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THE EVOLUTION OF ANIMAL SOCIETIES: MONOGAMY, GROUP-LIVING AND CONFLICT IN A CORAL-DWELLING FISH

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Marian Y.L. Wong

ABSTRACT

One of the fundamental goals of behavioural ecology is to understand the evolution of mating systems and determine how they influence the formation, structure and stability of animal societies. Monogamous mating systems are particularly challenging to understand, since one sex generally has a higher potential reproductive rate than the other and should therefore be selected to mate multiply. In addition, monogamous mating systems often occur within the context of social groups. The presence of other group members and hence potential mates makes the evolution of monogamy even more perplexing, since an individual's immediate opportunities for polygamy are greatly enhanced. Monogamy within the context of social groups also poses problems for understanding why other group members tolerate group-living, given that they are excluded from reproduction, and how conflict over reproduction between group members is resolved if groups are to be stable. In this thesis, I investigated the ecological determinants of the evolution of monogamy and its consequences for the formation, structure and stability of social groups in a coral-dwelling goby, *Paragobiodon xanthosomus* (Gobiidae).

In chapter 2, I quantified the social structure of natural groups and determined the mating system of *P. xanthosomus*. Regardless of group size, groups consisted of one mature male and female plus several smaller immature females that were organised into a size-based dominance hierarchy. Observations of breeding behaviour confirmed that *P. xanthosomus* exhibits a monogamous mating system in which only the mature male and female breed at the expense of the other female group members. To address the evolution of monogamy despite the immediate availability of multiple females within groups, I used field and aquarium experiments to test whether competition between females over limiting nest sites, food or paternal care constrained

males to monogamy. Supplemental feeding increased the fecundity of breeding females, suggesting that food is a limited resource for reproduction. Supplemental feeding did not however result in the maturation of other female group members. This suggests that monogamy has evolved because dominant females suppress the reproduction of subordinate females because they are competitors for limited food. Finally, males in pairs that received supplemental food exhibited a diminishing ability to care for enlarged clutches laid by similarly-sized breeding females. This suggests that constraints on paternal care provide additional benefits for females from maintaining a monogamous mating system.

In chapter 3, I investigated the consequences of the monogamous mating system on the maintenance of group-living in *P. xanthosomus*. Specifically, I applied cooperative breeding theory to determine the factors promoting group-living by non-breeding subordinates given that they obtain no current reproduction within groups due to the monogamous mating system. Using field and aquarium experiments in which coral saturation, costs of movement between corals and the size of available corals was manipulated, I demonstrated that non-breeding group members tolerate group-living because of high costs of movement and benefits from inheriting a territory of high quality. In contrast, coral saturation and position in the hierarchy appeared to have no effect on the occurrence of subordinate dispersal.

In chapter 4, I investigated the mechanisms involved in resolving conflict over rank between group members, and thus promoting the stability social groups given the unequal distribution of reproduction between group members due to the monogamous mating system. Specifically, I tested whether the combination of punishment by dominants in the form of eviction from the group, and cooperation by subordinates in the form of growth regulation had evolved as a means of conflict resolution. Firstly, I conducted a removal experiment in the field and showed that non-breeding subordinate group members form size-based queues in which they wait to inherit dominant breeding status. Conflict over reproduction should be particularly intense in queues since subordinates gain no reproduction while they wait and face the prospect of dying before inheriting a breeding position. Detailed quantification of size ratios between individuals of adjacent rank in groups revealed a prevalence of a specific size ratio between individuals, and analysis of individual growth rates in the field demonstrated that the specific size ratio is maintained over time via the regulation of subordinate growth rates. Staged contest experiments in aquaria between individuals of adjacent rank revealed that the specific size ratio represents a threshold above which subordinates can evict their immediate dominant from the group, but are much more likely to be evicted by their immediate dominant themselves. This suggest that threshold size ratios are being maintained by subordinates as a form of cooperation whereby they avoid becoming a threat to their immediate dominants, and that such cooperation arises in response to the threat of punishment by dominants. Societies in *P. xanthosomus* are therefore being stabilised as a result of punishment and cooperation acting in concert to promote the resolution of conflict over rank between group members.

Finally in chapter 5, I used a supplemental feeding and removal experiments to explicitly test whether: (1) disproportional acquisition of food resources as a result of differential competitive ability contributed to the maintenance of size differences between individuals within size-based queues, and (2) subordinates are capable of regulating their own growth to avoid inflicting costs on dominants. Supplemental feeding resulted in elevated growth rates of subordinates in both the presence and absence of the breeding female, suggesting that food limitation on subordinates due to competitive exclusion by dominants promotes the maintenance of size differences within size-based queues. Feeding of rank 4 subordinates resulted in some restraining their own growth by reducing their food intake, demonstrating that subordinates are capable of regulating their own growth to avoid breaching the threshold size ratio. The remaining rank 4 subordinates grew in breach of the threshold size ratio and were consequently evicted from the group, suggesting that food availability may also influence the stability of queues. Taken together, both competition over food and subordinate growth restraint in response to the threat of eviction appear to be important in regulating subordinate growth rates and in maintaining well-defined size differences within size-based queues, although social regulation is likely to be the primary factor ensuring the stability of *P. xanthosomus* societies over time.

In summary, this thesis represents a quantitative and systematic investigation into the mechanisms responsible for, and the links between, the evolution and maintenance of monogamy, social groups, and conflict resolution in a monogamous, group-living fish. In so doing, it provides a comprehensive picture of the mechanisms underlying the formation, structure and stability *P. xanthosomus* societies, and contributes to a greater understanding of the processes governing the evolution and maintenance of animal societies in general.

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DECLARATION

I declare that this thesis is my own work that has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

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Marian Y.L. Wong

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CHAPTER 1

GENERAL INTRODUCTION

The field of behavioural ecology is concerned with answering the question: why do animals behave the way they do? Or more specifically, why has natural selection favoured the evolution and maintenance of a particular behaviour over other alternatives? To investigate this, behavioural ecologists study the ecology of animals, since the environmental and social 'stage' on which animals play out their lives will have a major influence on determining whether a particular behaviour is favoured or penalised by natural selection (Orians, 1969; Wilson, 1975; Emlen & Oring, 1977; Axelrod & Hamilton, 1981; Maynard Smith, 1982). In essence therefore, behavioural ecologists are concerned with exploring the links between the ecology and evolution of an animal's behaviour, with the ultimate aim of explaining and predicting behavioural variation. Much of the focus in behavioural ecology is also concerned with understanding the causes and consequences of conflicts of interest between different individuals, since in many cases, the evolution and expression of a particular behaviour in one animal will impose costs on others (Parker, 1970; Trivers, 1974; Davies, 1992).

The types of animal behaviour explored by behavioural ecologists are extremely diverse, ranging from reproductive, social, survival, foraging, life history, parental and predatory behaviours (Wilson, 1975; Krebs & Davies, 1993). Among these, the evolution of reproductive and social behaviours has attracted considerable scientific interest and this interest has been sustained over many years (Gross, 1994; Owens, 2006). Investigations pertaining to these particular behaviours can be subdivided into the following central questions: 1) What ecological factors promote the evolution of mating systems? 2) What ecological factors promote the evolution of group-living? and 3) Why are there often conflicts of interest between individuals in social groups and how are they subsequently resolved? These inter-related questions are integral to a complete understanding of the structure, functioning and maintenance of animal societies.

Mating systems in animals can be broadly categorised into two main forms. Firstly, they can be monogamous, in which males and females essentially breed with just one other partner (Wittenberger & Tilson, 1980). Secondly, mating systems can be polygamous, in which males and/or females mate multiply with more than one other partner (Emlen & Oring, 1977). In comparison to polygamy, monogamy has received relatively little scientific attention which may partly be due to its relative rarity amongst animal taxa in general (Kleiman, 1977; Wickler & Seibt, 1981). In addition, monogamy may have received less attention because it has generally been viewed as a constraint on the process of sexual selection, owing to the lower variation in mating success among individuals in comparison to polygamous mating systems (Mock & Fujioka, 1990). Monogamy however, poses a fundamental challenge to our understanding of mating system evolution, since according to theory, one sex (usually the male) generally has a higher potential reproductive rate than the other (usually the female) and hence should be selected to mate with multiple partners (Clutton-Brock & Vincent, 1991; Clutton-Brock & Parker, 1992). From an evolutionary viewpoint, monogamy is a more intriguing mating system than polygamy, and begs the question of what ecological factors are responsible for promoting the evolution and maintenance of reproductive exclusivity when at least one sex is expected to mate polygamously.

Traditionally, the evolution of monogamy has been viewed as a product of the need for both parents to successfully raise offspring (the ‘bi-parental care’ hypothesis) (Lack, 1968; Wilson, 1975). This hypothesis arose from observations of the predominance of monogamy in birds (~90% of species being so classified) of which the majority of species also exhibit bi-parental care (Mock & Fujioka, 1994). However, increasing evidence from other taxonomic groups has since demonstrated that bi-parental care is far from an essential precondition for monogamy. For example, Komers & Brotherton (1998) conducted a quantitative analysis of the relationships between paternal care, female dispersion and monogamy across a wide range of mammals, and found monogamy in the absence of paternal care was actually more common that in its presence. In addition, of the 18 families of coral-reef fish known to exhibit monogamy, only one species exhibits bi-parental care (Barlow, 1986; Whiteman & Côté, 2004a). Finally, bi-parental care is also a poor correlate of monogamy in reptiles (Bull, 2000) and crustacea (Wickler & Seibt, 1981; Mathews, 2002). Although bi-parental care may be the primary reason for monogamy in birds, the collective evidence clearly indicates that it can not provide a general explanation for monogamy in other taxa.

Various alternative non-mutually exclusive hypotheses have been proposed to explain monogamy in the absence of bi-parental care. The majority of these ultimately invoke the role of resources (e.g. breeding sites, food and mates) and how their distribution, availability or quality promotes monogamy. The most influential of these resource-based hypotheses focuses on how the distribution and availability of resources influences the distribution and availability of females, and hence the ability of males to defend multiple females from other males (Emlen & Oring, 1977; Kleimann, 1977; Clutton-Brock, 1989; Shuster & Wade, 2003). Specifically, when limiting resources are sparse and uniformly distributed in space, there exists a low environmental potential for polygamy (EPP) as females will also tend towards an even and widespread distribution. Under these circumstances, males have little opportunity to defend multiple females from other males and thus monogamy should be favoured (Emlen & Oring, 1977). Conversely, if limiting resources are unevenly distributed in space, there exists a high EPP as females will also tend towards an uneven and clumped distribution. Under these circumstances, a subset of males would be able to defend groups of females or the resources they use from other males, resulting in the evolution of polygyny (Emlen & Oring, 1977). Despite being the key alternative hypothesis for monogamy, empirical support for the EPP model has been surprisingly mixed in a variety of different taxa (birds: Davies & Lundberg, 1984; fish: Fricke, 1980; Donaldson, 1989; Vincent *et al.,* 2004; Thompson *et al.,* In Press; insects: Trumbo & Eggert, 1994; mammals: Van Schaik & Dunbar, 1983; Kishimoto & Kawamichi, 1996; Brotherton & Manser, 1997).

One potential reason for these empirical discrepancies may relate to the fact that the EPP model strongly emphasises the role of male competition over females in determining the mating system, without invoking the potential role that females may play. An alternative perspective on monogamy invokes the role of female competition over limiting resources in determining the mating system (Ahnesjo *et al.,* 1993; Berglund *et al.,* 1993). In many cases, particularly within the context of social groups, one female will be dominant over others (e.g. Holecamp *et al.,* 1996; Henson & Warner, 1997; Clutton-Brock *et al.,* 1998; Monnin & Peeters, 1999). If resources are limiting, the dominant female would benefit from suppressing the reproductive status or behaviour of more subordinate females since this would minimise competition and ensure her monopoly over the limiting resources (Wasser & Barash, 1983; Clutton-Brock & Albon, 1985). As a result, the presence of only a single, reproductive female would constrain males to monogamy. Potential limiting resources that could underlie such female competition, reproductive suppression and hence monogamy include breeding sites (e.g. Lindstrom & Seppa, 1996) and food (e.g. Woodroffe & MacDonald, 1995). For species exhibiting paternal care of offspring, female competition could also arise if limits and costs of paternal care prevent males from caring for more than a certain number of offspring simultaneously (Kuwamura *et al.,* 1993; Whiteman & Côté, 2004a). If the dominant female is capable of providing males with all the offspring he can care for at each reproductive bout, then dominant females would benefit from suppressing the reproduction of subordinate females resulting in the evolution and maintenance of monogamy (Whiteman & Côté, 2004a).

Females may also play a key role in promoting the evolution of monogamy if they experience costs of polygyny, for example due to competition over limiting resources, and there is a low variance in the quality of resources defended by males (Verner & Willson, 1966). Under these circumstances, females are expected to choose a monogamous mating option since the benefits they may gain from breeding with an already mated male, in the form of access to higher quality resources, are too small to compensate for the costs of polygyny (Verner & Willson, 1966; Orians, 1969; Pribil & Searcy, 2001). In addition, females may prevent males from acquiring other mates if they benefit from male assistance in territory defence or maintenance, since this allows females more time to feed and increase their fecundity (Hourigan, 1989; Kokita & Nakazono, 1999). Finally, females may also promote the occurrence of monogamy for reasons that are not directly related to resources. For example, females in a population may employ a strategy where they all become receptive in synchrony. This strategy effectively constrains males to monogamy because a deserting male has little chance of finding another receptive female before his original mate is ready to reproduce again (Knowlton, 1979; Lobel, 1989; Takegaki, 2000).

The wealth of theoretical information combined with growing empirical support for the various alternative hypotheses is beginning to shed some light on why monogamy has evolved in a range of different animals. However, most empirical studies addressing the evolution of monogamy have focused solely on species in which monogamous males and females only occur in pairs. Monogamy frequently occurs within the context of highly organised and stable social groups in which group members co-occur together within the same habitat or territory (i.e. ‘animal societies’) (Taborsky, 1985; Komdeur, 1992; Emlen, 1995; Bourke, 1997; Clarke & Faulkes, 1997; Clutton-Brock *et al.,* 1998). The evolution of monogamy in social groups is even more perplexing since the presence of other group members, and thus potential mates, should greatly increase an individual's immediate opportunities for polygamy. Under these circumstances, the key alternative hypothesis for monogamy, namely the EPP model (Emlen & Oring, 1977), is unable to explain monogamy since females living within a social group are necessarily clumped in space and thus polygyny as opposed to monogamy would be expected. Why then should both males and females confine the majority of their reproduction with just one other partner when other mates are readily available within the group? Could the occurrence of monogamy in these societies be the product of competition between female group members? Currently, little is known about how well the alternative hypotheses for monogamy explain the evolution and maintenance of monogamy within group-living animal societies.

Despite being intriguing purely from the viewpoint of understanding mating system evolution, the occurrence of monogamy within the context of animal societies lies at the heart of another key question in behavioural ecology: *what ecological factors promote the evolution of group-living?* It is well recognised that social animals face a trade-off between various benefits and costs of group-living. Benefits of group-living include increased survival or reproduction through predator deterrence (e.g. Pitcher & Parrish, 1993; Hayes, 2000; Lingle, 2001), shared vigilance leaving more time for feeding or escape (e.g. Bertram, 1980; Semeniuk & Dill, 2004) and risk dilution from a reduction in an individuals' probability of being attacked by predators or parasites with increasing group size (e.g. Wrona & Dixon, 1991; Mooring & Hart, 1992; Ratti *et al.,* 2006). Costs of group-living include greater attraction of predators to larger groups (e.g. Uetz & Hieber, 1994), increased parasite transmission (e.g. Poulin, 1991; Moore, 2001), and increased competition for resources such as food, space and mates between group members (Sale, 1972; Wrangham *et al.,* 1993; Krause & Ruxton, 2002; Hollis *et al.,* 2004). Only when the benefits outweigh the costs will the evolution of group-living be favoured (Krebs & Davies, 1993).

In many of these situations, the pay-offs from group-living are similar for all group members. This would occur if, for example, all group members are able to breed within the group (Sale, 1972; Poulin, 1991; Pitcher & Parrish, 1993; Hayes, 2000; Lingle, 2001), or if costs of resource competition are similar for all group members (Pitcher & Parrish, 1993; Uetz & Hieber, 1994; Krause & Ruxton, 2002). In other societies however, the pay-offs from living within a social group differ markedly and consistently between group members (e.g. Craig *et al.,* 1982; Clutton-Brock *et al.,* 1998; Webster & Hixon, 2000; Buston & Cant, 2006). Such situations typically arise in species where group members are organised into dominance hierarchies. Within a dominance hierarchy, dominant group members monopolise the majority of resources, at the expense of subordinate group members that may be completely excluded from access to important resources when they are limiting (Ranta & Lindstrom, 1992; Faria *et al.,* 1998; Stahl *et al.,* 2000; Webster & Hixon, 2000). Consequently, dominants typically suffer lower costs of group-living as evidenced by their generally enhanced growth and/or survival rates compared to subordinate group members (Stamps, 1984; Buston, 2003b; Gilmour *et al.,* 2005).

Among animal societies, there exists great variation in the degree to which reproduction is distributed amongst group members (Keller & Reeve, 1994). In some societies, subordinates engage in reproduction within the group and as such, reproduction is shared amongst group members (e.g. Fricke, 1980; Jamieson, 1997; De Luca & Ginsberg, 2001; Reeve & Keller, 2001). At the other extreme, a pair of dominant group members excludes all subordinates from reproduction, for example through aggressive suppression of subordinate behaviour (Creel *et al.,* 1992; O'Riain *et al.,* 2000) or reproductive physiology (Faulkes & Bennett, 2001; Young *et al.,* 2006), resulting in an unequal distribution of reproduction between group members. Inequalities in the pay-offs from group-living between dominants and subordinates are most apparent in these societies where the mating system is essentially monogamous (Komdeur, 1992; Creel & MacDonald, 1994; Bourke, 1997; Balshine-Earn *et al.,* 1998; Faulkes & Bennett, 2001; Buston, 2003a; Griffin *et al.,* 2003). Inequalities are further enhanced within these societies if non-breeding subordinates are un-related to their dominant counterparts, since they can expect to gain no indirect reproductive benefits from group-living via the production of non-descendent kin (Hamilton, 1964). Why then do subordinates in such 'monogamous societies' tolerate group-living as opposed to dispersing to breed independently elsewhere? Clearly, non-breeding subordinates that remain in their current group suffer an automatic fitness cost in terms of missed reproductive opportunities elsewhere (Emlen, 1995). Thus, to answer this question, the ecological factors promoting compensation for this cost need to be identified.

The theory of cooperative breeding (Brown, 1974) has the potential to provide an insight into the ecological factors compensating for this cost and thus help to explain why non-breeding subordinates tolerate group-living. Although this framework is ultimately focused on explaining why non-breeding offspring provide help to their parents, an integral theoretical component is concerned with assessing the factors affecting the pay-offs to subordinates of dispersing to breed elsewhere versus remaining in their current group (Brown, 1974; Emlen, 1991). Specifically, non-breeding subordinates are expected to remain in their current group if costs from dispersing are high, for example if all alternative habitats in the environment are saturated (Selander, 1964; Pruett-Jones & Lewis, 1990, Komdeur, 1992; Bergmüller *et al.,* 2005) or if movement between available habitats is costly in terms of significant energy expenditure or predation risk (Emlen, 1982; DuPlessis, 1992; Russell, 2001; Heg *et al.,* 2004b). In addition, non-breeding subordinates are expected to remain in their current group if they stand to gain future fitness by doing so (Stacey & Ligon, 1987). For example, subordinates may be favoured to stay within a group if the territory on which they reside is of high quality and they stand to inherit it from more dominant group members in the future (Stacey & Ligon, 1987; Komdeur 1992; Walters *et al.,* 1992; Zack & Stutchbury, 1992; Pen & Weissing, 2000; Ekman *et al.,* 2001; Covas *et al.,* 2003). Thus, cooperative breeding theory serves as a useful framework with which to assess the factors affecting the pay-offs to non-breeding subordinates of group-living when they are excluded from reproduction within the group.

The very existence of animal societies in which reproductive benefits are unequally distributed as a result of monogamous mating systems underlies a third key question in behavioural ecology: *why are there conflicts of interest between group members and how are they subsequently resolved?* Potential conflict arises whenever there are asymmetries in the reproductive benefits accrued between individuals within a society (Ratnieks & Reeve, 1992). Failure to resolve potential conflict would lead to the expression of overt or actual conflict between group members over prolonged periods of time, which would impose significant and repeated costs on the fitness of all individuals involved (Ratnieks *et al.,* 2006). Ultimately, this could culminate in the de-stabilisation or dissolution of the society altogether (Wiley & Rabenold, 1984). Therefore, a fundamental challenge for behavioural ecologists is to understand the mechanisms that diffuse potential conflict within a society.

In the majority of cases, conflicts of interest between group members revolve around gaining access to reproduction (Ratnieks *et al.,* 2006). In societies where subordinates gain no current reproductive benefits, are un-related to dominant group members and confer little or no benefits to dominants from their presence (e.g. Mitchell, 2003; Buston, & Cant, 2006), the incentive for conflict over reproduction on the part of both subordinate and dominant group members is likely to be particularly magnified (Johnstone, 2000; Ratnieks *et al.,* 2006). This potential for conflict is further enhanced if non-breeding group members queue to inherit the top-ranked breeding position within the group, but face the prospect of dying before ever achieving this status (Field *et al.,* 1999; Buston, 2004a; Mitchell, 2003). Under these circumstances, subordinate group members would maximise their fitness by challenging and attempting to overthrow more dominant group members, since this would elevate their rank in the group and thus their position in the queue for reproduction (Wiley & Rabenold, 1984). Dominants on the other hand, would maximise their fitness by ensuring that their rank is maintained, which they could do by aggressively suppressing the challenges of subordinates or even evicting them from the group altogether (Buston, 2004a; Stephens *et al.,* 2005). Yet despite this potential for conflict between subordinate and dominant group members, aggression between group members is generally infrequent and queues surprisingly stable over time (e.g. Schwagmeyer & Parker, 1987; Field *et al.,* 1999; East & Hofer, 2001; Buston, 2004a; Mitchell, 2005), which suggests that potential conflict over rank and hence access to reproduction between dominant and subordinate group members is somehow being resolved within these societies (Ratnieks *et al.,* 2006).

