Disentangling the determinants of reproductive success in a temperate reef fish, *Forsterygion lapillum*

By

McKenzie Grace Tornquist

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Abstract

Identifying sources of individual variation in reproductive success has been a longstanding challenge for evolutionary ecologists. Reproductive success among individuals can be due to several factors such as competition between conspecifics for nest sites and mating partners, mate choice, or by the physical environment. Reproductive success, particularly among males, can be extremely diverse both within and between species and determining which components contribute to success can be particularly challenging. In this thesis, I investigated patterns and drivers of reproductive success in a temperate marine reef fish, *Forsterygion lapillum* (the common triplefin). Specifically, I examined how male quality, nest quality, and female choice influence male reproductive success. Additionally, I quantified male reproductive success during the winter and summer of the breeding season to examine the temporal dynamics of breeding success in *F. lapillum*.

Selection of mates by females can be driven by the quality and behavioural attributes of the male or by the quality of resources offered. In Chapter 2, using field-based observations, combined with a lab-based study, I evaluated the effects of different male traits and nest characteristics on female choice and male reproductive success. Specifically, I observed egg guarding males in the field during the breeding season and recorded their phenotypic traits, behaviours, and nest characteristics. I then examined their influence on 3 different components of male reproductive success (brood size, individual egg size, and mate attraction). Additionally, I conducted dichotomous choice tests in the laboratory to evaluate female preference for different sized males, holding different sized nests. In the field, I did not detect a significant relationship between male mating success and male total length or nest size. Brood size and individual egg size were highly variable among sampled males, however, further factors such as courtship frequency, and the number of interactions with potential predators did not explain any additional variation. The number of agonistic displays performed by egg guarding males was the only factor to influence egg size, however, it had no direct impact on brood size or mate attraction. On the contrary, results from the laboratory experiment suggested that male total length and nest size were important during female choice. Females were attracted to and spawned more frequently with larger males holding larger nests. Additionally,

females showed a particular preference towards males that displayed intense courtship behaviours. These results suggest that variation in reproductive success among individuals is not random in the common triplefin (*F. lapillum*) and may be due to a range of complex factors.

In natural systems, individual variation in mating success is known to be highly dynamic and vary over time. In Chapter 3, I addressed 3 questions related to reproductive success in male common triplefin: 1) Does the operational sex ratio (OSR) and the density of individuals change predictably within the breeding season? 2) Does male reproductive success change within the breeding season? And 3) Does the age and growth rate of successful males change within the breeding season? To address these questions, I sampled a population of *F. lapillum* during two periods of the breeding season and quantified a set of morphological and physical traits. Furthermore, I reconstructed individual life histories from the otoliths of egg guarding males. My results show that the density of individuals in the population increased during the summer months, but the operational sex ratio (OSR) remained male-biased. Male reproductive success in terms of brood size and average egg size did not fluctuate during the sampling period. However, the size of males and the size of the nest (cobblestone) held by males was significantly larger in summer compared to winter. Interestingly, successful males sampled in the winter had hatched significantly earlier than successful males sampled in the summer, but their average growth rate remained similar. These findings indicate that variation in male traits across the breeding season plays an important role in female mate choice. The mating system and pool of mating individuals in the common triplefin (*F. lapillum*) is highly dynamic over the year and has the potential to shape the success of individuals.

Overall, this study emphasizes the importance of considering multiple cues and temporal dynamics when disentangling the determinants of individual reproductive success. These findings suggest that male-male competition and female mate choice have a significant influence on male reproductive success. The reproductive ecology of *F. lapillum* is highly complex and my research has provided valuable insight into its dynamic nature. These results may apply to other species with male parental care and provides an important contribution towards understanding sexual selection and the evolution of mating systems with male parental care.

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General Introduction

The determinants of individual reproductive success for a wide variety of organisms and systems are still not well understood (Carlo et al. 2001). Variation in mating success among individuals forms the foundation of evolutionary biology (Andersson 1994), and determining what causes variation among individuals is crucial to our understanding of sexual selection (Reynolds 1996, Alonzo 2008). A large body of work seeks to evaluate the determinants of success in a wide variety of taxa including birds (Stacey 1982, Goodnow and Reitsma 2011), insects (Segoli and Rosenheim 2013, Rotenberry and Zuk 2016), mammals (Armitage 1987, Jones et al. 2012), and fish (Hastings 1988a, Bose et al. 2018). Reproductive success, particularly among males, can be extremely variable both within and between species (Fessehaye et al. 2006). Consequently, patterns of mating behaviour and male success are poorly known for many species.

Among vertebrates, fish show the greatest diversity in cooperative and competitive reproductive behaviours (Taborsky 2001). In fish that exhibit male parental care, male reproductive success is often dependent upon the acquisition and defence of a nesting site, resulting in intense male-male competition and female selectivity (Hastings and Petersen 2010). Traits associated with male reproductive success often include both male characteristics (dominance, courtship behaviour, colour, body size, age) and nest characteristics (nest size, location, depth) (Downhower et al. 1983, Thompson 1986, Clutton-Brock and Vincent 1991, Andersson 1994). Yet few studies evaluate the influence of these components together. To advance our understanding of male reproductive success, it is important to study multiple factors simultaneously. Selection may act differentially on each factor, and this may change over an individual's lifetime (Bose et al. 2018). By evaluating potential environmental and phenotypic factors that correlate with reproductive success, one can gain insight into how particular mating systems are shaped.

1.1 Sexual selection

Sexual selection has long been recognised by biologists as a powerful and predominant evolutionary force that has the potential to influence reproductive success by shaping physiological, morphological, and behavioural traits (Trivers 1972, Andersson 1994). In 1871, the theory of sexual selection was first proposed by Darwin and was defined as the reproductive advantage an individual possesses over another individual of the same sex and species (Darwin 1871). Sexual selection arises from variation in reproductive success, which itself is the product of competition for mates within a sex (intra-sexual selection) and differential mate choice (inter-sexual selection) (Andersson and Iwasa 1996). Although the role of sexual selection as an evolutionary force has been widely accepted, the relative roles of male-male competition and mate choice as driving mechanisms for sexual selection are not as clear (Bradbury and Gibson 1983).

Intra-sexual selection is usually strongest in the sex with the highest reproductive rates, and mate choice is employed by the opposite sex (Andersson and Iwasa 1996). Both sexes may be choosy, but in most cases males will compete for females, and females will show a mating preference (Trivers 1972). Female success is usually not restricted by mate access. Thus, selection frequently acts on females to choose mates based on one or more phenotypic traits that vary among competing males (Andersson 1982, Andersson 1994). Mate choice can impose sexual selection on the opposite sex and is responsible for the evolution of intricate ornaments and displays that influence female mating decisions (Wootton and Smith 2015). If a resource held by a male increases the fitness of a female or her offspring, selection will shift towards the tendency of females to prefer males holding such resources (Wootton and Smith 2015). Thus, male reproductive success is usually restricted by their ability to access limited resources (i.e. territories, females), which often leads to intense intra-sexual competition (Trivers 1972, Berglund and Pilastro 1996). Competition between males often selects for traits that maximize fighting capabilities, including large body size, strength, and weapons. These traits may also be favoured by females. For instance, females typically show preference for large males, which can often lead to sexual dimorphism (Demartini 1988, Andersson 1994, Stoltz and Neff 2006).

Female choice and male-male competition often act synergistically to cause variation in male reproductive success, and many studies focus on which component has the greatest impact. The ultimate cause of variation in reproductive success among individuals is often difficult to predict because multiple traits can contribute simultaneously to sexual selection (Demartini 1988, Oliveira et al. 2000, Candolin and Voigt 2001). Male quality and resource quality are also typically highly correlated, making it difficult to determine whether a female is choosing mates based on resource qualities, male qualities, or both (Bart and Earnst 1999). This in turn makes it difficult to determine what actually drives the evolution of many male traits.

1.2 Male reproductive success in fish

Fish exhibit an unparalleled diversity of social and reproductive behaviours (Taborsky 1994, 1998). There is strong evidence that both male-male competition and female mate choice have been critical in the evolution of many fish species, especially among Blennioid fishes (Wootton and Smith 2015). Blennioid fish are found in numerous coastal regions of the world and play an important role in reef communities in both tropical and temperate areas (Hastings and Petersen 2010). In recent years, the reproductive biology of blennioids has received increased interest and attention. However, published research is profoundly biased towards a few well-studied species and certain families (Hastings and Petersen 2010). Consequently, for many species a robust framework to explain the relative success of individuals is still undefined. An example of this is in species exhibiting male parental care, where males care for clutches of fertilised eggs (Wootton and Smith 2015). So far, it is known that male guarding of eggs is exhibited in all species of blennioids with external fertilization, which often takes the form of defence against predators and fanning of eggs (Hastings and Petersen 2010). The significance of male guarding behaviour is often reflected in the preference of females (Hastings and Petersen 2010). Females in these systems are expected to be choosy in regard to which male she mates with due to his direct impact on offspring survival (Andersson 1994, Wootton and Smith 2015). Likewise, females are expected to be choosy in regard to territory or nest quality because the resources in which the male is defending can impact offspring survival (Oliveira et al. 2000). Successful males in these systems are therefore predicted to be the males that acquire and defend the highest quality resources and attract females (Emlen and Oring 1997, Oliveira et al. 2000).

1.2.1 Male-male competition and reproductive sites

A form of male-male competition commonly seen in mating systems with male parental care is the competition between males for the establishment and defence of a territory or oviposition site (Gross and Sargent 1985, Lindström 1992, Emlen and Oring 1997). Territoriality occurs whenever the defence of a site provides access to a limiting resource (Given 1988). Territories can serve as a location for courtship, breeding, parental care, and can even regulate which individuals in a population breed (Gross and Sargent 1985, Wootton and Smith 2015). Territories of the highest quality are frequently contested and dominated by males with the greatest competitive abilities (Lindström 1992, Wootton and Smith 2015). As a result, the determinants of male success are expected to include traits that influence their ability to compete. A number of characteristics of fish can increase their fighting capabilities, such as large body size, robust muscles, and large jaws (Faria and Almada 2001, Hastings 2002). For many blennioid species, body size is a key determinant of dominance and plays an important role in territorial contests (Candolin and Voigt 2001). A larger body can place a male at a competitive advantage over smaller males when competing for territories (Takegaki et al. 2008). Taking part in aggressive interactions can result in high energetic costs, so typically the winners of such encounters can display agonistic behaviours for longer (Ros et al. 2006). Body size may act as a cue to deter smaller individuals from participating in a contest with a larger male, resulting in a positive correlation between male body size and resource holding potential, and consequently reproductive success (Kelly 2008, Takegaki et al. 2008).

The selection of a nest site is an important determinant of reproductive success (Mainwaring et al. 2014). If variation in the quality of male territories is large, this will lead to high levels of variation in male reproductive success (Friedl and Klump 2000). Males are predicted to compete for the larger/higher quality nest sites as these have the potential to carry more eggs (Wong et al. 2008, Carriço et al. 2014). The size or quality of a male's territory can influence the number of clutches he is able to obtain and can influence female mating decisions, limiting his overall reproductive success (Demartini 1988, Carriço et al. 2014). Males that defend and hold the best quality nests or largest territories tend to have the highest levels of reproductive success (Part 2001, Casalini et al. 2013). For instance, Bose et al. (2018) found that male plainfin midshipman fish, *Porichthys notaus,* preferred more enclosed and larger nests, and nest size was positively correlated with the number of eggs. However, a trade-off may occur

between the benefits of larger nests for more offspring with the cost of defending and maintaining them (Kvarnemo 1995). Size-assortative nest choice, where males choose nests according to their own body size, has been found in several fish species (Kvarnemo 1995). In sand gobies, *P. minutus,* males choose smaller and more defendable nests when nest takeover is high, but when the risks are low they prefer larger nests (Björk and Kvarnemo 2012). This assortative mating often leads to a positive correlation between body size and nest size. Hence, larger males tend to have the greatest reproductive success as they are often able to defend and compete for the largest nest sites.

1.2.2 Female choice

In addition to male-male competition, female choice can have a large impact on the reproductive success of males as constraints on female access can reduce the number of potential breeding opportunities (Andersson 1994). Consequently, determinants of male reproductive success often include characteristics that influence female choice (Trivers 1972). Female choice may be based on either direct or indirect fitness benefits (Andersson 1994). Direct benefits include male resources such as nuptial gifts, nest sites, absence of parasites, quality of parental care, and size or quality of a territory (Moller and Thornhill 1998). Indirect fitness benefits include genes for offspring survival or sexual attractiveness (Moller and Thornhill 1998). Mate choice is complex and optimal choice requires a female to correctly evaluate the benefits provided by the chosen male (Andersson 1982, Hanson and Cooke 2009). For this to happen, there needs to be a cue related to the status of the male that the female desires (Wiegmann and Baylis 1995, Hanson and Cooke 2009).

In systems where males provide resources in addition to sperm, females are predicted to choose males based on resource qualities rather than on male traits, particularly in systems that display male parental care (Hastings 1988a, Lehtonen et al. 2007, Carriço et al. 2014). Using cues related to resource quality may provide the female with direct benefits, such as food and shelter from predators or harmful environments (Jones and Reynolds 1999, Gotelli 2007, Hermann et al. 2015). For example, in the spinyhead blenny, *Acanthemblemaria spinosa*, females preferred males from shallower sites due to the increased availability of planktonic prey (Clarke and Tyler 2003). However, several factors may also cause male traits to be important cues during mate choice (Lehtonen et al. 2007). For instance, female preference for large males has been

demonstrated in several blennioid species (i.e. *Forsterygion varium*, Thompson 1986; *Coralliozetus angelica*, Hastings 1988b). Body size of potential mates can provide females with information on their likely parental effort (Wiegmann and Baylis 1995). Large males may possess larger energy stores, enabling them to invest more effort into offspring over longer periods of care because feeding is frequently decreased or stopped while care is provided (Wiegmann and Baylis 1995) Additionally, in mating systems with dominance hierarchies, large males often have a higher social status which can reduce the possibility of egg predation from conspecifics (Bisazza et al. 1989). Thus, the frequently observed correlations between female preference and male body size, and male reproductive success and male body size have been hypothesised as a result of greater parental abilities by larger males. Subsequently, females may directly drive the evolution of male size and in turn reinforce the selection pressure imposed by intraspecific competition for nest sites (Hermann et al. 2015).

Characteristics favoured by females may contradict the traits that are associated with a male's competitive ability. For example, in sand gobies, males that are successful competitors were not preferred by females, rather females favoured 'good fathers' (Forsgren 1997a). Females may not discriminate between subordinate and dominant individuals, or may even prefer subordinates (Smith and Spence 2006). Hence, in some cases the high reproductive success acquired by dominant individuals may simply be a result of dominant males monopolizing access to resources, diminishing the opportunity for female choice to operate (Reichard et al. 2005, Reichard et al. 2007). Additionally, in many species that display parental care, a male's mating history, including the presence of offspring, can influence female mate choice. Females have been shown to mate with males that already guard nests containing eggs, especially when those eggs are at an early stage of development (Kraak and Groothuis 1994). Spawning with males that already have eggs can increase the survival of their own eggs due to the reduced probability of male filial cannibalism or egg predation (i.e. 'the dilution effect') (Matsumoto et al. 2011). Additionally, many eggs from multiple females are less likely to be abandoned by the male as increased egg numbers are predicted to increase a male's parental investment (Gross and Sargent 1985).

Disentangling the determinants of success amongst competing males is often complex and requires multiple factors to be investigated simultaneously. From a theoretical point of view,

competition for limited resources is straightforward and easy to demonstrate (Wootton and Smith 2015). However, male-male competition may not only influence reproductive success through the accessibility of limited resources, but can also influence the process of female choice (Takegaki et al. 2008). Body size can maximise a males fighting ability and in turn, smaller males can become excluded from gaining access to nesting sites and females (Borg et al. 2006, Hastings and Petersen 2010). Therefore, the mating success of larger males may not be a true reflection of optimal female choice (Takegaki et al. 2008). Furthermore, when body size is an important contributor of resource holding potential, nest characteristics and body size are expected to correlate (Kelly 2008). This in turn makes it difficult to disentangle the relative contributions of both components on female choice. Despite the extensive literature on the independent effects of male quality and resource quality, very little is known about their joint and interactive effect. Females often base mating decisions on one or more mate choice cues, therefore, it is vital that their interactions are considered carefully. Additionally, the degree of female preference may vary within a breeding season. This is especially true in shorter lived species due to changes in the cohort of available males and their ability to sequester more successful sites. This leads to a number of unanswered questions: Do females assess male quality and resource quality simultaneously? Is one component of greater importance than the other? Does female preference change within a breeding season?

1.3 Study species

My thesis evaluates the determinants of male reproductive success in the common triplefin, *Forsterygion lapillum*. *F. lapillum* is a small temperate reef fish (maximum total length 70 mm) of the family Tripterygiidae. Tripterygiids are highly diverse and well represented in New Zealand, with 27 known endemic species in 12 genera (Hickey et al. 2009). *F. lapillum* are found widely distributed along the coast of New Zealand, inhabiting shallow (0-10 m), rocky, intertidal reefs (Feary and Clements 2006). Throughout the breeding season, sexually mature males often defend small cobbled breeding territories (~ 1 m^2) (Feary and Clements 2006, Wellenreuther et al. 2007). Females will lay eggs, in a single layer, on the underside of cobblestones within the nest of a chosen male and the male will fertilize each egg as it is laid (Thompson 1986, Moginie and Shima 2018). Males are highly territorial and will remain with and vigorously defend their nests throughout the breeding season, leaving occasionally for short-distance foraging (Thompson 1979, 1986). Males provide sole parental care of the eggs

until hatching, including defence from conspecific and heterospecific egg predators, and fanning of the eggs (Feary and Clements 2006). Larvae hatch after ~ 2-3 weeks and have a pelagic larval phase of ~ 40-60 days (Shima and Swearer 2009). Males can guard up to 2000 eggs from multiple females and care for numerous clutches during their reproductive lifetime (Mensink et al. 2014). Reproductive males actively court nearby females with extravagant displays that include looping in and out of the nest and moving rapidly from side to side (Mensink et al. 2014). Unlike males, females do not defend territories and travel to find mating opportunities (Feary and Clements 2006). Throughout most of the year, males and females share the same light brown body colouration. When males are reproductively active, they develop a uniform black colouration, making them distinguishable from females and other non-reproductive males (Fig.1.1) (Wellenreuther et al. 2007). *F. lapillum* are easy to observe in the wild and to maintain in laboratory settings, making them a perfect study species for this research (Wellenreuther et al. 2007).

1.4 Aims and thesis structure

This thesis aims to disentangle the relative importance of different mechanisms underlying reproductive success by investigating male mating success and female choice in a temperate reef fish.

