investments as their fecundity increases, resulting in more but slower developing young (Wootton & Smith, 2015). While development times clearly increased in larger broods, juvenile body sizes clearly increased only when hatched from larger eggs. Plainfin midshipman eggs are extraordinarily large (*c.* 7 mm and up to 9.9 mm diameter in our study) compared to modal values of 1.0–2.2 mm for other marine teleost eggs (Wootton & Smith, 2015). These differences in initial egg diameter translated into an 8.7-mm range in free-swimming body length, where the smallest juveniles reached lengths only 64 % of the largest juveniles. Thus, larger mothers laying larger eggs produce offspring that are bigger when they reach independence, and that likely survive in higher numbers (Sargent et al., 1987).

3.4.3 Benefits associated with different rearing environments are not equal across all mothers

When mothers were in better condition, the proportion of their eggs that survived to the independent, free-swimming juvenile stage was similar across all rearing environments. In contrast, eggs laid by mothers in diminished body condition survived to the free-swimming stage in much higher proportions when reared in warmer water rather than colder water, suggesting that nesting higher in the intertidal zone, where average incubation temperatures are higher, might arise from conditional strategies in female plainfin midshipman. In many teleosts, larger egg diameter is associated with greater yolk reserves and higher survival (Wootton & Smith, 2015); yet, any relationships between egg size and maternal condition or offspring survival to independence were unclear in our study. As we observed in female plainfin midshipman, higher maternal condition in Ambon damselfish (*P. amboinensis*) correlates with larger clutches but not substantially larger eggs. Instead, maternal body condition correlates to provisioning of energy reserves in the oil globule (Gagliano & McCormick, 2007). Smaller newly hatched *P. amboinensis* offspring with larger yolk sac reserves enjoy lower pre-settlement mortality (Gagliano et al., 2007b), likely explaining why oil globule size but not overall egg size (which correlates to larger larval body size; McCormick, 1999) predicts juvenile survival in this species. Similarly, condition-dependent mortality could act on plainfin midshipman juveniles as they leave their intertidal nests to settle in seagrass meadows or macroalgae beds if better condition young exhibit less risky foraging behaviours. We propose that females in poorer condition are likely constrained to produce poorly provisioned eggs with less yolk/nutritional stores but can still enhance their reproductive success by ovipositing in nests high in the elevation gradient. Such nests will have warmer water temperatures and more frequent emersion, and so offspring laid by low condition mothers would have faster development and thus be more likely to survive long enough to hatch, detach, and go to sea. This might be a *best of a bad job* strategy because their young will still be in poor body condition when they go out to the seagrass beds, but at least they will reach this stage of life (Luttbeg & Sih, 2010). Finding sites with warm water temperature and emersion might be less important for females in better condition because their young survived in relatively high proportions regardless of the temperature of the water in the rearing environment; however, young of mothers in good body condition enjoyed an even more rapid development rate when reared in warm water.

Further studies are now necessary to test the idea that mothers can compensate for lower investment in young by providing poorly provisioned offspring with an alternative advantage in the form of a rearing area with rapid development (Ratikainen & Kokko, 2010). In black-headed gulls (*Larus ridibundus*), females provision higher concentrations of antioxidants and immunoglobins to earlier-laid eggs, but compensate for reduced immunity in later-laid eggs by provisioning them with more testosterone, which enhances development rates and growth (Eising et al., 2001; Groothuis et al., 2006). Oviposition site selection might similarly allow mothers to compensate for diminished investment in young. Female diamondback terrapins (*Malaclemys terrapin*) that lay smaller eggs—which produce females that take longer to reach sexual maturity (Roosenburg & Kelley, 1996)—preferentially select cooler nest sites that bias sex ratios toward males, whose maturation times are not affected by their hatchling body size (Roosenburg, 1996). Similarly, snapping turtles (*Chelydra serpentine*) preferentially lay eggs that hatch into larger offspring farther inland, where they are safer from egg predators, because larger neonates can disperse more quickly and thus suffer lower mortality when migrating back to water (Delaney & Janzen, 2019). In our study, we did not provide females with a choice of where to nest; instead, we experimentally dispersed eggs from the same mother among several rearing environments. This allowed us to determine the payoffs of different oviposition decisions and show that they are intertwined with maternal body condition. Crucial next steps are to uncover the mechanism driving this effect by investigating how energy reserves and yolk sac composition vary with maternal condition, and to determine whether these patterns translate to similar survival differences in the wild.

3.4.4 Possible influences of paternal traits on female oviposition site decisions and on offspring

Young plainfin midshipman in the wild are cared for by males during a long 2-month development period (Cogliati et al., 2013; **Chapter 2**). In our laboratory study, we removed the caregiver from the eggs. Phenotypic traits of the guarding male likely contribute equally or even to a greater extent to a female's oviposition selection (Bose et al., 2018). Offspring can be profoundly impacted by paternal as well as maternal effects (Bernardo, 1996; Badyaev & Uller, 2009; Crean & Bonduriansky, 2014), and, in some traits such as growth, paternal effects account for more variation than maternal effects (Green & McCormick, 2005). Fathers can affect their young through provisioning parental care and through compounds transmitted in their sperm and seminal fluids (Crean & Bonduriansky, 2014). Parental plainfin midshipman males are active caregivers (**Chapter 2**), and parental care greatly increases offspring survival compared to no care (Bose et al., 2016). Further, guarder males invest heavily in reproductive organs known as accessory glands that could contain anti-microbial compounds that protect eggs against bacterial or fungal infections (Miller et al., 2019), as seen in other fishes (Giacomello et al., 2006; Pizzolon et al., 2010). While our results highlight the importance of rearing conditions for females in poor body condition, it will be important for future studies to consider how male body size, condition, and care behaviour might modify or reverse the benefits of abiotic conditions present at certain nesting sites.

3.4.5 Conclusions

Our aim was to uncover whether plainfin midshipman young accrue differential advantages according to their rearing environment or maternal allocation of resources, and whether certain rearing environments might benefit some mothers more than others. Development rates and juvenile physical performance were enhanced by incubating in warmer water temperatures and, to a lesser extent, by emersion during development. Young that emerged from larger eggs were larger as free-swimming juveniles, and more fecund (possibly older) mothers produced young that developed faster. However, rearing environment benefits varied with maternal condition, such that mothers in poorer condition appeared to accrue greater benefits from abiotic conditions similar to those found higher in the intertidal zone. These findings illustrate how parents might employ different, complementary strategies to maximize their reproductive success. Mothers that are in poor condition produce fewer eggs for their size but could compensate for lower survival rates by seeking out warmer, frequently emersed nests higher in the intertidal. Mothers in good condition, on the other hand, do not suffer greater offspring mortality in colder water, and could thus afford to deposit their eggs in lower intertidal nests, where average nest temperatures are cooler, but males are larger and likely better nest guarders (**Chapter 2**).

3.5 Acknowledgements

We thank P. Walker, R. Shepard, and Capt. B. Cogswell as well as Mr. Warren Johnny and the Stz'uminus First Nation, the Snaw-naw-as First Nation, and Fisheries and Oceans Canada for allowing us access to field sites. A. Bose provided insightful feedback on the study design. M. Martin, M. Gordon, and K. Cable contributed invaluable support and advice for rearing and housing the experimental animals, which was realized impeccably by the laboratory animal technicians at the University of Victoria’s Outdoor Aquatic Unit. We also thank N. Houpt, H. Davies, L. Agagnier, and K. Innes for their assistance during experimentation, and M. Siddiqui, S. Matthew, and A-L. Nguyen for assisting in data collection. This work was funded by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery and Accelerator grants to SB and FJ, a Liber Ero Foundation grant to FJ, and Animal Behaviour Society, American Museum of Natural History, Sigma Xi Foundation grants to NB. Additional funding was provided to NB from the Department of Psychology, Neuroscience, and Behaviour at McMaster University, Ontario, Canada.

3.6 References

Arora, H.L. (1948). Observations on the habits and early life history of the Batrachoid fish, *Porichthys notatus* Girard– Copeia 1948: 89–93.

Badyaev, A. V. & Uller, T. (2009). Parental effects in ecology and evolution: Mechanisms, processes and implications– Philos. Trans. R. Soc. B Biol. Sci. 364: 1169–1177.

Bates, D., Mächler, M., Bolker, B.M., & Walker, S. (2015). Fitting linear mixed-effects models using lme4– J. Stat. Softw. 67: 1–48.

Bernardo, J. (1996). Maternal effects in animal ecology– Am. Zool. 2: 83–105.

Bonduriansky, R. & Crean, A.J. (2018). What are parental condition-transfer effects and how can they be detected?– Methods Ecol. Evol. 9: 450–456.

Bose, A.P.H., Borowiec, B., Scott, G., & Balshine, S. (2019). Nesting on high: reproductive and physiological consequences of breeding in the intertidal zone– Evol. Ecol. 33: 21–36.

Bose, A.P.H., Cogliati, K.M., Luymes, N., Bass, A.H., Marchaterre, M.A., Sisneros, J.A., Bolker, B.M., & Balshine, S. (2018). Phenotypic traits and resource quality as factors affecting male reproductive success in a toadfish– Behav. Ecol. 29: 496–507.

Bose, A.P.H., Kou, H.H., & Balshine, S. (2016). Impacts of direct and indirect paternity cues on paternal care in a singing toadfish– Behav. Ecol. 27: 1507–1514.

Brantley, R.K. & Bass, A.H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae)– Ethology 96: 213–232.

Brown, C., Jones, F., & Braithwaite, V. (2005). In situ examination of boldness-shyness traits in the tropical poeciliid, *Brachyraphis episcopi*– Anim. Behav. 70: 1003–1009.

Brown, D. & Cox, A.J. (2009). Innovative uses of video analysis– Phys. Teach. 47.