Punishment is one of the key factors proposed to promote conflict resolution within hierarchical societies (Clutton-Brock & Parker, 1995). Dominants usually have greater ‘power’ than subordinates owing to factors such as greater size, age, experience, information or ability to form alliances with other group members (Beekman *et al.,* 2003). Therefore, dominant group members would generally be capable of forcing subordinate group members to refrain from acting selfishly, or at least reducing the benefits gained by subordinates from doing so, by threatening them with punishment. As a result, subordinates may have little choice but to behave cooperatively towards dominants at the expense of realising their own reproductive potential, thus promoting the resolution of conflict. Indeed, there is growing theoretical and empirical evidence that dominants, by employing the threat of punishment in the form of eviction from the group, are capable of inducing subordinates to provide costly help in the form of territory defense, maintenance and offspring care (Mulder & Langmore, 1993; Balshine-Earn *et al.,* 1998;

Kokko *et al.,* 2002; Bergmüller & Taborsky, 2005; Hamiton & Taborsky, 2005). In addition, theoretical studies strongly invoke the threat of eviction by dominants as a key factor in regulating the share of subordinate reproduction within groups (e.g. Johnstone & Cant, 1999). However, there is currently little empirical evidence to support the role of the threat of punishment in the restraint of subordinate challenges over rank within queue-based societies, despite theoretical references to this question (Wiley & Rabenold, 1984; Buston, 2004a).

Potential conflict between individuals could also be resolved as a result of constraints acting on subordinates such that they simply lack the ability to behave selfishly (Ratnieks *et al.,* 2006). For example, potential conflict over caste fate is prevalent in social hymenoptera, since individuals would prefer to be reared as queens as opposed to workers, but an excess of queens results in a reduction in colony productivity (Bourke & Ratnieks, 1999). Conflict is resolved because larvae are unable to acquire sufficient food to develop into queens since their food is rationed by workers and they are physically incapable of leaving their brood cells to obtain more food for themselves (Ratnieks *et al.,* 2006). Constraints on the ability of subordinates to successfully overthrow dominants arising from differential access to food resources may similarly have the potential to play a role in conflict resolution in other hierarchical societies. Indeed, differential access to food within hierarchies as a result of competitive interactions with dominants has been reported in a wide range of animal societies, including fish (e.g. Webster & Hixon, 2000), birds (e.g. Stahl *et al.,* 2001), and mammals (e.g. Pruetz & Isbell, 2000). Further investigations into the threat of punishment and constraints on subordinates in a wide range of taxa would therefore be beneficial for improving our understanding of the roles of both processes in the resolution of conflict over rank and the stability of hierarchical societies.

Clearly, the three questions often posed by behavioural ecologists regarding the evolution of reproductive and social behaviour are integrally linked. Although certain aspects of an animal's ecology will promote the occurrence of monogamy, group-living and conflict resolution independently, the evolution of monogamous mating systems will itself have an impact on the pay-offs to subordinates from living in groups since monogamy affects the distribution of reproductive benefits between group members. In turn, the evolution and maintenance of societies in which reproductive benefits are skewed as a consequence of monogamy will generate reproductive conflict between group members and influence how the conflict is subsequently resolved. Determining the ecological factors underlying these integrally related issues will provide a more complete picture of the formation, structure, functioning and maintenance of animal societies.

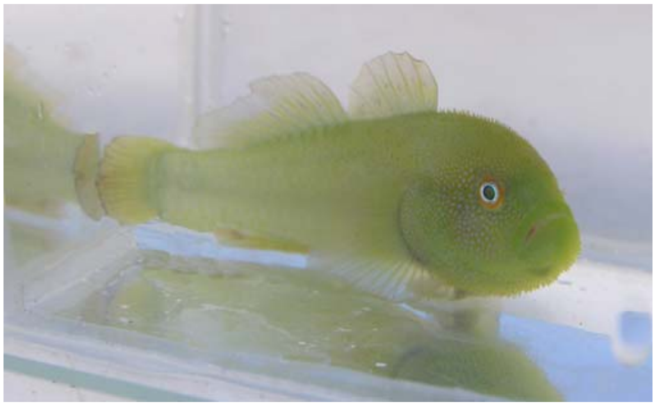
Coral-reef fish have the potential to provide a unique insight into the evolution of reproductive and social behaviour. Reef fishes exhibit a wide range of mating systems, including species with promiscuous mating systems in which both males and females mate with multiple partners (e.g. *Dascyllus marginatus*, Fricke, 1980; *Apogon spp*, Kuwamura, 1985; *Chaetodon spp*, Hourigan, 1989), and polyandrous mating systems whereby females mate with multiple males (e.g. *Syngnathus spp,* Jones & Avise, 2001). Many other species exhibit polygynous mating systems where males guard a permanent group of females with which they mate (e.g. *Canthigaster valentini,* Gladstone, 1987; *Centropyge interruptus*, Moyer & Nakazono, 1978; *Labroides dimidiatus,* Robertson & Hoffman, 1977, Kuwamura, 1984), or guard particular resources which themselves attract multiple females (e.g. *Dascyllus marginatus*, Fricke, 1980; Godwin, 1995; *Pseudolabrus celidotus*, Jones, 1981; *Thalassoma bifasciatum*, Warner, 1987). Variation in the mating system also occurs within species of reef fishes (Warner, 1991). For example, in the coral-dwelling damselfish, *Dascyllus marginatus*, the mating system shifts from monogamy to harem polygyny to multi-male harem polygyny and finally to promiscuity as the size of coral colonies increases (Fricke, 1980). This diversity of mating systems within and among species provides opportunities for comparing and contrasting the factors that might be responsible for the evolution of different mating system.

The capacity for variation in relation to the reproductive behaviour and mating systems exhibited by coral-reef fish is likely to lead to a relatively frequent and widespread occurrence of monogamy compared to some other groups of animals. Indeed, there have been increasing reports of monogamy occurring in species spanning a surprisingly diverse range of families amongst coral-reef fish (Whiteman & Côté, 2004a). In addition, the evolution of mating systems in coral-reef fish has been linked to an extremely diverse suite of potential factors, such as population size (e.g. Warner & Hoffman, 1980; Petersen, 1990), resource size or distribution (e.g. Lassig, 1976; Neudecker & Lobel, 1982), female spacing and behaviour (e.g. Jones, 1981; Baird, 1988; Ishihara & Kuwamura, 1996), breeding frequency (e.g. Warner, 1998), predation pressure (e.g. Robertson & Hoffman, 1977; Gladstone & Westoby, 1988), and phylogenetic (e.g. Godwin, 1995) or physical constraints (e.g. Hess, 1993). The diverse array of factors contributing to mating system evolution, in addition to the absence of bi-parental care, makes coral-reef fish an ideal group for assessing the various alternative hypotheses for monogamy.

A number of these monogamous species of coral-reef fish also form stable social groups that are organised into dominance hierarchies (e.g. *Amphiprion spp*, Fricke & Fricke, 1977, Buston, 2003a; Mitchell, 2003; *Paragobiodon spp,* Lassig, 1977, Thompson *et al.,* In Press). This provides an opportunity to investigate the factors promoting the maintenance of group-living in the face of reproductive asymmetries between group members. The occurrence of such societies in which monogamy prevails also provides an excellent opportunity for assessing the mechanisms promoting the resolution of conflicts over rank and reproduction between group members. Unlike most animal groups, hierarchies in coral-reef fish are usually size-based with larger individuals being dominant over smaller individuals (Fricke & Fricke, 1977, Forrester, 1991; Buston, 2003a; Mitchell, 2003). Resolution of conflict therefore hinges on mechanisms regulating individual growth rates within hierarchies over time since a subordinate only poses a challenge to its dominant if allowed to grow sufficiently large (Buston, 2004a). Therefore, coral-reef fishes have great potential to reveal new mechanisms underlying the process of conflict resolution in comparison to what is currently known in other animal societies.

In this thesis, I investigate the evolution and maintenance of an animal society by addressing three key questions pertaining to the evolution of reproductive and social behaviours: 1) what ecological factors promote the evolution of mating systems? 2) what ecological factors promote the evolution of group-living? and 3) why are there often conflicts of interest between individuals in social groups and how are they subsequently resolved? This investigation focuses on one species of coral-reef fish, the obligate coral-dwelling goby, *Paragobiodon xanthosomus* (Gobiidae) (Figure 1.1). This species resides permanently within colonies of one species of host coral, *Seriatophora hystrix* (Pocilloporidae), which provides as source of food, shelter and breeding sites for individuals (Figure 1.2). Previous studies have reported that *P. xanthosomus* exhibits a monogamous mating system that is fixed despite forming social groups of up to 11 individuals (Lassig, 1976; Thompson *et al.,* In Press). The breeding male and female partners produce a clutch of eggs that is laid at the base of a coral branch on a specific nest site, and eggs are cared for solely by the breeding male (Lassig, 1977). Subordinate non-breeders appear to provide no assistance to the dominant breeders (Lassig, 1977) and are unlikely to be related to dominants given that newly hatched larvae spend several weeks in a well-mixed pelagic environment before recruiting to the benthic coral habitat (Sale, 1991).

In Chapter 2, I describe the mating and social system of *P. xanthosomus*, confirming the occurrence of monogamy irrespective of social group size, and describing the size-based dominance hierarchy within groups. I then conduct experimental manipulations to test whether monogamy might have evolved because of competition among females in response to limited nest sites, food or paternal egg care. In Chapter 3, I determine why group-living has evolved in *P. xanthosomus* given the unequal distribution of reproductive benefits within groups, by applying the theory of cooperative breeding to elucidate the factors affecting the pay-offs to non-breeding subordinates from staying versus leaving groups. In Chapter 4, I demonstrate that size-based dominance hierarchies act as queues in which subordinates can inherit dominant breeding status, and use a combination of field and laboratory experiments to assess whether the threat of punishment promotes the resolution of conflict over rank between group members. In Chapter 5, I experimentally assess the relative influence of the threat of punishment versus competition over food in the resolution of conflict over rank and hence the stability of *P. xanthosomus* societies over time. This thesis therefore provides experimental demonstrations of the ecological factors promoting the evolution of monogamy, group-living and conflict resolution within an animal society, and enhances our current understanding of the ultimate reasons underlying the formation, structure and stability of animal societies. Published research on the social and mating systems of other coral-dwelling fishes conducted during my PhD tenure is presented in Appendix 1.



Photograph by Marian Wong

Photograph by Marian Wong

Figure 1.1: Photographs of study species, *Paragobiodon xanthosomus* (Gobiidae), showing side (top) and front view (bottom).

Photograph by Marian Wong

Photograph by Marian Wong

Figure 1.2: Photographs of a colony of *Seriatophora hystrix* (top) and its branches (bottom).

CHAPTER 2

THE EVOLUTION OF MONOGAMY: FOOD LIMITATION, PATERNAL CARE CONSTRAINTS AND FEMALE COMPETITION IN A CORAL-DWELLING FISH

2.1. ABSTRACT

Monogamy within social groups of three or more individuals poses a fundamental challenge to our understanding of the evolution of mating systems. The traditional explanation that monogamy evolves in response to a wide dispersion females, affording males little opportunity to defend multiple females, cannot apply. In social groups, monogamy potentially arises in response to female competition over resources such as breeding sites, food and paternal care. In this chapter, I investigated this hypothesis for the obligate coral-dwelling goby, *Paragobiodon xanthosomus* (Gobiidae) by conducting manipulative experiments to determine whether resource competition amongst females promotes monogamy in social groups. Within social groups, there was a size-based dominance hierarchy consisting of a dominant, breeding male and female, plus 1-15 smaller subordinate females that were reproductively immature. Breeding males and females behaved competitively towards individuals of their own sex – they were more aggressive towards consexual versus heterosexual intruders. Breeding females also evicted subordinate females that were large and reproductively mature from the group. Experimental removal of nest sites did not result in the cessation of reproductive activity by the breeding pair, demonstrating that nest sites were abundant and not the cause of female competition. Supplemental feeding of social groups did not result in the maturation of non-breeding subordinate females. Supplemental

feeding did, however, result in an increase in the fecundity of the breeding female, demonstrating that the reproductive success of females was food limited. This suggests that dominant, breeding females suppress the maturation of non-breeding subordinate females in order to minimise competition over limiting food resources, which in turn promotes monogamy within social groups. Finally, supplemental feeding of pairs demonstrated that the number of eggs hatched by males appeared to be a decelerating function of the number of eggs laid by females, suggesting that males experience constraints on paternal egg care. Therefore dominant, breeding females might also suppress the maturation of subordinate females to maintain a monopoly over limiting paternal care. These results provide support for the evolution and maintenance of monogamy in *P. xanthosomus* as a result of female competition over limiting resources.

2.2: INTRODUCTION

Monogamous mating systems, in which one male and female confine the majority of their reproduction with one another, are widespread across a diverse range of animal taxa (Kleiman, 1977; Wittenberger & Tilson, 1980; Rutberg, 1983; Bull, 2000; Rahman *et al.,* 2002; Whiteman & Côté, 2004a). The occurrence of exclusive relationships involving breeding pairs poses specific challenges to our understanding of mating system evolution, since one sex (usually the male) generally has a higher potential reproductive rate than the other (usually the female) and hence should be selected to mate with multiple partners (Clutton-Brock & Vincent, 1991; Clutton-Brock & Parker, 1992). The evolution and maintenance of monogamous mating systems has been the focus of considerable theoretical attention (e.g. Kleiman, 1977; Wickler & Seibt, 1981; Wittenberger & Tilson, 1980; Mock & Fujioka, 1992; Shuster & Wade, 2003; Whiteman & Côté, 2004a). Monogamy is particularly perplexing since it often occurs when multiple partners are immediately available. For example, many species form stable social groups, yet only one male and female group member breed with each other (e.g. Creel *et al.,* 1992; Komdeur, 1992; Clarke & Faulkes, 1997; Griffin *et al.,* 2003; Buston 2003a). The factors restricting reproduction to only two individuals within such animal societies are poorly understood.

One of the traditional hypotheses for the occurrence of monogamy rests on the tenet that the distribution of limiting and essential resources ultimately determines the evolution of mating systems (Emlen & Oring, 1977). Specifically, monogamous mating systems are expected to occur whenever females are widely distributed in space such that it is uneconomic for males to defend multiple females from other males (i.e. when there is a low environmental potential for polygyny (EPP)) (Emlen & Oring, 1977, Kleimann, 1977, Clutton-Brock, 1989). Conversely, polygynous mating systems are expected to occur whenever females are clumped in space allowing males to defend multiple females from other males (i.e. when there is a high EPP). However, this hypothesis alone cannot account for monogamy in species where individuals form stable social groups, since group members occur in close proximity to each other and share resources. In this situation, multiple females should be immediately available to males and polygyny as opposed to monogamy would be expected to evolve. In addition, this hypothesis emphasises the role of competition between males over resources and/or females in determining the mating system. However, it is likely that females also play an active role in determining the mating patterns in animals (Wasser & Barash, 1983; Warner, 1990; Ahnesjo *et al.,* 1993; Henson & Warner, 1997).

A more recent perspective considers the role of female competition over limiting resources for breeding in determining the mating system (Ahnesjo *et al.,* 1993; Berglund *et al.,* 1993). Within social groups, females are typically organised into some form of dominance hierarchy with more dominant females being competitively superior over their subordinates (e.g.

Holecamp *et al.,* 1996; Clutton-Brock *et al.,* 1998; Monnin & Peeters, 1999). If resources are limiting within groups and the reproductive success of females depends on access to these resources, then the most dominant female would benefit from monopolising resources and preventing subordinates from reproducing (Wasser & Barash, 1983; French, 1997; Moelman & Hofer, 1997). Since the dominant female would be the only one capable of reproducing, males would be constrained to monogamy despite the presence of additional females in the group.

Monogamy could arise in response to female competition over three potentially limiting resources. Firstly, the reproductive success of dominant females may be limited by a shortage of suitable breeding sites with which to successfully breed and rear offspring (e.g. Newton, 1994; Borg *et al.,* 2002; Kokko *et al.,* 2004). As a result, dominant females would benefit by preventing subordinates from reproducing, since this would ensure their exclusive access to the limited breeding sites.

Secondly, the reproductive success of dominant females may be limited by the availability of food resources (Wasser & Barash, 1983; Ali & Wootton, 1999; Berglund *et al.,* 1993; Clutton-Brock *et al.,* 1998). If so, then the reproductive success of dominant females is likely to be a decreasing function of the number of other breeding females within the same group. As a result, dominant females would stand to benefit by preventing subordinates from reproducing since this would minimise competition over food and thereby ensure they produced the maximum number of offspring possible under the given food conditions (Wasser & Barash, 1983; Clutton-Brock & Albon, 1985; Woodroffe & MacDonald, 1995). Alternatively, since reproductive status is often determined by food availability (Bernardo, 1993), a limitation of food could also prevent subordinates from breeding, without invoking any direct suppression of reproduction by dominant females. This could occur if dominant females out-compete their subordinates for limited food resources, enabling only themselves to mature and thus breed within their group. In response to food limitation, monogamy may therefore evolve because dominant females suppress subordinate reproduction to reduce competition over food, and/or because dominant females simply out-compete subordinates for food they need to sustain reproduction.

A third potential source of female competition applies to species exhibiting paternal offspring care. If there are constraints on the number of offspring a male can care for, females would be expected to compete for paternal care (Kuwamura *et al.,* 1993). Paternal care may be constrained if body size restrictions prevent males from caring for more than a certain number of offspring at a given time (Hess, 1993; Kuwamura *et al.,* 1993; Whiteman & Côté, 2004a), or if the quality of male care decreases with an increasing number of offspring (Whiteman & Côté, 2004a). If a single female can produce all the offspring that a single male can care for at a given time, then dominant females would maximise the number of offspring they produce by preventing subordinates from reproducing. Monogamy is therefore expected to evolve because constraints on paternal care have the potential to limit female reproductive success, which in turn selects for females to suppress the reproduction of others. Although paternal care constraints has been invoked as one of the key factors promoting monogamy (Whiteman & Côté, 2004a), there has yet to be an experimental verification of its role in monogamous animals.

Amongst the various taxonomic groups, monogamy is particularly widespread in coral-reef fishes occurring in a surprisingly diverse range of species (Barlow, 1986; Whiteman & Côté, 2004a). The occurrence of monogamy within the context of social groups is particularly striking amongst species that reside solely within small patches of coral or reef habitat (*Paragobiodon spp*, Lassig, 1976, 1977; Kuwamura *et al.,* 1993; *Gobiodon spp*, Cole & Hoese, 2001; Thompson *et al.,* In Press; *Amphiprion spp*, e.g. Fricke & Fricke, 1977; Buston, 2003a). Such habitat-specialist reef fish are characterised by small body size, extreme site-attachment to discrete habitat patches that provide access to food and breeding sites, and in most cases, a demersal spawning mode where eggs are laid onto nests within the habitat patch and are cared for by the breeding male (Munday & Jones, 1998). In these species, there is usually a positive correlation between the size of the habitat patch and the size of the group residing within the habitat patch, yet monogamy occurs irrespective of group size (*Paragobiodon spp*, Lassig, 1976, 1977; Kuwamura *et al.,* 1994; *Gobiodon spp*, Thompson *et al.,* In Press; *Amphiprion spp*, e.g. Fricke & Fricke, 1977; Mitchell & Dill, 2005). Given that multiple females would be present in a group, monogamy in these species is not a consequence of a low EPP and thus the inability of males to defend multiple females from other males. Alternatively, monogamy could have arisen and be maintained in these species in response to female competition over limiting resources for breeding. However, experimental tests of this alternative hypothesis and the critical resources involved are currently lacking.

In this study, I investigated the social and mating system of the obligate coral-dwelling goby, *Paragobiodon xanthosomus* (Gobiidae), and conducted experimental manipulations of the potential resources limiting the reproduction of females to determine whether female competition promotes monogamy in this species. *P. xanthosomus* is particularly well-suited to this investigation. It is a small (<40mm standard length (SL)), site-attached goby that resides specifically in one species of host coral, *Seriatophora hystrix* (Pocilloporidae). *P. xanthosomus* has previously been reported to be monogamous even though it forms large social groups within single coral colonies (Lassig 1976; Thompson *et al.,* In Press). Coral colonies provide a potentially limiting source of food and breeding sites. In addition, *P. xanthosomus* exhibits paternal egg care (Lassig, 1977) which could also serve as a limiting resource for females.

Prior to the experimental manipulations, I investigated the social system of *P. xanthosomus* and the potential for female competition by quantifying the body size-structure of social groups and conducting observations of natural agonistic encounters within groups. I confirmed the occurrence of monogamy within groups by conducting behavioural observations of reproduction and histological examinations of the gonads of individuals from a range of different group sizes. In addition, I assessed whether coral size determines the EPP by analysing correlations between coral colony size and group size. I then experimentally assessed whether males and females compete with other individuals of the same sex by analysing agonistic responses to intruders of different sex, size and reproductive status.

A series of field experiments were then conducted to test whether: 1) nest site limitation, 2) food limitation, and/or 3) a limitation of paternal egg care were responsible for female competition and hence monogamy within groups of *P. xanthosomus*. In the first experiment, nest sites were experimentally removed. I hypothesised that if nest sites are limiting, then removal of an established nest site should result in the cessation of any further reproduction within the group. In the second experiment, breeding pairs were given supplemental food. I hypothesised that if female fecundity is food limited, then feeding should result in an increase in the number of eggs laid by breeding females. In the third experiment, all members of social groups were given supplemental food. If the breeding female competitively excludes subordinates from limiting food supplies enabling only herself to mature and reproduce, then supplemental feeding of all group members should result in the maturation and breeding of subordinates. In the final experiment, clutch size was experimentally manipulated to assess the ability of males to care for larger clutches. I hypothesised that if paternal care is limiting, the clutch size hatched by males caring for an experimentally enlarged clutch laid by their female partner should be similar in size to the clutch size hatched by males caring for a normal clutch laid by their female partner.