In Chapter two, I investigate the relationship between male traits, nest characteristics, female choice, and reproductive success in adult *F. lapillum.* I haphazardly sampled and observed males in the field to evaluate the relative importance of different aspects of male quality and nest characteristics on 3 components of male reproductive success: brood size, average egg size, and mate attraction. Additionally, I conducted laboratory experiments to evaluate female choice in terms of male size, nest size, and the interaction between the two.

In Chapter three, I quantified male reproductive success early and late in the breeding season and examined the temporal dynamics of breeding success in *F. lapillum*. I sampled individuals in the field and quantified a set of morphological traits and reconstructed individual life histories of nest holding males across the breeding season.
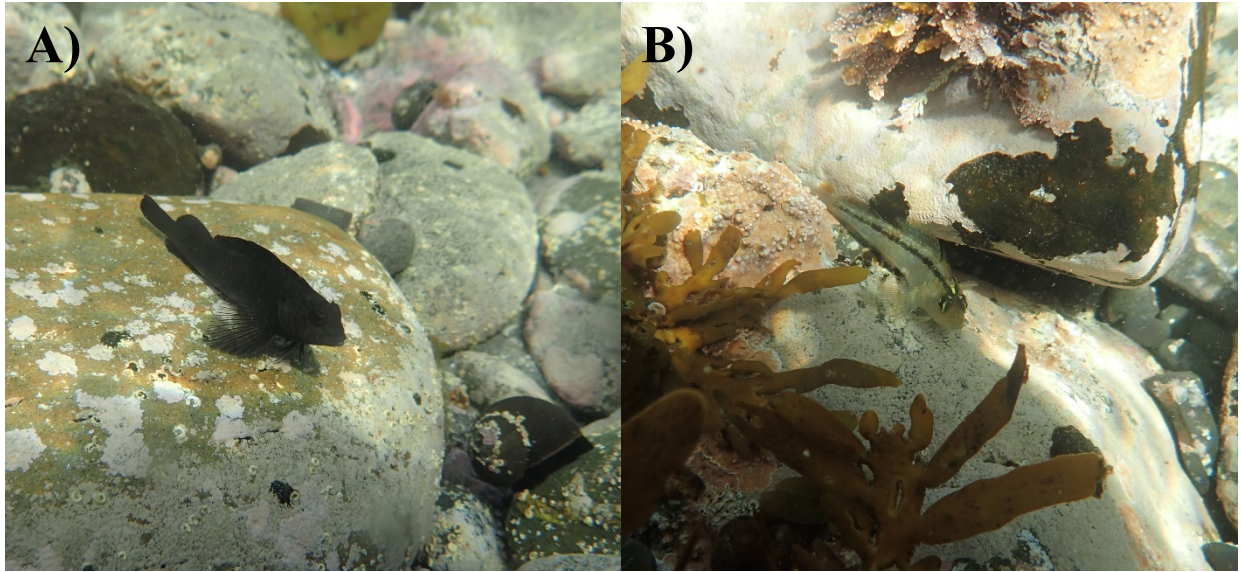
Figure 1.1 A) Adult Male *F. lapillum* displaying black breeding colouration, resting on top of nesting site (cobblestone). **B)** Adult *F. lapillum* displaying normal, non-breeding, colouration, resting on top of cobblestone amongst seaweed.

CHAPTER TWO

Female choice and male reproductive success in a temperate reef fish, *Forsterygion lapillum*

2.1 Introduction

In most mating systems, females are typically the choosy sex, preferentially mating with certain male phenotypes to maximise their breeding success (Andersson 1994). Females often base their mating preference on more than one mate choice cue and will preferentially choose mates with resources or traits that increase offspring survival (Candolin 2003, Carriço et al. 2014). Sexual displays are often highly complex and involve multiple signal components (Candolin 2003). In polygamous species with resource defence polygyny, territorial males mate with multiple females and provide resources, such as nest sites or food, that females require for reproduction (Friedl and Klump 2000, Neff and Pitcher 2005, Casalini et al. 2013). In these systems, males compete for mating opportunities either directly through male-male competition (intra-sexual selection), or indirectly through female choice (inter-sexual selection) (Andersson and Iwasa 1996). The quality of resources provided by males can vary substantially in a population, thus, female choice is expected to be driven by both the quality of resources offered and by the quality of the prospective mate (Clutton-Brock 1988, Lehtonen et al. 2007). The effects of male and resource characteristics on female choice may not be additive, and selection may act differentially on each factor, yet few studies evaluate the influence of these factors together (Clutton-Brock 1988, Bose et al. 2018). Consequently, little is known about the joint and interactive effect of both components on mate choice.

If reproductive fitness is linked to the quality of resources, females should primarily base their mating decision on the quality of resources offered (Kelly 2008). In many marine fish that display male parental care, females deposit eggs directly onto the nest site guarded by the male (Hastings and Petersen 2010). By seeking the best available nest site, females may gain direct fitness benefits as resource quality can directly impact offspring survival (Oliveira et al. 2000). Some nest sites may be less prone to predation and shield the parents or eggs from harmful environments (Carriço et al. 2014). Additionally, some nest sites may receive more parental care from the male (Jones and Reynolds 1999, Carriço et al. 2014, Hermann et al. 2015). For

example, larger nest sites have the potential to carry more eggs with multiple clutches, and higher egg numbers are predicted to increase a male's parental investment (Gross and Sargent 1985, Wong et al. 2008). However, several mechanisms may also cause male traits to be important cues during mate choice (Lehtonen et al. 2007). Traits such as body size can be indicative of social status and can provide information on the likely parental investment of the potential mate (Wiegmann and Baylis 1995). Males in these systems often compete with one another for territories and larger males frequently obtain and monopolize the favoured nest sites, which can reduce the possibility of egg predation from conspecifics (Hamilton et al. 1997, Hastings and Petersen 2010). Additionally, larger males may possess higher energy stores enabling them to invest more effort into offspring over longer periods (Wiegmann and Baylis 1995). Therefore, when making mating decisions, females often integrate information from both resource quality and male phenotypic cues (Candolin 2003, Lehtonen et al. 2007).

The quality of a resource or mate is unlikely to be determined by a single cue, and mate choice decisions often integrate several different components (Johnstone 1996, Candolin 2003). Despite this, a lot of research investigates the independent effects of a single male trait or resource characteristic (Côte and Hunte 1989, Jamieson and Colgan 1989, Bose et al. 2018). Consequently, little is known about the use of multiple cues during mate selection and whether they have a joint or interactive effect. Male body size is the most commonly studied male trait, particularly in fish that display male parental care, however, this tends to account for little variation in resource value and reproductive success (Kelly 2008). Additionally, a correlation between male body size and resource quality is often found due to the importance of male size when competing for a nest site. This leads to a difficult distinction between the relative importance of the two when evaluating their contributions during mate selection (Sargent 1982, Kodric-Brown 1983, Candolin and Voigt 2001). Correlating a single characteristic with mating success explains only part of the problem to determine what attracts a female to a particular mate or location (Candolin 2003). Investigating the effects of multiple mate choice cues together in a controlled laboratory environment and the field can provide insight into how male quality and resource quality interact to explain variation in reproductive success among males in a population.

In this study, I investigated relationships between male traits, nest characteristics, female choice, and reproductive success in a temperate marine fish, the common triplefin (*Forsterygion lapillum*). *F. lapillum* exhibit male territoriality and parental care, and females exhibit mate choice. I observed males in the field to evaluate the relative importance of different aspects of male quality and nest characteristics on different components of male reproductive success. Additionally, I conducted laboratory experiments to address whether females select mates based on male size, nest size, or both. I examined the decision-making process of females during the 20-minute laboratory trials and investigated whether additional male traits influenced female choice. Specifically, I addressed 3 questions: 1) Which mate choice cues predict differential reproductive success between males? 2) Do females show a mate preference? and 3) Does male size and nest size predict male reproductive success?

2.2 Methods

2.2.1 Study species and system

Forsterygion lapillum (the common triplefin) is a small-bodied temperate reef fish found commonly along the shores of New Zealand (Wellenreuther et al. 2007, 2008). *F. lapillum* establish and defend small territories (~ 1 m^2) within cobble habitats that contain potential nesting sites (Wellenreuther et al. 2007, Mensink et al. 2014). During the breeding season, males assume a black nuptial colouration and display intricate courtship behaviours towards passing females. Unlike males, females display a consistent colouration throughout the year, indistinguishable from the colouration of non-reproductive males (Wellenreuther et al. 2008). *F. lapillum* are asynchronous continuous spawners, laying eggs daily throughout the breeding season (Mensink et al. 2014). Adult females travel to find mating opportunities, depositing eggs in a single layer on a chosen male's nest, typically underneath cobbles within the male's territory (Feary and Clements 2006). Males guard the nest entrance and provide sole parental care of the eggs until hatching, after ~ 3 weeks, consisting of defence against predators and fanning of the eggs (Francis 2012). Males often defend eggs laid by multiple females throughout the breeding season (Mensink et al. 2014). *F. lapillum* is known to be reproductively active from July to February (Wellenreuther et al. 2007).

2.2.2 Field study

To explore how male morphological traits and resource quality influence male reproductive success in the field, I sampled and observed reproductive males and their broods during the breeding season. A total of 21 nesting males were observed from a single site (Moa point in Wellington Harbour, 41°20'34" S, 174°48'42" E) during low tide in December 2019 and January 2020. Moa point is a south-facing site and was selected because of its abundance of suitable habitat for common triplefin reproduction (loose cobblestones). Each nest was randomly located by gently overturning cobblestones to uncover the male and eggs beneath. When eggs were located, the associated male was classified by its colouration and nest guarding behaviours. Overturning the nest often startled the fish, but they quickly acclimated and returned to their usual activities within minutes. Once I was satisfied that the fish were no longer reacting to my presence, I began my observations. Each male was observed for 10 minutes, during which I recorded the number of visits by females and used this as a measure of mate attraction. I also recorded the number of spawning attempts and the frequency of courtship displays (i.e. lead displays, lateral displays; Appendix A). As a proxy for male dominance and aggression, the frequency of agonistic displays towards conspecifics (males and females) was recorded (i.e. chasing/biting, parallel swimming, and lateral threats; Appendix A). The number of interactions with potential predators was also recorded.

Measures of male reproductive success

To quantity each male's reproductive success, I measured both brood size (the surface area of eggs present in the nest) and average egg size for each nest. Egg size can be affected by the female's nutritional condition and can be an important determinant of larval survival and growth (Brooks et al. 1997). Therefore, egg size may differ among competing males due to the variation in females they may attract (Mensink et al. 2014). In the field, I briefly photographed the underside of the cobblestone on which the eggs were laid (in a single layer), with a scale bar *in situ* (Fig 2.1). I then calculated the surface area of each brood by tracing the outline of the eggs using the photo analysis software, Image-Pro Premier (9.3.3). This method only offers an estimation of brood size as eggs may not be laid continuously and may be broken by small empty spaces (Mensink et al. 2014). Due to image quality constraints, it was not possible to reliably measure around and exclude empty patches. I assume that the error introduced by this

is small relative to effect sizes of interest (e.g., Fig 2.1, gives an indication of the issue). Egg size was estimated by haphazardly selecting 15 eggs from the photograph and measuring two diameters of each egg and then averaging to find the overall egg size (cm). I was unable to estimate egg size for two males (of the 21 sampled) due to poor image quality. Common triplefin males often lay eggs daily from multiple females and hence nests may contain eggs of various age cohorts (Mensink et al. 2014). It is possible to classify embryos into different developmental stages from photographs, however, this is not viable when eggs have been laid within hours or days of each other (Mensink et al. 2014). Therefore, I focused my measurement of reproductive success on brood size and egg size, rather than on the number of broods received.

As a measure of nest quality, I measured the surface area available for egg-laying within each nest (cobblestone) by multiplying the length of each stone's major axis by its perpendicular width (minor axis). Additionally, as a measure of male quality, the total length (from the tip of snout to end of the caudal fin, TL) of each male was estimated visually to the nearest 0.5 cm. Before each sampling date, I calibrated my visual size estimates in the field by approximating the size of various live individual fish in the laboratory. These were then compared with more precise measurements from callipers.

I made all observations over a 2-month period, sampling individuals only once. I acknowledge that my method provides a 'snapshot' approach and may be subject to unknown errors generated by temporal variation. However, because common triplefin are daily spawners (Mensink et al. 2014), I believe that this method of sampling was sufficient to evaluate reproductive success in this species.

Figure 2.1 *F. lapillum* eggs on the underside of a cobblestone ('nest'), with a scale bar in situ, at Moa Point, Wellington Harbour (41°20'34" S, 174°48'42" E).

2.2.3 Statistical analysis and models for field study

To examine if male size, nest size, or their interactions contribute to mate attraction (number of female visits observed during behavioural observations), I fit a generalised linear mixed effects model (GLMM), assuming a Poisson error distribution. I fit the number of female visits as the response variable with the fixed effects of male total length (TL), nest size (surface area cm^2), and the interaction between male TL and nest size. Sampling date was included as a random intercept effect to account for correlations between individuals that were sampled on the same date. The interaction term was not significant and was dropped from the final model.

Once I accounted for the hypothesised effect of male size and nest size, I examined whether any further variation was explained by male courtship displays, male agonistic displays, and number of interactions with potential predators. I extracted and examined the residuals from the above male size/nest size model and analysed a second regression. I fit a linear model with the male size/nest size model residuals as the response variable with the number of courtship displays, the number of agonistic displays, and the number of interactions with potential predators as fixed effects.

Additionally, I fit two linear mixed models to analyse the factors that contribute to 1) brood size; and 2) average egg size. I fit brood size and average egg size as the response variables, for individual models respectively, with the fixed effects of male TL, nest size, and the interaction between male TL and nest size. Again, sampling date was included as a random intercept effect. The interaction term was not significant for both models and was dropped from the final model.

As with mate attraction, to examine whether any further variation is explained by male courtship displays, male agonistic displays, or by the number of interactions with potential predators, I extracted and examined the residuals from the above brood size and the egg size models. I fit two linear regression models, one with the residuals from the brood size model and the other with the residuals from the egg size models, as the response variables. Each model had the fixed effects of the number of courtship displays, the number of agonistic displays, and the number of interactions with potential predators.

For parameters to be on a comparable scale, all predictors were standardized to a mean of 0 and standard deviation (SD) of 0.5. To obtain confidence intervals on my models I used parametric bootstrapping; I used Wald-z scores for statistical significance.

Due to the constraints imposed by my small sample size, comparisons between all variables based on multivariate methods (i.e. multiple regression) was not possible. Therefore, analysing the residuals in this stepwise approach allowed me to formally test my primary hypothesis (i.e. the effect of nest size and/or male size), as well as some additional effects as a secondary and distinct analysis in a more *post hoc* way. However, I do understand that this method makes an implicant assumption about how the biological processes might be structured.

All statistical analyses were performed using R Statistical Software 3.6.2 (R Core Team 2019). Generalised linear mixed effects models and linear mixed effects models were performed using the R function "glmer" and "lmer", respectively, from the package "lme4". Generalised linear models and simple linear models were performed using the R function "glm" and "lm", respectively. Standardization of parameters was achieved using the "arm" package in R.

2.2.4 Laboratory study

To investigate female preference during mate selection, I conducted choice experiments in the laboratory from September 2019 to January 2020. Adult males (n = 120) and females (n = 60), of differing sizes, were collected from two sites on the Miramar peninsula: Shark Bay (41°18'08.70" S, 174°49'00.60" E) and Kau Bay (41°17'11.74" S, 174°49'43.37" E) between August and December 2019. Fish were collected using hand nets, with the aid of snorkel, and transported back to Wellington University Coastal Ecology Laboratory (WUCEL) in buckets filled with seawater. Before the experiment, fish were stocked in multiple single-sex laboratory aquaria (54 L) and fed once daily on a diet of blended frozen mussels (*Mytilus edulis*). Fish were maintained in the laboratory for at least 2 weeks prior to use in an experiment. Each experimental trial was conducted in an indoor glass, 54 L, aquaria (L 60 cm x W 30 cm x H 30 cm), with continuous flow-through seawater and exposure to natural light.

To assess the preference of females for male size, nest size, and the interaction between male and nest size, females were given the opportunity to choose between two males holding an

artificial nest site. The preference test consisted of two stages: initial preference and mating preference.

2.2.5 Initial preference: Does nest size or male size influence a female's initial preference, measured by the percent of time spent in front of the chosen male?

During stage one, the test tanks (54 L) were divided into 3 equal compartments (20 cm across) by two removable transparent plexiglass dividers with holes to allow water movement. Water was pumped from one male compartment and flowed out from the other male compartment (Fig 2.2). Two males were placed randomly on opposite sides of the aquaria and after a 24-hour acclimation period, a gravid female was introduced into the centre compartment of the aquaria. To control for possible side biases, male/nest size combinations were randomly assigned to sides of the arena. The female was able to see both males and the female compartment separated the two males from each other. The two males showed no obvious interactions and signs of aggression towards one another. Although external cues (e.g. from observers) were possible, fish showed no signs of disturbance from my presence. The female was placed into the experimental tank and was given a 15-minute acclimatization period. After this, I recorded the position of the female and any courtship displays of both males every 30 seconds by scan sampling for a total of 20 minutes. Inside the female compartment, 3 cm preference zones were marked out in front of each male compartment. Females were considered to be associated with a male when her body was oriented towards the male and was inside the preference zone, i.e. the distance between her and the male compartment was no more than 3 cm.

The males used in this experiment were classified as either small ($\leq$ 45.5 mm) or large ($\geq$ 50 mm), reflective of the bimodal size distribution of males in my sample (Fig 2.3). Males were provided with bricks, which had the middle compartment cut out into a hemicylindrical shape and closed at one end with different size openings, serving as a large (6 cm diameter opening with 140 cm^2 available roof and side space for egg laying) or small (4 cm diameter opening with 80 cm^2 available roof and side space for egg laying) nest site (Fig 2.4). Altogether, six experimental treatments were assessed containing the following male and nest size combinations;

1) A small male and a large male both with a small nest (males: n = 20, females: n = 10)
2) Small male and a large male both with a large nest (males: n = 20, females: n = 10)

3) Two large males, one with a small nest and one with a large nest (males: n = 20, females: n = 10)

4) Two small males, one with a small nest and one with a large nest (males: n = 20, females: n = 10)

5) A small male with a small nest, and a large male with a large nest (males: n = 20, females: n = 10)

6) A small male with a large nest, and a large male with a small nest (males: n = 20, females: n = 10). Each individual was used once.