Buchanan, K., Burt de Perera, T., Carere, C., Carter, T., Hailey, A., Hubrecht, R., Jennings, D., Metcalfe, N., Pitcher, T., Peron, F., Sneddon, L., Sherwin, C., Talling, J., Thomas, R., & Thompson, M. (2012). Guidelines for the treatment of animals in behavioural research and teaching– Anim. Behav. 83: 301–309.

Burgess, S.C. & Marshall, D.J. (2014). Adaptive parental effects: The importance of estimating environmental predictability and offspring fitness appropriately– Oikos 123: 769–776.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and multimodel inference. Springer, New York, NY, Second edition.

Chapman, B.B., Morrell, L.J., & Krause, J. (2010). Unpredictability in food supply during early life influences boldness in fish– Behav. Ecol. 21: 501–506.

Cogliati, K.M., Neff, B.D., & Balshine, S. (2013). High degree of paternity loss in a species with alternative reproductive tactics– Behav. Ecol. Sociobiol. 67: 399–408.

Crean, A.J. & Bonduriansky, R. (2014). What is a paternal effect?– Trends Ecol. Evol. 29: 554–559.

Dantzer, B., Newman, A.E.M., Boonstra, R., Palme, R., Boutin, S., Humphries, M.M., & Mcadam, A.G. (2013). Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal– Science 1215: 1215–1218.

Delaney, D.M. & Janzen, F.J. (2019). Offspring dispersal ability covaries with nest-site choice–

Behav. Ecol. 30: 125–133.

DeMartini, E.E. (1988). Spawning success of the male plainfin midshipman. I. Influences of male body size and area of spawning site– J. Exp. Mar. Bio. Ecol. 121: 177–192.

DeMartini, E.E. (1990). Annual variations in fecundity, egg size and condition of the plainfin midshipman (*Porichthys notatus*)– Copeia 1990: 850–855.

DeMartini, E.E. (1991). Spawning success of the male plainfin midshipman. II. Substratum as a limiting spatial resource– J. Exp. Mar. Bio. Ecol. 146: 235–251.

Domenici & Blake (1997). The kinematics and performance of fish fast-start swimming– J. Exp. Biol. 200: 1165–78.

Doty, M.S. (1946). Critical tide factors that are correlated with the vertical distribution of marine algae and other organisms along the Pacific coast– Ecology 27: 315–328.

Einum, S. & Fleming, I.A. (2000). Highly fecund mothers sacrifice offspring survival to maximize fitness– Nature 405: 565–567.

Einum, S. & Fleming, I.A. (2002). Does within-population variation in fish egg size reflect maternal influences on optimal values?– Am. Nat. 160: 756.

Eising, C.M., Eikenaar, C., Schwabl, H., & Groothuis, T.G.G. (2001). Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: Consequences for chick development– Proc. R. Soc. B Biol. Sci. 268: 839–846.

Ferrière, R., Le Galliard, J.-F., & Clobert, J. (2004). Physical performance and Darwinian fitness in lizards– Nature 432: 502–505.

Gagliano, M. & McCormick, M.I. (2007). Maternal condition influences phenotypic selection on offspring– J. Anim. Ecol. 76: 174–182.

Gagliano, M., McCormick, M.I., & Meekan, M.G. (2007a). Temperature-induced shifts in selective pressure at a critical developmental transition– Oecologia 152: 219–225.

Gagliano, M., McCormick, M.I., & Meekan, M.G. (2007b). Survival against the odds: Ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish– Proc. R. Soc. B Biol. Sci. 274: 1575–1582.

Giacomello, E., Marchini, D., & Rasotto, M.B. (2006). A male sexually dimorphic trait provides antimicrobials to eggs in blenny fish– Biol. Lett. 2: 330–333.

Green, B.S. & McCormick, M.I. (2005). Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish– Mar. Ecol. Prog. Ser. 289: 263–272.

Griffiths, S.W. & Armstrong, J.D. (2002). Rearing conditions influence refuge use among over-wintering Atlantic salmon juveniles– J. Fish Biol. 60: 363–369.

Groothuis, T.G.G., Eising, C.M., Blount, J.D., Surai, P., Apanius, V., Dijkstra, C., & Müller, W. (2006). Multiple pathways of maternal effects in black-headed gull eggs: Constraint and adaptive compensatory adjustment– J. Evol. Biol. 19: 1304–1313.

Hanssen, S.A., Hasselquist, D., Folstad, I., & Erikstad, K.E. (2005). Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction– Proc. R. Soc. B Biol. Sci. 272: 1221-1039–1046.

Hijazi, R.H. & Jernigan, R.W. (2009). Modelling compositional data using Dirichlet regression models.– J. Appl. Probab. Stat. 4: 77–91.

Horn, M.H., Martin, K.L.M., & Chotkowski, M.A., eds (1999). Intertidal Fishes: Life in Two

Worlds– Encycl. Ocean Sci. Academic Press, San Diego, CA.

Jones, B.C. (1972). Effect of intertidal exposure on the survival and embryonic development of Pacific herring spawn– J. Fish. Res. Board Canada 29: 119–1124.

Kamler, E. (2002). Ontogeny of yolk-feeding fish: an ecological perspective– Rev. Fish Biol. Fish. 12: 79–103.

Kamler, E. (2005). Parent-egg-progeny relationships in teleost fishes: An energetics perspective– Rev. Fish Biol. Fish. 15: 399–421.

Kenyon, R.A., Haywood, M.D.E., Heales, D.S., Loneragan, N.R., Pendrey, R.C., & Vance, D.J. (1999). Abundance of fish and crustacean postlarvae on portable artificial seagrass units: Daily sampling provides quantitative estimates of the settlement of new recruits– J. Exp. Mar. Bio. Ecol. 232: 197–216.

Lindström, J. (1999). Early development and fitness in birds and mammals– Trends Ecol. Evol. 14: 343–348.

Luttbeg, B. & Sih, A. (2010). Risk, resources and state-dependent adaptive behavioural syndromes– Philos. Trans. R. Soc. B Biol. Sci. 365: 3977–3990.

Marshall, D.J. & Uller, T. (2007). When is a maternal effect adaptive?– Oikos 116: 1957–1963.

Martin, K.L.M., Van Winkle, R.C., Drais, J.E., & Lakisic, H. (2004). Beach-spawning fishes, terrestrial eggs, and air breathing– Physiol. Biochem. Zool. Ecol. Evol. Approaches 77: 341–356.

McCormick, M.I. (1999). Experimental test of the effect of maternal hormones on larval quality of a coral reef fish– Oecologia 118: 412–422.

McCormick, M.I. (2006). Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish.– Ecology 87: 1104–9.

McCormick, M.I. (2012). Lethal effects of habitat degradation on fishes through changing competitive advantage– Proc. R. Soc. B Biol. Sci. 279: 3899–3904.

McCormick, M.I., Fakan, E., & Allan, B.J.M. (2018). Behavioural measures determine survivorship within the hierarchy of whole-organism phenotypic traits– Funct. Ecol. 32: 958–969.

Miller, J.S., Mazzoldi, C., Rasotto, M.B., & Balshine, S. (2019). Differential investment in male accessory glands: lessons from a marine fish with alternative reproductive tactics– Mar. Biol. 166: 1–12.

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change– Philos. Trans. R. Soc. B Biol. Sci. 363: 1635–1645.

Mousseau, T.A. & Fox, C.W. (1998). The adaptive significance of maternal effects– Trends Ecol. Evol. 13: 403–407.

Olfert, E.D., Cross, B.M., & McWilliam, A.A., eds (1993). Guide to the care and use of experimental animals. Canadian Council on Animal Care, Ottawa, ON, 2nd ed., Vol. 1.

Pizzolon, M., Giacomello, E., Marri, L., Marchini, D., Pascoli, F., Mazzoldi, C., & Rasotto, M.B. (2010). When fathers make the difference: Efficacy of male sexually selected antimicrobial glands in enhancing fish hatching success– Funct. Ecol. 24: 141–148.

R Core Team (2016). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ratikainen, I.I. & Kokko, H. (2010). Differential allocation and compensation: Who deserves the silver spoon?– Behav. Ecol. 21: 195–200.

Refsnider, J.M. & Janzen, F.J. (2010). Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice– Annu. Rev. Ecol. Evol. Syst. 41: 39–57.

Resetarits, W.J.J. (1996). Oviposition site choice and life history evolution– Am. Zool. 36: 205–215.

Robinson, C.L.K. & Yakimishyn, J. (2013). The persistence and stability of fish assemblages within eelgrass meadows (*Zostera marina*) on the Pacific coast of Canada– Can. J. Fish. Aquat. Sci. 70: 775–784.

Roosenburg, W.M. (1996). Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination?– Am. Zool. 36: 157–168.

Roosenburg, W.M. & Kelley, K.C. (1996). The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin*– J. Herpetol. 30: 198–204.

Sargent, R.C., Taylor, P.D., & Gross, M.R. (1987). Parental care and the evolution of egg size in fishes– Am. Nat. 129: 32–46.

Scharf, F.S., Buckel, J.A., McGinn, P.A., & Juanes, F. (2003). Vulnerability of marine forage fishes to piscivory: Effects of prey behavior on susceptibility to attack and capture– J. Exp. Mar. Bio. Ecol. 294: 41–59.

Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients– Methods Ecol. Evol. 1: 103–113.

Somero, G.N. (2002). Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living– Integr. Comp. Biol. 42: 780–789.

Stearns, S.C. (1992). The evolution of life histories. Oxford University Press, Oxford, UK.

Walters, C.J. & Juanes, F. (1993). Recruitment limitation as a consequence of natural selection for use of restricted feeding habitats and predation risk taking by juvenile fishes– Can. J. Fish. Aquat. Sci. 50: 2058–2070.

Wapstra, E. (2000). Maternal basking opportunity affects juvenile phenotype– Funct. Ecol. 345–352.