2.3: METHODS

Study site and species

The study was conducted at Lizard Island (14° 40’S, 145° 28’E) on the northern Great Barrier Reef, Australia between March 2004 – November 2005 (Figure 2.1). Field observations, experiments and coral collections were conducted on two reefs, Palfrey and Loomis (Figure 2.1). Aquarium observations and experiments were carried out at the Lizard Island Research Station (Figure 2.1). *P. xanthosomus* is an obligate coral-dwelling reef fish that inhabits colonies of just one species of coral, *Seriatopora hystrix* (Pocilloporidae) outside of which it faces high risks of mortality (Lassig, 1981). *P. xanthosomus* spawns demersal eggs within the coral onto a nest site created by the breeding male (Lassig, 1977). The breeding male also provides the vast majority of parental care (Lassig, 1977). *P. xanthosomus* is also a protogynous hermaphrodite – if the male of a group dies, the female changes sex to become the breeding male and the largest non-breeder becomes the breeding female (Lassig, 1977).

Social system of *P. xanthosomus*

To determine the social system of *P. xanthosomus*, I quantified the body size distribution of individuals within social groups. A total of 54 colonies of *S. hystrix* were haphazardly collected from the reef and each one immediately transferred to a waiting boat. Most colonies of *S. hystrix* are attached to loose rubble allowing corals to be easily collected. Gobies were never seen to depart from corals during the collection process. Resident gobies were removed from each coral by inverting the coral over a bucket of seawater and allowing the gobies to fall into the bucket. This process rarely took longer than 2 minutes after which the ‘emptied’ coral was returned back to the reef. This process was repeated for all groups collected. All gobies were returned to the laboratory and were anaesthetised by placing them into a small beaker containing a clove oil solution (Munday & Wilson, 1997). Body size of each fish was measured (standard length (SL) ± 0.1mm) using calipers, and sex determined by the shape of the genital papilla (Lassig, 1977). Males have a long, conical papilla and females have a short, blunt papilla (Lassig, 1977). Each group member was designated a size rank based on its size relative to other group members, with rank N being larger than rank N+1 etc. All gobies were released back onto their original corals in the field unless they were retained for histological analysis or further experimental tests (see below). The body sizes of breeding males and females within each group were compared using Paired T-tests after confirming the assumptions of normality (Kolmogorov-Smirnov tests) and homogeneity of variances (plots of predicted versus residual values and Levene's tests). The relationship between the body sizes of breeding males and females across groups were compared using a Reduced Major Axis Regression (RMA). RMA is more appropriate than the standard Ordinary Least Squares (OLS) regression when the X variable is not fixed i.e. both X and Y variables are random and measured with error (Quinn & Keough, 2002). This analysis was conducted since if paternal care is limited, breeding pairs are predicted to be size-matched given that the body size of both males and females is important for the reproductive success of the pair (Kuwamura *et al.,* 1993; Whiteman & Côté, 2004a).

To determine whether size rank reflects dominance rank, patterns of agonistic interactions were observed between group members in natural groups. If size reflects dominance, agonistic interactions should be strongly size-based – dominance displays should only be directed from larger to smaller group members and subordinate displays from smaller to larger group members. Seven coral colonies each containing five gobies were placed into separate aquaria. Gobies from each group were anaesthetised, measured, sexed under a dissecting microscope and uniquely tagged by injecting fluorescent elastomer (Northwest Technologies Inc.)

into the dorsal musculature. These tags have high retention rates with no adverse effects on growth or survival (Malone *et al.,* 1999). For the following 3 days, each group was observed for two 15 minute periods between 0830 and 1700, with a minimum of 4 hours in between observation periods for each group. A total of 6 observation periods were conducted per group. During each observation period, dominance and subordinance behaviours were recorded and the identity of the group member exhibiting the behaviour was scored whenever a pair-wise interaction was observed. A dominance display consisted of a direct approach by one individual to another whilst maintaining a head-on or side-on profile and erected fins, and a subordinate display consisted of a head-on profile and flattened fins, and/or a flee response (Lassig, 1976).

To determine the sex ratio and reproductive status of individuals in social groups, a random selection of 24 social groups was assigned for histological analysis of gonads. All individuals in these groups were euthanased with a lethal dose of clove oil anaesthetic. Gonads were dissected from the body and fixed in vials containing FAACC (4% formaldehyde, 5% acetic acid, 1.3% calcium chloride) for 7 days and then transferred and stored in 70% ethanol. Whole gonads were embedded in paraffin wax, transverse sections made at 5μm using a rotary microtome, mounted onto glass slides and stained with Mayer's alum haemotoxylin and Young's eosin-erythrosin. Thin sections were viewed by light microscopy. In females, germ cells were categorised into the following 5 stages based on descriptions by West (1990): 1) Chromatin nucleolar, 2) Perinucleolar, 3) Cortical alveolar, 4) Vitellogenic and 5) Ripe. Females with pre-vitellogenic oocytes i.e. stages 1 and 2 were classed as immature and those with developing and vitellogenic oocytes i.e. stages 3, 4 and 5 classed as mature (West, 1990). In males, germ cells were categorized into two stages as described by Cole & Hoese (2001): 1) Spermatocytes and 2) Spermatozoa. Only males with spermatozoa were considered mature.

Mating system of *P. xanthosomus*

To verify the mating system as suggested from the histological evidence, field observations of the spawning behaviour of individuals in natural groups were conducted. A total of 10 groups each containing 5-6 gobies were randomly selected in the field. Gobies were removed from each group, measured, tagged using fluorescent elastomer, and released back into their coral colony. The following day, each group was observed for one 15 minute period between 0900 - 1700 and the occurrence of any courtship and reproductive behaviour performed by group members recorded. Courtship behaviour was recorded whenever reciprocal shivering between a pair of individuals combined with energetic activity around the vicinity of a nest site was observed (Lassig, 1977). Reproductive behaviour was recorded whenever egg clutches were present in a nest site (Lassig, 1977). The position of the nest site and the presence or absence of eggs was readily inferred from the behaviour and location of the mature male, because nest-tending males are highly immobile and rarely venture away from the nest site (Lassig, 1977). Observations were repeated every other day for a period of 2 weeks for each group.

Determinants of the EPP

If coral colony size influences the dispersion of females and hence the EPP, I predicted that there would be a positive correlation between coral colony size and group size. To test this prediction, the length (L), width (W) and height (H) of 100 coral colonies were measured, and the average diameter (AD = (L+W+H)/3) was used as an indicator of coral size (Kuwamura *et al.,* 1994). The number of gobies within each coral was counted and the relationship between coral size and social group size examined using Pearson’s correlation. The assumption of bi-variate normality was checked using Kolmorov-Smirnov Tests for normality.

Intrasexual aggression

Two laboratory experiments were carried out to determine: 1) whether males and female compete with other individuals of the same sex, and 2) the mechanism by which female competition occurs. In the first experiment, breeding males and females were presented with other individuals of the same and opposite sex. I hypothesised that if males and females compete with members of the same sex, the frequency of intrasexual aggression by males and females would be significantly greater than the frequency of heterosexual aggression. To test this prediction, 5 coral colonies each containing a pair of gobies were collected from the reef and placed into a separate aquaria. Gobies were measured, sexed, tagged, and placed back onto their original corals as previously described. Different coloured tags were used for males and females so they could be visually distinguished. Pairs were left to acclimatise overnight. The following day, 5 additional gobies were collected and returned to the lab where they were sexed and tagged. At the start of an aggression trial, one goby ('intruder') was randomly selected and placed into a transparent plastic tube (5 x 3cm) covered at both ends with cloth gauze. The gauze allowed for the circulation of any chemical cues that may be used by individuals to determine sex. This 'aggression trap' (Fricke, 1986) was then placed directly on top of one of the experimental coral colonies such that it was at an equal distance between the resident male and female. Trials commenced as soon as one resident partner approached the intruder and each trial lasted for 10 minutes. During a trial, the frequency of aggressive displays by both partners towards the intruder was recorded. Aggressive displays consist of a direct approach by one individual to another whilst maintaining a head-on or side-on profile and erected fins (Lassig, 1976). On completion of a trial, the aggression trap was removed from the coral and the intruder released back into a separate aquaria. Intruders were never used more than once. In total, 5 trials were conducted per day (1 trial per pair). This process was repeated until each resident pair had been tested against both a male and female intruder. The mean percentage of intrasexual versus heterosexual displays made by males and females were compared using non-parametric Mann-Whitney U tests due to non-normality and heterogeneity of variances.

The second experiment assessed the mechanism by which females compete within groups. Specifically, I predicted that breeding females may employ an eviction strategy whereby they evict other females from the group that represent a threat as resource competitors and tolerate those that pose no threat. To test this prediction, 13 coral colonies each containing 5 group members were collected from the reef and each placed into a separate aquarium. The rank 3 female (largest immature female) was experimentally removed from these groups and was replaced with a female intruder that was: 1) immature and the same size as the previous rank 3, 2) immature and larger than the previous rank 3, 3) mature and the same size as the previous rank 3, or 4) mature and larger than the previous rank 3. The order in which the 4 different classes of intruder were presented was random with respect to each group. The responses of the breeding female towards the new rank 3 female were observed for 15 minutes immediately after the new fish was introduced, during which time the intruder female was either evicted or tolerated in the group. Evictions were scored when the intruder female was chased out of the coral and entered the dead coral base or the side or bottom of the aquaria. Groups were left overnight and the outcome re-scored the following morning. The experiment was repeated to produce the following levels of replication per treatment: 1) n = 13, 2) n = 12, 3) n = 12 and 4) n = 12.

Log-linear analysis of frequency tables was used to assess the relative effects of maturity and size on the occurrence of eviction. In this analysis, a series of models was constructed to test whether the occurrence of eviction was: 1) dependent on an interaction between maturity and size, 2) dependent on maturity alone, 3) dependent on size alone and 4) dependent on maturity and size but not on an interaction between these factors (Table 2.1). The method of model constructions follows Munday *et al.,* (2001) where the factors manipulated in the experiment are included in every model and it is the interaction between these factors and the response variable that is sequentially tested. Models were tested by fitting them in decreasing order of complexity until there was no further significant reduction in the goodness-of-fit statistic (chi-squared value) from one model to the next. By doing so, the simplest model to explain the observed data was found.

Nest site limitation

To determine if nest sites are limiting, I experimentally removed established nest sites from corals and monitored the behavioural responses of group members. If nest sites are limiting, I hypothesised that nest site removal should result in the complete cessation of reproductive activity within the group. Alternatively, if nest sites are not limiting, pairs should commence breeding on a new nest site elsewhere in the coral colony. Seven coral colonies, each containing a breeding pair of *P. xanthosomus*, were randomly selected. Each coral colony was observed for 5 minutes in order to locate the position of the established nest site and to confirm that only one active nest site was present. Nest sites are characterised by a small patch of algae at the base of a coral branch on which eggs are laid (Lassig, 1977) (Figure 2.2). The coral branch on which the nest was located was gently removed by positioning a screwdriver at the base of the branch and gently tapping the screwdriver with a small hammer. This resulted in a clean break of the particular branch and minimal damage to the rest of the coral. The broken branch with attached egg clutch was removed from the coral and returned to the lab where the egg clutch was photographed with a digital camera. The number of eggs in each clutch was counted using a digital image of the clutch projected on a computer screen. After a period of 2 weeks, each group from which the nest site had been removed was surveyed, and the occurrence of reproductive behaviour and the presence of new nest sites recorded. New nest sites were removed and the number of eggs counted as before. The clutch size on old nests was compared to that on new nests on the seven corals using a Paired T-test. Clutch size data was checked for normality using Kolmogorov-Smirnov Tests and for homogeneity of variances using plots of predicted clutch size values versus residual values and a Levene's Test.

Food limitation

Supplemental feeding experiments were used to test whether: 1) the fecundity of breeding females in groups was food limited, and 2) food availability was limiting the number of females that were mature and reproductively active within each group. If food supply limited reproductive behaviour, I predicted that supplemental feeding would increase the fecundity of the breeding female and also result in the maturation and breeding of the other female group members. To test whether the fecundity of breeding females was food-limited, I collected a total of 16 coral colonies each containing a breeding pair of gobies. Corals were placed 2m from the edge of the reef at 3m intervals from each other, and their relative positions mapped. Each coral colony was uniquely tagged by affixing a numbered cable tie around its base. Pairs were removed from each coral as described above, anaesthetised, measured (SL ± 0.1mm), and sexed by the shape of the genital papilla and tagged using fluorescent elastomer as described above. Gobies were returned to their original corals and left undisturbed for 2 days.

Eight of the 16 pairs were randomly assigned to the control treatment ('unfed' pairs), and the remaining 8 pairs to the supplemental feeding treatment ('fed' pairs) (Figure 2.3 - treatments A and B respectively). Pairs in the supplemental feeding treatment were fed high nutrient commercial fish pellets twice daily (INVE NRD pellets, size 5/8). Food pellets were discharged into each coral by expelling them from a 50ml syringe, ensuring an excess of pellets. Pellets were trapped in the coral by the coral polyps which ensured that they were retained in the coral. Observations of each pair were made following feeding to confirm that they were consuming the pellets. Pairs were fed in this way for 3 weeks.

To compare female fecundity in the unfed and fed pairs, the first new egg clutch laid by each pair was collected within 24 hours of the eggs being laid at the end of the feeding period. Eggs are white (day 1), turning grey (day 2-3), black (day 3-4) and finally black and silver (day 4-5) whereupon hatching occurs (Wong, *pers. obs.*). All gobies were also collected, their body size re-measured and then released back onto their corals. Egg clutches were placed in vials filled with 70% ethanol and photographed using a digital camera from which clutch size was determined as previously described. The size of clutches laid by females in the unfed versus fed treatments were statistically compared using a t-test. Prior to this, a t-test was used to compare the body sizes of females between these treatments since female size is commonly linked to female fecundity (Bagenal, 1967). In addition, linear regression analyses were conducted for both treatments to ensure there were no significant relationships between female body size and clutch size laid. Data was checked for normality and homogeneity of variances as previously described.

To test whether supplemental feeding would result in the maturation and breeding of other female group members, 10 coral colonies each containing 5 group members were colleted from the reef and placed into separate aquaria in the laboratory. Each aquarium was supplied a continuous flow of fresh seawater. Gobies from each group were removed, measured and tagged as described above, and placed back into their original coral colony. Five groups were randomly assigned to the supplemental feeding treatment. These groups were fed twice daily with pellets for 3 weeks. During each feeding bout, food pellets were dispensed over the entire coral such that there was an excess of food available. The remaining 5 groups did not receive supplemental feeding. These control groups only had access to natural planktonic food arriving in the flow of seawater to their aquariums. On completion of the experiment, all individuals from each group were re-measured, euthanased and their gonads examined histologically as previously described.

Paternal care constraints

To determine whether paternal care was limiting, I added two supplemental experimental treatments to the 'Food limitation' experiment (see previous section). Fourteen more coral colonies each containing a breeding pair were collected. Eight of these pairs were assigned to an unfed treatment and the other 8 to a supplemental fed treatment (Figure 2.3 – treatments C and D respectively). In both these new treatments, egg clutches were collected just prior to hatching (day 4-5). Eggs turn black/silver within 1 day of hatching, which provided a reliable way to assess the correct time for collection. This resulted in a total of 4 treatments for the experiment (Figure 2.3). If male care is limiting and costly, the clutch size hatched by males should be similar in the unfed and fed treatments, even though the clutch size laid by females would be greater in the fed compared to unfed treatments (i.e. female fecundity is food limited). Conversely, if male care is not limiting and costly, the clutch size hatched by males in the fed treatments should be significantly greater than that in the unfed treatments, given that the clutch size laid by fed females is greater than for unfed females.

The size of clutches hatched by males in unfed versus fed treatments (treatments C and D) was compared using a t-test. Prior to this, a t-test was used to compare the body sizes of males between these treatments since male size is commonly linked to paternal care ability (e.g. Kuwamura *et al.,* 1993; Sunobe & Nakazono, 1999). In addition, linear regression analyses were conducted for both treatments to ensure there were no significant relationships between male body size and clutch size hatched. All data was checked for normality and homogeneity of variances as described previously.

2.4: RESULTS

Social system of *P. xanthosomus*

A total of 24 natural groups were collected, ranging in size from 5 - 17 individuals. Histological examination of the gonads of group members (N = 199) from the collected groups revealed that there was only ever one mature male and female per group, independent of group size. All other group members were immature females (the gonads of group members ranked 14 and upward were not analysed because they were considered too small for histological analysis). The gonads of mature males (n = 24) were characterised by the presence of spermatozoa (Figure 2.4a), and those of mature females (n = 24) characterised by a high density of vitellogenic oocytes (Figure 2.4b). The gonads of all immature females (n = 151) were characterised by a predominance of pre-vitellogenic oocytes (Figure 2.4c). Of the 151 immature females analysed, the gonads of 20 contained a very small number (1-3) of more developed oocytes (Figure 2.4d). These were mainly found in rank 3 females (n = 8), and then in rank 4 (n = 4), rank 5 (n = 4), rank 6 (n = 2), rank 7 (n = 1) and rank 8 (n = 1) females. The very small number of maturing oocytes and the absence of vitellogenic or ripe oocytes in these gonads indicates that the fish were not functional, immature females.

Within each group, the mature male and female (rank M and F) were the largest two individuals (Figure 2.5). The remaining immature female group members (rank 3 upwards) were smaller than the breeding pair (Figure 2.5). Furthermore, immature females exhibited a step-wise reduction in their body sizes throughout the group, indicating the presence of a size-hierarchy

(Figure 2.5). The body sizes of the mature males and females ranged from 24.0 to 32.0mm SL, and that of immature (ranks 3 – 13) females ranged from 12.0 to 28.0mm SL, demonstrating that some immature female group members were of reproductive size. There were no significant differences between the body sizes of the mature males and females within groups (Paired t-test; $t = 1.2$, $n = 50$, $p = 0.237$). There was a significant positive correlation between the body sizes of the mature males and mature females in each pair (Figure 2.6) (Reduced Major Axis Regression; $R^2 = 0.5$, $n = 50$, $p < 0.001$). Patterns of agonistic interactions observed in groups confirmed that the size-based hierarchy reflects a dominance hierarchy. A total of 278 pair-wise agonistic interactions were observed across the 7 groups. During each observed interaction, the initiator of a dominance display was always larger than the receiver, and the initiator of a subordinate display and/or flee response was always smaller than the asserter. Group members are therefore organised into a linear size-based hierarchy with size rank reflecting dominance rank.

Mating system of *P. xanthosomus*

Observations of social behaviour supported the histological data confirming social monogamy in *P. xanthosomus*. Courtship behaviour was confined to the largest 2 individuals within each observed group (n = 10) i.e. the mature male and female. Eight of these groups exhibited reproductive behaviour as evidenced by the presence of eggs at a nest site (Figure 2.2), and in each case, only the breeding male and female were observed in the vicinity of the nest site. Together with the histological evidence, these results provide confirmation of social monogamy in *P. xanthosomus* groups.

Determinants of the EPP

Coral sizes and group sizes from a total of 100 groups were measured to determine if coral size influenced the EPP. There was a significant positive correlation between coral colony size and group size (Figure 2.7) (Pearson's correlation; $R^2 = 0.517$, n = 100, $p < 0.001$). An increase in coral size is therefore associated with an increase in the number of females within the group, and thus an increase in the EPP.

Intrasexual aggression

In the first experiment, breeding males and females exhibited a significantly higher mean percentage of aggressive displays towards intruders of the same sex compared to intruders of the opposite sex (Figure 2.8) (males: mean ± S.E. = 97.1% ± 1.8 intrasexual versus 2.9% ± 1.8 heterosexual displays; Mann-Whitney U test, Z = 2.6, $p = 0.007$; females; mean ± S.E. = 98% ± 2 intrasexual versus 2% ± 2 heterosexual displays; Mann-Whitney U test, Z = 2.6, $p = 0.009$). Breeding males and females displayed similar mean percentages of intrasexual (Figure 2.8) (Mann-Whitney U test; Z = 0.31, $p = 0.75$) and heterosexual displays (Figure 2.8) (Z = 0.31, $p = 0.75$).

In the second experiment, all evictions of intruder females were carried out by the breeding female (n = 24). Breeding males were never observed to evict intruder females. Both maturity and size significantly affected the frequency with which a rank 3 intruder female was evicted (Figure 2.9) (Table 2.1a - Model 2 provided the best fit to the observed data) (Table 2.1b) (Step-wise testing of log linear model, $X^2 = 0.03$, df = 1, $p > 0.95$). The best fitting model includes an interaction between maturity and eviction, and interaction between size and eviction, but not an interaction between maturity, size and eviction. However, the removal of maturity from the model resulted in a much poorer fit of the model (Table 2.1b) (comparison of model 2 v

3, $X^2 = 13.6$, df = 1, $p < 0.01$) than did the removal of size (Table 2.1b) (comparison of model 2 v 3, $X^2 = 6.9$, df = 1, $p < 0.01$). This indicates that maturity had a much greater effect on the frequency of eviction than did size (Figure 2.9). Taken together, these results suggest that immature females that grow too large, but particularly if they mature, are more likely to be evicted from the group by the breeding female than those that remain small and immature.

Nest site limitation

Established nest sites were removed from 7 coral colonies to determine whether monogamy may arise as a result of female competition over limiting nest sites. In all cases, a new nest site was established by the breeding male and female within a period of 2 weeks, and eggs were observed on each new nest site. This result therefore demonstrates that nest sites were not limiting.

Food limitation

To determine whether supplemental feeding increased the fecundity of breeding females, I compared clutch sizes laid (i.e. female fecundity) between breeding pairs that received supplemental food to those that received no supplemental food. Clutches laid by females in the fed treatment (mean ± S.E. = 301.9 ± 29.4 eggs) were 48% larger than those in the unfed treatment (204 ± 22.2 eggs) (Figure 2.10) (T-test; $t_{14} = -2.65$, $p = 0.019$), demonstrating that the fecundity of breeding females is food limited. Since female fecundity often correlates with female size in fishes, I also assessed whether female size (mm SL) might have influenced the clutch sizes laid by females in the unfed and fed treatments. There was no significant difference in the body sizes of females in the unfed versus fed treatments at the time when clutches were collected (T-test; $t_{14} = -2.08$, $p = 0.06$). In addition, there was no significant relationship between female size and clutch size in both unfed (Linear Regression: $R^2 = 0.007$; $p = 0.95$) and fed treatments ($R^2 = 0.175$; $p = 0.3$). Therefore, female body size was unlikely to have influenced the comparison of clutch sizes between treatments.