Before each trial, I measured the standard length (from tip of snout to start of caudal fin, SL), to the nearest 0.05 mm using callipers, and wet weight, to the nearest 0.0001g, of all experimental males (n = 120) and females (n = 60). I then calculated a metric of body condition using Fulton's K:

$$K = \frac{wet\ weight\ (g)}{Standard\ length^3 (cm)}$$
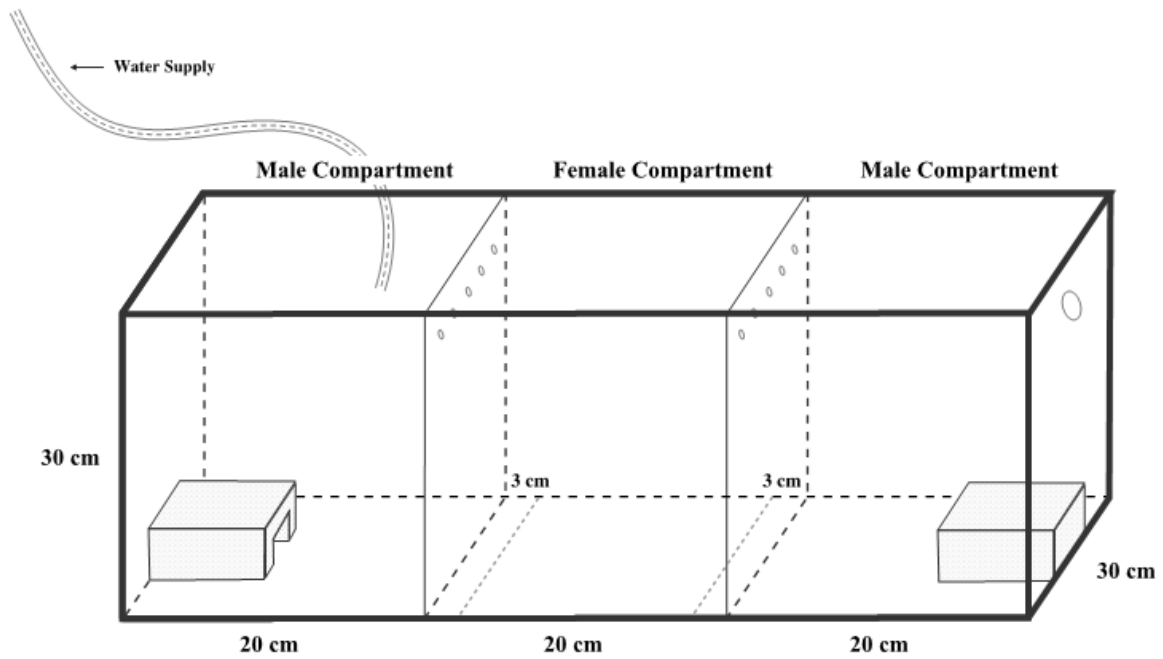
Figure 2.2 Diagram of the experimental arena used to evaluate the effects of male size and nest size on female choice. The arena consists of a 54 L aquarium with 3 equal compartments (2 male compartments, and 1 central female compartment). Water was pumped from one male compartment and flowed out from the other male compartment. The male compartments were isolated from the central female compartment via removable transparent plexiglass dividers, with small holes to allow water movement. An artificial nest site (small or large) was placed into each of the male compartments. Grey dotted lines in the central female compartment indicate the marked 3 cm male preference zones.
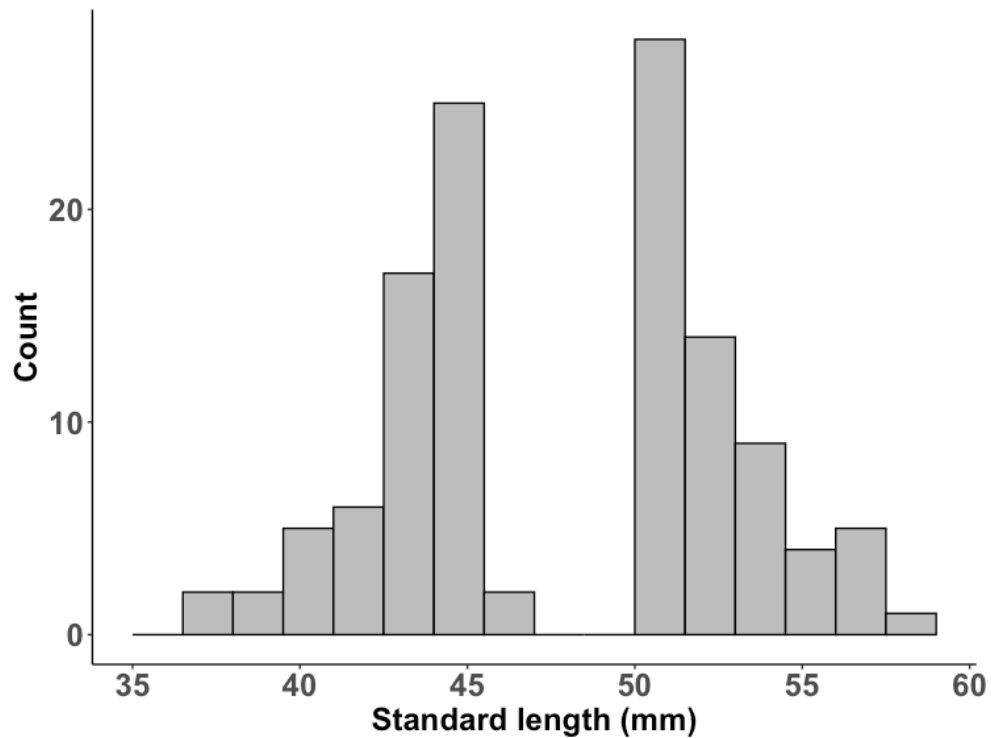
Figure 2.3 Histogram of the standard length (mm: from the tip of snout to start of caudal fin, SL) of males used in the laboratory experiment. Reflective of the large ($\geq$ 50 mm) and small ($\leq$ 45) categories of male SL.
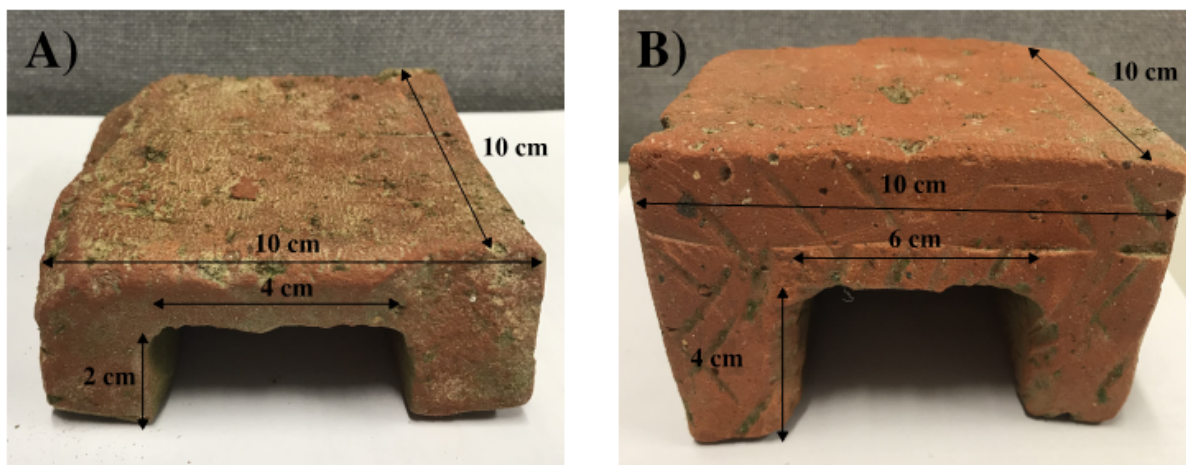
Figure 2.4 Small and large artificial nest sites provided during the dichotomous choice tests. Nests were made from red bricks which had the middle compartment cut out into a hemicylindrical shape, which were closed at one end. **A)** Demonstrates the small artificial nest site with a 4 cm diameter opening, and 80 cm^2 available internal roof and side space for egg laying. **B)** Demonstrates the large artificial nest site with a 6 cm diameter opening and 140 cm^2 available internal roof and side space for egg laying.

2.2.6 Mating preference: Does nest size or male size influence female spawning preference?

During this stage, females were permitted free access to both males, allowing her to spawn with her preferred male. Immediately after stage one, the two plexiglass dividers were removed from the test tank allowing male-female and male-male interactions to occur simultaneously. I then monitored which male the female chose to spawn with. I checked for the presence of eggs daily, and if no eggs had been laid within 5 days, the experimental trial was stopped and discarded.

F. lapillum do not exhibit sexual dimorphism, meaning females can be the same size as the males. Because of this, the plexiglass dividers needed to be fully removed to allow interactions between males and females. Consequently, males were able to move freely around the tank and interact with each other. Therefore, the effects of male-male competition cannot be ruled out.

2.2.7 Statistical analysis and models for the laboratory study

Initial preference:

Pairwise comparisons between the 4 different combinations of male size and nest size

During the initial phase of the experiment, where females were physically separated from the males, the distribution of female preference (measured by the percent of time spent in front of each of the two male preference zones) appeared to be strongly bimodal. This reflected the female's choice when presented with the two males during the dichotomous choice test. I, therefore, fit a binomial response for female preference (1 = chosen, 0 = not chosen). If the female spent more than 60 % of her time in front of one of the two males presented to her, then this was classified as a choice ('1'), and the other male in the test was assigned '0' (not chosen).

To explore whether females preferred one male over the other during this phase, I conducted a chi-square test of association for all pairwise differences between the 4 possible combinations of male size/nest size treatments (i.e. large male/ large nest, large male/ small nest, small male/ large nest, and small male/ small nest). The binomial response for female preference (chosen/not chosen) and the different male/nest size combinations were used as categorical variables.

Does male size or nest size or both influence female preference?

To explore the individual and interactive effects of male size and nest size on female choice, I ran a logistic regression. To do this, I pooled the data among all 6 treatment groups. I fit the binomial term for female preference as the response variable with the fixed effects of male size (small or large), nest size (small or large), and the interaction between male size and nest size.

Exploration of additional male traits on female choice

During the laboratory study, I measured male courtship frequency and male body condition, which can be considered as important mate choice cues (Jennions and Petrie 1997). Therefore, to explore the effects of these additional traits on female choice, I ran an additional logistic regression. I fit the binomial term for female preference as the response variable with the categorical effects of male size (small or large), nest size (small or large), male courtship frequency, male body condition, and the interaction between male size and nest size.

Exploration of female preference throughout the 20-minute dichotomous choice tests:

To explore the decision-making process by females throughout the 20-minute trial for all pairwise differences between the 4 possible combinations of male size/nest size treatments, the trial was divided into 4-time categories: 0-5 mins, 5-10 mins, 10-15 mins, and 15-20 mins. Female choice was measured as the proportion of time spent in front of the two presented males during these 4-time categories. The assumptions of equal variance and normality were not met even after various transformations. Therefore, a non-parametric Friedman test, in place of a repeated measures ANOVA, was performed to analyse whether the proportion of time spent with each male changed over the 20-minute trial for all pairwise choices.

Mating preference:

Does the time spent by a female in front of a male during the dichotomous choice test correlate with female spawning preference?

To determine if female choice in the initial stage, measured by the time spent in the male preference zones, was correlated with the acquisition of eggs during the second stage of the experiment, I performed a chi-square test of association.

Pairwise comparisons between the 4 different combinations of male size and nest size

As in the initial preference stage, I conducted a chi-square test of association for all pairwise differences between the 4 possible combinations of male size/nest size treatments to explore whether females spawned with one male over the other. The binomial response for female preference (received eggs: yes or no) and the different male/nest size combinations were used as categorical variables.

Does male size or nest size or both influence female mating preference?

To explore the individual and interactive effects of male size and nest size on female spawning preference, measured by the acquisition of eggs, I also performed a logistic regression. I fit the binomial term for female preference (received eggs: yes or no) as the response variable with the categorical effects of male size (small or large), nest size (small or large), and their interaction.

All statistical analyses were performed using R Statistical Software 3.6.2 (R Core Team 2019). All confidence intervals were calculated from parametric bootstrapping.

2.3 Results

2.3.1 Field study

Of the total 21 reproductive males surveyed, the average total length $\pm$ standard error (SE) was 57 $\pm$ 1.23 mm, with an average nest size of 224.4 $\pm$ 29.75 cm^2 (mean $\pm$ SE).

Mate attraction models

The average number of female visits to the reproductive male's nest over the 10-minute observation period was 1 $\pm$ 0.27 females (Mean $\pm$ SE).

Female visits to the nest were not influenced by the total length of the male or by the size of the nest site (Table 2.1). Additionally, when controlling for all factors in the model, residual mate attraction was not significantly affected by the number of agonistic displays ($t_{1,20} = -1.620$, $p = 0.124$), courtship frequency ($t_{1,20} = 1.388$, $p = 0.183$), or by the number of interactions with potential predators ($t_{1,20} = -0.455$, $p = 0.655$). The random effect of sampling date did not explain any additional variation.

Brood size models

Male *F. lapillum* showed a large variation in brood size. The average brood size $\pm$ SE of the 21 reproductive males sampled was 30.67 $\pm$ 5.72 cm^2, the smallest brood size guarded by a male was 0.87 cm^2 and the largest brood size guarded by a male was 71.65 cm^2. As with mate attraction, male total length and nest size were not significantly correlated with brood size (Table 2.1). The random effect of sampling date explained 36 % of the variation in brood sizes amongst sampled males.

When accounting for the hypothesised effect of male size and nest size, brood size was not significantly affected by the number of agonistic displays ($t_{1,20} = -0.839$, $p = 0.413$), courtship frequency ($t_{1,20} = -1.156$, $p = 0.264$), or by the number of interactions with potential predators ($t_{1,20} = -0.464$, $p = 0.648$).

Egg size models

Of the 21 reproductive males sampled the average egg size $\pm$ SE (measured as diameter) was 0.103 $\pm$ 0.003 cm. Egg size ranged between 0.084 cm to 0.126 cm. Egg size was not influenced by male total length or by the size of the nest site (Table 2.1). The random effect of sampling date accounted for 15.5 % of the variation in egg sizes amongst sampled males.

Controlling for other main effects in the model, residual egg size (accounting for the hypothesised effect of male size and nest size) increased with the number of agonistic displays ($t_{1,15} = 2.119$, $p = 0.05$). However, courtship frequency ($t_{1,20} = 0.964$, $p = 0.3506$) or the number of visits from predators ($t_{1,20} = -0.793$, $p = 0.4403$) did not have a significant effect on residual egg size.

Table 2.1 Standardised parameter estimates (scaled to mean of 0 and standard deviation of 0.5) from regression analysis to examine the effects of male size and nest size on the 3 measures of reproductive success: brood size (surface area of eggs in a nest at sampling); average egg size of reproductive males at sampling; and mate attraction (number of female visits to the nest at sampling). P-values are based on Wald z scores. Confidence intervals were constructed from parametric bootstrapping.

Component of reproductive success	Parameter	Estimate	Unconditional SE	Confidence Interval	p-values
Brood size (cm^2)	Intercept	30.346	8.033	(15.716, 45.747)	0.0122
	Male Total Length (TL)	2.791	11.965	(-23.804, 27.596)	0.8182
	Nest Size	7.987	9.863	(-11.782, 26.879)	0.4305
Egg size (cm^2)	Intercept	0.103	0.004	(0.095, 0.110)	2.34×10^{-5}
	Male Total Length (TL)	-0.0002	0.007	(-0.012, 0.012)	0.982
	Nest Size	0.002	0.006	(-0.010, 0.015)	0.717
Mate attraction	Intercept	0.139	0.213	(-0.509, 0.449)	0.512
	Male Total Length (TL)	0.616	0.416	(-0.156, 1.741)	0.139
	Nest Size	-0.626	0.576	(-2.703, 0.390)	0.277

2.3.2 Laboratory study

2.3.3 Initial preference:

Pairwise comparisons between the 4 different combinations of male size and nest size

All females (n=60) spent substantially more time (> 60 %) with one of the males during the dichotomous choice test. On average, females spent 87.9 ± 1.5 % (mean $\pm$ SE) of their time with the preferred male and 8.1 ± 1.3 % (mean $\pm$ SE) of their time with the non-preferred male.

From the 6 pairwise comparisons between the 4 different male size/nest size combinations, females were seen to have a significant preference for a particular male in only 2 pairwise choices (Fig. 2.5). Females showed a significant preference for a small male holding a large nest over a small male holding a small nests ($\chi^2 = 12.8$, $p = 0.00035$), choosing a small male holding a large nest in 90 % of trials (Fig. 2.5). Additionally, a significant preference was found for large males holding a large nest over small males holding a large nest ($\chi^2 = 7.2$, $p = 0.0073$). Large males holding a large nest were chosen by a female in 80 % of trials (Fig 2.5).

Does male size or nest size or both influence female preference?

Female choice was not based on the interaction between male body size and nest size (Table 2.2). Male body size had the only significant main effect on female choice, where the odds of being chosen for smaller males is less than the odds of being chosen for larger males, when adjusting for nest size and the interaction between male and nest size (Table 2.2). Nest size did not have a significant effect on female choice (Table 2.2).