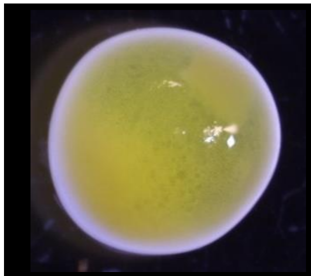
Wapstra, E., Olsson, M., Shine, R., Edwards, A., Swain, R., & Joss, J.M.P. (2004). Maternal basking behaviour determines offspring sex in a viviparous reptile– Proc. R. Soc. London. Ser. B Biol. Sci. 271: 230–232.

Webb, P.W. (1976). The effect of size on the fast-start performance of rainbow trout *Salmo cairdneri*, and a consideration of piscivorous predator-prey interactions.– J. Exp. Biol. 65: 157–77.

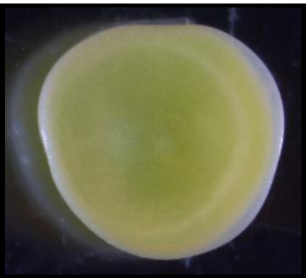
Wootton, R.J. & Smith, C. (2015). Reproductive Biology of Teleost Fishes. John Wiley & Sons, Ltd., West Sussex, UK.

Table 3.1 Discrete, identifiable developmental stages in plainfin midshipman. Specimens are representative of the embryonic, larval and early juvenile life stages observed across all the rearing environments. Developed by Nicholas AW Brown.

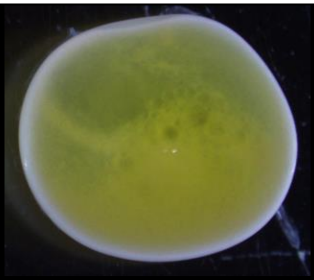
1. New egg
A white blastodisc is visible on the surface of the yolk sac (upper-right of egg).



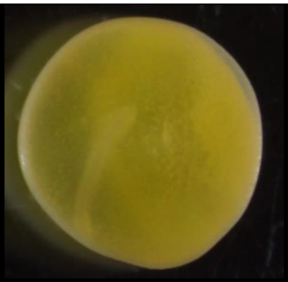
2. Gastrula
Epiboly results in the formation of a thin, crescent-shaped blastoderm.



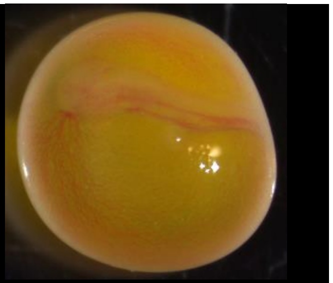
3. Early neurulation
The gastrula converges into a thin notochord (middle-left of egg).



4. Late neurulation
Dorso-ventral axis becomes apparent with the formation of a head at one end of the notochord.



5. Heart
Organogenesis is clear: veins appear; a faint heartbeat can be detected with the naked eye.



6. Eye
Advanced visceral organ development, most notably the darkly coloured eye.

7. Newly hatched larva
The new larva emerges from its yolk sac casing.

8. Swim bladder
The silver organ appears in the abdominal region. Coincides with flexion.

9. Melanophores
Darkening, striped pigmentation spreads across the dorsal surface, starting at the head.

10. Free-swimming juvenile
Attains all adult features. Yolk sac is nearly or fully absorbed. Can remain attached for some days before free-swimming.

Figure 3.1 A) Artificial nest set up for spawning tanks in the lab (see Experiment 1), including small (right) and large (left) artificial nests. The larger darker fish in the nest opening is a male, and the smaller, lighter fish is a gravid female. B) Start chamber and arena used for physical assays in Experiment 2. C) A view from the underside of the arena showing how a glass rod was used to tap the tail of a juvenile and elicit a fast-start response, from which burst swimming velocities were scored.

Figure 3.2 Development times of young raised under different combinations of water temperature and emersion. Smaller dots along the x-axis represent the day on which the final juvenile in each replicate reached the free-swimming life stage. Larger dots show the median values (written above the dots) for each group—lines connecting these dots show the direction of the interaction between water temperature and emersion.

Figure 3.3 (A) Proportion of offspring on each brick that survived to the free-swimming juvenile stage as a function of female condition (RSC). (B) Body lengths (SL) of juveniles on bricks that reached the free-swimming life stage as a function of female (maternal) body condition. In both panels, Smooth black lines and the shaded areas around them show fitted model predictions and 95% CIs, respectively.

Figure 3.4 The proportion of juveniles from each rearing environment that were counted in each of five habitat types. Dot sizes represent the number of juveniles counted in the corresponding habitat. Solid lines show predicted values from a Dirichlet regression.