To determine whether the maturation or breeding of subordinate female group members was being constrained due to food limitation, I analysed the gonads of immature female group members that were fed (n = 15) and unfed (n = 15). The vast majority of oocytes in the gonads of both fed and unfed females were pre-vitellogenic oocytes. Only 2 of 15 fed and 2 of 15 unfed females contained a few (1-3) maturing oocytes in their gonads. In addition, there were no observations of breeding or courtship behaviour by these females during the experimental period. This suggests that the maturation of subordinate females is not directly affected by food availability.

Paternal care constraints

Given that the clutch size laid by fed females was found to be greater than that of unfed females, I compared the size of clutches hatched by males that were unfed to the clutch sizes hatched by males that were fed to determine whether limiting male care might favour monogamy. Since male size often correlates with male care ability in fishes, I firstly assessed whether male body size (mm SL) could be affecting the sizes of clutches hatched in the unfed versus fed treatments. There was no significant difference in the body size of males between treatments (T-test: $t_{12} = -0.83$, $p = 0.42$), and no significant relationship between male size and clutch size hatched in the unfed (Linear Regression: $R^2 = 0.76$, $p = 0.51$) and fed treatments ($R^2 = 0.431$, $p = 0.16$). Therefore, male size was unlikely to influence comparisons of clutch sizes hatched between treatments. On average, the clutches hatched by males in the fed treatment (mean ± S.E. = 157.2 ± 67 eggs) were 24% larger than males in the unfed treatment (126.1 ± 25.1 eggs) – this is only half the magnitude of the increase in clutch sizes laid by females in the fed compared to unfed treatments. The difference between clutch sizes hatched by males in unfed versus fed treatments was not significant (Figure 2.10) (T-test: $t_{12} = -0.48$, $p = 0.64$). However, power to detect a 50% increase in clutch sizes hatched (i.e. the approximate magnitude of the increase in clutch sizes laid by females in fed compared to unfed treatments) was low (post-hoc power calculation with $\alpha = 0.05$, $n_{(unfed)} = 8$, $n_{(fed)} = 6$; $P = 0.24$). This result therefore provides tentative support for a diminishing ability of males to care for eggs over and above that laid by a similarly-sized female partner.

Table 2.1: a) Log-linear analysis of relationships between intruder maturity and size on the probability of intruder eviction. M = Maturity (immature or mature); S = size (smaller or larger); E = eviction (evicted or not evicted), and **b)** results of step-wise model testing. The best fitting model is underlined.

a)

Model	**Chi^2 Likelihood ratio**	**df**	***p***
1) M+S+E + MxS + MxE + SxE + MxSxE	0.00	0	1.00
<u>2) M+S+E + MxS + MxE + SxE</u>	0.003	1	0.954
3) M+S+E + MxS + MxE	6.93	2	0.03
4) M+S+E + MxS + SxE	13.66	2	0.001
5) M+S+E + MxS	19.19	3	0.0002

b)

Compare models	**Differential Chi^2 Likelihood ratio**	**df**	***p***
1 v 2	0.003	1	>0.95
2 v 3	6.927	1	<0.01
2 v 4	13.657	1	<0.01
3 or 4 v 5	19.187	2	<0.01

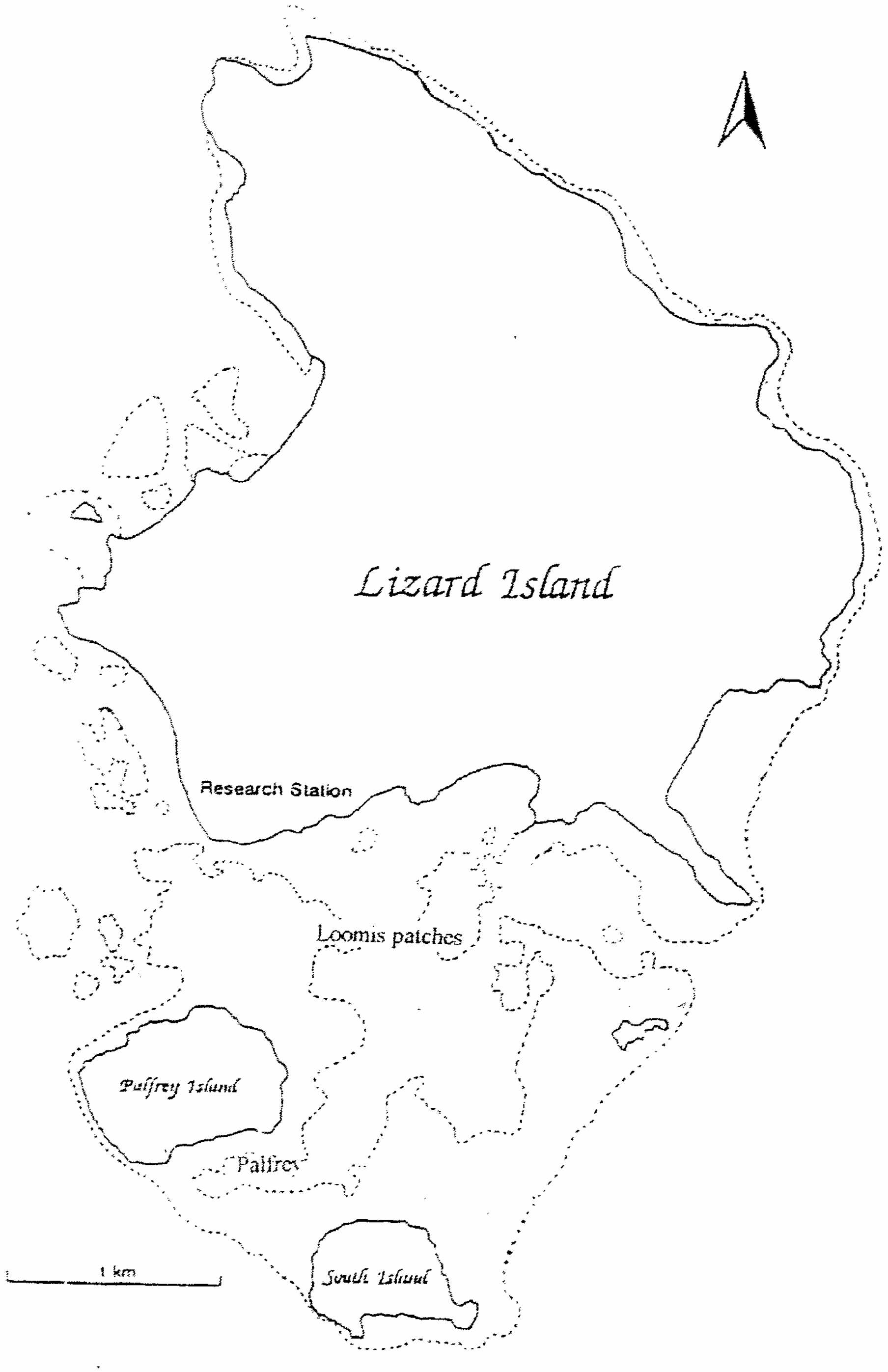
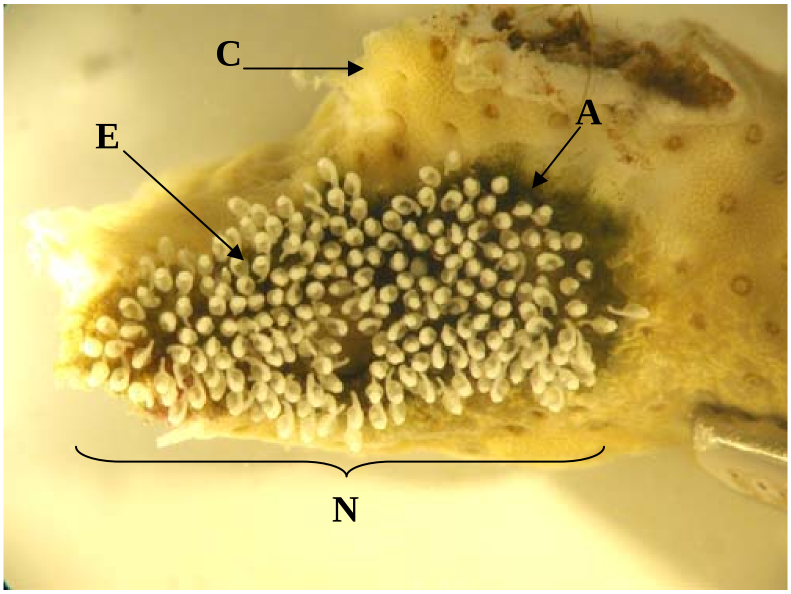


Figure 2.1: Map of Lizard Island showing research station and reefs where field observations, experiments and collections were conducted. Dotted lines demarcate reef zones; Solid lines demarcate land zones.



Photograph by Marian Wong

Figure 2.2: Nest site (N) of *P. xanthosomus* found at the base of a coral branch (C), consisting of eggs (E) laid onto a patch of algae (A) that covers the nest site.

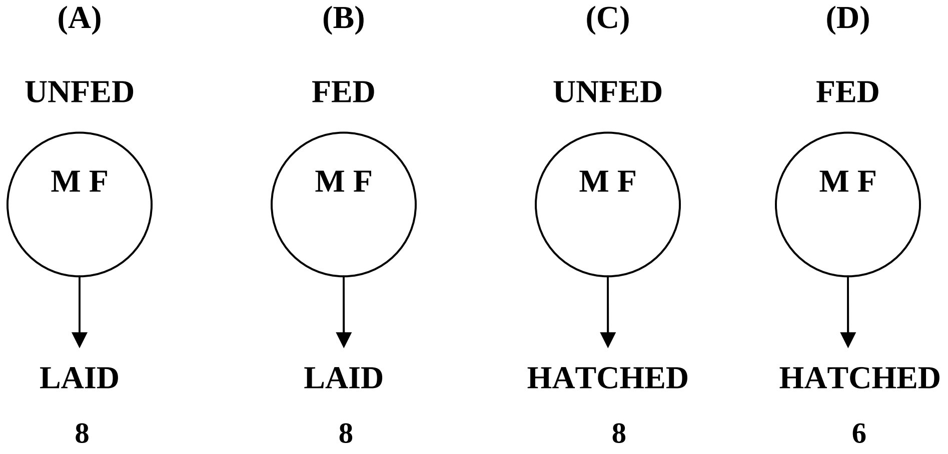
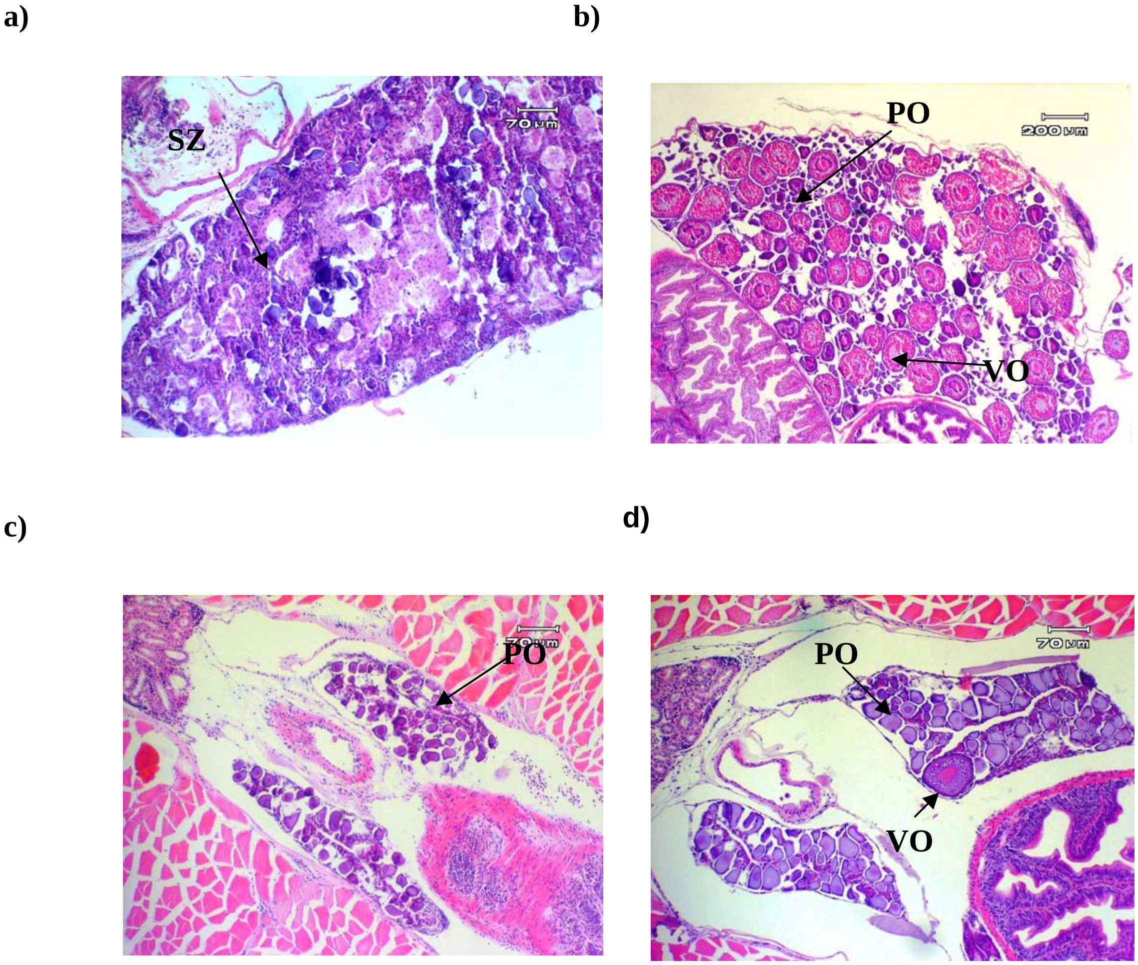


Figure 2.3: Experimental set-up for the paternal care constraints experiment. Treatments A and B were simultaneously used to test whether the fecundity of the breeding female was food limited ('Food limitation' section). Treatments C and D were used to test whether males were capable of caring for an enlarged clutch. Round circles represent coral colonies. M = breeding male; F = breeding female. Numbers under each treatment represent the sample size of pairs per treatment.



Photographs by Marian Wong

Figure 2.4a-d: Sex cell allocation of *P. xanthosomus*. a) Cross section of a testis lobe from a mature male showing spermatozoa (SZ); b) Detail of an ovarian lobe from a mature female showing pre-vitellogenic (PO) and vitellogenic oocytes (VO); c) Ovarian structure from an immature female, showing only pre-vitellogenic oocytes (PO); d) Ovarian structure from a 'maturing' female containing primarily pre-vitellogenic oocytes (PO) and one vitellogenic oocyte (VO).

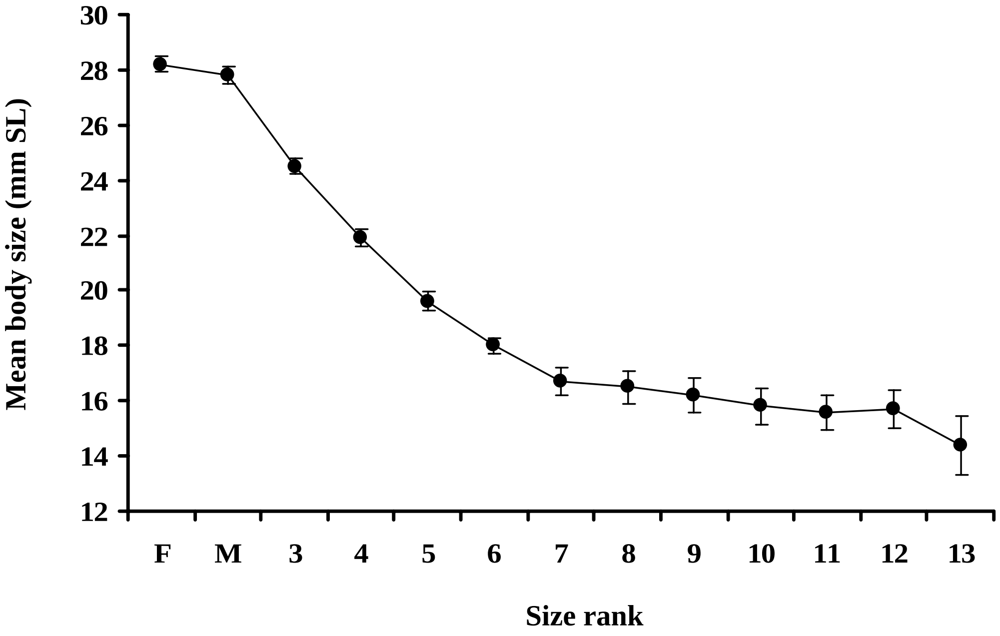


Figure 2.5: Mean body sizes ± S.E. of group members in relation to their size rank within the group. F = mature female, M = mature male, ranks 3 -13 = immature females. Ranks greater than 13 omitted due to small sample sizes.

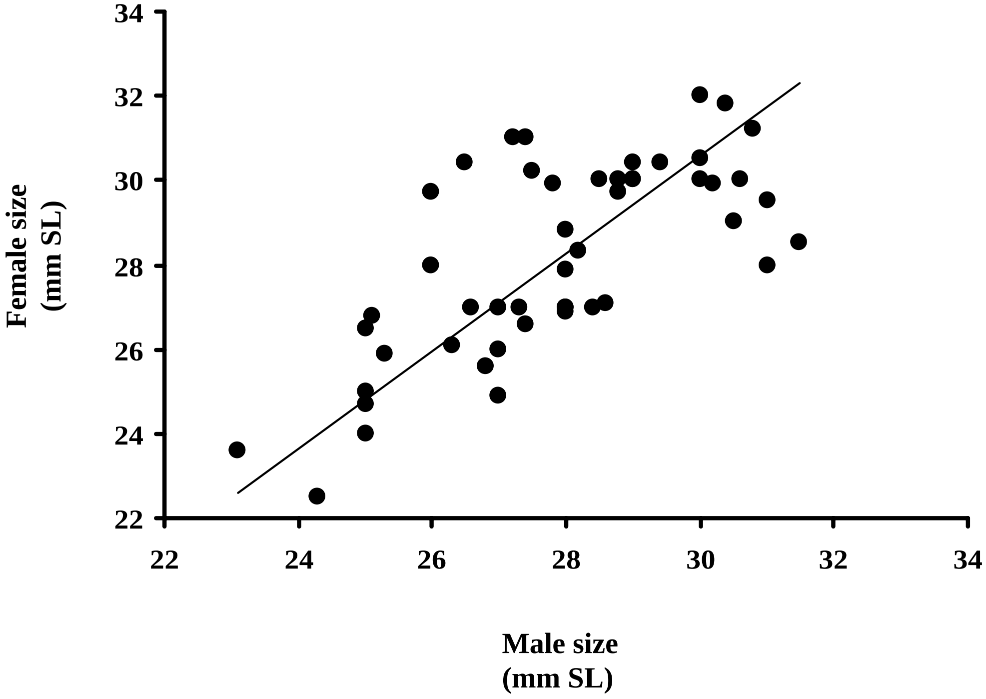


Figure 2.6: Relationship between the body sizes of breeding males and females. Diagonal line shows reduced major axis regression (y = -4.044 + 1.155x).

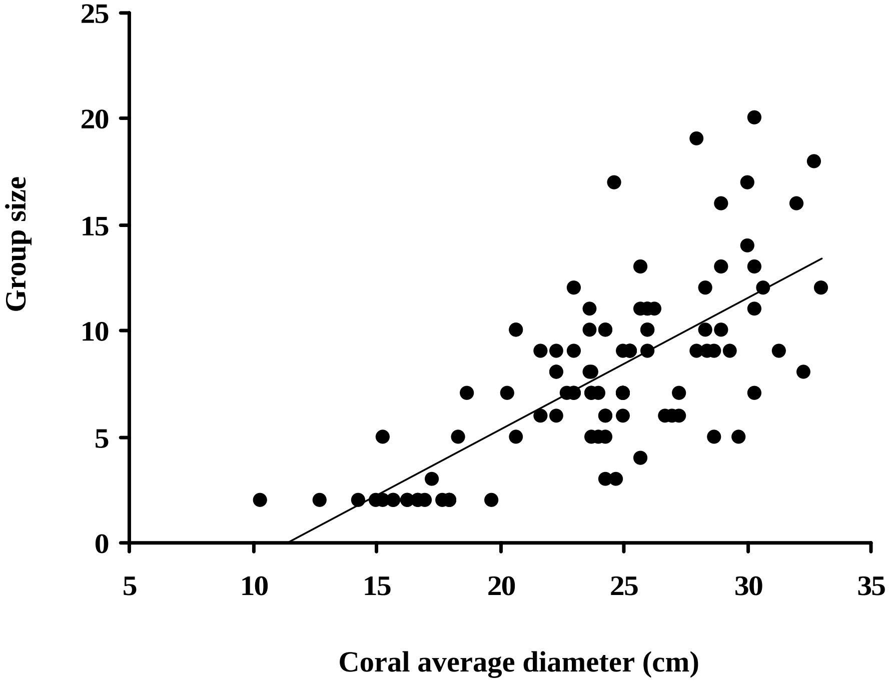


Figure 2.7: Relationship between coral size and social group size. Diagonal line shows linear regression of group size on coral size (y = - 7.0088 + 0.6179x).

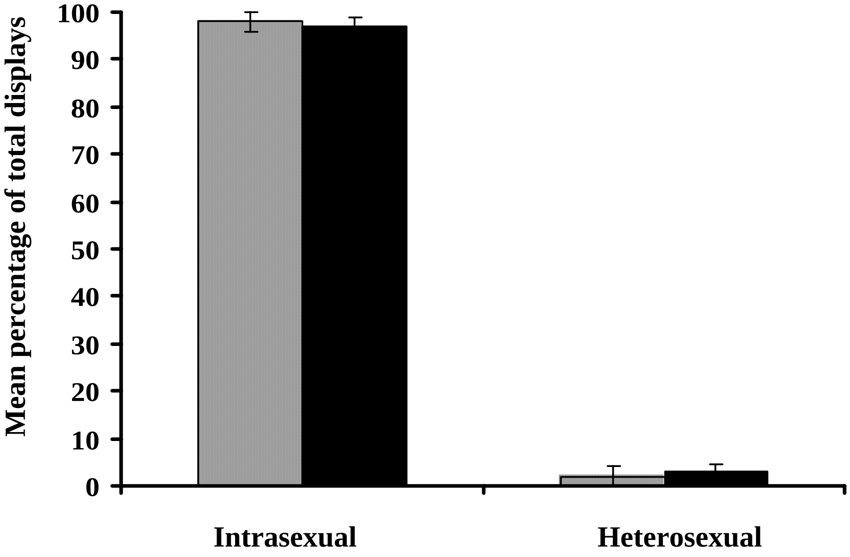


Figure 2.8: Mean percentage ± S.E. of agonistic displays by female (stripe bars) and male (filled bars) residents towards intruders of the same and opposite sex.