The estimated probability of being chosen was highest for large males holding a large nest site and the lowest estimated probability of being chosen was for small males holding a small nest site (Fig. 2.6).
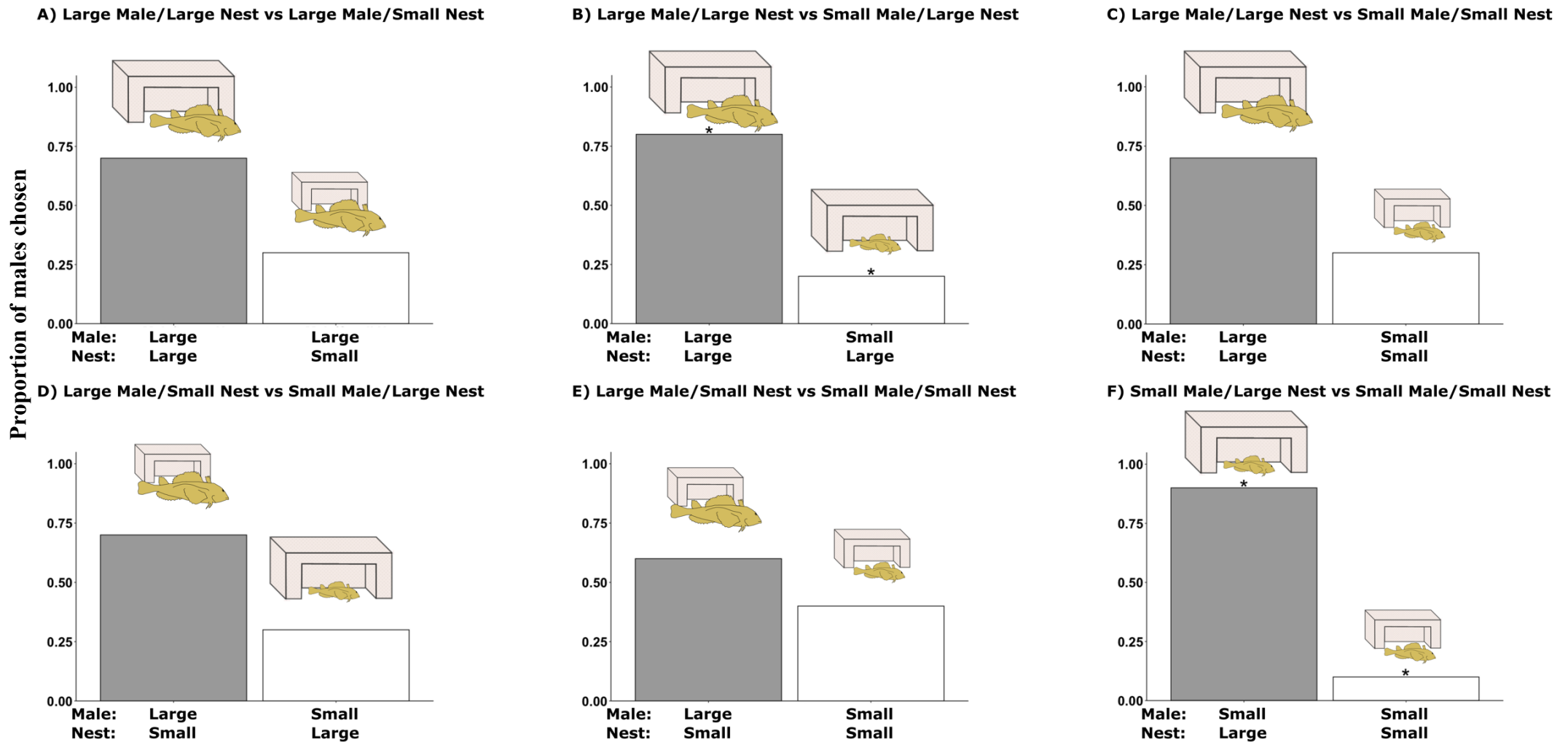
Figure 2.5 Proportion of males chosen by a female for the 6 pairwise comparisons between the 4 different male size and nest size combinations. A male was classified as 'chosen' if a female spent more than 60 % of her time in the male's preference zone during the 20-minute dichotomous trial. Female choice between **A)** large male with a large nest versus a large male with a small nest, **B)** large male with a large nest versus a small male with a large nest, **C)** large male with a large nest versus a small male with a small nest, **D)** large male with a small nest versus a small male with a large nest, **E)** large male with a small nest versus a small male with a small nest, and **F)** small male with a large nest versus a small male with a small nest. Significant deviations from null distributions ($P < 0.05$) are indicated with a '*'.
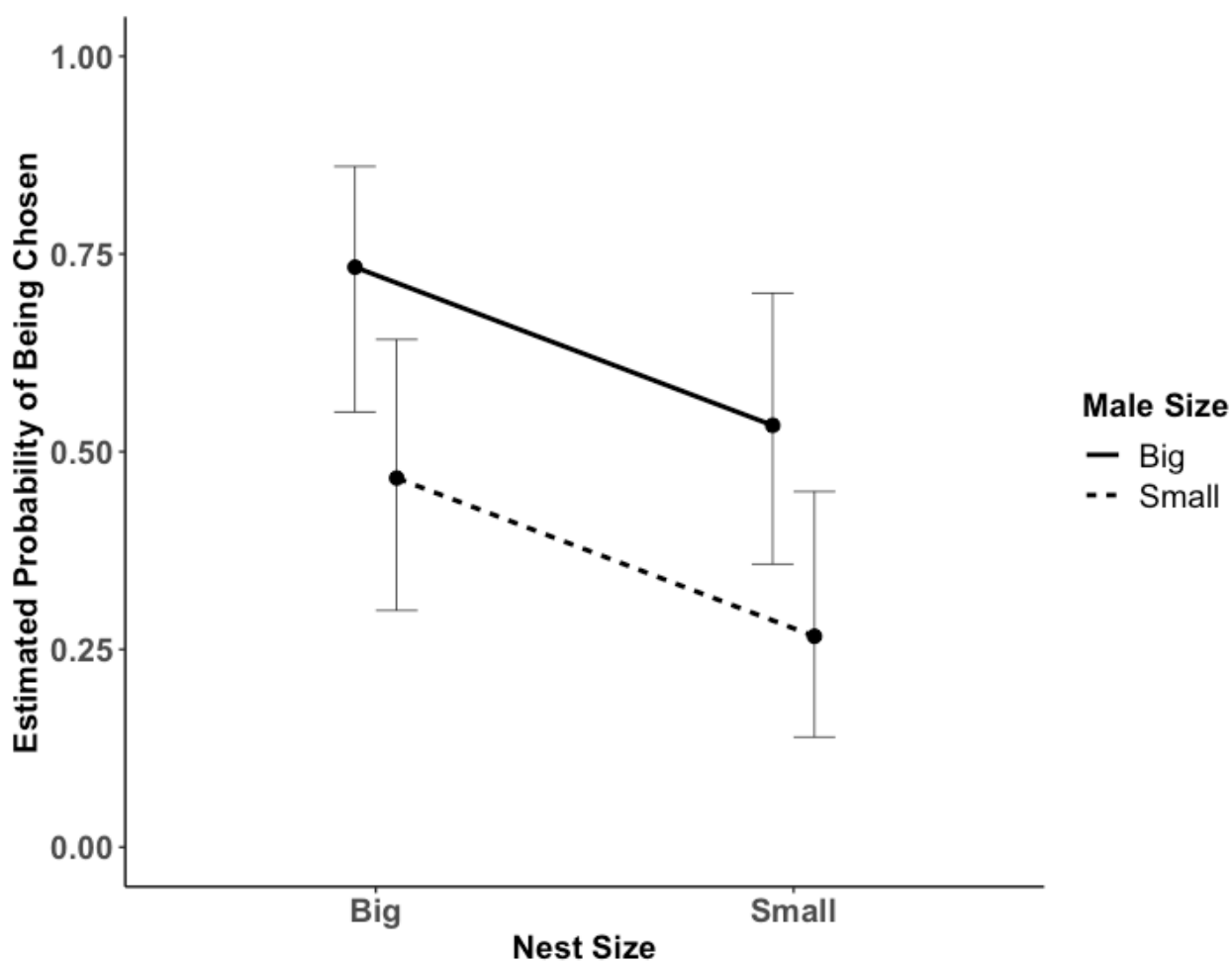
Figure 2.6 Estimated probability of a male being chosen by a female for the 4 different male size and nest combinations: large male holding a large nest, large male holding a small nest, small male holding a large nest, and a small male holding a small nest. A male was classified as 'chosen' if a female spent more than 60 % of her time in the male's preference zone during the 20-minute dichotomous trial. Probabilities are estimated from the logistic regression model using 'Emmeans' package in R. Error bars represent 95 % confidence intervals.

Exploration of additional male traits on female choice

With the addition of male courtship frequency and male body condition to the model, the significant effect of male size seen in the above analysis was diminished (Table 2.2). Courtship frequency was the only male trait to have a significant effect on female choice (Table 2.2). An increase in male courtship displays by 10 % is associated with a 70% increase in the odds of being chosen by a female (Table 2.2).

Exploration of female preference throughout the 20-minute dichotomous choice tests:

Throughout the 20-minute dichotomous female choice trials, the average time spent by a female with each male did not vary over the 20-minute trial for all male size/nest size treatment groups, apart from one combination. For all other combinations, female preference, measured by the proportion of time spent with a particular male, was held constant over time, demonstrating that females select their chosen male almost immediately. The only significant difference between the proportion of time spent with a particular male occurred for the treatment group that included a large male holding a small nest and small male holding a large nest (Table 2.3, Fig. 2.7). The proportion of time spent by the female with the large male holding a small nest differed between 10-15 mins and 15-20 mins ($\chi^2 = 21$, $p = 0.031$). Females increased the amount of time spent in front of the large male holding a small nest between 10-15 minutes, and then this significantly decreased between 15-20 minutes (Fig. 2.7).

Table 2.2 Parameter estimates from the logistic regression analysis for the initial preference phase of the laboratory experiment. Model **a)** Does male size or nest size or both influence female choice (measured by the proportion of time spent in front of the preferred male). Model **b)** Do additional male traits (body condition and courtship frequency) influence female choice. Parameter estimates are for small males and small nest sites, with the intercept representing the reference level (large males, large nest sites). 95 % confidence intervals were constructed from parametric bootstrapping. Results in bold represent significant deviations from null distributions ($P < 0.05$) .

Model	Parameter	Estimate	Standard Error	Confidence Interval	z value	p-values
	Intercept	1.012	0.413	(0.242, 1.885)	2.450	0.0143
a) Does male size or nest size	**Male size (small)**	**-1.145**	**0.552**	**(-2.266, -0.087)**	**-2.076**	**0.0379**
influence female choice?	Nest size (small)	-0.878	0.552	(-1.995, 0.186)	-1.592	0.1115
	Male size* Nest size	-1.38×10^{-15}	0.780	(-1.536, 1.536)	0.000	1.000
b) Do additional male traits influence	Intercept	-2.248	1.277	(-4.843, 0.253)	-1.761	0.0782
female choice?	Male size (small)	-0.859	0.824	(-2.509, 0.757)	-1.042	0.2972
	Nest size (small)	-0.920	0.821	(-2.586, 0.672)	-1.121	0.2621
	Courtship Frequency	**0.068**	**0.012**	**(0.048, 0.094)**	**5.834**	**5.4×10^{-9}**
	Body condition	428.774	589.301	(-771.606, 1604.911)	0.728	0.4669
	Male size* Nest size	0.061	1.121	(-2.156, 2.294)	0.054	0.9566
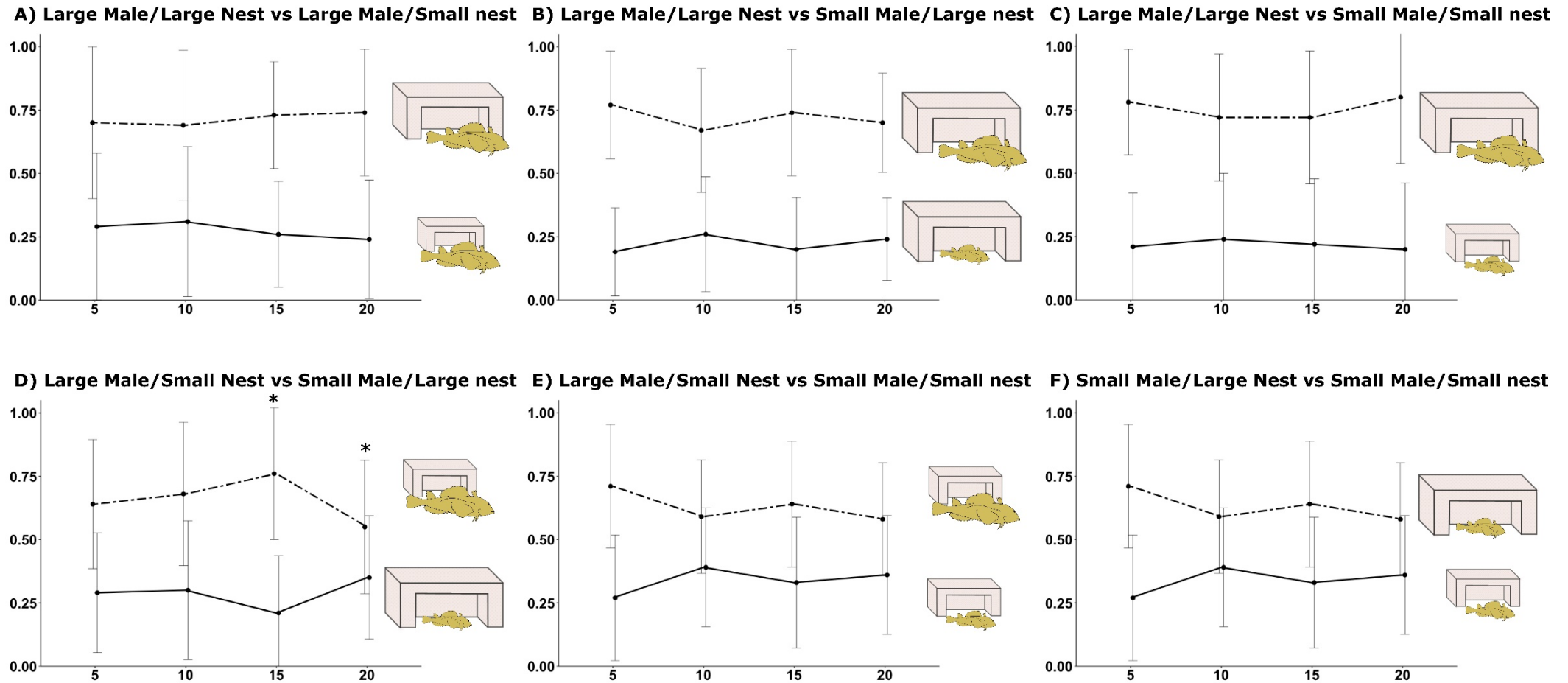
Figure 2.7 Line graph representing the average proportion of time spent by a female with each male during 5-minute time blocks (0-5 minutes, 5-10 minutes, 10-15 minutes, and 15-20 minutes) during the 20-minute dichotomous female choice test for all pairwise comparisons. A) Dashed line represents a large male holding a large nest, and the solid line represents a large male holding a small nest. B) Dashed line represents a large male holding a large nest, and the solid line represents a small male holding a large nest. C) Dashed line represents a small male holding a large nest, and the solid line represents a small male holding a small nest. D) Dashed line represents a large male holding a small nest, and the solid line represents a small male holding a large nest. E) Dashed line represents a large male holding a large nest, and the solid line represents a small male holing a small nest. F) Dashed line represents a large male holding a small nest, and the solid line represents a small male holding a small nest. Error bars represent 95% confidence intervals. Significant deviations from null distributions ($P < 0.05$) are indicated with a '*'

Table 2.3 Friedman test output examining whether the proportion of time a female spends with a male changes across 4-time blocks during the 20-minute dichotomous female choice test for the 6 pairwise comparisons. The 4-time blocks include 0-5 minutes, 5-10 minutes, 10-15 minutes, and 15-20 minutes. Output includes the test statistic, p values, and associated degrees of freedom. Results in bold represent significant deviations from null distributions ($P < 0.05$) .

Pairwise combinations	Friedman Test statistic	Degrees of Freedom	p-values
Big Male/Big Nest	1.08	3	0.781
Big Male/Small Nest	1.54	3	0.673
Big Male/Big Nest	4.12	3	0.248
Small Male/Big Nest	3.38	3	0.337
Small Male/Big Nest	1.79	3	0.617
Small Male/Small Nest	0.831	3	0.831
Big Male/Small Nest	**9.33**	**3**	**0.0252**
Small Male/Big Nest	2.39	3	0.496
Big Male/Big Nest	3.52	3	0.318
Small Male/Small Nest	0.778	3	0.855
Big Male/ Small Nest	5.72	3	0.126
Small Male/Small Nest	4.91	3	0.179

2.3.4 Mating preference

Does the time spent by a female in front of a male during the dichotomous choice test correlate with a female's spawning preference?

Female choice during the initial phase of the experiment, measured by the proportion of time spent with a chosen male, was associated with the probability of a male receiving eggs during the mating phase of the experiment ($\chi^2 = 26.606, \ p = 2.494 \ x \ 10^{-7}$). Of the 60 trials, 67 % of males chosen in the initial phase laid eggs with a female during the mating phase.

Pairwise comparisons between the 4 different combinations of male size and nest size

Fifty-two females (of 60) spawned with a chosen male and all eggs were laid with one of the two presented males. Females showed a significant preference for one male for 4 of the 6 pairwise comparisons. Females showed a significant preference for large males holding a large nest over large males holding a small nest ($\chi^2 = 16.364$, $p < 0.00001$), laying eggs with a large male holding a large nest in 90% of trials (Fig. 2.8). Additionally, a significant preference was found for large males holding a large nest over small males holding a large nest ($\chi^2 = 38.182$, $p < 0.00001$). Again, in 90 % of trials females spawned with the large male holding a large nest (Fig. 2.8). A significant difference also occurred for treatments including large males holding small nests vs small males holding large nests ($\chi^2 = 7.2$, $p = 0.00617$). In 70 % of trials females spawned with a large male holding a small nest (Fig. 2.8). Finally, females showed a significant preference for large males holding a small nest over small males holding a small nest ($\chi^2 = 7.2$, $p = 0.000729$), laying eggs with a large male holding a small nest in 80 % of trials (Fig 2.8).

Does male size or nest size or both influence female preference?

As seen in the initial preference test, female spawning preference was not based on the interaction between male body size and nest size (Table 2.4). However, on its own, male body size had a significant effect on female spawning choice (Table 2.4). The odds of receiving eggs for small males is less than the odds of receiving eggs for large males, when adjusting for nest size and the interaction between male size and nest size. Additionally, nest size had a significant effect on female choice (Table 2.4). The odds of receiving eggs for males holding small nests

is less than the odds of receiving eggs for males holing large nest sites, when adjusting for other factors in the model.