Figure 3.1

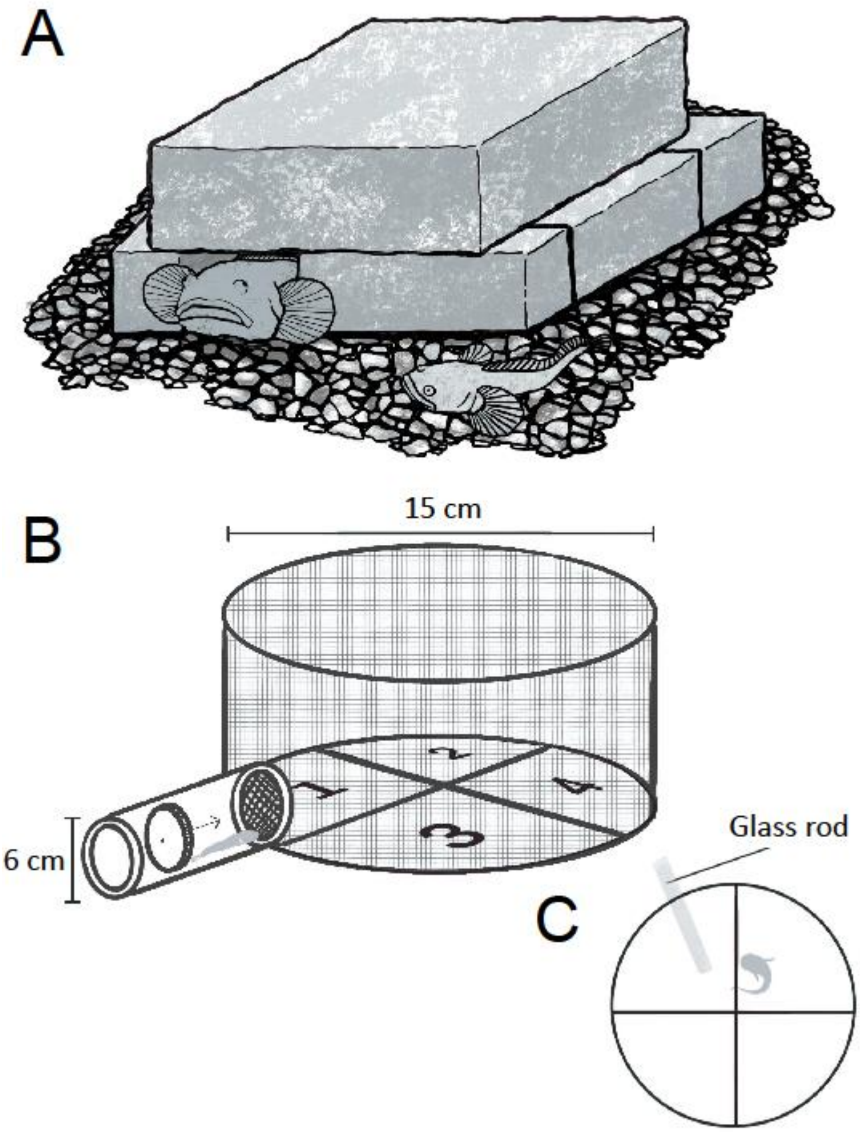


Figure 3.2

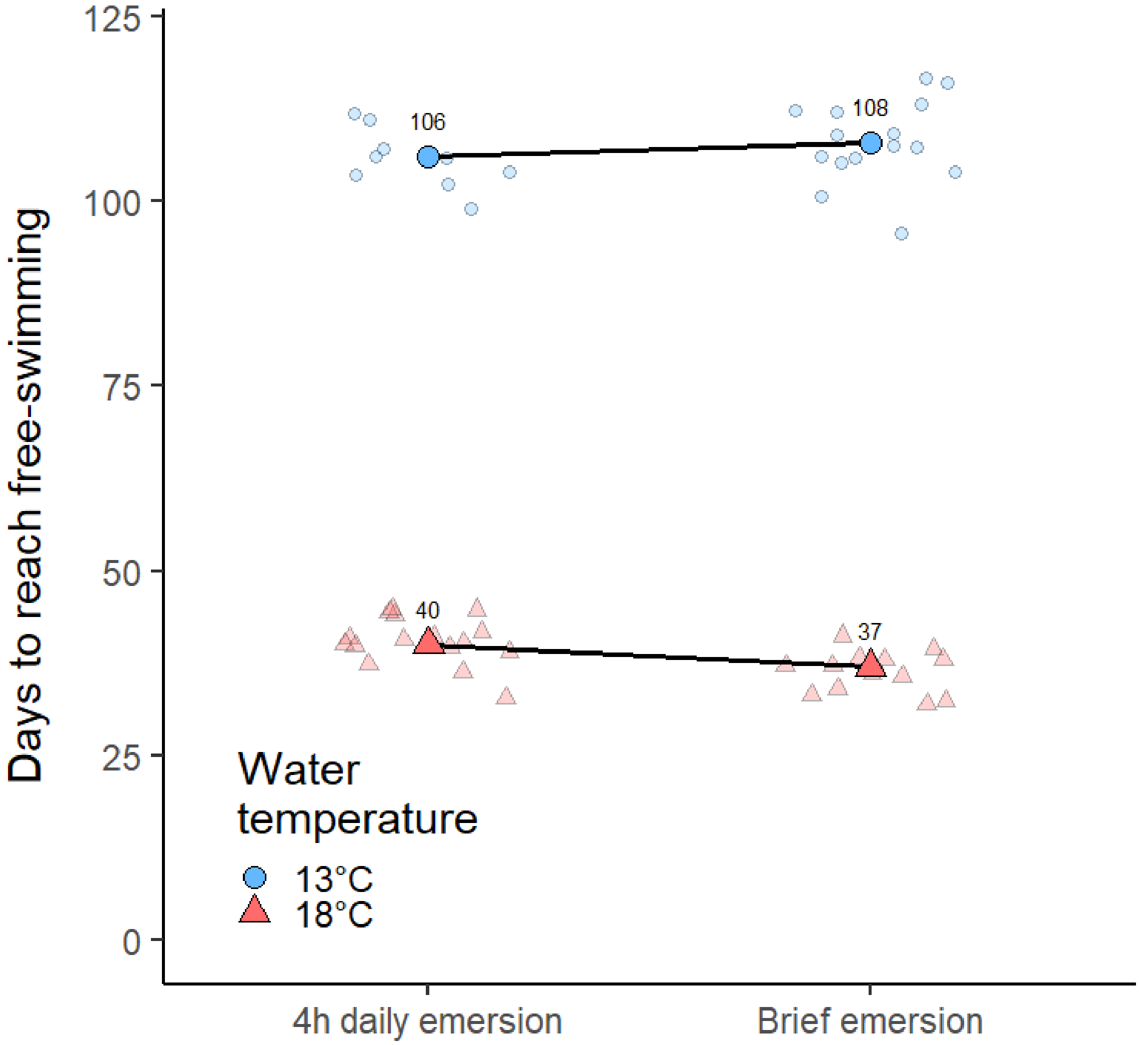

Days to reach free-swimming
125
100
75
50
25
0
106
108
40
37
Water temperature
13°C
18°C
4h daily emersion
Brief emersion

Figure 3.3

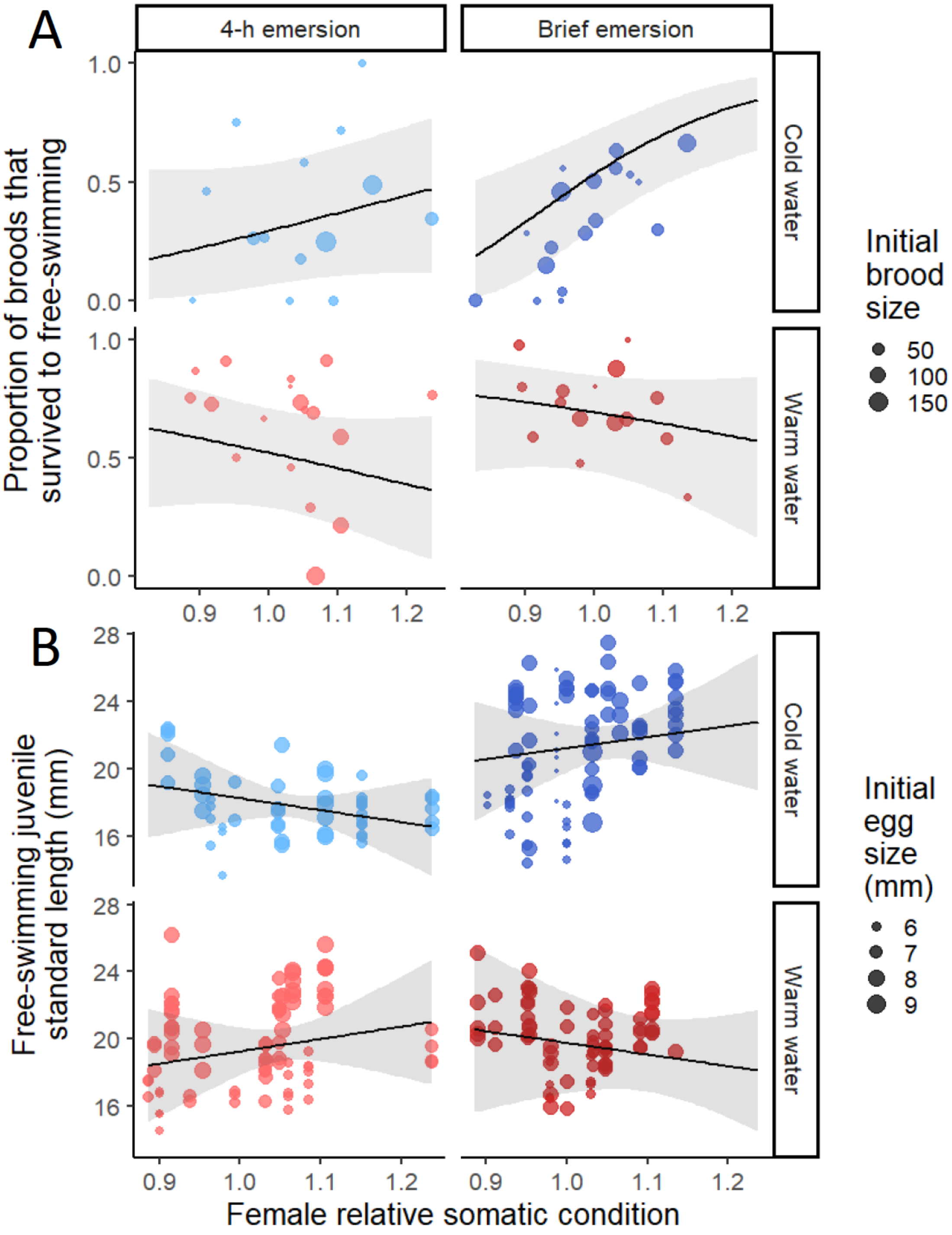
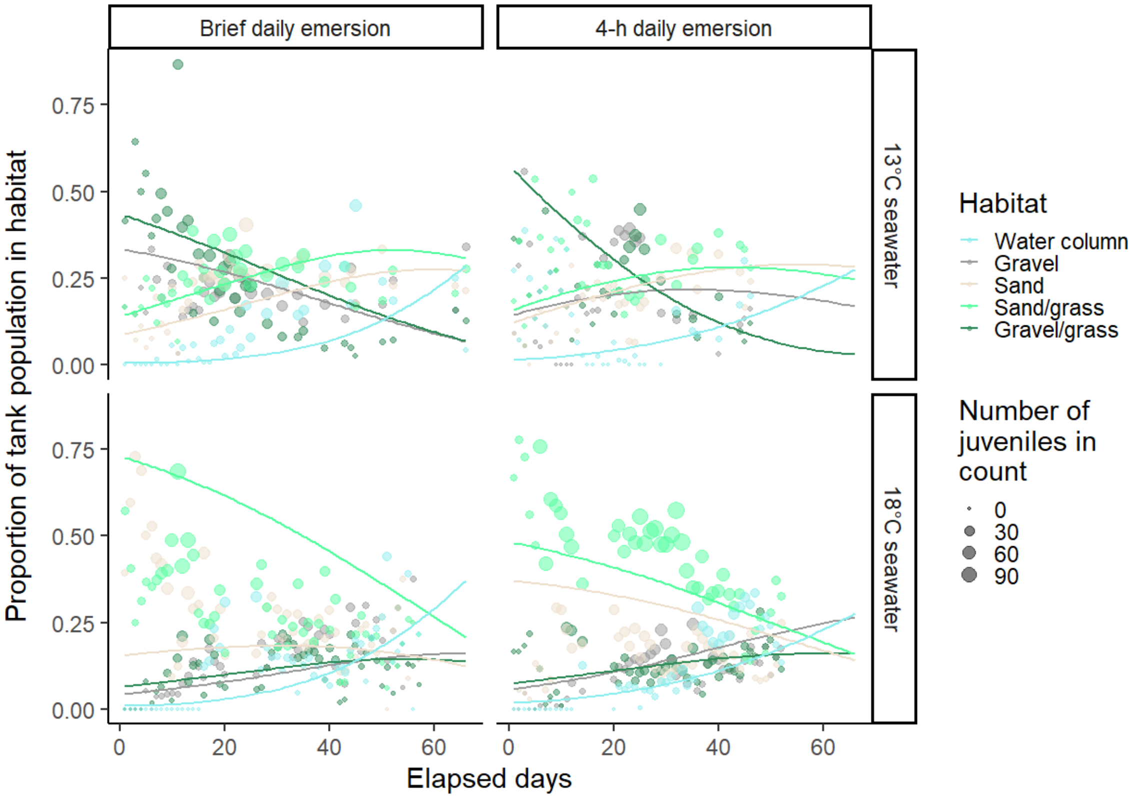

A
4-h emersion
Brief emersion
Cold water
Warm water
Proportion of broods that survived to free-swimming
0.0
0.5
1.0
0.9
1.0
1.1
1.2
Initial brood size
50
100
150
B
Free-swimming juvenile standard length (mm)
16
20
24
28
Initial egg size (mm)
6
7
8
9
Female relative somatic condition

Figure 3.4


Brief daily emersion
4-h daily emersion
13°C seawater
18°C seawater
Proportion of tank population in habitat
0.00
0.25
0.50
0.75
0
20
40
60
Elapsed days
Habitat
Water column
Gravel
Sand
Sand/grass
Gravel/grass
Number of juveniles in count
0
30
60
90

Chapter 4: General discussion

4.1 Summary

During my M.Sc, I carried out field (**Chapter 2**) and laboratory (**Chapter 3**) experiments to measure how the costs and benefits incurred by plainfin midshipman fish (*Porichthys notatus*) vary between nesting sites and across tidal elevations according to abiotic conditions and individual traits. Specifically, I addressed two questions: 1) how do parents balance the benefit–cost ratio of care when selecting a nest site? and 2) how do the benefits conferred to offspring vary according to abiotic conditions and in relation to maternal traits? I investigated these questions in **Chapters 2 & 3**, respectively. Here in the last chapter of my thesis, I discuss the answers to these questions, while highlighting the most promising avenues for future research. Finally, I provide some overarching conclusions of my work as well as their broader implications in the context of life history evolution.