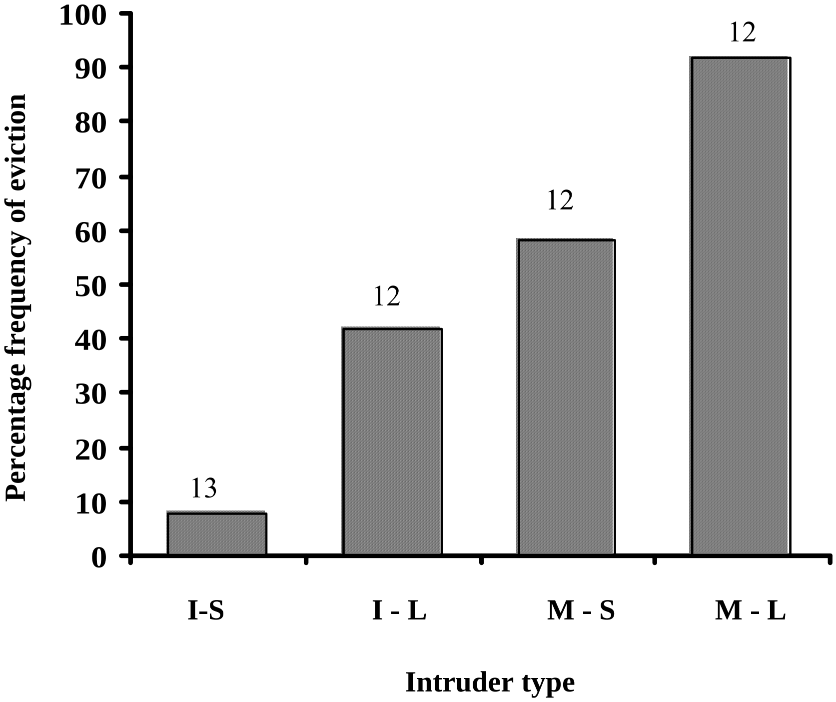


Figure 2.9: Percentage frequency of trials in which female intruders were evicted by dominant, breeding females in relation to the reproductive status and size of the female intruders. I = immature female intruder; M = mature female intruder; S = immature female intruder the same size as the previous rank 3 female; L = immature female intruder larger than the previous rank 3 female. Numbers above bars are the total number of trials carried out for each type of subordinate female intruder.

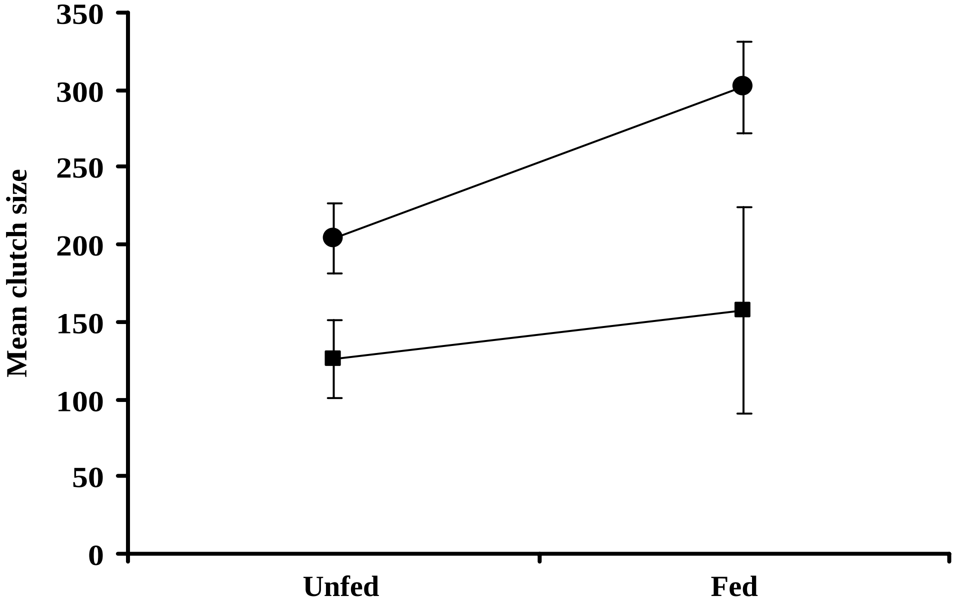


Figure 2.10: Mean clutch sizes ± S.E. laid by females (circles) and hatched by males (squares) in unfed and fed treatments.

2.5: DISCUSSION

Monogamous mating systems in animals are usually thought to occur whenever males are unable to defend more than one female due to a low environmental potential for polygyny (Emlen & Oring, 1977). For *P. xanthosomus*, coral size was positively correlated with group size indicating that coral size is a key determinant of the magnitude of the EPP in this species. Yet despite the large variation in EPP, there was only ever one mature, breeding male and female within each social group. These monogamous partners were always the two largest members of the group, with all remaining subordinate group members being immature females that were smaller than the breeding pair and organised into a size-based dominance hierarchy. Although immature, some of these females, particularly those of high rank, were of reproductive size. Clearly, monogamy in *P. xanthosomus* is not a simple product of the distribution of females.

Breeding males and females were significantly more aggressive towards members of the same sex. The occurrence of intrasexual aggression indicates that males and females potentially compete with members of the same sex within social groups. Males are likely to suffer costs from tolerating other males in the group, and hence compete with each other, because one male is theoretically capable of fertilizing the eggs of all the females within the group (Trivers, 1972). Females on the other hand, are likely to suffer costs from tolerating other females in the group, and hence compete with each other, due to a limitation of essential resources for breeding (Wasser & Barash, 1983). Results from resource manipulations in this study indicate that nest sites were abundant within the coral, suggesting that females were not competing over nest sites. The abundance of suitable nest sites is consistent with other studies on monogamous marine gobies that have also demonstrated an abundance of nest sites (*Valenciennea strigata*, Reavis & Barlow, 1998; *Gobiosoma evelynae*, Harding *et al.*, 2003; *Elacatinus evelynae*, Whiteman &

Côté, 2003). The size of individual nests has been found to limit the extent of polygyny in the sand goby, *Pomatoschistus minutus,* since males given large nests were able to mate with and care for the egg clutches of more females than males with small nests (Lindstrom & Seppa, 1996). Although I did not test the effects of nest size, it is unlikely that nest size limitation would apply to *P. xanthosomus* since males create nest sites themselves by removing coral tissue from the base of a branch (Lassig, 1976) and do not have to rely on any pre-existing substrate that may potentially constrain nest size. The abundance of nest sites therefore indicates that monogamy in *P. xanthosomus* has not evolved because female group members compete over limiting breeding sites.

Supplemental feeding had a positive effect on the clutch sizes laid by breeding females, demonstrating that the fecundity of breeding females is limited by food. This finding is consistent with other studies demonstrating that experimental enhancement of food increases the fecundity of females in fishes (e.g. Siddiqui *et al.,* 1997; Ma *et al.,* 1998; Jones & McCormick, 2002). As a result, the reproductive success of females is likely to be a decreasing function of the number of other breeding females within the group. This therefore suggests that dominant, breeding females would stand to benefit by preventing subordinates from maturing and breeding. Consequently, the dominant female becomes the sole reproductive female within the group, thereby promoting the occurrence of monogamy despite the presence of multiple females.

This hypothesis is supported by the observation that subordinate female group members did not mature and breed even though they were fed an excess of food over a 3-week period. Although a removal of food-limited fecundity (through feeding) may be expected to cause a removal of suppression (and thus subordinate maturation) if food-limited fecundity promotes monogamy, it is unlikely that such a direct link can be experimentally demonstrated since food limitation is likely to have been a continual constraint on the reproductive output of females resulting in weak selection on females to modify their propensity to suppress the reproduction of subordinates in response to present-day variation in food availability. Reproductive suppression can only be removed by removing the dominant female from groups – this has been shown to result in the subsequent maturation of subordinates in less than 2 weeks following removal (chapter 4). In light of this, if the maturation and breeding of subordinate group members was simply a result of limited food availability due to competitive exclusion by dominants, supplemental feeding should have had an observable effect within 3 weeks. Individuals are likely to retain a high degree of plasticity in terms of the timing of maturation given the intense conflict over rank within groups and the possibility of inheriting breeding status (chapter 4). This would select for individuals with rapid and flexible growth and maturation responses to changes in their social environment, as seen in other species of fish (Fricke & Fricke, 1977; Borowsky, 1978; Hattori, 1994; Hobbs *et al.,* 2004). Therefore it seems that the absence of subordinate reproduction does not simply arise because dominants out-compete subordinates for food. Rather, it seems that dominant females are suppressing the reproductive status of subordinates because food is a limited resource.

In conjunction, these two results provide support for the role of food-limited fecundity in promoting female competition, the suppression of subordinate reproduction by dominants, and hence the occurrence of monogamy in *P. xanthosomus*. Food-limited reproductive success of dominant females also appears to be the cause of reproductive suppression in subordinates in other social animals in which females are organised into dominance hierarchies. For example, in the cooperative breeding meerkats (*Suricata suricatta*), dominant females that are pregnant frequently kill pups born to subordinates (Clutton-Brock *et al.,* 1998). Since the amount of food obtained by the dominant's pups is a decreasing function of the pup : helper ratio within the group, dominant females are likely to maximise their reproductive success by suppressing the reproduction of their subordinates via infanticide (Clutton-Brock *et al.*, 1998).

Supplemental feeding of breeding pairs revealed that paternal care is also likely to be a limiting resource for female *P. xanthosomus*. Females in fed treatments laid clutches that were approximately 50% larger than in unfed treatments, yet clutches hatched by males in fed treatments were only 24% larger than in unfed treatments. Therefore, the number of eggs hatched by males appears to be a decelerating function of the number of eggs laid by females. Given that the feeding experiment was designed to induce similarly-sized female partners to lay unnaturally enlarged clutches, this result suggests that males experience a diminishing ability to care for an increasing number of eggs over and above that which a similarly-sized female partner would usually lay. It also suggests that dominant, breeding females are capable of providing males with all the eggs males can successfully care for at a given time. Consequently, dominant females stand to benefit by preventing their subordinates from breeding since this would maximise the survival of their own offspring. This result therefore provides preliminary support for the role of limiting paternal care in promoting female competition, the suppression of subordinate reproduction by dominants, and hence the occurrence of monogamy in *P. xanthosomus*.

The diminishing characteristic of paternal egg care might be related to three possible factors. Firstly, increased energetic demands of fanning enlarged clutches (Perrone & Zaret, 1979; Takahashi *et al.,* 2004; Karino & Arai, 2006) may constrain the ability of males to care for clutches larger than that which a similarly-sized female would lay. This could be particularly relevant for *P. xanthosomus* since eggs are laid in a single layer and enlarged clutches would necessarily be spread over a larger area (Wong, *pers. obs.*). Males might be able to care for enlarged clutches if they increase their food intake in order to increase their energy reserves for fanning (Lindstrom, 1997). However, this is unlikely to be the case for male *P. xanthosomus* since under natural conditions, nest-tending males are rarely observed to leave the vicinity of their nest site in order to forage throughout the coral (Wong, *pers. obs.*). In light of this, the average clutch size hatched by males obtained from the current study may infact be an over-estimation of the ability of males to care for enlarged clutches, since both males and females were allowed access to supplemental food. A repeat of the current experiment where only the female but not male partner is fed may therefore result in smaller average clutch sizes hatched by males in comparison to that obtained in the current experiment.

Secondly, males may experience a decreasing ability to care for increasingly large clutches if large clutches are more susceptible to or attract more egg predators, such as the crabs which also inhabit coral colonies (Wong, *pers obs.*). Thirdly, it is possible that males may even cannibalize a proportion of their own eggs as clutch sizes increase. According to theory, partial filial cannibalism is an adaptive means by which males can invest in both current and future reproduction – by eating some of their current clutch, and thus reducing its size, males conserve energy which could subsequently be allocated to enhancing their survival or reproductive capacities in the future (Sargent, 1992; Manica, 2002). Specifically, the number of eggs eaten by males is expected increase with clutch size (Manica, 2002), which would therefore explain why the number of eggs hatched by males is a diminishing function of the number of eggs laid by females, as in the current study. The three potential sources of egg loss could even be related. For example, there may be a trade-off between fanning and egg defense such that even if males are able to ventilate an enlarged clutch, the number of eggs hatched may still be a diminishing function of clutch size since more eggs would be lost to egg predators (Lissaker & Kvarnemo, 2006). Additionally, increased effort in both fanning and egg defense may result in increased rates of filial cannibalism by males as a means of restoring spent energy (Lissaker & Kvarnemo, 2006), resulting in a reduction in the clutch size hatched. Regardless of the potential sources of egg mortality due to paternal care of enlarged clutches, dominant females would still benefit from suppressing the reproduction of subordinate females since this would keep clutches sufficiently small and thus minimize the mortality of their own eggs.

The observation that breeding partners were size-matched is also consistent with the occurrence of constraints on paternal egg care. If paternal care is constrained, size-matched pairs are generally predicted to arise because: 1) females maximise their reproductive success by mating with males that are large since male size is commonly linked with paternal care ability (Kuwamura *et al.,* 1993; Sunobe & Nakazono, 1999), and 2) given that breeding females guard males, males maximise their reproductive success by mating with females that are large given that female fecundity is usually correlated with female size (Bagenal, 1967). This observation is important because it suggests that food limitation on females is unlikely to be the sole mechanism promoting monogamy in *P. xanthosomus.* If food were the only resource limiting female reproductive success, one would expect the occurrence of monogamous pairs in which the female is considerably larger than the male, as seen in some anemonefish (e.g. Fricke & Fricke, 1977). Various mechanisms have been proposed to explain how size-matched pairing can arise in animals (Crespi, 1989; Harari *et al.,* 1999; Rahman *et al.,* 2002). Recently, Munday *et al.,* (2006) demonstrated that for a related coral-dwelling goby *Gobiodon histrio,* monogamous partners cooperatively regulated their growth and converged on approximately the same size over time, and that this occurred because it apparently enhanced the reproductive success of both individuals in the pair. Taken together, these results support the role of female competition over limiting paternal egg care in promoting the reproductive suppression of subordinates and thus the evolution and maintenance of monogamy in *P. xanthosomus*.

Further support for the role of limiting paternal care in promoting monogamy comes from the pattern of sex change reported for *P. xanthosomus.* This species is a protogynous hermaphrodite – if the male of a group dies, the large, breeding female subsequently changes sex to become the male and the largest non-breeding subordinate female matures to become the new breeding female (Lassig, 1977). If only female but not male size was important for the reproductive success of both sexes, then the rank 3 subordinate female as opposed to the dominant breeding female might be expected to change sex to become the male, since this would maximize the egg production of the breeding pair. Therefore, the fact that the largest remaining group member (i.e. the breeding female) becomes the new male suggests that the reproductive success of pairs may be a stronger function of the quality of male care than the number of eggs produced (Warner, *pers. comm.*).

What are the mechanisms by which breeding females prevent subordinates from breeding in *P. xanthosomus*? Breeding females directed almost all their aggressive displays towards other females as opposed to other males, suggesting that aggressive stress-related suppression of subordinates females could be one such mechanism. Such stress-related suppression of subordinate reproduction due to agonistic encounters with dominants has been reported for other species (e.g. Borowsky, 1972; Sohn, 1977; Faulkes & Bennett, 2001; Young *et al.,* 2006). In addition, breeding females evicted intruder females that were, in order of increasing severity, large, mature and both large and mature. Breeding females would benefit from evicting mature subordinates since they are likely to represent the individuals with whom competition over limiting resources is most intense (Bernardo, 1993) and their competitive dominance is least assured, since size equates to competitive ability (Webster & Hixon, 2000; Whiteman & Côté, 2004b). Given: 1) the absence of subordinates that are mature or of similar size to the breeding female in natural groups, 2) the occurrence of subordinate eviction in direct proportion to the threat posed to breeding females, and 3) the high costs of being evicted from a group (Lassig, 1981; Munday, 2002; current chapter), it is likely that breeding females employ the threat of eviction to suppress the reproductive physiology and size of subordinates. Specifically, any subordinate that matures or grows too large faces the threat of being forcibly evicted from the group by the breeding female, a fate that is likely to result in death of the subordinate (Lassig, 1981; Wong, *pers. obs.*). Consequently, subordinates should respond to the threat of eviction by suppressing their own reproductive status and size. Both subordinates and breeding females would benefit since subordinates are ensured continued membership within the group, and breeding females ensured minimal competition over limiting resources. Although direct evidence for subordinate self-regulation of reproductive status and size is lacking, studies on other coral-dwelling gobies have demonstrated that juveniles have the ability to control the timing of their maturation in relation to their social environment (Hobbs *et al.,* 2004). In addition, there is growing empirical evidence supporting the ability of subordinates to regulate their own growth and body size in relation to the size of their immediate dominant (e.g. Heg *et al.,* 2004a; Buston & Cant, 2006), as well as demonstrations of the links between the threat of eviction and subordinate growth regulation (chapter 4 and 5), lending further support to the occurrence of subordinate self-suppression.

In animals, the suppression of subordinate reproduction has been shown to occur via a variety of mechanisms, including behavioral suppression through interference with mating or expulsion of mature subordinates (Reyer *et al.,* 1986; Creel *et al.,* 1992; Bennett *et al.,* 1996; Clutton-Brock *et al.,* 1998; O'Riain *et al.,* 2000; Cant *et al.,* 2001), or via physiological suppression of the reproductive functioning of subordinates mediated through stress or other dominant control mechanisms (Creel *et al.,* 1992; Molteno & Bennett, 2000; Faulkes & Bennett, 2001; Fitzpatrick *et al.,* 2005; Young *et al.,* 2006). However, the threat of eviction has yet to be invoked in the physiological suppression of subordinate reproductive functioning in social animals. In the cooperative breeding meerkats (*Suricata suricatta*), subordinate females are temporarily evicted by dominant breeders (Clutton-Brock *et al.,* 1998) resulting in stress-related inhibition of their reproductive physiology (Young *et al.,* 2006), but there is no evidence to suggest that subordinate females pre-empt this possibility of being evicted by suppressing their own reproductive physiology (Young, *pers. comm.*). The threat of eviction may play little role in reproductive suppression in such cooperatively breeding species because of high costs of evicting subordinates relative to the costs incurred by subordinates of being evicted (Hamilton & Taborsky, 2005). This likely to occur because dominant breeders in cooperatively breeding species accrue significant benefits from the presence of subordinates which act as helpers in the group (Mumme, 1992; Boland *et al.,* 1997), and thus would not benefit as greatly from evicting subordinates as would dominant *P. xanthosomus* whose subordinates do not help (Wong, *pers. obs.*). In addition, although subordinate birds and mammals may incur costs from being evicted from the group (Cant *et al.,* 2001; Young *et al.,* 2006), individuals that or expelled from or leave groups frequently have the option of returning back to their group (Clutton-Brock *et al.,* 1998; Young *et al.,* 2006), assuming a new albeit lower quality territory elsewhere (Koenig *et al.,* 1992), or becoming 'floaters' that wait in marginal areas for an available territory (Zack & Stutchbury, 1992). In contrast, the spatially discrete nature and unpredictable distribution of coral colonies, combined with high rates of mortality outside corals results in extremely high costs to subordinate *P. xanthosomus* from being evicted (Lassig, 1981; Munday, 2002). Therefore, the size and spatial distribution of the coral habitat combined with the extreme habitat specialisation of *P. xanthosomus* enable the threat of eviction to be an effective strategy in the suppression of subordinate maturation and hence in maintaining a monogamous mating system.

Given that subordinate females represent competitors over limiting resources, why then do dominant, breeding females not simply exclude subordinates from the group permanently? Dominants may tolerate the presence of subordinates, despite being potential competitors over resources, if they accrue benefits from the presence of subordinates, for example through a reduced risk of predation, enhanced foraging efficiency or if subordinates represent future potential mates (Krause & Ruxton, 2002). Alternatively, dominants may suffer higher costs from having to continuously evict settling subordinates rather than from tolerating their presence, for example if evicting is energetically costly and diverts resources away from reproduction (Hamilton & Taborsky, 2005). As a result, tolerating the presence of subordinates but ensuring they do not breed within groups may represent the optimal strategy given the costs and benefits of tolerating and evicting subordinates for dominants. This strategy would be particularly effective since dominant *P. xanthosomus* appear to have complete control over the reproductive status and size of subordinates, given that all subordinates group members are always immature and smaller in size than the dominant female. This contrasts with other animal societies in which subordinates do engage in reproduction even though resources such as food appear to be limiting (e.g. Fricke, 1980, Forrester, 1991; Clutton-Brock *et al.,* 2001). This suggests that dominants in these species may lack complete control of subordinate reproduction, being able to skew the distribution of reproduction in favour of themselves but not completely suppress subordinate reproduction altogether (Clutton-Brock, 1998). Control in such species may be incomplete because subordinates can retaliate against dominants (De Waal & Luttrell, 1988; Clutton-Brock & Parker, 1995) or avoid dominants (Berard *et al.,* 1994; Creel *et al.,* 1997) and thereby avoid dominant suppression. In contrast, dominant control is likely to be complete in *P. xanthosomus* because dominants always have a size and thus competitive advantage over subordinates and the extremely low mobility of subordinates that reside within the confines of a coral colony would enable dominants to effectively monitor the activities of subordinates (Wong, *pers. obs*.).

To conclude, the dispersion of resources and females, and hence the ability of males to compete over females does not account for the evolution of monogamy in *P. xanthosomus* – this species is monogamous even though individuals form large social groups in which one male and multiple females reside in close spatial proximity. Instead, this study suggests that a combination of factors, including limitation of food resources and constraints on paternal egg care, generates competition between females which is likely to select for the suppression of subordinate reproduction by dominants. The mechanism by which subordinate reproduction is suppressed could involve the self-suppression of maturation in response to the threat of eviction by dominants, although further tests of this mechanism are required. This study demonstrates that female tolerance of reproductive sharing is not an inevitable consequence of spatial aggregation and group-living, particularly when there is a strong asymmetry in competitive ability. Although the EPP model is a key framework for understanding the environmental correlates of monogamy, it is clear that the pay-offs from different mating systems experienced by females need also be considered when assessing the evolution of mating systems, particularly within the context of social groups.