The estimated probability of receiving eggs was highest for large males holding a large nest and the estimated probability of receiving eggs was lowest for small males holding a large nest (Fig. 2.9).
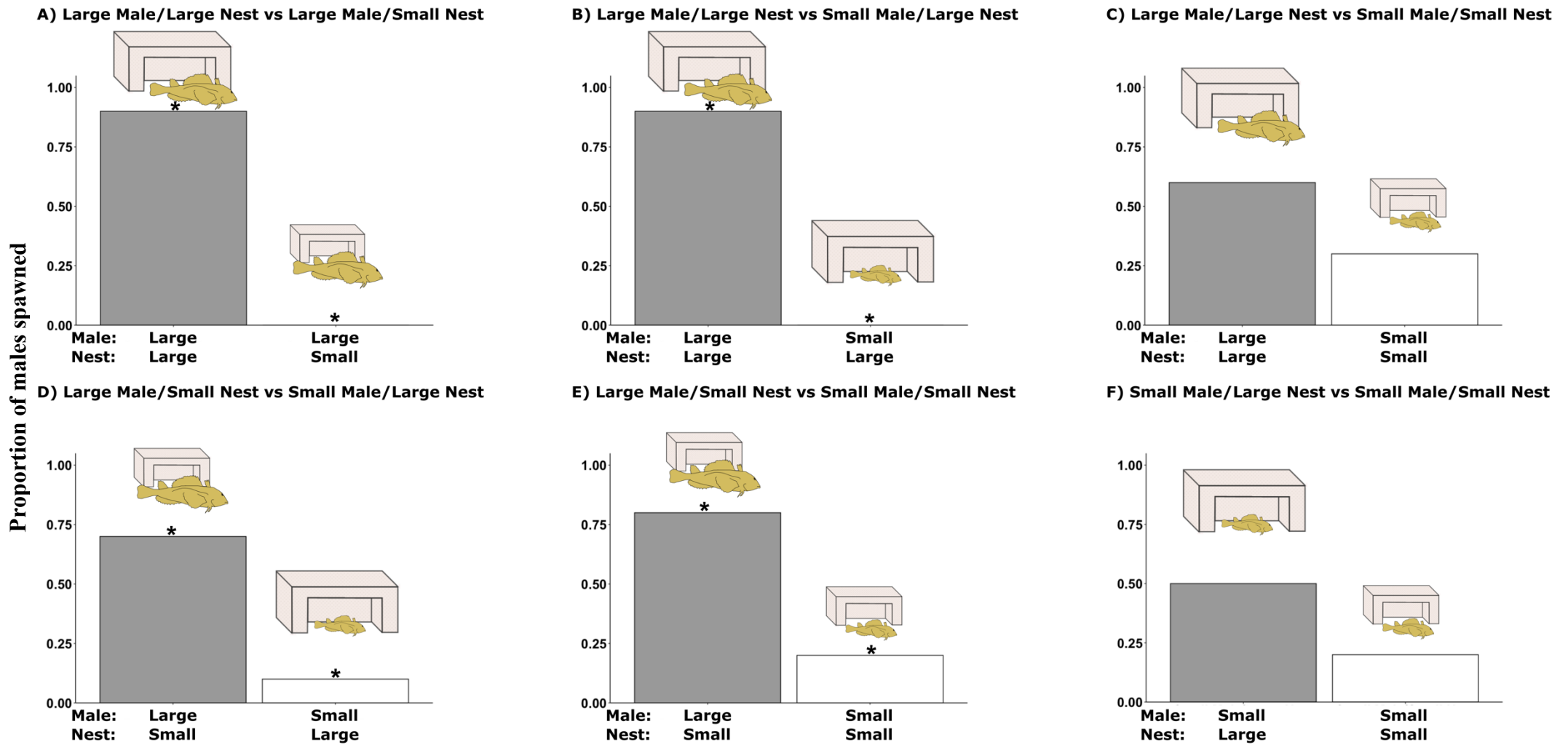
Figure 2.8 Proportion of males that spawned with a female for the 6 pairwise comparisons between the 4 different male size and nest size combinations. A spawning event was classified when a female laid all her eggs within a chosen male's nest site. Proportion spawned between **A)** large male with a large nest versus a large male with a small nest, **B)** large male with a large nest versus a small male with a large nest, **C)** large male with a large nest versus a small male with a small nest, **D)** large male with a small nest versus a small male with a large nest, **E)** large male with a small nest versus a small male with a small nest, and **F)** small male with a large nest versus a small male with a small nest. Significant deviations from null distributions ($P < 0.05$) are indicated with a '*'
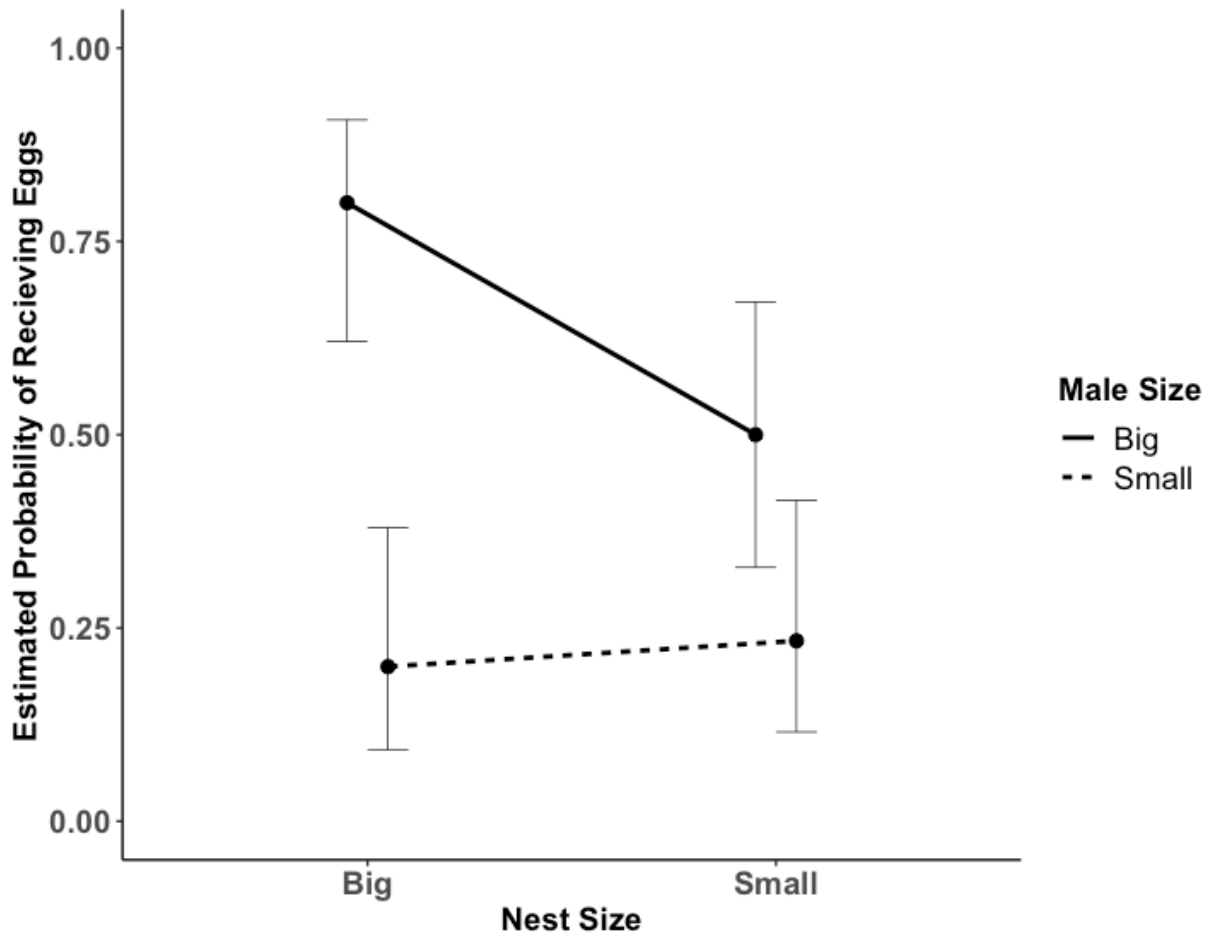
Figure 2.9 Estimated probability of a male receiving eggs by a female for the 4 different male size and nest combinations: large male holding a large nest, large male holding a small nest, small male holding a large nest, and a small male holding a small nest. Probabilities are estimated from the logistic regression model using 'Emmeans' package in R. Error bars represent 95 % confidence intervals.

Table 2.4 Parameter estimates from the logistic regression analysis for the mating phase of the laboratory experiment to examine whether male size or nest size or both influence female choice (measured by spawning preference: laid eggs with preferred male). 95 % confidence intervals were constructed from parametric bootstrapping. Results in bold represent significant deviations from null distributions ($P < 0.05$) .

Model	Parameter	Estimate	Unconditional SE	Confidence Interval	z value	p-values
Mating preference	Intercept	1.386	0.456	(0.129, 2.114)	3.037	0.00239
Does male size or nest size	**Male Size**	**-2.772**	**0.645**	**(-2.391, 0.097)**	**-4.295**	**1.74 x 10^{-5}**
influence female choice?	**Nest Size**	**-1.386**	**0.584**	**(-3.742, -1.011)**	**-2.372**	**0.01771**
	Male Size: Nest Size	1.583	0.858	(-0.117, 3.283)	1.845	0.06507

2.4 Discussion

Analysing potential male and nest quality factors which correlate with male reproductive success and female choice can provide an understanding of how particular mating systems are shaped and can help predict patterns of individual mating success. In systems with male parental care, female choice is said to be primarily based on the quality of the male and/or some attribute of the nest (Downhower and Brown 1980, Lindström and Hellström 1993, Hamilton et al. 1997). To evaluate the relative importance of male quality and nest quality on the reproductive success of the common triplefin (*F. lapillum*), I combined field-based observations with controlled laboratory-based experiments. Male reproductive success was highly variable among individuals in the field, however, I did not detect a significant relationship between male mating success and male total length or nest size. Furthermore, additional factors such as courtship frequency, number of interactions with potential predators, and the number of agonistic displays did not explain any additional variation for all measures of reproductive success apart from egg size. However, results from the laboratory experiment suggested that these factors were important during female choice. Male body size significantly influenced female choice, and both male size and nest size correlated with male spawning success. Additionally, females were more attracted to males that displayed a higher frequency of courtship displays. These results suggest that variation in reproductive success among individuals is not random in the common triplefin (*F. lapillum*) and may be due to a range of complex factors.

Body size has been well documented as an important mate choice cue in a variety of fish species (Thompson 1986, Oliveira et al. 2000, Lehtonen et al. 2007, Passos et al. 2013, Carriço et al. 2014). Larger males should be preferred as they can secure and defend more desirable resources for reproduction, and have the capability of providing greater parental care than smaller sized males (Breitburg 1987, Pollock et al. 2008). Large males may be more successful at defending eggs from potential egg predators, increasing egg survival, and consequently providing direct benefits to the female (Lehtonen et al. 2007, Pollock et al. 2008). In the laboratory study, females were seen to prefer larger males and spawned more frequently with larger males. However, in the field, I found no relationship between male body size and reproductive success in terms of brood size, egg size, and mate attraction. This is contrary to other findings in closely

related subtidal triplefin species. For example, body size has been shown to play a role in female choice in *Ruanoho spp*. (Wellenreuther et al. 2008) and was positively correlated with reproductive success in *F. varium* (Thompson 1986). In nest brooding fish with male parental care, body size can play an important role in the ability to monopolize resources (Faria and Almada 2001, Hastings 2002). Due to their size advantage, large males may be able to exclude smaller males from preferred areas in which courtship and spawning take place (Rowe et al. 2008). In the field, I surveyed males that were already defending a nest site with eggs. Body size may influence the ability of a male to obtain a suitable nest site and may be less important in influencing variation in egg size and brood size amongst already mated males (Mensink et al. 2014). Female preference may also change depending on the males mating status. Females may make different decisions when choosing mates with eggs versus without eggs (Hale and St Mary 2007). Furthermore, relative male size rather than absolute male size may have more of an influence on female choice. *F. lapillum* females are mobile and circulate amongst male territories, spawning with chosen males throughout the breeding season. Females may only circulate amongst males in a limited area and choose to spawn with the largest available male. This could explain why females preferred large males when presented with two different sized males in the laboratory and could explain why no relationship was found with previously mated males in the field.

In the laboratory study, females were less likely to spawn with a male holding a small nest site. However, nest size did not influence female preference in the first stage of the dichotomous choice test and did not predict male reproductive success in the field. In many fish species, male mating success is related to the quality of the nest or oviposition site (Jennions and Petrie 1997, Oliveira et al. 2000). The size of a nest can impose an upper limit on the number of mating's a male can receive, limiting his lifetime reproductive success (Demartini 1988, Bisazza et al. 1989). Females evaluate the size of a nest to determine if there is enough room to hold a high number of eggs, and in doing so may select males with higher parental investment (Bisazza et al. 1989). It is predicted that parental care is proportional to brood size and smaller broods are more susceptible to whole clutch cannibalism by the parental male (Trivers 1972, Bisazza et al. 1989, Wootton and Smith 2015). Initial female choice in the dichotomous choice test was not influenced by nest size, however, when females were presented with two small males holding different sized nests, they preferentially chose males holding the larger nest. Additionally, when females were able to interact and assess the male and his nest site, nest size

significantly influenced male spawning success. Females preferred and spawned more frequently with larger males holding a larger nest. Hence, female choice was not based upon nest size and male size independently, but rather on their combined and additive effect. Female *F. lapillum* may use male body size as an initial mate choice cue when first evaluating a potential male and then when given the opportunity, they will evaluate nest quality and size before they spawn with the male in question.

Males that displayed higher courtship activity during the dichotomous choice test had an increased chance of being chosen by female *F. lapillum*. Frequency of courtship displays can indicate sexual motivation and can be used by females during mate selection (Oliveira et al. 2000). In several fish species, male courtship intensity is used by females as a mate choice cue, where highly active males are preferred over inactive males (Knapp and Kovach 1991, Oliveira et al. 2000, Reichard et al. 2005, Steinwender et al. 2012). Male courtship intensity is usually more intense in larger males and can be a reliable indicator of male parental investment (Oliveira et al. 2000). Male behaviours can also be indicative of male quality due to the energetic costs of performing such displays (Hale and St Mary 2007). Females that preferentially mate with males that court more frequently may gain indirect benefits in regards to offspring viability (Oliveira et al. 2000). *F. lapillum* females may assess male quality through courtship intensity. Therefore, male body size may be under indirect selection by females due to its association with other influences that better explain variation in male reproductive success. This could explain why the significant effect of body size was diminished when courtship intensity was added to the regression analyses when investigating factors that influence female preference in the dichotomous choice test.

In addition to courtship behaviours, frequency of attacks against conspecifics can be reliable indicators of male sexual motivation and can play an important role in determining male reproductive success (Oliveira et al. 2000). Competition for the establishment of a territory or nesting site is a form of male-male competition often seen in species with male parental care (Gross and Sargent 1985, Lindström 1992). The best quality nest sites are frequently contested and occupied by males with the highest competitive ability (Lindström 1992, Wootton and Smith 2015). Interactions among males may determine the initial mating pool in which females exert mate choice (McGhee et al. 2007). However, there is evidence of direct costs to females

who partner with dominant males, because aggressive males also have the potential to be aggressive towards females (McGhee et al. 2007). In the field study, the number of agonistic displays against conspecifics was found to be the only factor to explain any additional variation in egg size. In many fish species, egg size is often influenced by the female's size and nutritional condition (Brooks et al. 1997, Mazzoldi et al. 2002). Females who are of better condition may be able to withstand and spawn with more aggressive and dominant males (Mazzoldi et al. 2002). Hence, dominant males may have larger eggs due to their ability to attract larger, higher conditioned females. Male-male competition and aggressive behaviours may have also played a role during the mating phase of the dichotomous choice tests where interactions between males were allowed. Males were able to interact and compete, which increases the cost of defending a nest site. This in turn could have enlarged the differences between the various male/nest size combinations. This could explain why female choice was more apparent during the mating phase of the experiment and could explain why nest size had more of an influence.

Results from both the laboratory and field study suggest that female reproductive decisions are plastic with respect to the factors and traits used to select mates. Factors that influenced female choice and male reproductive success in the laboratory study did not influence male reproductive success in the field. This could be due to variability in the degree of female choosiness, mate preference functions, genetic differences, or immediate environmental factors (Jennions and Petrie 1997). Females are usually choosier when there are higher levels of variation in male traits and female choice is often based on the most variable signal (Forsgren 1992, Jennions and Petrie 1997). Consequently, it is often difficult to determine what causes variation in male reproductive success under natural conditions (Jennions and Petrie 1997). This in turn makes it difficult to distinguish which tactics are being used by females. This could explain why the results from both the laboratory study and field study did not complement each other. In the laboratory study, I could independently manipulate variables that are often highly correlated under natural conditions. The males and nest sites presented to females during the laboratory study represented the two size extremes found in natural populations. However, when females are circulating among different males under natural conditions, differences in male sizes and nest sizes might not be as extreme and variable, and instead, females may be using a more variable and obvious cue. Additionally, mate sampling is often costly, which puts constraints on the ability to perform optimal mate choice (Gibson and Bachman 1992). In the

field, females may be using tactics that confer fewer costs and add or discard mate choice criteria depending on environmental conditions (Forsgren 1992, Jennions and Petrie 1997). At times females may even mate with the first male encountered. This was seen to be the case during the laboratory study where females made their mate choice decisions almost immediately. Furthermore, I sampled a small number of males that were already guarding an oviposition site and visited each male only once. This 'snapshot' sampling approach could be subject to unknown errors caused by temporal variation and the small sample size may have reduced the power of my analyses. Sampling date accounted for a small percentage of variation in both brood size and egg size. This could suggest that mating may be more punctuated, and some sampling dates may have coincided with more reproductive activity than others.

Overall, my study indicates that reproductive success in male *F. lapillum* is highly variable which can be attributed to female choice and male-male competition. Female choice was apparent in *F. lapillum*, however, I could not conclude that one factor was more important than another during mate selection. Females were influenced by a range of male traits and nest quality factors. This highlights that multiple cues and environmental factors beyond male size and nest quality should be carefully considered when studying reproductive success. It is difficult to determine what criteria females use to weight different cues, and it is possible that other cues not considered in this study could also affect female mating decisions and male reproductive success (Jennions and Petrie 1997). Traits that show no relationship with male mating success may be dismissed as being irrelevant to female choice (Jennions and Petrie 1997). However, females may only consider males as potential mates when they reach a certain threshold value. Future studies should sample all reproductive males in the study population to determine if female mate choice criteria is absolute or relative. Additionally, these males should be sampled and assessed over the entire breeding season to reduce any errors caused by temporal variation. Investigating the relative importance of different mate choice cues and signals has important implications for understanding population dynamics and provides an understanding of how sexual selection shaped parental care in males.

Temporal variation in the reproductive success of a marine reef fish with male parental care

3.1 Introduction

Characterising variation in reproductive success is central to our understanding of sexual selection and population dynamics (Andersson 1994). In many mating systems variation in male reproductive success is often relatively large and male fitness is frequently constrained by the number of matings obtained (Clutton-Brock and Vincent 1991, Andersson 1994). Many studies on reproductive success are often temporally and spatially constrained (Breitburg 1987, Casalini et al. 2013, Hermann et al. 2015). However, in natural systems, reproductive success is known to be highly dynamic and vary over time (Flanagan et al. 2016). Reproductive fluctuations can be affected by ecological variables such as climatic factors (McAllan and Geiser 2006, Wacker et al. 2014), the distribution and availability of nest sites/territories (Forsgren et al. 1996), variation in mate quality (Kvarnemo and Forsgren 2000), the density of individuals (Eshel 1979, House et al. 2019), or by the operational sex ratio (OSR) (Reichard et al. 2008, Wong et al. 2018). Throughout a breeding season, changes in such variables can cause fluctuations in the intensity and strength of sexual selection and can also interact to affect mate choice or mate quality (Reichard et al. 2008, Milner et al. 2010, Flanagan et al. 2016). Year to year variation in sexual selection and reproductive success has been well studied and recognized. However, variation within the breeding season has often been overlooked (Reichard et al. 2008, Wacker et al. 2014).