4.2 How do parents balance the benefit–cost ratio of care when selecting a nest site?

The plainfin midshipman fish exhibits one of the most remarkable reproductive life histories among teleost fishes. Parental males withstand harsh conditions in intertidal nests to tend and guard broods of eggs for periods sometimes exceeding 60 days (Arora, 1948; Cogliati et al., 2013; Bose et al., 2019). Although guarder males possess an impressive battery of physiological mechanisms that mitigate the effects of intertidal stressors (Craig et al., 2014; LeMoine et al., 2014; Houpt et al. *in review*), their body condition deteriorates steadily while on the nest (Bose et al., 2016a; **Chapter 2**). The benefits of parental care are theoretically higher in animals with prolonged offspring development times, such as the plainfin midshipman fish (50–70 days; Cogliati et al., 2013; **Chapters 2 & 3**), because care greatly increases offspring survival during this vulnerable life stage (Klug & Bonsall, 2010). Accordingly, Bose et al. (2016b) observed complete mortality in plainfin midshipman broods that did not receive parental care over a 28-day period while in contrast approximately 40 % of broods survived if they received care. Therefore, guarder males that select nest sites where their own likelihood of mortality is high risk losing both their current and future broods. Accordingly, I predicted that males would compete most intensely for nesting sites that represent an optimal balance between fast offspring development rates and slow paternal body condition deterioration, which I expected to find in the middle intertidal zone.

In **Chapter 2** my coauthors and I quantified the extent to which offspring development rates, survival, and paternal body condition deterioration varied along a gradient from the shallow subtidal to the high intertidal zone in the wild. Despite increasing temperatures and periods of emersion higher up in the intertidal zone, we did not find clear evidence that body condition deteriorates faster in males nesting high compared to males nesting lower in the intertidal zone. However, broods suffered more rapid mortality rates in higher nests and surprisingly, despite warmer conditions, did not develop more quickly, suggesting thermal conditions in higher nests might be sub-optimal for offspring. Although we were unable to measure brood mortality rates in subtidal nests, the considerably slower development rates we observed at these sites suggest these nests might also be sub-optimal for offspring. Further, males in higher nests were observed providing more frequent parental care, which might imply these males increase their level of care to compensate for these sub-optimal conditions (Bonsall & Klug, 2011). Males in the lowest intertidal nests were notably larger (better competitors) than those in intermediate and high elevation nests; suggesting that the most competitive males are taking nesting spots in the low intertidal zone, which confer the greatest survival benefits to offspring, potentially allowing males in these nests to relax their parental effort.

These findings agree with previous studies and with two predictions drawn from life history theory (LHT). Oviposition sites should be carefully selected to maximize the benefits received by offspring when: 1) the costs to parents of being choosy are low, and 2) the benefits conferred to offspring vary considerably among possible sites (Resetarits, 1996; Klug & Bonsall, 2010). In support of the first prediction, parents whose oviposition site choices carry high mortality risks have been shown to nest in locations that diminish their own mortality risk but increase the risks of nest predation or overheating in their offspring (Spencer, 2002; Amat & Masero, 2004; Tieleman et al., 2008). For example, female freshwater turtles (*Emydura macquarii*) nest closer to the shoreline, where nest predation risk is higher, and thus diminish their own exposure duration to terrestrial predation by foxes (*Vulpes vulpes*; Spencer, 2002). In support of the second prediction, several studies have shown that maternally chosen sites confer greater advantages to offspring than do randomly chosen sites in the same environment (*e.g.* Wilson, 1998;

Doak et al., 2006; Warner & Shine, 2008; Gall et al., 2012; Li et al., 2018; but see Warner & Mitchell, 2013). Reproductive site preferences of male and female *Edalorhina perezi* frogs shift seasonally according to fluctuating predator and conspecific densities in small pools, such that offspring are consistently fertilized and deposited in pools with the lowest mortality risk (Murphy, 2003a, 2003b). Our findings in **Chapter 2** also match both predictions. The costs of care for guarder males *did not* vary between nesting sites in the intertidal zone, but the benefits conferred to offspring—both survival and development—*did*. Accordingly, guarder males exhibited a size-assortative distribution with respect to intertidal elevation—the most competitive males were found in sites where the benefits to offspring were highest. However, less competitive males that were likely driven to higher nesting sites appeared to be physiologically equipped to make the best of a bad job and rear broods under harsher conditions.

4.3 How do the benefits conferred to offspring vary according to abiotic conditions and in relation to maternal traits?

Most intertidal spawning fishes do not possess amphibious adaptations and visit these semi-terrestrial locations only briefly for reproduction, suggesting the substantial benefits conferred to offspring by warmer temperatures and exposure to air are the primary reason that aquatic organisms evolved to oviposit in these environments (Martin et al., 2004; Ishimatsu et al., 2018). However, maternal effects, most notably relative allocation between fecundity and per-egg investment, also strongly affect the advantages offspring receive early in life (Bernardo, 1996). Investing in more versus larger eggs (by adjusting the amount of per-offspring nourishment) is a classic life history trade-off that is physiologically constrained (Stearns, 1992; Ricklefs & Wikelski, 2002). In contrast, oviposition site selection is a highly plastic trait (Resetarits, 1996). If the benefits of different nesting sites for offspring vary according to their relative size or degree of provisioning as embryos, a mother's oviposition decision might depend on how many eggs she has, the size of her eggs, and her body condition. Well-provisioned embryos (from larger eggs and/or with richer energy stores) take longer to develop but are more resistant to starvation and desiccation and hatch into larger, typically fitter young (Bernardo, 1996; Wootton & Smith, 2015). Mothers with a low per-offspring investment might accrue greater benefits from laying their eggs in warmer nesting sites higher in the intertidal zone. This strategy could allow mothers to circumvent the trade-off between post-hatching offspring performance and development time by positioning their eggs in environments where development is enhanced.

In **Chapter 3**, my coauthors and I investigated these ideas by employing a split-brood methodology where we partitioned one brood into rearing environments with different water temperatures and emersion regimes. Incubation in warm (18 °C) water allowed embryos to develop into free-swimming juveniles two and a half times faster than when the same batch of eggs was incubated in cold (13 °C) water. Juveniles reared and housed in warm water also exhibited faster burst swimming speeds (escape responses) during physical performance assays. Emersion attenuated the temperature effect on development rates, resulting in slightly faster development in emersed embryos incubated in cold water, but slightly slower development in emersed embryos incubated in warm water. Egg size had surprisingly little influence on offspring development times and on the proportion that survived to independence, but larger eggs did develop into larger juveniles, as expected from LHT and from numerous previous studies in a wide variety of taxa (Bernardo, 1996; Krist, 2011). However, contrary to these general trends, maternal fecundity, but not egg size, increased development times (broods with more eggs developed slower). Higher maternal body condition decreased development times, but only when young were reared in warm water. Further, water temperature and emersion altered the relationships we observed between maternal body condition and offspring survival and juvenile body size. Survival increased with maternal body condition in cold water and decreased with maternal body condition in warm water. We observed the same relationship between maternal body condition and juvenile body size when eggs were not emersed but experiencing a 4-h daily emersion reversed the effect of maternal body condition on juvenile size in the two water temperatures.

Our findings suggest that water temperature, emersion, and maternal body condition could impact oviposition decisions in the wild. Among the four rearing environments young experienced in the lab, two approximated ecologically relevant thermal and emersion conditions at the extreme ends of a natural intertidal gradient. Young reared in cold seawater without emersion experienced conditions similar to those measured in subtidal nests (see **Chapter 2)**, and young reared in warm seawater with emersion experienced conditions similar to those measured in middle or high intertidal nests. In the cold seawater without emersion condition (conditions approximating subtidal nests), mothers in good body condition produced offspring that survived in higher proportions and were larger as juveniles. In contrast, in warm seawater with emersion (conditions approximating middle to high intertidal nests), mothers in good body condition did not produce offspring that survived in higher proportions, but they did produce larger juveniles. Therefore, all else being equal, mothers in poor condition might accrue greater benefits from nesting higher in the intertidal zone. Roosenburg (1996) detected a similar phenomenon in female diamondback terrapins (*Malaclemys terrapin*), where a mother's nest site choice appears to depend on the size of her eggs. Smaller eggs that produce smaller, slower maturing female (but not male) hatchlings were deposited in cooler sites, which bias the sex ratios toward males (Roosenburg, 1996; Roosenburg & Kelley, 1996). Thus, female terrapins appear to select the nest sites that are most beneficial for their egg size. However, a follow-up study failed to detect the same pattern in painted turtles (*Chrysemys picta*) and the authors emphasize that other factors, such as predation and moisture conditions, might impose greater consequences on nest site selection in these turtles and thus override smaller advantages that could be gained from matching site conditions to egg size (Morjan & Janzen, 2003).

4.4 Future research directions

My findings open several exciting avenues for additional research on environmental and phenotypic determinants of oviposition site selection in the plainfin midshipman.

i. How do the costs and benefits of care vary along the full elevation distribution of nests in the wild?

In **Chapter 2**, I formally reported subtidal nesting in the plainfin midshipman for the first time (*note*: the phenomenon was known to other researchers; A. Bose, J. Sisneros, and E. DeMartini, personal communications), and I measured development rates of young in these nests. The following summer (2019), I returned to the same field site and further refined methodologies for working on submerged nests by snorkeling and freediving. Preliminary results from my work in 2019 (see **Chapter 5**) show that, 10 days after deployment of artificial nests, guarding males occupying subtidal and intertidal nests were of similar size, but later in the season (42 days after deployment) males in subtidal nests were generally longer, which might indicate competition for subtidal nests is higher than for some intertidal nests (**Chapter 2**). Females in subtidal nests might also be larger, but the small number captured at these sites ($N = 7$) precludes statistical confirmation (**Chapter 5**).