CHAPTER 3

EXPERIMENTAL TESTS OF THE CONSTRAINTS AND BENEFITS OF GROUP-LIVING IN A CORAL-DWELLING FISH

3.1. ABSTRACT

In many animal societies, subordinates tolerate group-living despite being excluded from reproduction and being unrelated to dominant breeders. The theory of cooperative breeding has the potential to explain the evolution of this phenomenon by specifying how the pay-offs to subordinates from dispersing versus staying in their current group vary in relation to certain ecological factors. In this study, I investigated how habitat saturation, costs of movement and habitat quality affect the dispersal and grouping decisions of non-breeding subordinates in the coral-dwelling fish, *Paragobiodon xanthosomus* (Gobiidae), as well as how social factors contribute to the maintenance of group-living in this species. Manipulations of dispersal distances to alternative coral colonies revealed that subordinate dispersal was strongly influenced by the cost of dispersal. Furthermore, in habitat choice experiments, subordinates showed a preference for group-living and non-breeding on larger corals versus pair-forming and immediate breeding on smaller corals. This preference became stronger as the size difference between the potential coral hosts increased. In contrast, subordinate dispersal was independent of coral saturation and social rank, which is likely to arise because of weak selection on individuals for facultative dispersal in relation to these factors. Finally, there were no detectable effects of interactions with dominants in promoting subordinate dispersal. These results demonstrate that costs of movement and variation in coral quality influence the pay-offs to non-breeding

subordinates from group-living. In addition, the results suggest that constraints imposed by a species' ecology over evolutionary time need also be considered to provide a complete picture of the factors influencing the maintenance of animal societies.

3.2. INTRODUCTION

In many animal societies, subordinate group members are excluded from direct reproduction by their dominants and gain no indirect reproductive benefits from group-living as a result of kinship (Emlen, 1991; Queller *et al.,* 2000; Faulkes & Bennet, 2001; Buston, 2002; Griffin *et al.,* 2002). To understand how these societies are maintained, a fundamental question needs to be addressed: why do non-breeding subordinates tolerate group-living as opposed to dispersing to breed independently elsewhere? Clearly, subordinates that remain in their current group suffer an automatic fitness cost in terms of missed reproductive opportunities elsewhere (Emlen, 1995). Thus for group-living to be tolerated by subordinates, this cost must somehow be compensated.

The theory of cooperative breeding (Brown, 1974) has the potential to provide an insight into how this cost may be compensated and thus help to explain how these societies are maintained. Although ultimately concerned with explaining why offspring delay dispersal and provide help to their parents (Emlen, 1991), an integral component of this theoretical framework is focused on describing the pay-offs to subordinates of dispersing to breed elsewhere versus remaining their current group (Brown, 1974). Furthermore, although the theory is usually applied to societies in which breeders and non-breeders are related, the decision of unrelated subordinates of whether to disperse or remain in their current group is likely to be determined by the cost of dispersing and the benefits of staying, just as it is for groups of related individuals (Gardner *et al.,* 2003). Therefore, cooperative breeding theory has the potential to act as a general framework for explaining the maintenance of sociality across taxa exhibiting a wide range of social, reproductive and genetic systems (Koenig *et al.,* 1992).

According to theory, non-breeding subordinates may tolerate group-living if there are high costs from dispersing to breed independently elsewhere (Emlen, 1982). This situation could arise if alternative territories or habitats in the area are saturated such that there are no available vacancies in which subordinates can breed or at least increase their chances of independent breeding (Selander, 1964). Additionally, group-living may be favoured regardless of the availability of breeding habitats if dispersal entails high costs, for example due to risks of predation or energy loss (Emlen, 1982). Implicit in these hypotheses is that group-living is a secondary option to dispersing and breeding independently for subordinates i.e. natural selection favours subordinates that live in groups because of reduced opportunities for breeding elsewhere (Emlen, 1982).

Subordinates may also tolerate group-living because they gain long term fitness benefits from doing so (Stacey & Ligon, 1987). Primary among the factors proposed to enhance the long-term benefits of group-living relates to variation in territory quality (Stacey & Ligon, 1987, 1991). Specifically, non-breeding subordinates currently residing in a high quality habitat may prefer not to disperse even if alternative habitat vacancies for breeding are available and costs of dispersal are low, if the alternative habitat is of inferior quality (Stacey & Ligon, 1987, 1991; Koenig *et al.,* 1992; Walters *et al.,* 1992; Zack & Stutchbury, 1992; Covas *et al.,* 2003). The influence of territory quality will become especially relevant in promoting group-living if non-breeding subordinates have the opportunity to inherit dominant, breeding status within their current habitat (Woolfenden & Fitzpatrick, 1978; Zack & Ligon, 1985; Komdeur, 1992; Zack & Stutchbury, 1992; Pen & Weissing, 2000; Ekman *et al.,* 2001; Buston, 2003a). If subordinates reside in habitats of high quality, and if they stand to inherit breeding status in the future, they may maximise their lifetime fitness by remaining as non-breeders in the current habitat as opposed to dispersing and reproducing immediately on available habitats of inferior quality (Stacey & Ligon, 1987, 1991; Zack & Stutchbury, 1992; Komdeur 1992; Ekman *et al.,* 2001). Similarly, subordinates (or even dominant breeders) currently residing on habitats of low quality may be favoured to disperse to habitats of high quality in which they accept subordinate non-breeding status within a group (Stacey & Ligon, 1991). Implicit in this hypothesis is that subordinates realise a greater overall fitness from remaining in and inheriting their current habitat, as opposed to making the 'best of a bad situation' by group-living (Emlen, 1995).

Finally, dispersal and grouping decisions of subordinates may also relate to various social factors (Pasinelli & Walters, 2002) in addition to environmental factors such as habitat availability, costs of movement and habitat quality (Koenig *et al.,* 1992). Recently, there has been growing theoretical and empirical emphasis on the importance of social rank in mediating dispersal decisions in species that form queues to inherit breeding status (Field *et al.,* 1999, Buston, 2002; Kokko & Ekman, 2002, Mitchell, 2005). These studies are directly relevant to determining why certain subordinates might tolerate group-living, and specifically highlight the fact that pay-offs from dispersing may vary from individual to individual rather than reflecting a generalised outcome of certain environmental conditions. In addition, the occurrence of dispersal may not just reflect a voluntary decision by subordinates in response to their environment, but an involuntary outcome in response to forcible eviction from the group by other group members (Balshine *et al.,* 1998; Johnstone & Cant, 1999; Dierkes *et al.,* 1999; Cant *et al.,* 2001; Buston & Cant, 2006; chapter 4). Greater consideration of these social factors is therefore important for providing a complete picture of the maintenance of group-living in social animals.

In this study, I investigated the role of environmental and social factors in promoting the maintenance of group-living in the obligate coral-dwelling goby, *Paragobiodon xanthosomus*

(Gobiidae). This species is particularly well suited for such an investigation for a number of reasons. Individuals reside within colonies of just one species of host coral, *Seriatophora hystrix* (Pocilloporidae), making it possible to compare among habitat patches without confounding effects of different habitat types. Coral colonies vary greatly in size and occur as spatially discrete units (chapter 2), which provides the opportunity to manipulate the cost and benefits of remaining in the current habitat patch versus dispersing to other habitat patches. Within each colony, gobies form social groups ranging in size from 2-20 individuals, but reproduction is monopolised by the largest mature male and female ('dominants') (chapter 2). The non-breeding subordinates are organised into size-based hierarchies that serve as queues to inherit breeding status (chapter 4). Therefore, the cost and benefits of group-living versus dispersal to breed elsewhere may differ between individuals in relation to their position in the social hierarchy (Field *et al.,* 1999; Buston, 2002). Finally, subordinate non-breeders provide no assistance to the dominant breeders (Wong, *pers. obs.*) and are unlikely to be related to each other given that newly hatched larvae spend several weeks in a well-mixed pelagic environment before recruiting to the benthic coral habitat (Sale, 1991).This means that kin relationships are unlikely to be important in decisions to remain in a group or disperse to breed elsewhere.

Three specific aims were addressed in this study. Firstly, I determined whether habitat saturation and costs of movement constrain subordinates to group-living by simultaneously manipulating coral saturation and distances to alternative corals and comparing the proportion of subordinates dispersing. If saturation and dispersal costs influence group-living, then the proportion of subordinates dispersing should be greater to unsaturated compared saturated corals and greater when dispersal distances are short compared to long. Secondly, I determined whether dispersal decisions of subordinates were influenced by the benefits of residing and breeding in a coral of high quality by experimentally manipulating the size differences between corals and assessing the habitat choices of subordinates. If subordinates base their dispersal decisions on habitat quality and coral size reflects coral quality, then there should be an increasing tendency for subordinates to favour group-living and non-breeding on a larger coral versus immediate breeding on a smaller coral as the size difference between corals increases. Finally, I compared the occurrence of subordinate dispersal in relation to their social rank and the size ratio between themselves and their immediate dominant to investigate whether social factors, in the form of rank and forcible eviction by immediate dominants, were also involved in the maintenance of group-living in *P. xanthosomus*.

3.3. METHODS

Study site and species

The study was conducted at Lizard Island (14° 40’S, 145° 28’E) on the northern Great Barrier Reef, Australia between March 2004 – November 2005. Field observations and experiments were conducted in the Lizard Island lagoon (Figure 2.1), and aquarium observations and experiments were carried out at Lizard Island Research Station (Figure 2.1). Colonies of *S. hystrix* in which *P. xanthosomus* resides provides individuals with a source of food, shelter and breeding sites, and gobies moving between coral colonies are subject to high risks of predation (Lassig, 1981). Non-breeding subordinates within the group are all immature females despite some being of reproductive size (chapter 2). Within groups, non-breeding subordinates queue to inherit breeding status, and individuals of adjacent rank tend to differ in size by a ratio of 0.93 (chapter 4). Any individual within the queue that grows and exceeds this size ratio faces a high probability of being evicted from the group by its immediate dominant (chapter 4).

Habitat saturation and costs of movement

To determine whether costs of dispersal, such as habitat saturation and risks of movement, play a role in the maintenance of group-living, I simultaneously manipulated the levels of saturation and the dispersal distances to alternative corals and compared the proportion of subordinates that dispersed. If habitat saturation alone promotes group-living, I predicted that the proportion of dispersing subordinates should be greater to an unsaturated versus saturated coral, regardless of dispersal distance. If costs of movement alone promotes group-living, then the proportion of dispersing subordinates should be greater when dispersal distances are low versus far, regardless of the levels of coral saturation. If habitat saturation and costs of movement influence dispersal, then the proportion of dispersing subordinates should be greater to an unsaturated versus saturated coral when dispersal distances are both low and far, but the overall proportion of dispersing subordinates should lower when dispersal distances are far.

To test these predictions, a total of 62 coral colonies were collected from the reef. Group sizes of resident gobies ranged from 5 -17 individuals. Each coral colony was paired with another that did not differ in group size by more than 2 individuals, such that a total of 31 ‘coral pairs’ were created. Each coral of a pair was numbered uniquely by fixing a numbered disc to the base. Both corals of a pair were placed on rubble platforms adjacent to one another in a sandy lagoon that was at least 5m away from any other reef habitat. Each coral pair was separated from the next pair by at least 300cm of sand to minimise movement between coral pairs. A previous pilot experiment in which corals were placed at 10cm, 50cm, 100cm, 200cm and 500cm distances apart on sand revealed that gobies did not move when distances were greater than 100cm (Wong *et al.,* unpub. data), thus 300cm was deemed a sufficient distance to ensure a lack of movement. Four experimental treatments were then established: 1) alternative coral unsaturated + short dispersal distance, 2) alternative coral unsaturated + long dispersal distance,

3) alternative coral saturated + short dispersal distance and 4) alternative coral saturated + long dispersal distance.

To create the first treatment, 10 of the 31 coral pairs were randomly selected and all subordinate females from one randomly selected coral of each pair were removed by inverting the coral over a bucket of water allowing the gobies to fall out. The dominant male and female were measured using calipers (standard length (SL) ± 0.1mm) and both were tagged by injecting a small spot of the same colour of fluorescent elastomer (Northwest Technologies Inc.) just under the skin. These tags have high retention rates with no adverse effects on growth or survival (Malone *et al.,* 1999). The dominant male and female were placed back into the unsaturated coral to control for any effects of potential reproductive behaviour on the dispersal decisions of subordinates. In this way, an 'unsaturated' coral was created. All gobies from the other coral of the pair were removed, measured, and tagged using a different colour to the breeding pair from the unsaturated coral, and replaced back into their original coral. Each coral of the pair was positioned so that the dispersal distance between them was 10cm (i.e. a short dispersal distance). To create the second treatment, 8 of the 31 coral pairs were randomly selected. The same procedure for treatment one was repeated except that each coral of the pair was placed 100cm apart (i.e. long dispersal distance). To create the third treatment, 8 of the 31 coral pairs were randomly selected. The procedure for treatment one was repeated except that no gobies were removed from either coral of the coral pair so that the alternative coral was left 'saturated'. To create the fourth treatment, 5 of the 31 coral pairs were randomly selected. The same procedure for treatment three was repeated except that each coral of the pair was placed 100cm apart.

The following day, each coral pair was observed on SCUBA and the occurrence of movement between corals of a pair detected by noting the presence of any gobies of the opposing tag colour. The size and thus dominance rank of gobies that moved from their original group was recorded, as well as the size and dominance rank they achieved in their new group. All corals were then left undisturbed for 7 days whereupon the occurrence of any further movement was scored in the same way.

Log-linear analysis of frequency tables was used to assess the relative effects of saturation and distance on the number of subordinates moving between corals. In this analysis, a series of models was constructed to test whether the occurrence of dispersal was: 1) dependent on an interaction between saturation and distance, 2) dependent on saturation alone, 3) dependent on distance alone and 4) dependent on saturation and distance but not on an interaction between these factors (Table 3.1). The method of model constructions follows Munday *et al.,* (2001) where the factors manipulated in the experiment are included in every model and it is the interaction between these factors and the response variable that are sequentially tested. Models were tested by fitting them in decreasing order of complexity until there was no further significant reduction in the goodness-of-fit statistic (chi-squared value) from one model to the next. By doing so, the simplest model to explain the observed data was found.

Habitat quality

To determine whether the benefits of staying in the current group in relation to the quality of habitat in which the subordinate resides influences why subordinates tolerate group-living, I presented subordinate females with the choice of breeding immediately as a dominant female on a small coral versus becoming a non-breeding rank 3 subordinate on a larger coral. If the benefits of staying in a current group are positively related to the quality of a subordinate's current coral, and the size of a coral reflects its quality, then the frequency with which a subordinate foregoes immediate reproduction on the smaller coral in preference for group-living on the larger coral should be positively correlated with the difference in size between the two corals.

To test this prediction, I used a binary choice experiment in which a subordinate female was allowed to choose between joining a small coral as a breeder or a larger coral as a non-breeding rank 3 subordinate (Figure 3.1). Four small coral colonies were collected from the reef and each placed into a separate circular aquarium supplied with a continuous flow of fresh sea water. Based on previous work, coral colonies were defined as small if they contained only a breeding pair and no subordinates, and if they were between 10 – 18 cm average diameter (L+W+H / 3) (chapter 2). Corals containing only 1 goby were not collected even if they were within this coral size range since it was necessary to ensure the coral was habitable for a breeding pair. The breeding pair was removed from each small colony and placed together in separate holding tanks, and the size of the coral calculated. A further 4 coral colonies were then randomly collected from the reef, each varying in size but larger than the 4 small colonies. All resident gobies were removed from these corals and placed into holding tanks. Each of these corals was measured and then randomly paired with one of the 4 small corals in the aquaria at a distance of 20cm apart (Figure 3.1).

To simulate a choice between immediate reproduction on the small coral versus group-living and delayed reproduction on a larger coral, a single mature male was introduced into each of the 4 small corals, and a mature male and female pair introduced into each of the larger corals (Figure 3.1). Mature males and females were collected from the reef by spraying a clove oil anaesthetic solution over a coral colony and gently extracting gobies by wafting water currents through the coral. Only the largest 2 group members per group were collected since I previously showed that these are the mature males and females (chapter 2). To create a breeding pair, the mature males and females collected from the reef were anaesthetised in clove oil solution, sexed by the shape of their genital papillae (Lassig, 1977) and tagged a unique colour. Four pairs of mature males and females were created by randomly matching males and females together. I ensured that there was a small size difference (< 3mm SL) between the matched partners since the breeding male and female are typically size-matched under natural conditions (chapter 2). Pre-existing breeding pairs were not used in order to control for any possible confounding effects of pair duration on the outcome of subordinate dispersal decisions or outcomes. A matched pair was then introduced into each of the larger corals set up in the aquaria (Figure 3.1), and a single mature male released onto each of the small corals set up in the aquaria (Figure 3.1). To control for any effects of male size on the dispersal decisions of subordinate females, the size of the single male in the small corals was closely matched to the size of the paired male in the larger corals, such that size differences between them never exceeded 1mm.

Four subordinate (immature) females were collected from the reef to act as the ‘choosing’ subordinate females. Each female was measured and randomly assigned to one of the 4 experimental aquaria. Previous work demonstrated that a subordinate greater than a size ratio of 0.93 relative to its immediate dominant (SL subordinate / SL immediate dominant) is likely to be evicted from the group by its dominant (chapter 4). Therefore, to ensure that dispersal decisions reflect a true choice by the subordinate females and not eviction by their immediate dominant, the size ratio between the choosing subordinate female and the mature males and females was always less than 0.93. Subordinate females were introduced into each aquaria by gently dropping them into a transparent plastic pipe placed an equal distance between the small and larger coral (Figure 3.1). Holes were cut into the pipe to allow circulation of olfactory cues. The subordinate female was not released from the pipe until she had observed both the small and larger coral, whereupon the pipe was gently lifted by pulling an attached piece of string. The choice of the subordinate female was recorded and then re-scored the following morning. The choice the following morning was used as the final choice of the subordinate female.

The protocol was repeated daily until 4 choice trials per difference in coral size was completed, and until 16 coral size differences had been replicated. For each new trial: 1) a new subordinate female was used, 2) the breeding male and female partners were re-matched such that in no two trials were the same breeding male and female paired together, 3) each breeding male and female was never used more than once in the same coral colony and 4) each single male was used in a different small coral colony. When all combinations of fish and coral colonies had been trialed, new fish and coral colonies were collected from the reef and the used fish and corals replaced.

The percentage frequency with which the subordinate female joined the group on the larger of the two corals was calculated for each coral size difference and plotted against coral size ratio (size of smaller coral / size of larger coral). This relationship was analysed using a Spearman's Rank Correlation.

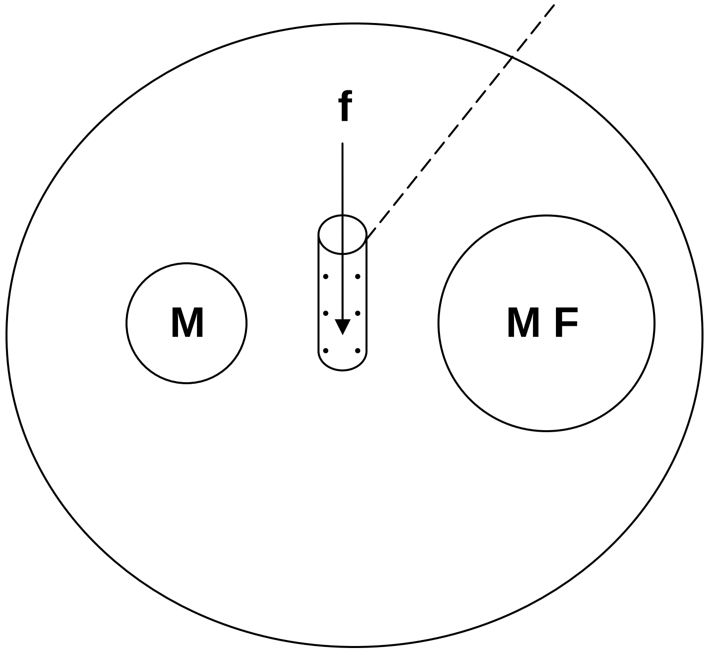


Figure 3.1: Experimental set up for the habitat choice experiment. The large round circle represents the choice aquaria and the two smaller circles represent the smaller and larger coral. M = mature male, F = mature female, f = subordinate choosing female. The subordinate choosing female was placed into a transparent pipe attached to a piece of string (dashed line). The subordinate female was released by pulling on the string to raise the pipe.

Social factors

To assess whether social rank of subordinates and interactions with their immediate dominant influenced the occurrence of subordinate dispersal and the maintenance of group-living, a logistic regression was used to simultaneously investigate the effects of rank and size ratio between subordinates and their immediate dominant on the dispersal behaviour of subordinates. Data from treatments 1 and 3 (corals 10cm apart) of the 'Habitat saturation and costs of movement' experiment was used in this analysis. Only these 10cm treatments were used since few individuals dispersed in the 100cm treatments. Size ratios were calculated as: SL subordinate / SL immediate dominant. Dispersal was considered the binary response variable (0, no dispersal; 1, dispersal) with rank and size ratio as the independent variables. Calculation of tolerances confirmed a lack of collinearity between rank and size ratio predictor variables (Quinn & Keough, 2002) therefore it was reasonable to consider these as independent variables. The criterion for backward elimination of an independent variable was set at $\alpha > 0.05$ and the significance of each variable and interaction assessed with a likelihood ratio test.