Within-season variation in reproductive success is often associated with species that have a prolonged breeding season or in species where individuals mate multiple times within one season (Wacker et al. 2014). Studies that sample such species once per breeding season could fail to detect important dynamics and drivers of reproductive variation (Kasumovic et al. 2008, Wacker et al. 2014). For example, one variable that could influence reproductive success over the breeding season is the operational sex ratio. The operational sex ratio (OSR, the ratio of available males and available females) is an estimate of mate availability and has the potential

to influence the level of choosiness and competition for mates (Emlen and Oring 1997, Borg et al. 2006). Within-season variation in the OSR has been described in several animal groups, such as birds (Colwell and Oring 1988), fish (Balshine-Earn 1996, Forsgren et al. 2004), and insects (Lawrence 1986, Pröhl 2002). As the OSR becomes progressively biased towards one sex, sexual selection and mate competition are expected to increase in the mate-limited sex (Emlen and Oring 1997, Wacker et al. 2014). Several studies have confirmed this prediction, showing that males compete more in male-biased OSRs and in some cases have increased their display of courtship behaviours (Forsgren et al. 2004, Carrillo et al. 2012, Aronsen et al. 2013, Wacker et al. 2013). Within-season change in the OSR is predominantly caused by changes in the density of individuals (House et al. 2019). Population density is predicted to influence reproductive success by altering mate availability and competition (Eshel 1979, Forsgren et al. 2004, House et al. 2019). At high densities, individuals may be able to monopolize matings and female choosiness may increase due to a greater variance in male quality and increased intersexual encounter rates (Zeh 1987, Owens and Thompson 1994, Borg et al. 2006). This monopolization of resources can give individuals direct or indirect access to mates and has a direct influence on the strength and intensity of sexual selection (Emlen and Oring 1997, Wong et al. 2018).

In many mating systems, male quality and body size are the most important determinants of reproductive success (Andersson 1994). Larger males often rank highly and as a result, can monopolize access to females and resources (Reichard et al. 2008). Male quality is often linked with temporal dynamics of mating systems (Flanagan et al. 2016). For instance, in fish, quality is often associated with hatch and settlement dates (Moginie and Shima 2018). Individuals born earlier often have a competitive advantage due to a longer growing period and higher juvenile survival, and as a result may obtain a larger size by the onset of breeding (Lindholm et al. 1994, Cargnelli and Gross 1996, Reichard et al. 2008). Consequently, early breeding individuals often have greater reproductive success (Oring and Lank 1982, Aebischer et al. 1996, Reichard et al. 2008, Parkos et al. 2011). Given the importance of the temporal dynamics in regulating reproductive success, this topic is surprisingly understudied in many systems.

In this study, I quantified male reproductive success during two periods of the breeding season in a population of temperate reef fish, *Forsterygion lapillum* (the common triplefin). *F.*

lapillum are asynchronous continuous spawners, laying eggs daily throughout the main breeding season (Mensink et al. 2014). At my study location, the main breeding season of *F. lapillum* occurs from July to February, however, they have been found to breed all year round (Wellenreuther et al. 2007, Moginie and Shima 2018). *F. lapillum* exhibit male territoriality and parental care, and females exhibit mate choice, providing an excellent model system to test mating system dynamics. I sampled individuals in the field and quantified a set of morphological traits and reconstructed individual life histories from otoliths of nest-holding males. I addressed 3 questions: 1) Does the operational sex ratio (OSR) and the density of individuals change predictably within the breeding season? 2) Does male reproductive success change within the breeding season? And 3) Does the age and growth rate of successful males change over the breeding season?

3.2 Methods

3.2.1 Study species and system

Refer to section 2.2.1

3.2.2 Field surveys

To explore how variation in male reproductive success changes over the main breeding season I conducted field surveys at Moa Point on the Wellington South coast (41°20'34" S, 174°48'42" E) during August and September 2019 and again in December and January 2019-2020. Moa point is a south-facing site with exposure to southerly winds and swells. This site contains high densities of *F. lapillum* and an abundance of suitable habitat for *F. lapillum* reproduction (loose cobblestones) (Mensink 2014). During each sampling date, I haphazardly placed 1 m^2 quadrats (n = 25) onto the seafloor and counted all individuals (adults and juveniles) and estimated their sizes (total length, TL) visually to the nearest 5 mm. I sexed each individual and characterised the reproductive status of sampled males (Territorial male guarding eggs; Territorial male without eggs; Non-territorial male). I determined whether males were associated with a nest or territory based on the display of aggressive and nest guarding behaviours (Appendix A). If eggs were located within the nest of any males (n = 18) these males were caught using a hand net and transported back to Wellington University coastal ecology lab (WUCEL). Before each sampling date, I calibrated my visual estimates of body

size by approximating the size of various live fish in the laboratory. These estimates were then compared with more precise measurements from callipers. All surveys were conducted with the aid of snorkel during low tide.

For each quadrat, I estimated the operational sex ratio as the number of ready-to-mate males (Territorial males guarding eggs and territorial males without eggs) divided by the number of ready-to-mate individuals (Territorial males + Females). The OSR is male biased when OSR > 0.5, and female biased when OSR < 0.5 (Emlen and Oring 1997).

$$OSR = M_T/(M_T + F)$$

3.2.3 Quantifying measures of male reproductive success

As a measure of male reproductive success, I quantified both average egg size and brood size (surface area of eggs present in the nest) for each male guarding eggs (n = 18). In the field, I briefly photographed the underside of the nest (cobblestone) on which eggs were laid (in a single layer), with a scale bar *in situ*. As a measure of brood size, I calculated the surface area of each brood by tracing around the outline of the egg patch using the photo analysis software Image Pro-Premier (9.3.3). This method offers an estimation of brood size as eggs may not be laid continuously or may be broken by small empty spaces due to inconsistencies on the rock surface (Mensink et al. 2014). Due to image quality constraints, it was not possible to reliably measure around and exclude empty patches. I assume that the error introduced by this is small relative to effect sizes of interest. To estimate egg size, I haphazardly selected 15 eggs from each photograph and measured two diameters of each egg and then averaged to obtain the overall egg size (diameter, cm). *F. lapillum* can care for broods contributed by multiple females over the breeding season and nests may contain eggs of various age cohorts. In this species, it was not possible to characterise embryos into different developmental stages due to the possibility of eggs being laid within hours or days of each other (Mensink et al. 2014).

As a measure of nest quality, I measured the surface area available for egg laying within each nest (cobblestone) by multiplying the length of each stone's major axis by its perpendicular width (minor axis). For all nesting males brought back to the lab (n = 18), I measured the

standard length (from tip of snout to start of caudal fin, SL) to the nearest 0.05 mm using callipers. Furthermore, I quantified wet weight to the nearest 0.0001 g using a microbalance. I then calculated a metric of body condition using Fulton's K:

$$K = \frac{wet\ weight\ (g)}{Standard\ length^3 (cm)} \times 100$$

3.2.4 Otolith analysis

From each nesting male (n = 18) I extracted sagittal otoliths to reconstruct individual life histories (growth rates and age). Otoliths are made from calcium carbonate and sit behind the eye, within the skull, of the fish (Popper and Coombs 1980). Otoliths are used by fish for hearing and to maintain balance (Popper and Coombs 1980). In many fish species (including *F. lapillum;* Shima and Swearer 2009) otoliths show the presence of daily growth increments which can be used to estimate growth rates, age, and hatch and settlement dates (Panella 1971, Shima and Swearer 2009).

To remove any tissue, otoliths were separated and washed with a 15 % H_2O_2 solution buffered with NaOH for 6 hours. Otoliths were then rinsed 3 times with deionised water (DI) and placed in the drying oven at 60° C overnight. I randomly selected a single otolith from each fish and mounted each otolith onto a glass slide using Crystal bond ^{TM} thermoplastic adhesive. The otolith was positioned so that the distal end was overhanging the edge of the slide with the core remaining on the slide. I then hand ground the overhanging portion of the otolith using a 9 μm (grit size) diamond lapping film until it reached the edge of the slide. I then repositioned the otolith onto a clear plexiglass disk so that the ground side was mounted facedown, and the proximal end of the otolith was directed upright. The proximal end was then ground down to produce a thin transverse section. The otolith section was polished using 9 and 3 μm lapping films until all daily rings and the central core were clear. Finally, to further clarify rings and diminish dark areas, I covered the surface of the otolith section with a drop of immersion oil for ~ 24 h before image analysis. I photographed each otolith using an optical LED microscope (Leica DM2500) which was connected to a computer and viewed through the Leica Application Suite (4.13.0) software, at 10 x magnification, to expose daily growth increments. I analysed the images using the Otolith M application on Image Pro-Premier (9.3.3) to count and measure daily increments.

To age fish, I counted and measured daily growth increments along a common growth axis for all individuals. For all otoliths, I identified the presence of a 'hatch check' (Shima and Swearer 2009) and calculated the hatch date by subtracting the number of daily increments to the 'hatch check' from the date the fish was caught and sacrificed. Hatch dates were then converted to Julian dates for analyses. I measured average lifetime growth rate by dividing the distance between the first visible ring and the otolith edge by the number of growth increments counted along this axis (Focht 2018).

3.2.5 Statistical analysis

To evaluate variation in fish densities, the OSR, and body size within the breeding season I conducted a Mann-Whitney U test. Additionally, I used a Kruskal Wallis test of association to evaluate size differences between the different male classifications (Territorial males guarding eggs; Territorial males without a nest; Non-territorial males) and females and calculated their pairwise comparisons using a Pairwise Wilcoxon Rank Sum test. For these analyses, the assumptions of normality and equal variance were not met, even after various transformations, hence non-parametric tests were chosen.

To assess whether body size, age, hatch date, growth rate, condition, brood size, egg size, and nest quality of reproductive males varied across the breeding season I used a t-test.

I conducted a set of ANCOVAs to evaluate the influence of nest quality, total length, and condition on brood size and egg size and tested whether this relationship changed over the season. The interaction terms were not significant and removed from the final models. Finally, I performed a set of simple linear regressions to evaluate the effect of age, hatch date, and growth rate on brood size and egg size. Additionally, I performed a simple linear regression to evaluate the relationship between hatch date and growth rate.

Because of the small sample size, comparisons between all variables based on multivariate methods (i.e. multiple regression) was not possible. Therefore, univariate methods were applied, and results below should be taken with caution as mating success may be influenced

by the entire profile of variables more than the characteristics individually. All statistical analyses were performed using R Statistical Software 3.6.2 (R Core Team 2019).

3.3 Results

3.3.1 Within-season fluctuations and population demographics

Across all surveys I sampled 113 *F. lapillum* individuals, 18 of which were males guarding eggs, 43 were territorial males without a nest, 34 were non-territorial males, and 18 were females. Densities of *F. lapillum,* within individual quadrats, ranged from 2–10 individuals/m². The density of *F. lapillum* was significantly higher in summer compared to in the winter months (W $= 37, p = 0.0271$; Fig. 3.1A). The density of territorial males (nest-holding and non-nest holding territorial males), however, did not change over the two sampling seasons (W $= 57.5, p = 0.286$).

I estimated the OSR, as the number of ready-to-mate individuals, during the winter and summer months of the main breeding season. There was no significant change in the OSR over the breeding season (W $= 95.5, p = 0.296$; Fig. 3.1B). During both winter and summer the OSR was male-biased (OSR > 0.5).

The total length (TL) of *F. lapillum* ranged from 20 – 70 mm and varied significantly between the different female and male classifications $(X^2 = 49.01, df = 3, p = 1.295 \times 10^{-11}$; Fig. 3.2). Territorial males guarding eggs were 1.8 times larger than non-territorial males $(p = 7.5 \times 10^{-8})$, and 1.2 times larger than territorial males not holding a nest site $(p = 4.7 \times 10^{-5})$. Both territorial males without a nest site and males guarding eggs were larger than females $(p = 0.038, p = 8.3 \times 10^{-7}$, respectively). The average size (TL) of all individuals sampled, male and female, did not vary over the two sampling seasons $(X^2 = 0.043, df = 1, p = 0.8366)$.
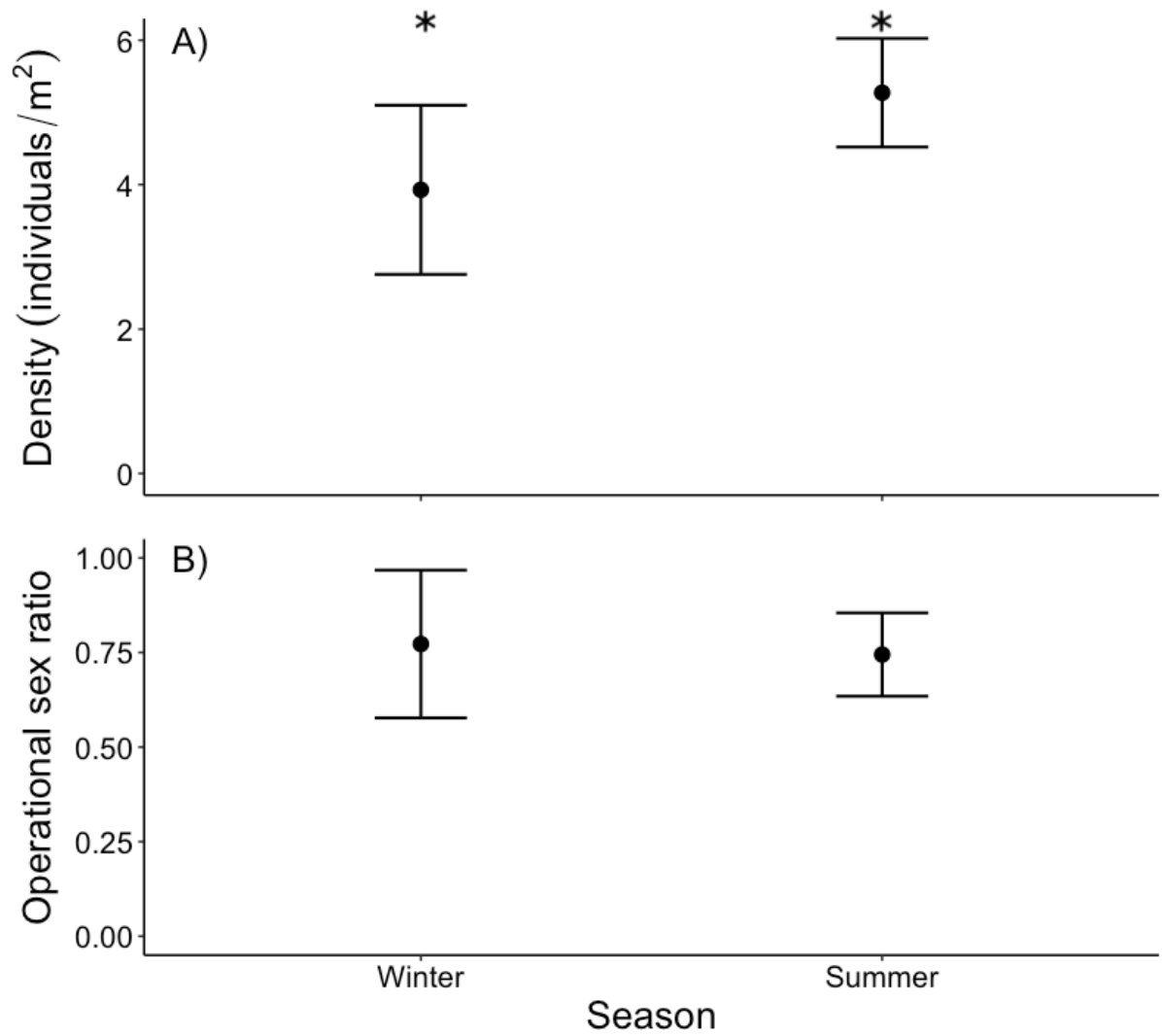
Figure 3.1 A) Average density (individuals/m2) of *F. lapillum* (male and female) during winter (August-September) and summer (December-January) of 2019. **B)** Average operational sex ratio (ratio of ready to mate males and females) of *F. lapillum* during winter (August-September) and summer (December-January) of 2019. Error bars represent 95 % Confidence Intervals. Significant deviations from null distributions (P < 0.05) are indicated with a '*'.
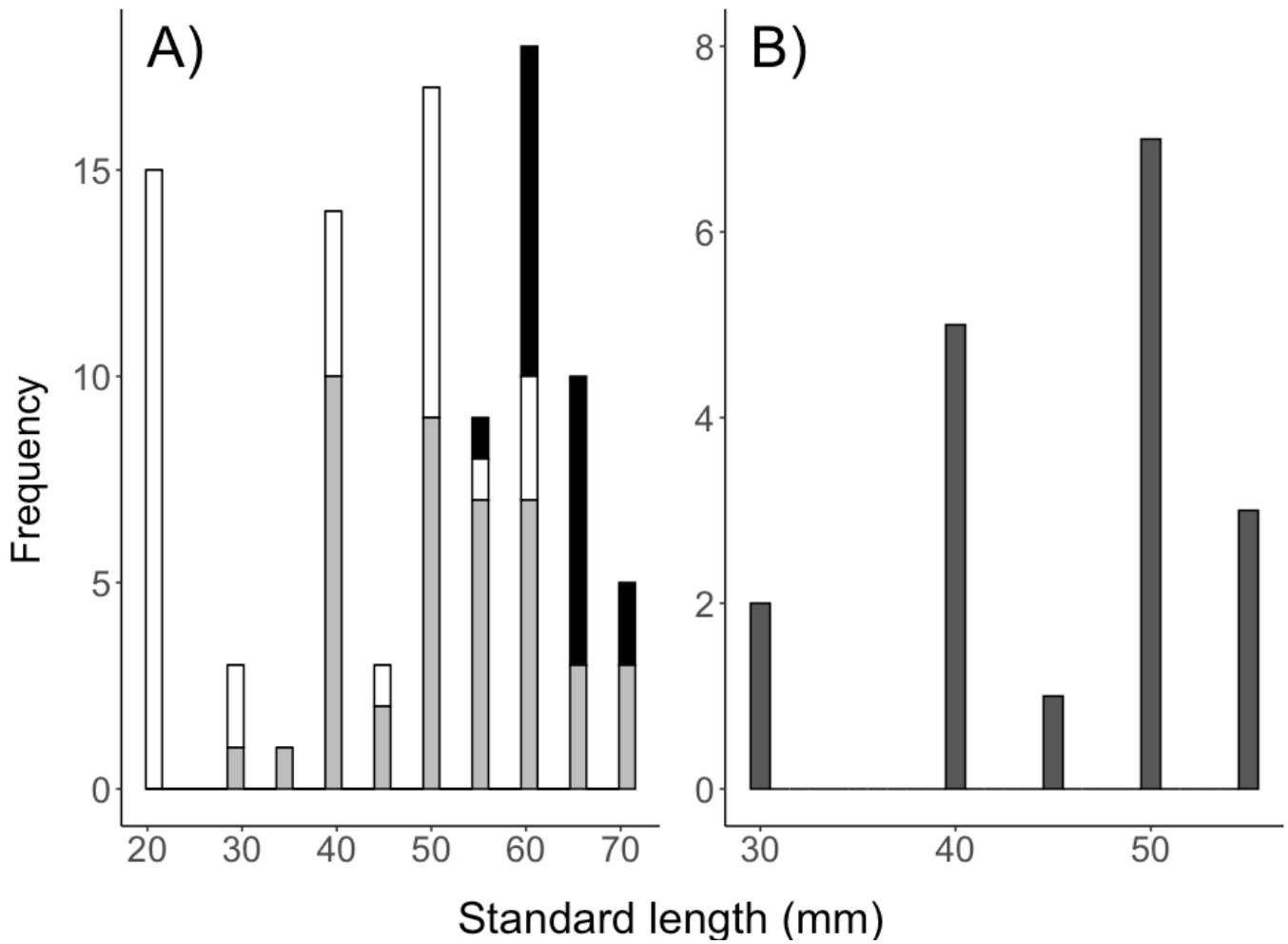
Figure 3.2 A) Size distribution of male *F. lapillum* sampled over the entire sampling period. Black bars represent territorial males holding a nest, grey bars represent territorial males without a nest, and white bars represent non-territorial males. **B)** Size distribution of female *F. lapillum* sampled over the entire sampling period.