During both years of my study (2018 & 2019), I placed the subtidal nests along a depth contour approximately 25 m offshore from the lowest intertidal nests to ensure I sampled a habitat with markedly different abiotic conditions. I also placed intertidal artificial nests at only three elevations. However, at the field site (and likely in other midshipman breeding sites), natural nests spanned the entire elevation gradient between the high intertidal and shallow subtidal. Future researchers should distribute artificial nests more evenly across the entire distribution of natural nests to determine the relative breadth of the optimal rearing zone. The tidal elevation of each intertidal nest (relative to heights reported in local tidal charts) could be quantified by recording its emersion time during a low tide and back-calculating the height from known high and low water heights and times (LINZ, 2017). For each subtidal nest this could be accomplished by measuring its depth below the water's surface at low tide and subtracting that depth from the known low tide height. Using this methodology, researchers could map nest uptake and spawning patterns, estimate nest success, and more precisely zero in on the phenotypic distributions in males and females along an emersion and temperature gradient.

My work in **Chapter 2** suggests peak reproductive success might be attained somewhere near the low water mark. Petranka and Petranka (1981) employed a design similar to that I described in the previous paragraph to map the distribution of marbled salamander (*Ambystoma opacum*) nests in seasonally filled freshwater ponds. These salamanders dig nests and deposit their eggs while ponds are dried up during the summer. Marbled salamander eggs delay hatching until submerged when seasonal rains fill the ponds. Petranka and Petranka (1981) found high densities of nests at intermediate depths with few nests at either extreme, suggesting middle depths in the ponds are the best locations for rearing young successfully. Eggs in the deepest nests are at risk of hatching before consistent rainfalls ensure the ponds stay filled and young can remain submerged. Eggs in the shallowest nests are at risk of complete mortality by freezing if water levels in the pool do not reach these nests before the winter (Petranka & Petranka, 1981). Measuring nests at all elevations along the tidal elevation gradient could allow researchers to uncover whether similar patterns exist in the plainfin midshipman.

ii. Why might rearing environment effects depend on maternal body condition, and do females exhibit condition-dependent oviposition site selection in the field?

In **Chapter 3,** we uncovered the possibility that females in lower body condition could suffer higher brood mortality if they lay their eggs in subtidal nests. Egg size did not differ between females in better or worse condition, which raises the question: which attributes of eggs vary with female body condition that might explain why their offspring performance differs across rearing environments? Gagliano and McCormick (2007) found that better maternal body condition in female damselfish (*Pomacentrus amboinensis*) improves energy reserves in their embryos' yolk sacs and oil globules without increasing overall egg size. The same phenomenon might well occur in the plainfin midshipman, and future researchers should determine whether, and to what extent, maternal body condition affects energy provisioning in embryos. In general, a better understanding of female midshipman behaviour and life history is needed.

Whether females exhibit body condition-dependant oviposition site selection in the wild is another exciting avenue for future research that stems from my work. Roosenburg (1996) provides some support for this phenomenon in a turtle (*M. terrapin*), but similar patterns were not detected in another turtle species (*C. picta*), leading to the conclusion that broad environmental effects that act on the entire population might sometimes supersede effects driven by individual variation in oviposition decisions (Morjan & Janzen, 2003). Similar broad effects might also sufficiently explain most variation in plainfin midshipman oviposition site selection. Because parental care is strongly related to offspring survival in this species (Bose, Kou, et al., 2016), females could simply go to where the best males are, regardless of their own body condition (DeMartini, 1988, 1991; Bose et al., 2018). In either case, two complementary field experiments could test the condition-dependant choice hypothesis. First, it would be useful to investigate whether there is a relationship between female body condition and nest tidal elevation in the field. Second, one could artificially seed high and low intertidal (or subtidal) nests with size-matched guarder males and with females in varying body condition to determine whether lower-condition mothers suffer higher brood mortality in low intertidal nests compared to high intertidal nests.

iii. How many times can an individual plainfin midshipman fish reproduce?

The total lifetime reproductive potential of an individual will dramatically affect its optimal nesting site conditions. Parents that make costly investments in their current brood ultimately diminish their future reproductive potential (Webb et al., 2002). Numerous studies have illustrated that animals with higher future reproductive potential reduce their levels of parental care (including choosing sub-optimal oviposition sites for offspring) to increase their likelihood of surviving to reproduce again (*e.g.* Szekely & Cuthill, 2000; Amat & Masero, 2004; Ward et al., 2009).

Plainfin midshipman guarder males spawn multiple times and thus raise several broods during a single breeding season (Cogliati et al., 2013). Within a season, a male will likely have the highest reproductive success in a nest where he can persist for as long as possible and thus mate as many times as possible (Dickerson et al., 2005; Cargnelli & Neff, 2006). However, a male should presumably also select a site based on his likelihood of surviving to return and breed again in the following season. Previous work failed to detect consistent genetic structure across different breeding sites (Suk et al., 2009), as would be expected if individuals and populations consistently return to the same site year after year. In a notable mark-recapture effort, Phillips (2007) tagged 176 guarder males in Tomales Bay, CA during the 2006 breeding season, but did not recapture any tagged individuals in 2007, further affirming that breeding site fidelity is low between years. Male Lusitanian toadfish (*Halobatrachus didactylus*), reach sexual maturity around 2.1 years of age and 160 mm body length and females around 3 years and 190 mm; males and females reach up to 12 and 10 years of age, respectively, with body sizes correspondingly reaching 405 and 366 mm (Palazón-Fernandez et al., 2010), suggesting these fish are reproductively mature for several years. A similar 2-fold increase in body length is observed between the biggest and smallest guarder males nesting on the beach (Bose et al., 2018). The largest males could therefore be up to several years older than the smallest, and possibly survive for multiple breeding seasons. Determining how many reproductive seasons males and females can complete during their lifespan, and whether individuals return to breed each year, will greatly enhance our understanding of nest site selection and other parental behaviours in this species.

4.5 Conclusions

Broadly, my findings in **Chapters 2 & 3** support the idea that there are benefits of intertidal compared to subtidal oviposition (Martin et al., 2004). Eggs develop faster if they can incubate in warmer temperatures; warmer water is accompanied by emersion in the intertidal. However, water temperature had far greater effects than emersion itself, challenging the notion that increased oxygen availability during emersion is a principal benefit of intertidal oviposition (Sayer & Davenport, 1991; Martin et al., 2004; Ishimatsu et al., 2018). The specific developmental benefit of enhanced oxygen availability during emersion of intertidal fish eggs has yet to be successfully decoupled from possible air temperature effects (Jones, 1972; **Chapter 3**), but the 2–11% differences in development time observed by Jones (1972) in herring (*Clupea pallasi*) and by me in midshipman suggest that effects of emersion on development are minor compared to those of temperature. We do not yet know if midshipman eggs can take up oxygen across the chorion. Additionally, Jones (1972) observed an 18% increase in prehatching mortality between herring eggs that were never emersed and those that were emersed for 8 h twice daily. In **Chapter 2**, I found that brood survival was highest in low intertidal nests where emersion was less frequent. In **Chapter 3**, emersion decreased survival in both demersal, developing young and free-swimming juveniles. Taken together, my findings in **Chapters 2 & 3** suggest higher temperatures are the principal benefit midshipman young receive from developing in intertidal rather than subtidal nests. Emersion might be detrimental to brood survival.

My work carries broad implications pertaining to life history evolution. Resetarits (1996) emphasizes the importance of interrelation between oviposition behaviour and principal life history traits—to maximize fitness, animals must match their life history phenotype (*e.g.* relative allocation between offspring number and size) to the environment in which its adaptive value is highest. Accordingly, each life history phenotype should have a corresponding optimal oviposition site. However, an oviposition site selection–life history nexus (*sensu* Ricklefs & Wikelski, 2002) has yet to be established. My findings in **Chapters 2 & 3** are consistent with Resetarits' (1996) nexus. The plainfin midshipman fish lies at the extreme end of the egg size–fecundity trade-off in fishes (Wootton & Smith, 2015). Compared to other fishes, they lay very few (≈ 70–230; DeMartini, 1990; **Chapter 3**) very large eggs (5.5–9.9 mm; **Chapter 3**). Extended parental care and a life history characterized by relatively large, slow developing young—as in the plainfin midshipman—likely arise through coadaptation because: 1) offspring survival in the absence of parental care decreases with development time, and 2) young that are more developmentally advanced when parental care is terminated are more likely to survive to reproduce (Shine, 1978; Sargent et al., 1987; Klug & Bonsall, 2010). In **Chapter 2**, I demonstrated that the largest males are found in sites where offspring survival is highest (low intertidal zone), and males at these sites exhibit less-active parental care. In **Chapter 3**, using a laboratory experiment, I confirmed that warmer incubation temperatures are the principal benefit offspring receive from near-shore nesting. I also uncovered the potential for fine-scale inter-individual variation in life history traits (*i.e.* degree of offspring provisioning) to affect which abiotic conditions might constitute an optimal oviposition site. Nest site selection in the plainfin midshipman appears to be driven by offspring benefits, which matches the predictions of life history theory given the species’ relatively high per-offspring investments, and its prolonged offspring development and parental care periods. However, much future work is needed to establish general relationships between life history traits and oviposition site decisions. Furthering our understanding of the life history–oviposition site selection nexus will improve conservation decision-making by refining predictions of species-specific responses to disturbances in oviposition habitats (Partridge & Harvey, 1988; Refsnider & Janzen, 2010).

4.6 References

Amat, J.A. & Masero, J.A. (2004). Predation risk on incubating adults constrains the choice of thermally favourable nest sites in a plover– Anim. Behav. 67: 293–300.

Arora, H.L. (1948). Observations on the habits and early life history of the Batrachoid fish, *Porichthys notatus* Girard– Copeia 1948: 89–93.

Bernardo, J. (1996). The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations– Am. Zool. 236: 216–236.