3.4. RESULTS

Habitat saturation and costs of movement

A total of 44 out of 264 subordinates (16.7%) dispersed from their home coral in the habitat saturation and costs of movement experiment. Across all 4 treatments, 28 of the 44 subordinates (63.6%) that dispersed did so by day 1, with remaining 16 (36.3%) dispersing by day 7. All subordinates that moved on day 1 were still present in their new group by day 7. All subsequent analyses are based on this day 7 data. The frequency with which subordinates dispersed was dependent on the distance between corals (Table 3.1a, model 3 provided the best fit to the observed data (Table 3.1b) (comparison of model 2 v 3, $X^2 = 1.46$, df = 1, $p > 0.1$). Specifically, subordinates were over 10 times more likely to disperse when the alternative coral was 10cm as opposed to 100cm away (Figure 3.2). In contrast, the proportion of subordinates dispersing was independent of coral saturation (Table 3.1b) (comparison of model 2 v 4, $X^2 = 27.4$, df = 1, $p < 0.01$). Specifically, the proportion of subordinates dispersing to a saturated coral close by was slightly but not significantly lower than the proportion dispersing to an unsaturated coral close by (Figure 3.2). The best fitting model did not include a significant interaction between the effects of saturation and distance on the proportion of subordinates dispersing (Table 3.1a). Therefore, it seems that costs of movement have a much greater influence on the probability of subordinates dispersing among social groups than does the saturation of neighbouring coral colonies.

Habitat quality

A total of 16 coral size differences were replicated, ranging from a difference of 1 – 15cm average diameter (Figure 3.3). For 4 of the 16 coral size differences, the choice of females were successfully replicated only 3 as opposed to 4 times. Therefore, 60 different subordinate females were successfully tested in total. In 28% of all choice trials, the choice of the subordinate female the following morning differed to the choice made the day of the trial i.e. the subordinate female had moved between corals overnight. The analysis is based on the dispersal outcomes recorded the following morning.

There was a significant negative relationship between coral size ratio and the percentage frequency with which subordinate females formed groups on the larger coral (Figure 3.3) (Spearman's rank correlation: R = -0.65, n = 16, $p = 0.006$). Thus, as the coral size ratio decreased i.e. the difference in coral size increased, subordinate females increasingly settled on the larger corals as non-breeding group members even when they had the opportunity of breeding immediately as dominant females on the smaller corals.

Social factors

The occurrence of dispersal was independent of social rank (Table 3.2), the size ratio between themselves and their immediate dominant (Table 3.2) and an interaction between rank and ratio (Table 3.2). Only cases of movement in treatment 1 and 3 (corals 10cm apart) were considered for this analysis because of the rare occurrences of dispersal in treatments 2 and 4 (corals 100cm apart).

Table 3.1: a) Log-linear analysis of the effects of coral saturation and dispersal distance on the frequency of dispersal by subordinates. S = saturation (unsaturated or saturated), D = distance (10cm or 100cm), d = dispersal (disperse or not disperse), **b)** results of step-wise model testing. The best fitting model is underlined.

a)

Model	**Chi^2 Likelihood ratio**	**df**	***p***
1) S+D+d + SxD + Dxd + Sxd + SxDxd	0.00	0	1.00
2) S+D+d + SxD + Dxd + Sxd	0.02	1	0.89
3) S+D+d + SxD + Dxd	1.48	2	0.48
4) S+D+d + SxD + Sxd	27.4	2	<0.001
5) S+D+d +SxD	28.1	3	<0.001

b)

Compare models	**Differential Chi^2 Likelihood ratio**	**df**	***p***
1 v 2	0.02	1	>0.1
2 v 3	1.46	1	>0.1
2 v 4	27.4	1	<0.01
3 v 5	26.62	1	<0.01

Table 3.2: Backward stepwise logistic regression assessing the effects of social rank and the size ratio between individuals of adjacent rank on the probability of subordinate dispersal.

	Variable	Log Likelihood	X^2	df	*p*
Step 1	Rank	-118.413	0.715	1	0.398
	Ratio	-118.293	0.475	1	0.491
	Rank*Ratio	-118.434	0.759	1	0.384
Step 2	Rank	-118.443	0.301	1	0.583
	Rank*Ratio	-118.489	0.393	1	0.531
Step 3	Rank*Ratio	-118.582	0.278	1	0.598

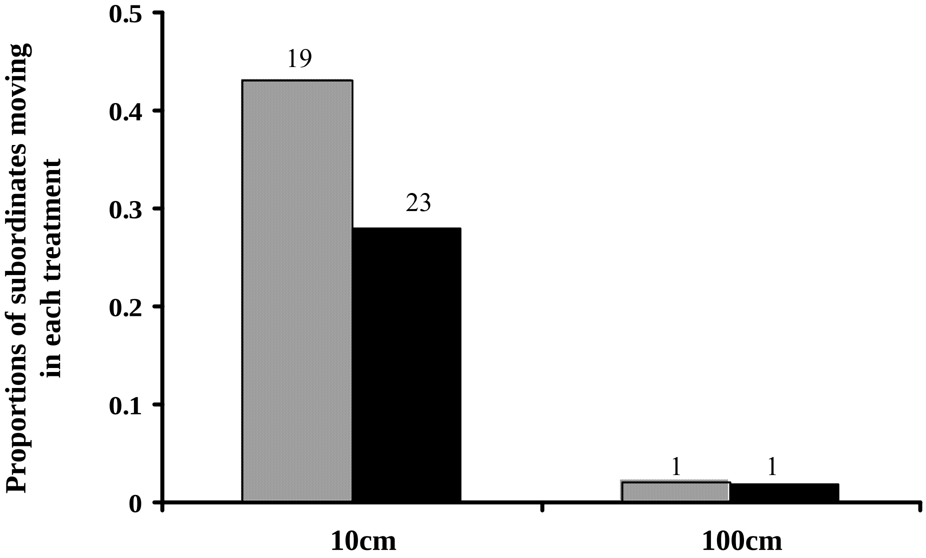


Figure 3.2: Proportions of subordinates that dispersed to unsaturated (diagonal lines) and saturated (filled) coral colonies that were placed either 10cm or 100cm away from their home coral. The number of subordinates that dispersed in each treatment is shown above the bars.

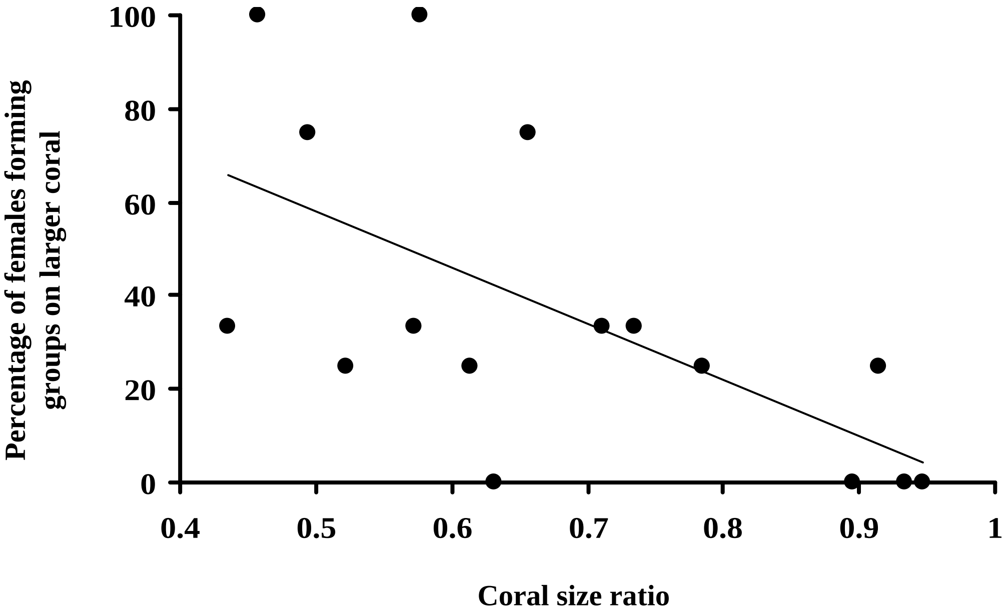


Figure 3.3: Relationship between the difference in coral size and the percentage of subordinate females choosing to form groups and delay breeding on the larger coral. Diagonal line shows spearman rank correlation between coral size ratio and the percentage of females forming groups ($y = 118.5 - 120.6x$).

3.5. DISCUSSION

Determining the ecological and social factors influencing the pay-offs to dispersing is essential for assessing why subordinates tolerate group-living when they are excluded from current direct reproduction, gain no kin-selected benefits, and accrue no benefits through helping behaviour within the group. In the current study, dispersal and grouping decisions of subordinate *P. xanthosomus* were unaffected by habitat saturation, social rank and interactions with dominants, but were strongly affected by costs of movement and the quality of available habitats.

Habitat saturation did not affect the dispersal behaviour of subordinates, suggesting that habitat saturation plays little role in the maintenance of group-living in *P. xanthosomus*. For habitat saturation to be relevant in the maintenance of group-living, there needs to be sufficient variation in the degree of habitat saturation in the environment at the spatial scale an individual can sample (Koenig *et al.,* 1992). In *P. xanthosomus* and other coral-dwelling fishes, the majority of coral colonies above a certain size are occupied (Kuwamura *et al.,* 1994; Hobbs *et al.,* 2004; Wong, *pers. obs.*) and group size is usually positively correlated with coral size (Lassig, 1976; Fricke, 1980; Kuwamura *et al,*. 1994; Munday *et al.,* 1998; Wong *et al.,* 2005; Thompson *et al.,* In Press; chapter 2). This suggests that corals in the environment are likely to be limiting and fully saturated. Given that adaptation to the most commonly encountered environment may preclude current day adjustments to an induced change in the environment (Godwin, 1995) it is unlikely that individual *P. xanthosomus* would be under strong selection to retain the capacity for facultative dispersal in relation to fluctuations in the levels of saturation of alternative corals. Dispersal strategies would still be maintained in the population however, because individuals would occasionally make successful dispersal events that result in a fitness increase (Ochi, 1989; Hattori, 1994; Mitchell, 2005; Manabe *et al.,* In Press). Therefore, although habitat saturation may have played a key role in the initial evolution of group-living strategies, saturation is likely to play an insignificant role in the current day maintenance of group-living due to weak selection on subordinates to respond to changes in levels of saturation in their immediate environment.

Further support for the occurrence of weak selection on subordinates to take advantage of potentially fitness-maximising opportunities (and hence a lack of behavioural flexibility) comes from the lack of effect of social rank on dispersal found in the current study. Social rank had no apparent effect since: 1) when an unsaturated alternative coral was close-by, many of the subordinates that did not disperse could have immediately improved their social rank by doing so, 2) subordinates that did disperse to a coral close-by did not significantly improve their rank by doing so, a result consistent with other studies examining dispersal patterns in habitat-specialist and group-living fishes (Mitchell, 2005; Manabe *et al.,* In Press), 3) despite recent theoretical analyses advocating a strong effect of rank on dispersal decisions (Field *et al.,* 1999; Buston, 2002; Kokko & Ekman, 2002), dispersal was unrelated to the social rank of subordinates in their original group, and 4) in the 10cm treatments, subordinates that dispersed on day 1 remained in their new group by day 7 even if they had decreased in rank by moving. If subordinates were under strong selection to improve their rank by moving, subordinates that had moved down in rank by dispersing would be expected to reverse their decision, since costs of dispersal were minimal. Selection for facultative dispersal in relation to social rank may be weak because it is likely that individuals are unable to accurately compare their potential rank in alternative groups relative to their rank in their current group due to the dispersed distribution of coral colonies and the high risk of moving between corals (Lassig, 1981; Munday, 2002). Subordinates in other social animals are known to use their home territory as a base from which they visit neighbouring territories and assess their respective social conditions before making a dispersal decision (e.g. Woolfenden & Fitzpatrick, 1978). For *P. xanthosomus* however, the low mobility and site-attached nature of individuals would generally preclude this sort of behaviour (Lassig, 1981; Munday, 2002).

There was also no indication that dispersal occurred as a result of interactions with a subordinate's immediate dominant i.e. forcible eviction of subordinates, since dispersal was independent of the size ratio between a dispersing subordinate and its immediate dominant. However, subordinates only stand to be evicted at ratios above 0.93 (chapter 4) and they regulate their growth to generally remain below a ratio of 0.93 in natural groups (chapter 4). Therefore, it is hardly surprising that no effect of eviction on dispersal was detected since it is relatively rare for subordinate *P. xanthosomus* to grow in breach of this threshold (chapter 4). Increasing reports of subordinate evictions by dominants in a wide range of social animals suggests that forcible eviction does play an important role in subordinate 'dispersal' behaviour (Taborsky, 1985; Balshine-Earn *et al.,* 1998; Dierkes *et al.,* 1999; Johnstone & Cant, 1999; Cant *et al.,* 2001; Young *et al.,* 2006; chapter 2). This is also the case for *P. xanthosomus*, but since subordinate growth is regulated so that they avoid coming into conflict with their immediate dominants within the group, an eviction effect would only be observed through experimental manipulations (chapter 4).

A greater proportion of subordinates dispersed from their home groups when the dispersal distance between corals was short compared to long, suggesting that costs of dispersal influence whether subordinates tolerate group-living. Costs of dispersal have been shown to constrain the dispersal decisions of subordinates in various cooperative breeding species (Du Plessis, 1992; Russell, 2001; Heg *et al.,* 2004b) as well as in non-cooperative but group-living species (Gardner *et al.,* 2003), suggesting that costs of dispersal are a key factor promoting delayed dispersal and group-living in general. Costs of dispersal are likely to be influencing the current day maintenance of group-living in *P. xanthosomus* since there exists considerable variation in inter-coral colony distances in the environment (Wong, *pers. obs.*). This would have ensured strong selection on subordinates to facultatively adjust their dispersal behaviour in relation to variation in dispersal distances and hence dispersal costs experienced. Distance is likely to be a suitable proxy for dispersal costs given that *P. xanthosomus* resides exclusively within coral colonies of *S. hystrix* which provide a source of food, shelter and breeding sites (Lassig, 1976). As such, individuals are unlikely to possess the adaptations required for efficient movement outside of coral colonies, resulting in increasing costs from energy expenditure with increasing dispersal distances between corals. In addition, increasing distance is also likely to result in increasing risks of mortality as a result of predation given that *P. xanthosomus* is small-bodied and readily consumed by other reef fish (Lassig, 1981; Wong, *pers obs*).

Subordinates rarely dispersed when alternative corals were more than 1 meter apart. However, this is likely to be an under-estimation of the true dispersal potential of *P. xanthosomus* since the current experiment was carried out using coral colonies artificially placed in the sand. Coral colonies are usually located on the reef and in this situation, dispersing individuals might be less conspicuous and have greater opportunities to hide and rest during dispersal (Wong, *pers. obs.*). Indeed, the maximum dispersal distance as yet recorded for this species under natural conditions is approximately 10m (Wong *et al.,* unpub. data). Thus, although costs of movement influence the dispersal behaviour of subordinates and hence play a key role in determining why subordinates tolerate group-living, this does not necessarily mean that subordinates are incapable of dispersing considerable distances if necessary.

Subordinate females showed an increasing preference for group-living as a non-breeder on large corals over immediate breeding on smaller corals as the size difference between coral colonies increased. Although the relationship between coral size and individual fitness was not measured in this study, correlations between territory/habitat size, quality and reproductive success of individuals are commonly found in many animal species (e.g. Goldschmidt & Bakker, 1990; Oring *et al.,* 1991; Brooker & Rowley, 1995). As such, this result suggests that residing as a non-breeding group member in a large coral somehow confers considerable fitness advantages to individuals such that they will forego immediate reproductive opportunities on a small coral. The benefits accrued from remaining in a current group in relation to the quality of the current habitat are therefore likely to be promoting the maintenance of group-living in *P. xanthosomus*. Habitat quality is likely to influence the current day maintenance of group-living in this way since there exists considerable variation in the quality of corals in the environment, ensuring strong selection on subordinates to adjust their dispersal behaviour in relation to variation in coral quality. This is supported by the observation that coral colony sizes of *S. hystrix* range from approximately 5-50cm average diameter (Thompson *et al.,* In press) which is greater than that of a closely related goby, *Paragobiodon melanosomus* (10-25cm average diameter) in which group-living by subordinates does not occur (Thompson *et al.,* In Press).

How might females benefit from group-living in large corals? Since non-breeding subordinates stand to inherit breeding status in the future (chapter 4), the fitness advantages of group-living on a large and thus high quality coral may reflect lifetime reproductive considerations, whereby subordinates incurring fitness costs from missed reproductive opportunities are compensated in the long-term by eventually being able to breed on a high quality habitat (Stacey & Ligon, 1987, 1991; Zack & Stutchbury, 1992; Pen & Weissing, 2000). Since female size is positively correlated with female fecundity in many fishes (Bagenal, 1967), females breeding on large corals may experience a greater reproductive output per breeding attempt if females on large corals grow larger than females on small corals. However, the size of breeding females is not positively correlated with the size of the coral colony (Wong *et al.,* unpub. data), and thus size-related fecundity benefits from living in a large group are likely to be minimal. Alternatively, females may benefit from breeding on larger corals if they have higher survival probabilities than females on smaller corals, resulting in a greater number of reproductive attempts per lifetime on larger corals (Kuwamura *et al.,* 1994). Individuals often have greater chances of survival in habitats or territories containing an abundance of shelter sites (Zack & Ligon, 1985; Shulman, 1985). Thus, survival of *P. xanthosomus* could be enhanced within larger corals (Kuwamura *et al.,* 1994), particularly because their greater depth allows gobies to shelter from predators that are able to pass through the outer branches of the coral (e.g. small wrasses, Wong, *pers. obs.*). Additionally, since group size is positively correlated with coral size (Lassig, 1976; Thompson *et al.,* In Press; chapter 2), females could be benefiting from the presence of other group members rather than the size of the coral per se (Clifton, 1990; Balshine *et al.,* 2001). Since subordinates provide no help to dominants, any benefits accrued from subordinate group members are most likely to arise as by-products of the subordinate's own selfish actions (Clutton-Brock, 2002). Such by-product benefits may include a reduced predation risk due to the predator 'dilution' effect from grouping or increased predator detection due to a greater number of vigilant individuals within the group (Krause & Ruxton, 2002). Further testing of these hypotheses would be required to confirm that females benefit from residing and breeding in large corals.

In conclusion, this study demonstrates that both costs of movement and the quality of alternative corals influence the pay-offs to non-breeding subordinates from group-living. Coral saturation did not affect subordinate dispersal decisions, although the general saturation of corals in the environment is likely to have played a key role in the initial evolution of group-living. Social rank and interactions with dominant individuals also failed to account for patterns of subordinate dispersal. This study lends weight to the idea that the theory of cooperative breeding can provide a useful framework for understanding why subordinates tolerate group-living even when unrelated and unhelpful subordinates are being excluded from current reproduction. In addition, this study emphasises that the strength of selection on subordinates to respond adaptively to potentially fitness-maximising opportunities may often be weak owing to constraints imposed by a species’ ecology over evolutionary time. Therefore, the specific ecological and evolutionary history of a species also needs consideration when evaluating the factors driving the current day maintenance of animal societies.

CHAPTER 4

THE THREAT OF PUNISHMENT ENFORCES COOPERATION AND STABILISES SIZE-BASED QUEUES IN A CORAL-DWELLING FISH

4.1. ABSTRACT

In many animal societies, individuals are organised into dominance hierarchies that serve as queues to inherit dominant, breeding status. Within queues, subordinates often gain no reproductive benefits while they wait and face the prospect of dying before reaching a top ranked breeding position. A natural consequence of queuing is therefore the occurrence of conflict over rank, and hence conflict over reproduction between group members, and this has the potential to de-stabilise societies if left un-resolved. Here I demonstrate that punishment and cooperation promote the stability of size-based queues in a coral-dwelling goby, *Paragobiodon xanthosomus* (Gobiidae). Using a removal experiment in the field, I showed that the size-based dominance hierarchy reflects a size-based queue to inherit breeding status. Quantification of size differences between individuals in groups revealed the prevalence of a specific body-size ratio between individuals of adjacent rank, and analysis of individual growth rates in the field demonstrated that this specific size ratio is maintained over time via the regulation of subordinate growth rates. Staged contest experiments in aquaria between individuals of adjacent rank indicated that the specific size ratio represents a threshold above which subordinates can evict their immediate dominant from the group, but are much more likely to be evicted by their immediate dominant themselves. Taken together, these results suggest that the threat of being evicted forces

subordinates to regulate their own growth to maintain threshold size ratios between themselves and their immediate dominant. Since eviction by dominants is a form of punishment and growth regulation by subordinates is a form of cooperation, queue stability is being achieved through the effects of punishment and cooperation acting in concert to promote the resolution of conflict over rank between group members.

4.2. INTRODUCTION

In many animal societies, individuals are organised into dominance hierarchies that function as social queues (Schwagmeyer & Parker, 1987; Poston, 1997; Field *et al.,* 1999; East & Hofer, 2001; Buston, 2004a; Mitchell, 2005). Within queues, subordinates wait in line for the death or disappearance of those ahead of them in the queue before they themselves ascend in rank and eventually inherit dominant breeding status (Kokko & Sutherland, 1998; Field *et al.,* 1999). Theoretical studies have demonstrated that queues are particularly likely to evolve if subordinates can outlive their dominants (Kokko & Johnstone, 1999) and if the pay-offs from dispersing to breed independently are low due to intense ecological constraints (Kokko & Johnstone, 1999; Ragsdale, 1999; Shreeves & Field, 2005; Buston, 2004a).

Although the evolution of queues is relatively well understood, few studies have addressed the processes involved in promoting the stability of queues over time (Wiley & Rabenold, 1984; Cant *et al.,* 2006; Mesterton-Gibbons *et al.,* 2006). Within social queues, selection should favour a subordinate that managed to challenge and overtake its immediate dominant in rank, since this would increase its probability of inheritance (Wiley & Rabenold, 1984). Such conflict over rank, and hence conflict over access to reproduction, should be particularly intense in queues where subordinates gain no reproductive benefits while they wait and face the prospect of dying before they reach the top ranked breeding position (Field *et al.,* 1999; Buston, 2004a; Mitchell, 2005). Conflict over rank therefore has the potential to undermine the stability of social queues unless mechanisms have evolved to resolve such conflict. Surprisingly, very little is known about the nature of these mechanisms despite the prevalence of stable queues in animal societies.

In some animals, particularly fish, social rank and hence position in the queue is based on an individuals' body size relative to other members of the same group i.e. larger individuals are competitively superior, more dominant, and thus further ahead in the queue than smaller individuals (Kuwamura, 1984; Forrester, 1991; Sakai & Kohda, 1997; Balshine-Earn *et al.,* 1998; Buston, 2003a; Mitchell, 2005). Within such size-based queues, conflict over rank would occur if a subordinate grew so that the size and thus competitive difference between itself and its immediate dominant was sufficiently reduced. Only then would the subordinate be capable of successfully challenging and overtaking its dominant in rank (Buston, 2004a; Buston & Cant, 2006). Therefore, the mechanisms involved in promoting the resolution of conflict over rank within size-based queues would necessarily entail the regulation of subordinate growth rates over time such that subordinates always remain sufficiently small and un-threatening (Buston, 2004a; Buston & Cant, 2006).