3.3.2 Correlates of reproductive success in nest guarding males

The total length of males guarding eggs ranged from 55 mm to 70 mm and increased significantly in summer ($W = 12$, $p = 0.0154$). Males during the summer months were 1.07 times larger than males during winter (Fig. 3.3A). Additionally, the size of the nest (as a measure of nest quality) guarded by reproductive males changed across the season ($t_{1,16} = -2.548$, $p = 0.02149$). Nest sizes ranged from 28 cm^2 to 300 cm^2 and were 1.75 times larger in summer compared to winter (Fig. 3.3B).

Male reproductive success remained constant within the two sampling periods with both brood size and egg size remaining the same across the year (Brood size: $t_{1,16} = -0.087$, $p = 0.932$; Egg size: $t_{1,16} = -1.127$, $p = 0.2765$). Male total length did not influence brood size or egg size, and this did not change across the two seasons (Table 3.1, Fig3.4A & D). However, there was a positive relationship between nest quality and both egg size and brood size (Table 3.1, Fig.3.4B &E). Despite the differences in slopes seen in figure 3.4B and E, this relationship did not change across the year and differences in slopes may be an attribute of the sampling effort. The condition of males guarding eggs did not change over the year ($t_{1,16} = 1.491$, $p = 0.1553$) and had no influence on brood size or egg size (Table 3.1, Fig. 3.4C & F).
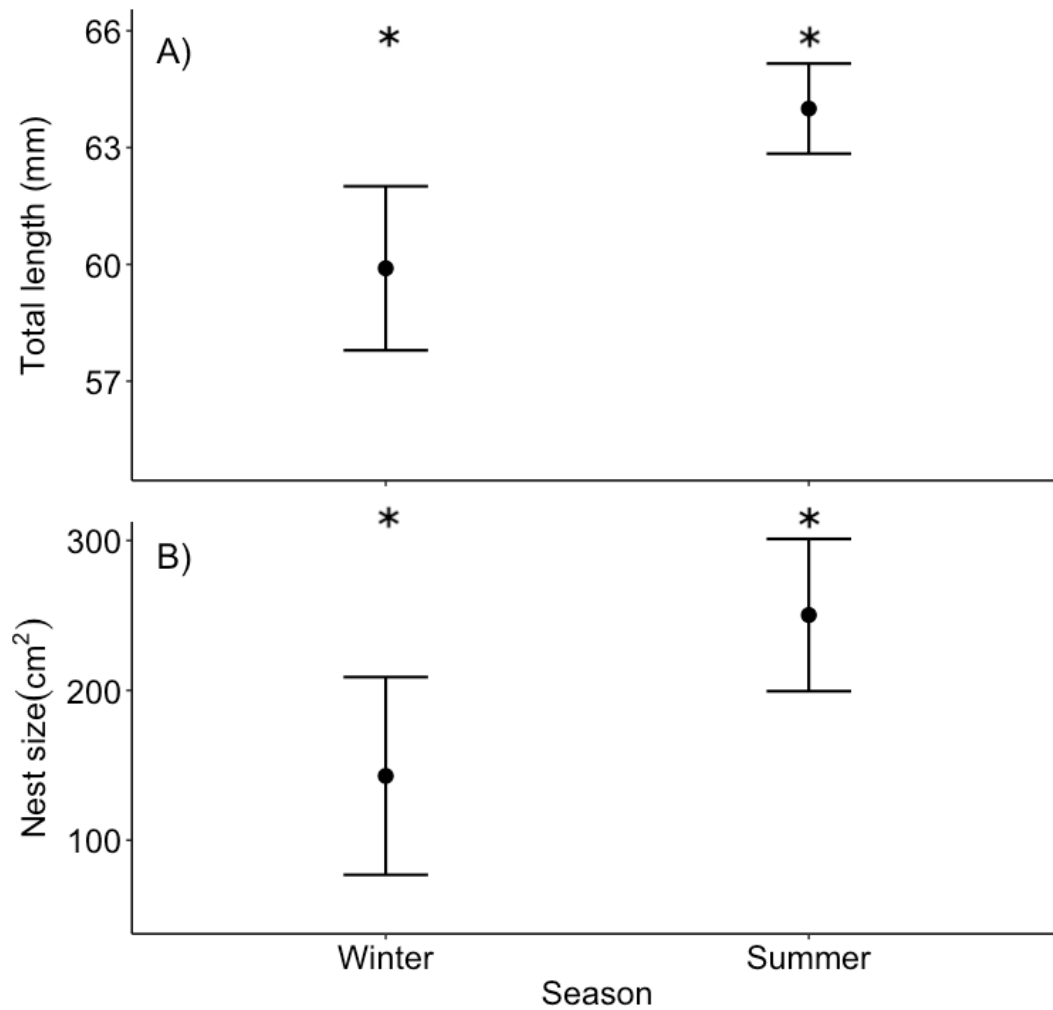
Figure 3.3 A) Average total length (mm) of males guarding eggs during winter (August-September) and summer (December-January) of 2019. **B)** Average size of the nest (cobblestone) held by males guarding eggs during winter (August-September) and summer (December-January) of 2019. Error bars represent 95 % confidence intervals. Significant deviations from null distributions ($P < 0.05$) are indicated with a '*'
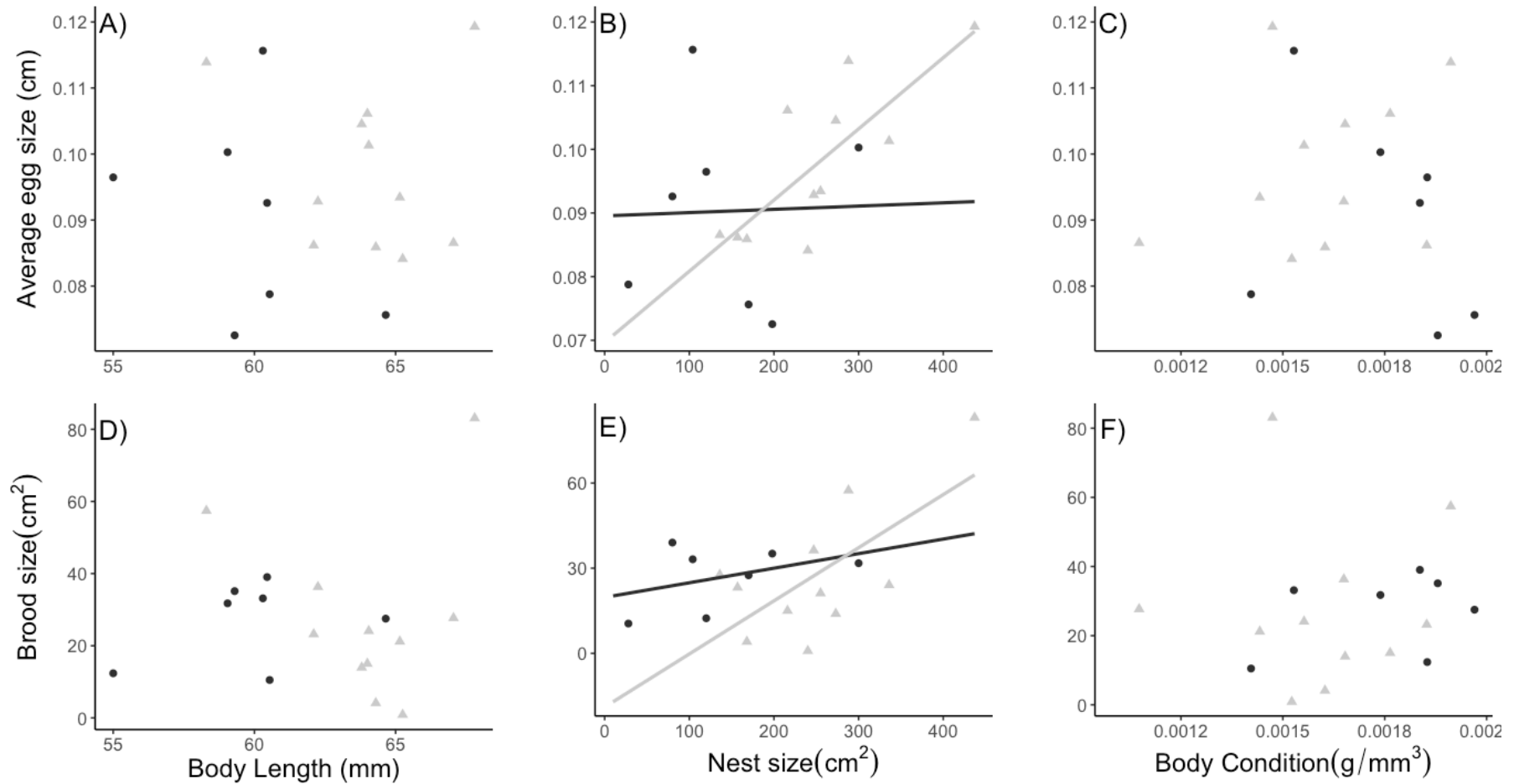
Figure 3.4 Relationship between average egg size and A) the total length of males guarding eggs, B) the nest quality, in terms of the overall surface area available for egg laying on the cobblestone guarded by nesting males, and C) the body condition of egg guarding males, and the relationship between brood size and D) the total length of males guarding eggs, E) the nest quality, and F) the body condition of egg guarding males. Full circles represent males sampled during winter and grey triangles represent males sampled during summer. Trend lines represent a significant relationship between the independent and dependent variables. Solid black lines represent males sampled during winter and solid grey lines represent males sampled during summer.

Table 3.1 ANCOVA output examining the influence of nest size, male total length, and season (winter and summer) on the average egg size (cm) of reproductive males at sampling, and the brood size (surface area of eggs in a nest at sampling, cm^2) of reproductive males at sampling. Results in bold represent significant deviations from null distributions ($P < 0.05$).

Model	Parameter	Df	Sum Sq.	Mean Sq.	F-value	P-value
Egg size	**Nest quality**	**1**	**8.29×10^{-4}**	**8.29×10^{-4}**	**5.327**	**0.0357**
	Season	1	7×10^{-8}	7×10^{-8}	0.0004	0.983
	Residuals	14	0.003	1.556×10^{-4}		
	Total length	1	1.1×10^{-7}	1.1×10^{-7}	0.001	0.981
	Season	1	3.895×10^{-4}	3.895×10^{-4}	2.107	0.167
	Residuals	15	2.77×10^{-3}	1.849×10^{-4}		
	Condition	1	1.96×10^{-5}	1.96×10^{-5}	0.100	0.756
	Season	1	2.138×10^{-4}	2.138×10^{-4}	1.095	0.312
	Residuals	15	2.923×10^{-3}	1.953×10^{-4}		
Brood size	**Nest quality**	**1**	**1628**	**1628.04**	**5.680**	**0.0308**
	Season	1	558.5	558.54	1.949	0.1830
	Residuals	15	4299.2	286.62		

Total length	1	24.4	24.41	0.057	0.815
Season	1	2.9	2.87	0.007	0.936
Residuals	15	6458.5	430.57		
Condition	1	48.6	48.65	0.114	0.741
Season	1	19.9	19.91	0.046	0.832
Residuals	15	6417.3	427.82		

3.3.3 Seasonal variation in growth history and age of reproductive males

Analysis of otoliths suggested that the age of surveyed males guarding eggs ranged from 172-252 days, with an average age of 197 days. The age of successful males did not vary over the two sampling periods ($t_{1,16} = -0.159, p = 0.876$) and did not influence the reproductive success of males in terms of brood size ($F_{1,16} = 0.345, p = 0.563$) and egg size ($F_{1,16} = 0.005, p = 0.946$).

Surveyed males guarding eggs hatched over a 7-month period from 22nd of January 2019 to the 20th of July 2019, with a median hatch date of the 24th of May (Fig. 3.5). Hatch dates (measured in Julian days) significantly differed between males surveyed in the winter of the breeding season compared to males surveyed in the summer of the breeding season ($t_{1,16} = 7.530, p = 1.206 \times 10^{-6}$). Successful males sampled in winter hatched significantly earlier than successful males sampled in summer. Hatch date did not influence the reproductive success of males in terms of brood size ($F_{1,16} = 0.251, p = 0.623$) and egg size ($F_{1,16} = 1.413, p = 0.252$).

The average lifetime growth rate of males did not differ between males guarding eggs sampled in the winter and summer of the breeding season ($t_{1,16} = 1.76, p = 0.098$) and this had no influence on male reproductive success in terms of brood size and egg size (Brood size: $F_{1,16} = 0.016, p = 0.901$; Egg size: $F_{1,16} = 1.122, p = 0.305$). However, it was found that the average lifetime growth rate increased significantly with hatch date ($F_{1,16} = 5.390, p = 0.00$). The increase in growth rate with hatch date appears to be larger in males sampled during the winter months (Fig.3.6), however, this pattern was not significant ($F_{1,16} = 0.223, p = 0.643$).
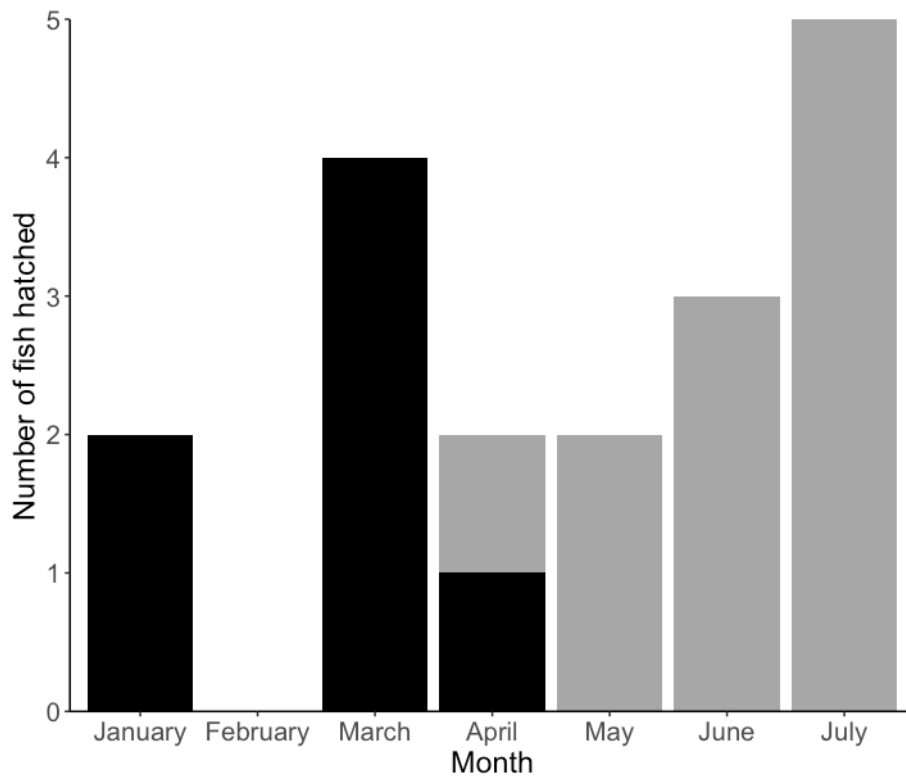
Figure 3.5 Hatching months of males guarding eggs sampled during winter and summer. Hatching months range from January to July 2019. Black bars represent males sampled during winter (August-September), grey bars represent males sampled during summer (December-January).
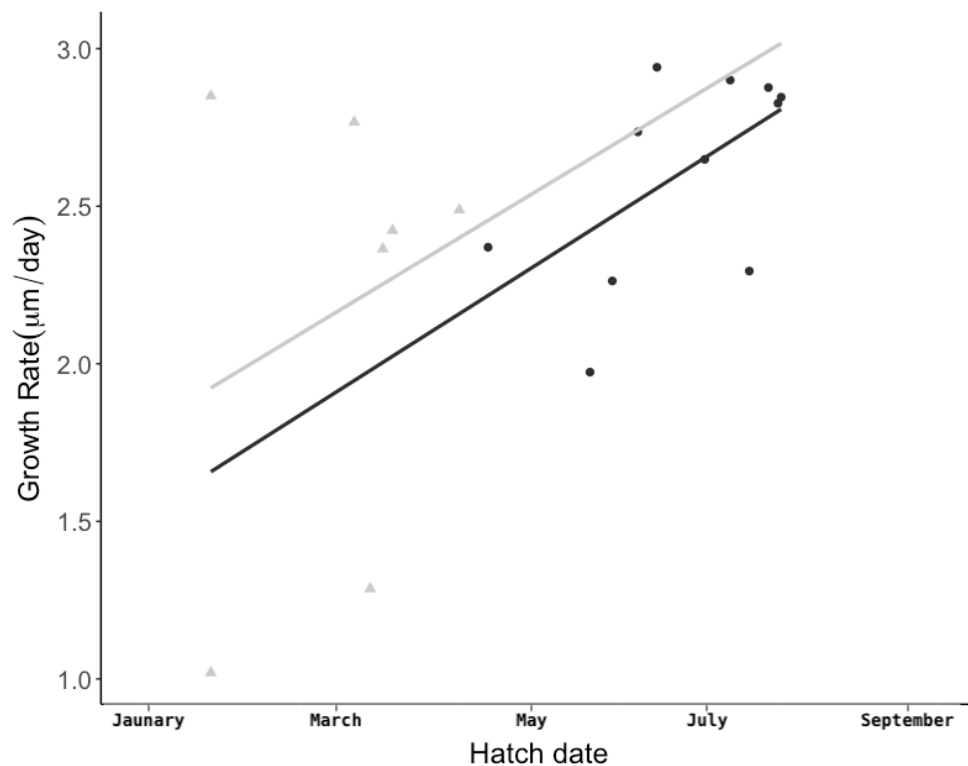
Figure 3.6 Relationship between hatch date and growth rate for males guarding eggs sampled during winter and summer. Full circles and black trend line represent males sampled during summer. Grey triangles and grey trend line represent males sampled during winter.