Bonsall, M.B. & Klug, H. (2011). The evolution of parental care in stochastic environments– J. Evol. Biol. 24: 645–655.

Bose, A.P.H., Borowiec, B., Scott, G., & Balshine, S. (2019). Nesting on high: reproductive and physiological consequences of breeding in the intertidal zone– Evol. Ecol. 33: 21–36.

Bose, A.P.H., Cogliati, K.M., Luymes, N., Bass, A.H., Marchaterre, M.A., Sisneros, J.A., Bolker, B.M., & Balshine, S. (2018). Phenotypic traits and resource quality as factors affecting male reproductive success in a toadfish– Behav. Ecol. 29: 496-507.

Bose, A.P.H., McClelland, G.B., & Balshine, S. (2016a). Cannibalism, competition, and costly care in the plainfin midshipman fish, *Porichthys notatus*– Behav. Ecol. 27: 628–636.

Bose, A.P.H., Kou, H.H., & Balshine, S. (2016b). Impacts of direct and indirect paternity cues on paternal care in a singing toadfish– Behav. Ecol. 27: 1507–1514.

Cargnelli, L.M. & Neff, B.D. (2006). Condition-dependent nesting in bluegill sunfish *Lepomis macrochirus*– J. Anim. Ecol. 75: 627–633.

Cogliati, K.M., Neff, B.D., & Balshine, S. (2013). High degree of paternity loss in a species with alternative reproductive tactics– Behav. Ecol. Sociobiol. 67: 399–408.

Craig, P.M., Fitzpatrick, J.L., Walsh, P.J., Wood, C.M., & McClelland, G.B. (2014). Coping with aquatic hypoxia: How the plainfin midshipman (*Porichthys notatus*) tolerates the intertidal zone– Environ. Biol. Fishes 97: 163–172.

DeMartini, E.E. (1988). Spawning success of the male plainfin midshipman. I. Influences of male body size and area of spawning site– J. Exp. Mar. Bio. Ecol. 121: 177–192.

DeMartini, E.E. (1990). Annual variations in fecundity, egg size and condition of the plainfin midshipman (*Porichthys notatus*)– Copeia 1990: 850–855.

DeMartini, E.E. (1991). Spawning success of the male plainfin midshipman. II. Substratum as a limiting spatial resource– J. Exp. Mar. Bio. Ecol. 146: 235–251.

Dickerson, B.R., Brinck, K.W., Willson, M.F., Bentzen, P., & Quinn, T.P. (2005). Relative importance of salmon body size and arrival time at breeding grounds to reproductive success– Ecology 86: 347–352.

Doak, P., Kareiva, P., & Kingsolver, J. (2006). Fitness consequences of choosy oviposition for a time-limited butterfly– Ecology 87: 395–408.

Gagliano, M. & McCormick, M.I. (2007). Maternal condition influences phenotypic selection on offspring– J. Anim. Ecol. 76: 174–182.

Gall, B.G., Brodie, E.D., & Brodie, E.D. (2012). Fine-scale selection by ovipositing females increases egg survival– Ecol. Evol. 2: 2763–2774.

Ishimatsu, A., Mai, H. Van, & Martin, K.L.M. (2018). Patterns of fish reproduction at the interface between air and water– Integr. Comp. Biol. 58: 1064–1085.

Jones, B.C. (1972). Effect of intertidal exposure on the survival and embryonic development of Pacific herring spawn– J. Fish. Res. Board Canada 29: 119–1124.

Klug, H. & Bonsall, M.B. (2010). Life history and the evolution of parental care– Evolution 64: 823–835.

Krist, M. (2011). Egg size and offspring quality: A meta-analysis in birds– Biol. Rev. 86: 692–716.

LeMoine, C.M.R., Bucking, C., Craig, P.M., & Walsh, P.J. (2014). Divergent hypoxia tolerance in adult males and females of the plainfin midshipman (*Porichthys notatus*)– Physiol. Biochem. Zool. 87: 325–333.

Li, S.R., Hao, X., Wang, Y., Sun, B.J., Bi, J.H., Zhang, Y.P., Janzen, F.J., & Du, W.G. (2018). Female lizards choose warm, moist nests that improve embryonic survivorship and offspring fitness– Funct. Ecol. 32: 416–423.

LINZ (2017). Method to find times or heights between high and low waters– In: New Zeal. Naut. Alm. (NZ 204). New Zealand Government, p. 32–34.

Martin, K.L.M., Van Winkle, R.C., Drais, J.E., & Lakisic, H. (2004). Beach-spawning fishes, terrestrial eggs, and air breathing– Physiol. Biochem. Zool. Ecol. Evol.

Approaches 77: 341–356.

Morjan, C.L. & Janzen, F.J. (2003). Nest temperature is not related to egg size in a turtle with temperature-dependent sex determination– Copeia 2003: 366–372.

Murphy, P.J. (2003a). Context-dependent reproductive site choice in a Neotropical frog– Behav. Ecol. 14: 626–633.

Murphy, P.J. (2003b). Does reproductive site choice in a neotropical frog mirror variable risks facing offspring ?– Ecol. Monogr. 73: 1–24.

Palazón-Fernandez, J.L., Potts, J.C., Manooch III, C.S., & Sarasquete, C. (2010). Age, growth and mortality of the toadfish, *Halobatrachus didactylus* (Schneider, 1801) (Pisces: Batrachoididae), in the Bay of Cádiz (southwestern Spain)– Sci. Mar. 74: 121–130.

Partridge, L. & Harvey, P.H. (1988). The ecological context of life history evolution– Science 241: 1449–1455.

Petranka, J.W. & Petranka, J.G. (1981). On the Evolution of nest site selection in the marbled salamander, *Ambystoma opacum*– Copeia 1981: 387.

Phillips, J.E. (2007). Parental ecology of the plainfin midshipman (*Porichthys notatus*). California State University, Sacramento.

R Core Team (2016). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Refsnider, J.M. & Janzen, F.J. (2010). Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice– Annu. Rev. Ecol. Evol. Syst. 41: 39–57.

Resetarits, W.J.J. (1996). Oviposition site choice and life history evolution– Am. Zool. 36: 205–215.

Ricklefs, R. & Wikelski, M. (2002). The physiology/life-history nexus– Trends Ecol. Evol. 17: 462–468.

Roosenburg, W.M. (1996). Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination?– Am. Zool. 36: 157–168.

Roosenburg, W.M. & Kelley, K.C. (1996). The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin*– J. Herpetol. 30: 198–204.

Sargent, R.C., Taylor, P.D., & Gross, M.R. (1987). Parental care and the evolution of egg size in fishes– Am. Nat. 129: 32–46.

Sayer, M.D.J. & Davenport, J. (1991). Amphibious fish: why do they leave water?– Rev. Fish Biol. Fish. 1: 159–181.

Shine, R. (1978). Propagule size and parental care: The “safe harbor” hypothesis– J. Theor. Biol. 75: 417–424.

Spencer, R.J. (2002). Experimentally testing nest site selection: Fitness trade-offs and predation risk in turtles– Ecology 83: 2136–2144.

Stearns, S.C. (1992). The evolution of life histories. Oxford University Press, Oxford, UK.

Suk, H.Y., Neff, B.D., Fitzpatrick, J.L., & Balshine, S. (2009). Isolation and characterization of polymorphic microsatellite loci in plainfin midshipman fish– Hereditas 146: 204–207.

Szekely, T. & Cuthill, I.C. (2000). Trade-off between mating opportunities and parental care: Brood desertion by female Kentish plovers– Proc. R. Soc. B Biol. Sci. 267: 2087–2092.

Tieleman, B.I., van Noordwijk, H.J., & Williams, J.B. (2008). Nest site selection in a hot desert: trade-off between microclimate and predation risk?– Condor 110: 116–124.

Ward, R.J.S., Cotter, S.C., & Kilner, R.M. (2009). Current brood size and residual reproductive value predict offspring desertion in the burying beetle *Nicrophorus vespilloides*– Behav. Ecol. 20: 1274–1281.

Warner, D.A. & Mitchell, T.S. (2013). Does maternal oviposition site influence offspring dispersal to suitable habitat?– Oecologia 172: 679–688.

Warner, D.A. & Shine, R. (2008). Maternal nest-site choice in a lizard with temperature-dependent sex determination– Anim. Behav. 75: 861–870.

Webb, J.N., Székely, T., Houston, A.I., & McNamara, J.M. (2002). A theoretical analysis of the energetic costs and consequences of parental care decisions.– Philos. Trans. R. Soc. B Biol. Sci. 357: 331–340.

Wilson, D.S. (1998). Nest-site selection: Microhabitat variation and its effects on the survival of turtle embryos– Ecology 79: 1884–1892.

Wootton, R.J. & Smith, C. (2015). Reproductive Biology of Teleost Fishes. John Wiley & Sons, Ltd., West Sussex, UK.

Appendix

Chapter 5: Preliminary results of 2019 fieldwork

5.1 Motivation

In **Chapter 2**, my coauthors and I sampled fish across an intertidal gradient but were unable to capture fish from subtidal nests owing to logistical difficulties. The temperature data we collected showed that the subtidal nesting locations constitute different thermal environments from those in the intertidal. So, in 2019, I returned to the field site with a new team to attempt to capture a sample of guarder males and females from subtidal nests. Our objective was to determine whether the pattern of increasing male body size with decreasing intertidal elevation continued into the subtidal, and whether there were patterns in how females were distributed between nests with respect to body size. The results presented here are one facet of a larger study comparing spawning success, competition, and parental care between the two nesting environments.

5.2 Brief methods

During low tides of the spring cycle from 23–25 April 2019 we constructed 30 artificial nests (929 cm^2 square concrete garden tiles) each along an intertidal and a subtidal contour (−1.3 and −4.0 ft relative to chart datum, respectively) at a private beach in Dabob Bay of the Hood Canal, WA, USA (47°76′N, 122°86′W). We left these nests for 10 days to allow guarder males to discover and excavate the nests, and potentially compete for preferred sites. Then, during the low tides of the following spring cycle (5–7 May), we sampled all nests in both the intertidal and subtidal groups by gently overturning each tile. For intertidal nests, we removed all male and females in the nest by hand and measured their masses (± 0.01 g) and standard lengths (SL; ± 1 mm). To capture fish from subtidal nests, two snorkelers worked in tandem to lift each tile and cover the nesting cavity below with a handheld net. Fish would typically swim up into the net while trying to escape, but occasionally one snorkeler had to reach under the net to startle fish up into the netting. On the rare occasion a fish escaped capture, we noted its presence in the nest. We returned to sample these nests again using the same methods one month later during low tides of the 3–4 June spring cycle.

All statistical analyses were carried out in R (R Core Team, 2016). We used binomial general linear models (GLMs) to examine differences between the number of nests that were occupied by males and received spawnings in both May and June. Differences in body sizes between intertidal and subtidal nests in May and June were evaluated using a 2 × 2 factorial ANOVA.

5.3 Preliminary results

Artificial nests along both the intertidal and subtidal contours were more likely to be taken up by males in June compared to in May (GLM, type II Wald chi-squared test; effect of month: estimate [est.] ± SE = 0.13 ± 0.04, $\chi^2(1) = 12.0$, $p < 0.001$). Intertidal nests were more likely to be occupied by a male than subtidal nests in both May and June (GLM, type II Wald chi-squared tests; effect of elevation: estimate [est.] ± SE = 0.10 ± 0.04, $\chi^2(1) = 5.8$, $p = 0.016$; elevation × month: $\chi^2(1) = 0.00$, $p = 1.0$). Similarly, more nests received eggs from at least one female in June compared to in May (GLM, type III Wald chi-squared test; effect of month: estimate [est.] ± SE = 0.47 ± 0.06, $\chi^2(1) = 51.7$, $p < 0.001$), and more intertidal nests than subtidal nests received eggs in both May and June (GLM, type II Wald chi-squared tests; effect of elevation: estimate [est.] ± SE = 0.23 ± 0.06, $\chi^2(1) = 13.7$, $p < 0.001$; elevation × month: $\chi^2(1) = 0.00$, $p = 1.0$).

Body sizes were similar among males in intertidal (mean ± SD SL = 244 ± 22 mm) and subtidal (mean ± SD SL = 239 ± 20 mm) nests in May, but in June, intertidal males (mean ± SD SL = 243 ± 20 mm) were smaller than subtidal males (mean ± SD SL = 259 ± 19 mm; 2 × 2 factorial ANOVA: elevation × month, $F_{1,99} = 9.74$, $p = 0.0023$; Fig. 2A). Intertidal females appeared smaller than subtidal females in both May and June (mean ± SD SL: intertidal, May =174 ± 16 mm; subtidal, May = 195 ± 10 mm; intertidal, June = 158 ± 23 mm; subtidal, June = 189 ± 25 mm; Fig. 2B), but we did not analyze these data for two reasons: 1) we collected a total of 7 females from subtidal nests during the May and June samples, and 2) smaller females might have escaped capture more easily if they could fit into small gaps between the edges of the net and the nesting cavity.

Figure 5.1 The proportion of nests (out of 30) A) occupied by a guarding male, and B) containing a brood of living eggs in both intertidal and subtidal nesting environments. Error bars in both panels show the 95% binomial CIs. Both measures were recorded during two sampling periods in 2019: 5–7 May and 3–4 June.

Figure 5.2 Body sizes (SL) of A) males and B) females captured in artificial intertidal and subtidal nests during two sampling events in 2019: 5–7 May and 3–4 June.

Figure 5.1

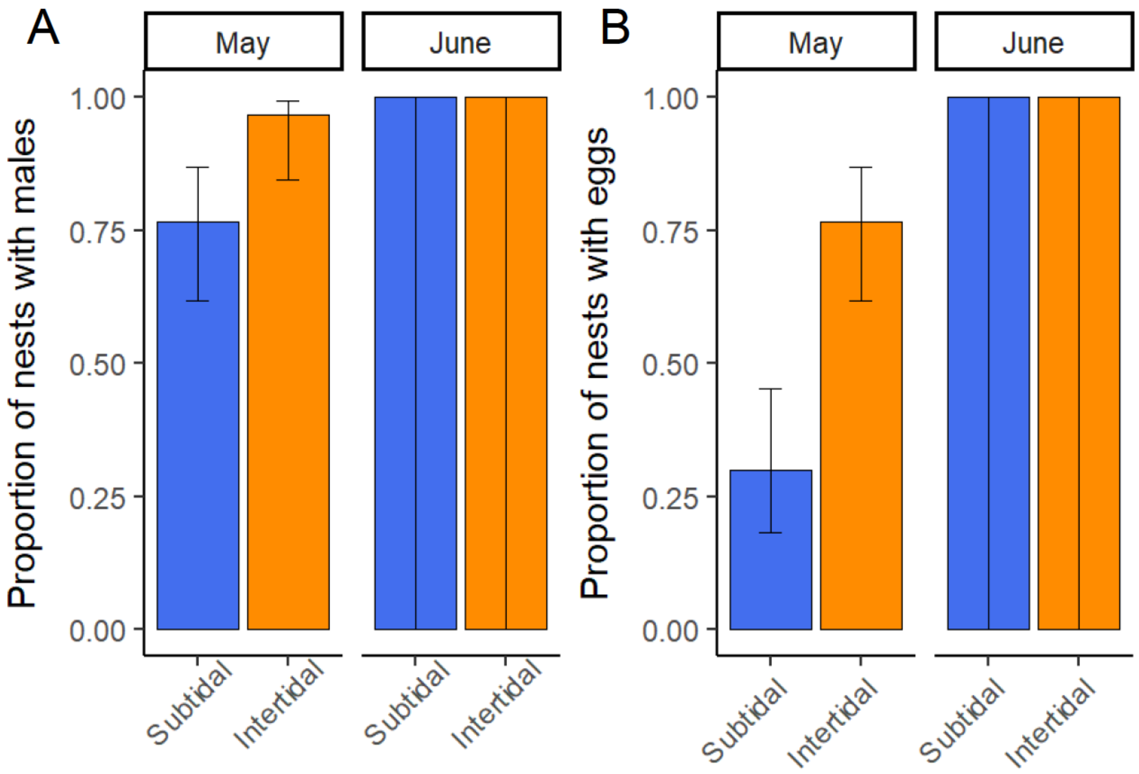
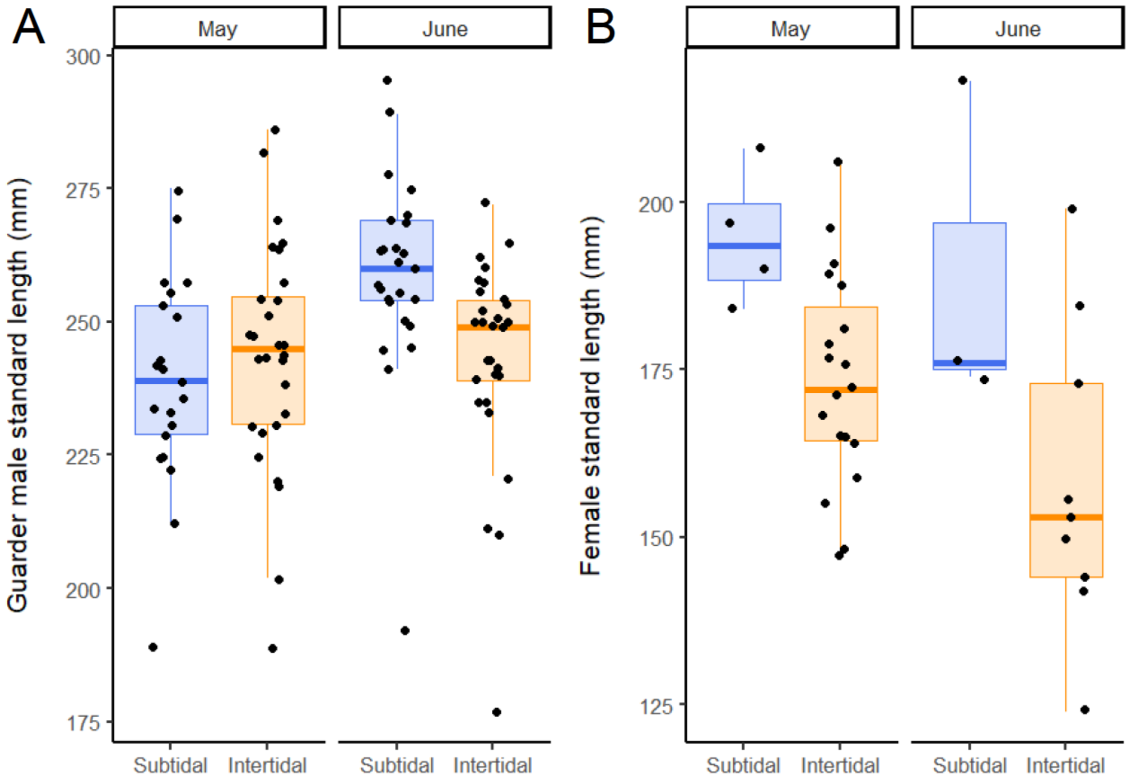

A
May
June
Proportion of nests with males
1.00
0.75
0.50
0.25
0.00
Subtidal
Intertidal
Subtidal
Intertidal
B
May
June
Proportion of nests with eggs
1.00
0.75
0.50
0.25
0.00
Subtidal
Intertidal
Subtidal
Intertidal

Figure 5.2


A
May
June
Guarder male standard length (mm)
300
275
250
225
200
175
Subtidal
Intertidal
Subtidal
Intertidal
B
May
June
Female standard length (mm)
200
175
150
125
Subtidal
Intertidal
Subtidal
Intertidal