3.4 Discussion

I measured the reproductive success of male *F. lapillum* at two points in time during the main breeding season and compared different morphological and population parameters during the winter and summer months. I found that although the density of individuals increased in summer, the OSR remained male-biased. Male reproductive success in terms of brood size and average egg size did not fluctuate across the year, however, the total length, and quality of the nest held by reproductive males increased in summer. Interestingly, I found that successful males sampled in winter had hatched significantly earlier than successful males sampled in summer. These results suggest that the mating system and pool of mating individuals in the common triplefin (*F. lapillum*) is highly dynamic and fluctuates throughout the year.

During both the winter and summer months, males guarding eggs were larger than the population average, indicating female choice and positive selection on male body size. Associations between body size and reproductive success are common among fishes, especially in species with male parental care (Demartini 1988, Oliveira et al. 2000, Lehtonen et al. 2007, Hanson and Cooke 2009). Size is typically correlated with male reproductive success as it can influence competition between males for access to breeding resources (Hastings and Petersen 2010). Larger males often have a competitive advantage over smaller sized individuals, granting them the ability to successfully acquire and defend more desirable resources for reproduction (Cole 1982, Lindström 1992, Wootton and Smith 2015). Throughout the entire sampling period, the operational sex ratio (OSR) in this population was male-biased and remained unchanged during the winter and summer months. Due to a shortage of sexually active females, male-biased OSRs are often associated with an increase in male-male aggression and courtship displays (Kvarnemo 1994, Wacker et al. 2013). Simultaneously, the opportunity for mate choice can increase (Kvarnemo 1994). Consequently, the male biased OSR, which continued over the entire sampling period, may have influenced the selection for larger *F. lapillum* males. In nest brooding species, like *F. lapillum*, reproductive success generally depends upon the availability of nest sites, and as a result, male-male competition can become intense, especially when resources vary in quality (Hastings and Petersen 2010). Body size can maximise a males fighting ability and in turn, smaller males can become excluded from gaining access to nesting sites and females (Borg et al. 2006, Hastings and Petersen 2010). Although an increase in the number of available mates can enhance the

opportunity for female choice, the constraints imposed by dominant males throughout the year may have limited female selectivity (Borg et al. 2006). I hypothesize that the selection for larger males is attributed to a combination of male-male competition and female choice, however, further studies are needed to fully disentangle this relationship.

While the selection and reproductive success of larger males did not change over the sampling period, the size of males guarding eggs was significantly larger in summer compared to winter. There may be several explanations for this increase in male body size. Firstly, towards the end of the year, there was a marked increase in the density of individuals in the population. Although male density remained approximately the same, the variance in male size may have been exaggerated due to an increase in smaller juveniles entering the population. Consequently, females may have increased their attraction towards larger males. This pattern has been observed in sand gobies where females were choosier and visited more males towards the end of the breeding season (Forsgren 1997b). This result, however, was not explained by the density of courting males. Instead, an increase in the variability of male courtship intensity towards the end of the season increased the opportunity for female choice (Forsgren 1997b). Courtship intensity is usually more intense in larger males and can be indicative of quality due to the energetic costs of performing such displays (Oliveira et al. 2000, Hale and St Mary 2007). Superior endurance held by larger *F. lapillum* males may have contributed to their increased selection in summer (Reichard et al. 2008). Finally, the potential increase in female choosiness over the season may be attributed to a decrease in the search costs of finding a mate (Crowley et al. 1991). Early in the breeding season, the cost of being preyed upon is predicted to be higher due to individuals having a higher residual reproductive value (Crowley et al. 1991, Forsgren 1997b). As the season progresses this residual reproductive value is predicted to decrease. This in turn reduces the potential costs of predation, allowing females to become more selective (Crowley et al. 1991).

Not only did the size of reproductive males increase in summer, but the size of the nest (cobblestone) held by males also increased. Furthermore, nest size was the only factor to influence brood size and average egg size. However, this did not correspond to an increase in brood size and egg size in the summer months. In many fish species, male mating success is related to the size of the nest or oviposition site (Jennions and Petrie 1997, Oliveira et al. 2000).

The size of the nest can impose an upper limit on the number of matings a male can receive, limiting his lifetime reproductive success (Demartini 1988, Bisazza et al. 1989, Wong et al. 2018). Females evaluate the size of a nest in order to determine if there is enough room to accommodate a high number of eggs, and in doing so often select males with the largest nest sites (Bisazza et al. 1989). As the season progresses and temperatures increase, the time needed for eggs to hatch decreases, shortening the period of parental care (Kvarnemo 1994). Male competition may become more intense due to a decrease in the energetic costs of parental care (Kvarnemo 1994). Consequently, competition for larger nests may have become more intense towards the end of the year, and females may be selecting males based on a combination of factors including nest quality, male quality, and competitive ability. The absence of an increase in brood size and average egg size in the summer months may be a consequence of an upper limit imposed on the maximum clutch size a male can and will effectively defend.

Interestingly, my results indicate that the hatch dates, but not the age, of successful males differed across the two sampling periods. Successful males sampled in winter hatched significantly earlier than successful males sampled in summer. Earlier hatching males will often arrive to the breeding grounds first and have an increased probability of obtaining and defending more desirable resources (Moginie and Shima 2018). Additionally, because they can commence mating earlier, they have the potential to increase the probability of fathering additional offspring (Flanagan et al. 2016). In many systems that reproduce seasonally, first arriving males are often of the highest quality and gain the highest reproductive success (Aebischer et al. 1996, Lozano et al. 1996, Candolin and Voigt 2003). In turn, later hatching males, that settle later in the season, often have faster growth rates due to a decreased growing season (Moginie and Shima 2018). Although growth rate increased with hatch date in my study population, growth rate did not differ between males sampled in the winter and summer. Furthermore, growth rate had no influence on brood size and average egg size. This is contrary to other studies which have suggested an associated cost with fast growth rates. For instance, in a previous study, *F. lapillum* males with faster growth rates had smaller broods than males with slower growth rates (Mensink 2014). Additionally, there was a presence of a negative relationship between early growth rate and reproductive success (Mensink 2014). Due to limitations on the quality of my otolith samples, I could only evaluate average growth rate over an individual's entire lifetime and could not differentiate between different periods of fast and slow growth. Additionally, due to the 'snapshot' approach I took when sampling males, I

cannot determine whether earlier breeding individuals were able to breed over the entirety of the breeding season and whether they had a higher lifetime reproductive success compared to later breeding individuals. Further research evaluating the same individuals over an entire breeding season could address these issues.

My results emphasize the importance of considering within-season variation when examining reproductive success and quantifying sexual selection. Temporal changes in the traits of individuals within a population can influence the degree and strength of sexual selection. Additionally, traits associated with the life history of an individual can have important consequences on the reproductive success of males in a population. While my results did find an increase in the body size and nest size of males guarding eggs over the season, I cannot firmly conclude which mechanisms underlie these changes. Furthermore, changes in female choosiness across the year cannot be characterised by ontogenetic changes or by an environmental response. These questions will need to be confirmed by additional studies. The physical and social environment is highly dynamic and can cause important changes in a mating system. More theoretical and empirical work is required for a complete understanding of the determinants and consequences of within-season change in reproductive success and sexual selection.

General Discussion

4.1 Overview

Over the last two decades, there has been an increase in the amount of theoretical and empirical work on mating system dynamics and individual reproductive success (Reynolds 1996). Mating systems are often complex and determining what factors correlate with reproductive success is often difficult, particularly in species with complex life cycles (Andersson and Iwasa 1996, Reynolds 1996). Consequently, patterns of mating behaviour and reproductive success are poorly defined in many species.

The primary goal of this thesis was to understand which factors correlate to the reproductive success of individuals in a marine reef fish. To accomplish this, I conducted a series of manipulative laboratory experiments, combined with field observations across the breeding season, on the common triplefin, *Forsterygion lapillum*. I focused on the influence of multiple mate choice cues on male reproductive success and female choice. Additionally, I examined the temporal dynamics of breeding success in *F. lapillum*. Overall, the findings in the preceding chapters have outlined the importance of considering multiple cues and temporal dynamics when determining which factors correlate with variation in reproductive success among individuals in a population. This research has provided valuable insight into the mating system of *F. lapillum,* highlighting the dynamic nature of individual reproductive success and sexual selection.

4.2 Male reproductive success

Reproductive behaviour among males can be extremely variable both within and between species (Taborsky 2001). Selection favours individuals exhibiting traits that enhance fitness and reproductive success (Andersson 1994). There is strong evidence that both male-male competition and female choice contribute to success in males, particularly in systems that display male parental care (Gross and Sargent 1985). To be successful, males must acquire

traits that promote both attraction to females and enhance fighting capabilities among conspecifics (Wootton and Smith 2015).

In many mating systems, body size is one of the most important determinants of dominance in males (Andersson 1994). Larger males often rank highly, and as a result, monopolize access to breeding resources, and females often show a preference for larger males (Reichard et al. 2005, Reichard et al. 2008). Results from both the field and laboratory studies indicate that male body size is a key determinant of reproductive success in *F. lapillum*. In Chapter 2, I conducted female choice experiments in the laboratory and found that females preferred and spawned more frequently with larger males. The estimated probability of receiving eggs for smaller males was considerably less than that of larger males. However, when females were provided with two sized matched individuals, they did not discriminate against smaller males. Female preference for large bodied males has been well documented in the literature in a variety of fish species and other closely related triplefin species (Thompson 1986, Oliveira et al. 2000, Lehtonen et al. 2007, Wellenreuther et al. 2007, Hermann et al. 2015). Females should prefer larger males as they have an enhanced ability to secure and defend more desirable resources for breeding and can provide greater parental care (Lee and Bass 2004, Pollock et al. 2008). Thus, when given the opportunity, female *F. lapillum* may prefer larger males as it represents a safer option for her to entrust her eggs, increasing her overall reproductive success.

Field observations in Chapter 3 corroborated laboratory results, as males defending eggs were significantly larger than other males in the population. However, results from field observations in Chapter 2 and 3 indicated that male reproductive success in terms of brood size and average egg size was not influenced by a male's total length. Body size may be more important prior to spawning, increasing the male's ability to obtain and defend a suitable nest site and increase his overall attractiveness towards a female. However, after a male has successfully acquired eggs, body size may be less important in influencing variation in average egg size and brood size amongst already mated males (Mensink 2014).

The quality of a nesting site can also have an important influence on a male's reproductive success. Nest size for instance can impose an upper limit on the number of matings a male can

receive (Bisazza et al. 1989, Oliveira et al. 2000). In turn, males will compete for the largest nests and females will often favour males holding these large nest sites (Bisazza et al. 1989). Results from Chapter 3 confirmed this prediction, revealing that variation in the size of a male's brood and individual eggs were explained by the size of the nest. Unexpectedly, this pattern was not reciprocated in field observations from Chapter 2, which could be a consequence of the 'snapshot' sampling approach and small sample size, reducing the power of my analyses. Nevertheless, results from the laboratory study revealed the importance of nest size on female choice, and in turn male reproductive success. Females preferred and spawned more frequently with larger males holding a larger nest site. This not only confirms the influence of nest quality on male reproductive success in *F. lapillum,* but also indicates the additive effect of male size and nest quality on female choice. The correlation between male body size, nest quality and female choice has been well established in the literature, particularly among species with male parental care (Oliveira et al. 2000, Candolin 2003). Most studies, however, evaluate these factors separately, leaving little knowledge on their joint or interactive effects. My study was unique as it combined these factors, allowing their interactive and additive effects to be acknowledged in both the field and in the laboratory.

Not only do female *F. lapillum* base their mating decisions on the size of the male and nest site, but they also use other cues related to the male's behaviour. For instance, females were more attracted to males with intense courtship behaviours during the dichotomous choice test. Courtship frequency can be a reliable indicator of sexual motivation and male quality due to the energetic costs of performing such displays (Oliveira et al. 2000, Reichard et al. 2005, Hale and St Mary 2007). Females can gain indirect benefits, through offspring viability, when breeding with intensely courting males (Oliveira et al. 2000). Hence, female *F. lapillum* may be assessing male quality through courtship intensity. However, in these settings, where females can interact with the two mates in question, it is likely that they influence each other's behaviours. It can, therefore, be difficult to separate whether the female associated with the male because of his courtship behaviours, or whether he began courting because of her association with him. Further studies to disentangle the cause and effect of courtship behaviour is worth investigating more thoroughly.

In addition to courtship behaviours, the frequency of attacks towards conspecifics can also be a reliable indicator of sexual motivation and can play an important role in determining success among males (Lindström 1992). The best quality nest sites are frequently contested and occupied by males with the highest competitive abilities (Wootton and Smith 2015). Interactions among males will ultimately determine the initial mating pool in which females exert mate choice, signifying the importance of male-male competition. Results from field observations in Chapter 3 revealed that the operational sex ratio (OSR) was male-biased during both sampling periods. Male-biased OSRs are often associated with an increase in male-male aggression and courtship intensity (Kvarnemo 1994, Wacker et al. 2013). Consequently, the OSR, causing an increase in male aggression, may have contributed to the selection of larger *F. lapillum* males by females. However, the relative contribution of both male-male competition and female choice on male reproductive success in *F. lapillum* requires further investigation, potentially through a controlled laboratory experiment.

4.3 Seasonal change in reproductive success

Natural mating systems are highly dynamic and there is growing evidence of the importance of considering variation in sexual selection and reproductive success within a breeding season (Forsgren et al. 2004, Reichard et al. 2008). My results in Chapter 3 add to this emerging literature suggesting that changes in male quality and individual life histories over the breeding season should be carefully considered when evaluating mating system dynamics. My results found an increase in male total length and nest size (as a measure of nest quality) from winter to summer. However, the specific mechanisms underlying these changes remain unclear. This raises some intriguing questions such as: Are these changes due to individual life histories? Are females changing their preference over the breeding season? And are these changes in female choosiness characterised by an ontogenetic change or an environmental response? Testing these questions would require further female choice experiments, occurring throughout the entire breeding season.

Reconstructed individual life histories from otoliths of egg guarding males indicated a change in hatch dates amongst males sampled in the winter and summer months. This 'birth date effect' predicts that earlier hatching males, who arrive at the breeding grounds first, will have an

increased probability of obtaining desirable resources and an increased probability of fathering multiple offspring (Flanagan et al. 2016, Moginie and Shima 2018). Additionally, first arriving males are often of the highest quality and later hatching males often have faster growth rates to compensate for the shorter growing season (Aebischer et al. 1996, Lozano et al. 1996, Moginie and Shima 2018). Although growth rate increased with hatch date in my study, growth rates did not influence brood size or average egg size. This is contrary to previous studies whom have suggested an associated cost with fast growth rates (Mensink 2014). In my study I did not evaluate males over the entire breeding season and could not determine whether growth rates had an influence on the lifetime reproductive success of individuals with different hatching dates. A more in-depth study, occurring across the entire year, would provide valuable information on the influence of growth rates on lifetime reproductive success and consequently the dynamics of this population over the year.

4.4 Limitations

Due to time and health restrictions, field observations made in Chapter 2 and Chapter 3 were constrained to one population over a single breeding season. In Chapter 2, I made all observations over 2 months, sampling individuals only once. Tagging and resampling trials proved unsuccessful, therefore, I was unable to follow individuals across the breeding season. This 'snapshot' approach may be subject to unknown errors generated by temporal and spatial variation across the breeding season. Furthermore, in Chapter 3, I could only sample during two time periods (end of winter and start of summer). This restricted the amount of data I could collect, limiting my ability to evaluate patterns across the entire breeding season. The literature indicated that *F. lapillum* breed intensely from July to early February (Mensink et al. 2014, Moginie and Shima 2018). However, back-calculated birthdates from captured fish indicated that the breeding season extends far beyond this. By sampling across the entire year, I may have detected greater within-season variation than what was concluded in this study.

The dichotomous choice tests conducted in chapter 2 were subject to several limitations. For instance, during the mating phase of the experiment, individuals were free to move around and physically interact with one another. It is possible that the results seen were due to larger males restricting smaller males from accessing females. Although I did not observe these behaviours

specifically, *F. lapillum* are known to be highly territorial and aggressive towards conspecifics. Incorporation of video recordings in future studies would address these questions enabling the assessment of all interactions and courtship behaviours leading up to spawning. During the initial phase of the experiment, males were separated by Plexiglass dividers, preventing any physical interactions. However, because water was pumped from one male compartment and flowed out of the other, chemical signals may have been exchanged by the two males. Therefore, the effect of male-male competition cannot be ruled out.

I recognize that some assumptions are built in the decision to calculate the operational sex ratio with the chosen formula in Chapter 3. For instance, it is assumed that all females recorded were gravid and ready to mate. Although, there are no defining features distinguishing a gravid female from a non-reproductive female in *F. lapillum,* the behaviours displayed by the male towards an approaching female lead me to believe these were actively courting females. Therefore, I believe the error caused by this is minimal. Additionally, this calculation assumes that all males are available to mate and in turn have enough room in their nest to accommodate more eggs. All nests sampled had room to accommodate more eggs, however, it is unknown whether there is a maximum clutch size that a male will and can effectively defend.

Finally, I acknowledge that my study was limited by its small sample size in both Chapter 2 and Chapter 3. This reduced the power of my statistical analyses and limited its generalizability. I was unable to make comparisons between all variables based on multivariate methods (i.e. multiple regression). Therefore, my results should be taken with caution as reproductive success may be influenced by the entire profile of variables more than the characteristics individually.

4.5 Conclusion

Overall, this thesis emphasizes the importance of considering multiple cues and temporal dynamics when disentangling the determinants of individual reproductive success. Previous studies often focus on mate choice cues individually, failing to reveal the significance of their combined and interactive effects. This study aimed to determine how multiple factors drive the

reproductive success of individuals in a temperate reef fish, *F. lapillum*. My results emphasize the dynamic and complex nature of studying mating systems and reproductive ecology. Highlighting the need for future studies to consider multiple cues and environmental factors beyond male size and nest quality when studying reproductive success. Taken as a whole my results have contributed towards an increased understanding of sexual selection and the evolution of mating systems with male parental care.

Appendix A: Ethogram describing male reproductive behaviours used in field and laboratory observations

Table A1: Description of male *F. lapillum* reproductive behaviours observed in field and laboratory experiments in Chapter 2 and Chapter 3.

Measure	Behaviour	Description	Reference
Courtship	Lead displays	Male swims towards female and back to the nest in a figure 8 orientation, with exaggerated fin and body movements.	(Wellenreuther et al. 2008)
	Lateral display	Male spreads all 3 dorsal fins and displays the lateral portion of the body towards female.	(Teresa and Gonçalves-de-Freitas 2011)
Aggression	Chasing	Fish swims towards opponent and opponent swims in the opposite direction.	(Neat 2001)
	Biting	Fish bites opponent usually on top of dorsal fin.	(Helfman et al. 2009)
	Lateral threat	Fish approaches opponent displaying lateral portion of body and spreads all fins.	(Teresa and Gonçalves-de-Freitas 2011)
	Parallel swimming	Two fish swim together with fins spread, orientated either antiparallel (head to tail) or parallel (head to head). Fish may beat body vigorously. Combat finishes when loser swims away.	(Helfman et al. 2009)

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