Here I propose that the threat of punishment by dominants (Clutton-Brock & Parker, 1995) could play a key role in promoting the regulation of subordinate growth rates within size-based queues. Specifically, when a subordinate grows beyond a specific body-size ratio with respect to its immediate dominant, it becomes capable of challenging and overtaking its dominant in rank. Consequently, the dominant punishes its immediate subordinate by evicting it from the group. The specific size ratio therefore represents a threshold above which subordinates face the threat of punishment by eviction. Provided that being evicted is costly, subordinates would be forced to regulate their growth so that they maintain the threshold size ratio with respect to their immediate dominant. By regulating their growth to maintain the threshold size ratio, subordinates avoid becoming a threat to their immediate dominant and are said to be peacefully cooperating (Buston, 2004b; Buston & Balshine, *In Review*). Therefore, queue stability would ultimately be achieved through the effects of punishment and cooperation acting in concert to ensure the regulation of subordinate growth rates and hence the resolution of conflict over rank between group members. Although various studies of social fishes have demonstrated that subordinate growth rates are influenced by the size of their immediate dominant (Buston, 2003a, 2004a; Heg *et al.,* 2004a), and that dominants sometimes evict subordinates that are large (Taborsky, 1985; Balshine *et al.,* 1998), there is yet no experimental demonstration of the combined effects of dominant punishment by eviction, and subordinate cooperation by growth regulation, on the stability of size-based queues in any species.

Here I tested the ‘punishment-cooperation’ hypothesis in the coral-dwelling goby, *Paragobiodon xanthosomus* (Gobiidae). Firstly, I assessed whether size-based hierarchies act as queues to inherit breeding status, and then tested 4 key predictions arising from the punishment-cooperation hypothesis: 1) there should be a prevalence of a specific size ratio found between group members of adjacent rank in natural groups and this ratio should differ significantly from that obtained from an expected random distribution of size ratios, 2) the growth rates of subordinates should be regulated such that the specific size ratio is maintained between themselves and their immediate dominants over time, 3) subordinates should be capable of challenging and evicting their immediate dominants if they have breached the specific (threshold) size ratio, and 4) dominants should punish immediate subordinates that breach the specific (threshold) size ratio by evicting them from the group. By testing these predictions, I ascertained whether the interplay of punishment and cooperation serves to resolve conflict over rank and hence enhance the stability of these societies over time.

4.3. METHODS

Study site and species

The study was conducted at Lizard Island (14° 40'S, 145° 28'E) on the northern Great Barrier Reef, Australia between March 2004 – November 2005. Field observations and experiments were conducted in the Lizard Island lagoon (Figure 2.1) and aquarium observations and experiments were carried out at Lizard Island Research Station (Figure 2.1). *P. xanthosomus* is a small coral-dwelling fish that inhabits just 1 species of coral, S*eriatophora hystrix* (Pocilloporidae). Coral colonies provide the fish with a source of food, shelter and breeding sites (Lassig, 1976). Coral colonies are spatially discrete units, and within each colony there is a group of gobies consisting of a breeding pair and up to 15 non-breeding females (chapter 2). The breeding male and female (ranks M and F respectively) are the largest group members and similar in size (chapter 2). The remaining females (rank 3 upwards) are smaller than the breeding pair, and are organised into a size-based dominance hierarchy (chapter 2). Subordinate non-breeders appear to provide no assistance to the dominant breeders (Lassig, 1977) and are unlikely to be related to dominants given that newly hatched larvae spend several weeks in a well-mixed pelagic environment before recruiting to the benthic coral habitat (Sale, 1991). *P. xanthosomus* is also a protogynous hermaphrodite, with the dominant female changing sex to male if the male dies or is removed from the group (Lassig, 1977).

Do size-based hierarchies act as queues to inherit breeding status?

To assess whether the size-based dominance hierarchy acts as a queue for breeding, the occurrence of courtship and reproductive behaviour was compared between groups where the breeding female was removed versus groups where the breeding female was not removed. Only the breeding female was removed in this experiment because removal of the male would result in sex change by the dominant female (Lassig, 1977). If group members queue for top ranked breeding positions, removal of the breeding female should result in the occurrence of courtship and reproduction between the breeding male and the largest non-breeding female (i.e. rank 3) only. Twenty coral colonies each containing 5-7 gobies were collected and brought to a waiting boat where they were immediately placed in a large bucket of fresh seawater. Each coral colony was tagged with a numbered ribbon tied around its base and the resident gobies removed by inverting the coral over a bucket of water allowing the gobies to drop out. Gobies were collected from the bucket with a hand net and transferred to labeled zip-lock bags. Coral colonies were then returned to the reef where they were placed along the reef edge with distances of approximately 5m between colonies. A map of the tagged corals along the reef edge was then made. Gobies from each coral colony were returned to the laboratory where they were anaesthetised with clove oil solution (Munday & Wilson, 1997). Body size of each group member was measured (standard length (SL) ± 0.1mm) using calipers. Sex was determined by inspecting the shape of the genital papilla – males have a long, conical papilla and females have a short, blunt papilla (Lassig, 1977). Finally, gobies were uniquely tagged by injecting a small spot of fluorescent elastomer (Northwest Technologies Inc.) into the dorsal musculature. These tags have high retention rates with no adverse effects on growth or survival (Malone *et al.,* 1999).

Ten groups of gobies were randomly assigned to the female removal treatment. For each of these groups the breeding female was removed from the group before releasing all other group members back into their original coral colony. The 10 remaining groups were assigned to the control treatment. For each of these groups all group members including the breeding female were released back into their original coral colony. Behaviour of individuals in each group was observed for ten minutes every other day for 2 weeks, and the occurrence of courtship and reproductive behaviour recorded. Courtship behaviour was defined as the occurrence of reciprocal shivering and energetic activity by partners around the nest site, and reproductive behaviour was defined by the presence of eggs in the nest site (Lassig, 1976*)*.

Is there a prevalence of a specific size ratio?

To assess whether a specific size ratio exists between group members of adjacent rank, 54 natural groups containing a total of 420 individuals were collected and used to create a frequency distribution of body-size ratios. If there is a prevalence of a particular size ratio between group members of adjacent rank in natural groups, the observed distribution of body-size ratios of individuals adjacent in rank should differ from the distribution of body-size ratios expected under a null model (Gotelli & Graves, 1996). Ratios between the breeding male and female within each group were excluded from the analysis since they are effectively of equivalent rank and are no longer queuing for breeding positions (chapter 2). Ratios between ranks 8 upwards were also excluded since these individuals represent the most recently arriving group members that have not yet established a regular size-based hierarchy amongst themselves (Wong, *pers. obs.*). The body size (mm SL) of each group member was measured in order to calculate body-size ratios between group members of adjacent rank. These were expressed as: SL rank N+1 / SL rank N. However, body size measurements were subsequently found to be subject to measurement error since there were considerably more body size measurements to the nearest whole number than to the first decimal place, suggesting that measurement accuracy was not

0.1mm (Figure 4.1). To correct for any effects of this measurement error on the size ratio calculations and resulting size ratio frequency distribution, each value was rounded to its nearest whole number and a random number between -0.5mm to +0.5mm was added to the rounded value. This correcting procedure eliminated bias in the data (Figure 4.1), whilst retaining a continuous distribution for the estimation of size ratios between individuals. Size ratios between adjacent individuals were calculated and a frequency distribution of body-size ratios generated. The whole procedure was iterated 100 times, generating 100 size ratio frequency distributions. The final observed frequency distribution was obtained by taking the mean ± SD of the 100 ratio frequency distributions.

To test whether the observed frequency distribution of size ratios differed from a random distribution of size ratios, a random distribution of size ratios expected under a null model was constructed using a Monte Carlo procedure programmed in MATLAB. This procedure involved the random selection of individuals from the pool of 420 size-corrected individuals and combining them into groups with the same distribution of group sizes found in the original sample. The randomly selected individuals allocated to each group were then ranked according to relative size and the size ratios between group members of adjacent rank calculated. This procedure was iterated 100 times, generating a final expected ratio frequency distribution against which the observed distribution was compared. This null model design was appropriate since it excludes the factor of interest (social interactions between adjacent ranked individuals) whilst retaining all other factors (body size and group size distribution) (Gotelli & Graves, 1996).

Ten of the 100 original frequency distributions were statistically compared to ten of the 100 expected frequency distributions using Kolmogorov-Smirnoff (KS) tests. A frequency distribution of the p-values resulting from these ten comparisons was then compared to a uniform distribution of p-values using a final KS test. If a significant difference exists between the observed and expected frequency distributions, the distribution of p-values should be skewed due to an abundance of p-values close to zero, and the skewed distribution of p-values should differ significantly to that of a uniform distribution of p-values. If no significant difference exists between the observed and expected distributions, the distribution of p-values should range more evenly from zero to one, and thus there should be no significant difference between this and a uniform distribution of p-values.

Is subordinate growth being regulated to maintain the specific size ratio?

To determine whether subordinates regulate their growth to maintain the specific size ratio between themselves and their immediate dominant, the growth rates of subordinates and dominants through time were assessed in relation to the initial size ratio between them. If it is only subordinates that regulate their growth to maintain the specific size ratio, then the growth rate of subordinates (percentage increase in SL per day) through time should be negatively correlated with the initial size ratio between themselves and their immediate dominant, and there should be no correlation between the growth rate of dominants (percentage increase in size per day) and the initial size ratio between themselves and their immediate subordinate. Furthermore, if the growth of subordinates is being regulated to maintain a specific threshold size ratio with respect to their immediate dominant, subordinate growth rate should be equal to that of their immediate dominant when the initial size ratio between them is equal to the threshold ratio. Growth rates were expressed as a percentage increase in body size per day to control for differences in absolute body size. Twelve natural social groups each containing 5-7 gobies were collected. Each goby was measured, sexed and uniquely tagged as previously described and replaced back into its coral colony. Size ratios between ‘subordinates’ (ranks 3-7) and their immediate dominants (ranks 2-6) were then calculated and termed ‘initial size ratios’. Fish were left undisturbed in the field for 6 months whereupon they were collected and re-measured to determine growth rates. Growth rates of subordinate and dominants (percentage increase in SL per day) were calculated from the increase in body size that occurred within this 6 month period, and square-root transformed to reduce skew in the data. A Linear Mixed Effects (LME) analysis was used to test the relationship between individual growth rate and initial size ratio. It was also predicted that an individual's growth rate would not only be affected by initial size ratio, but by the growth rates of other group members, particularly those closest to them in rank. Therefore, an Autoregressive order 1 covariance structure (AR1) was incorporated into the Linear Mixed Effects model to more accurately test the relationship between individual growth rate and initial size ratio. The AR(1) assumes that ranks are autocorrelated with their adjacent ranks, with an exponentially diminishing correlation with ranks further away. Thus any variation due to the growth rate of other group members on the final relationship between growth rate and initial ratio was accounted for. The equation for the LME model was:

$$\text{Growth rate} = \text{initial ratio} + \text{group}_{AR(1)} + \text{error}$$

with the fixed effect being initial ratio and the random effects being $\text{group}_{AR(1)}$ (the autocorrelated group effect) and error (random error). The analysis was first conducted to compare the growth rate of each fish in relation to the size ratio between itself and its immediate dominant. This tested whether subordinate growth rates were being regulated to maintain the specific body-size ratios. The analysis was then repeated to compare the growth rate of each fish in relation to the size ratio between itself and its immediate subordinate. This tested whether dominant growth rates were being regulated to maintain specific body-size ratios.

Can subordinates evict their immediate dominant at ratios above the threshold?

If subordinates regulate their growth to maintain threshold size ratios as a form of cooperation whereby they avoid becoming a threat to their immediate dominant, subordinates should be capable of evicting their immediate dominant from the group at ratios above the specific size ratio (found to be approximately 0.93). To test this, staged contest experiments were conducted to determine the size ratios at which subordinates could evict their dominants. Seven coral colonies, each containing 4-5 gobies were collected from the field and transferred to separate aquaria in the laboratory. Gobies from each group were removed, measured, sexed and uniquely tagged as previously described. The breeding male and female were returned to their original coral colony. Pairs of contestants were generated from the pool of available gobies (or from newly collected gobies). Contestants were matched so they fitted into one of 4 categories of size ratio: 0.85, 0.9, 0.95 and 1. Ratios between contestants were always assigned randomly. The larger fish was designated the dominant and the smaller the subordinate. Contestants were always immature females with no prior experience of each other, and not differing in their original rank by more than 1 rank position. The paired contestants were then released into a trial coral in which neither had prior residence. Contestants were observed continually for 15 minutes from the start of their first interaction and the occurrence of subordinate eviction, dominant eviction, or no eviction was then scored. Previous experiments had shown that contest outcome (i.e. eviction or no eviction) is resolved within 15 minutes (chapter 2). Eviction was scored whenever one contestant left the live part of the coral and either entered the dead base of the coral or a piece of coral rubble placed at the other end of their tank. Contestants were left in their corals overnight and contest outcome re-scored the following morning.

Do dominants punish their immediate subordinate at ratios above the threshold?

If subordinates regulate their growth in response to the threat of eviction by dominants, subordinates should suffer higher probabilities of being evicted at ratios above compared to below the threshold. To test this, the stage contest experiment described above was used to ascertain the size ratio at which subordinates faced a significant threat of being evicted from the group by their immediate dominant.

4.4. RESULTS

Do size-based hierarchies act as queues to inherit breeding status?

The female removal experiment demonstrated that *P. xanthosomus* forms a strict size-based queue for breeding positions within groups. In all experimental groups where the breeding female was removed (n = 10), courtship between the initial rank 3 and the male was observed within two days, and egg clutches were observed within two weeks in 6 of the 10 groups. In no experimental group was the breeding vacancy usurped by a non-breeder from another coral head, neither was there any evidence of courtship or reproduction between non-breeders of initial rank 4 and upward. In control groups where the breeding female was not removed (n = 10), there was no evidence of courtship or reproduction between the male and non-breeders rank 3 or upwards. These results demonstrate that *P. xanthosomus* forms a strict size-based queue for breeding positions within groups.

Is there a prevalence of a specific size ratio?

There was a highly significant difference between the frequency distribution of size ratios between group members of adjacent rank in natural groups compared to the expected frequency distribution generated under a null model (Figure 4.2a and 4.2b) (Kolmogorov-Smirnov test: p = 0.0007). Most noticeably, in the observed frequency distribution of body size ratios there was a peak in ratios between 0.90-0.95 but fewer ratios above 0.95, compared to a peak in ratios of 0.95-1 in the expected distribution (Figure 4.2a). Comparisons between the observed versus expected frequency distributions at each individual ratio category revealed that the observed relative frequency of body-size ratios was significantly greater than the expected relative frequency at ratios of 0.9 – 0.95 (Figure 4.2a) (T-test, $t_8 = 3.04$, $p = 0.016$). The observed relative frequency of body-size ratios was significantly lower than the expected relative frequency at ratios of 0.7 – 0.75 (Figure 4.2a) ($t_8 = -2.95$, $p = 0.018$), and slightly though not significantly lower than the expected relative frequency at the ratio category of 0.95 – 1 (Figure 4.2a) (Mann-Whitney U test, $Z = -1.77$, $p = 0.076$). A Mann-Whitney U test as opposed to a t-test was used for the latter comparison due to non-normality in the distribution of the observed relative frequency data at this ratio category. This result demonstrates that the distribution of body size ratios of individuals adjacent in rank is non-random, and suggests that the growth of individuals is being regulated such that group members adjacent in rank converge onto specific size ratios of 0.90-0.95 over time.

Is subordinate growth being regulated to maintain the specific size ratio?

To determine whether subordinate growth is being regulated to maintain the specific size ratio, the growth rates of subordinates and dominants in relation to the initial size ratio between them was analysed. There was a significant negative relationship between subordinate growth rate and initial size ratio (Figure 4.3 and Table 4.1*a*) (Linear Mixed Effects Model: $n = 38$, $df = 26$, $t = -4.79$, $p < 0.0001$) and a non-significant relationship between dominant growth rate and initial size ratio (Figure 4.3 and Table 4.1*b*) (Linear Mixed Effects Model: $n = 39$, $df = 21.5$, $t = 1.07$, *p*

= 0.297), after controlling for the growth rate of all other group members (Table 4.1*c* and 4.1*d*). Autoregressive group variation accounted for most of the random variation for both subordinate (Table 4.1*c*) and dominant (Table 4.1*d*) growth rates. In addition, the model predicted that the initial size ratio at which the growth of subordinates is equal to that of their immediate dominants is 0.93 (Figure 4.3), within the range of the prevalent size ratios (0.90-0.95) observed in natural groups (Figure 4.2a). Together, these results suggest that the growth of subordinates is being regulated so that they converge onto the specific size ratio of 0.93 with respect to their immediate dominants, but that dominants do not adjust their growth in relation to the size ratio between themselves and their immediate subordinates.

Can subordinates evict their immediate dominant at ratios above the threshold?

Staged contests were used to test the potential of subordinates to usurp the rank of their immediate dominant. When the size ratio between contestants was less than 0.93 (i.e. 0.85 and 0.9), dominants were never evicted by their immediate subordinate (Figure 4.4). However, dominants suffered a significantly higher risk of eviction at ratios above 0.93 (i.e. 0.95 and 1) (Figure 4.4) (Chi-squared test comparing probability of the dominant being evicted above and below 0.93: $X^2 = 12.5$, df = 1, $p = 0.0004$). Subordinates therefore become a threat to their immediate dominant at ratios above 0.93, thus by regulating their growth to maintain threshold body-size ratios of 0.93, subordinates are peacefully cooperating by avoiding becoming a threat to their dominants.

Do dominants punish their immediate subordinate at ratios above the threshold?

The probability of a subordinate being evicted by its immediate dominant increased as the size ratio increased (Figure 4.4), and more importantly, subordinates were approximately twice as much more likely to be evicted by their immediate dominant when the size ratio between them was greater than 0.93 compared to less than 0.93 (Figure 4.4) (Chi-squared test comparing probability of the subordinate being evicted above and below 0.93: $X^2 = 4.52$, df = 1, $p = 0.0335$). Eviction serves as an effective form of punishment for dominants since dominants had significantly greater chances of evicting their subordinate when the size ratio exceeded 0.93 than subordinates had of evicting their dominant (Figure 4.4) (Chi-squared test comparing probability of the dominant and subordinate winning a fight over eviction at 0.95; $X^2 = 6.8$, df = 1, $p = 0.0092$). This demonstrates that dominants punish subordinates that do not regulate their growth to maintain ratios of 0.93 with respect to their immediate dominant.

Table 4.1: Linear Mixed Effects Model of square root transformed growth rate (% increase SL per day) on initial size ratio. **a)** Fixed effects of initial ratio on subordinate growth rate; **b)** Fixed effects of initial ratio on dominant growth rate; **c)** Random effects variance estimates and 95% confidence limits for subordinate growth rate on initial size ratio; and **d)** Random effects variance estimates and 95% confidence limits for dominant growth rate on initial size ratio.

a)

Effect	Estimate	Standard Error	DF	t Value	P
Intercept	1.0602	0.1638	27.4	6.47	<.0001
ratio	-0.8911	0.1861	26	-4.79	<.0001

b)

Effect	Estimate	Standard Error	DF	t Value	P
Intercept	-0.00878	0.2085	22.5	-0.04	0.9668
ratio	0.2534	0.2368	21.5	1.07	0.2965

c)

Covariance Parameter	Subject	Estimate	Alpha	Confidence Limits
AR(1)	Group	0.5581	0.05	0.2774, 0.8389
Residual		0.01796	0.05	0.0110, 0.0344

d)

Covariance Parameter	Subject	Estimate	Alpha	Confidence Limits
AR(1)	Group	0.4927	0.05	0.1598, 0.8256
Residual		0.02140	0.05	0.0132, 0.0407

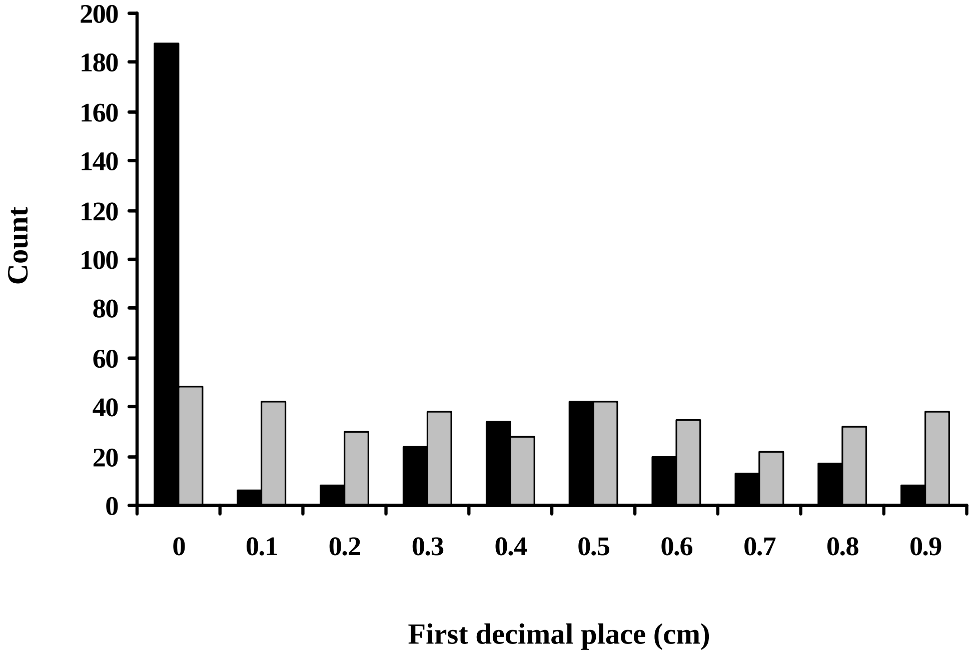


Figure 4.1: Bar graph illustrating the body size measurement bias, showing counts of first decimal places for original data with measurement error (black), and counts of first decimal places for original data with correction for measurement error (grey).

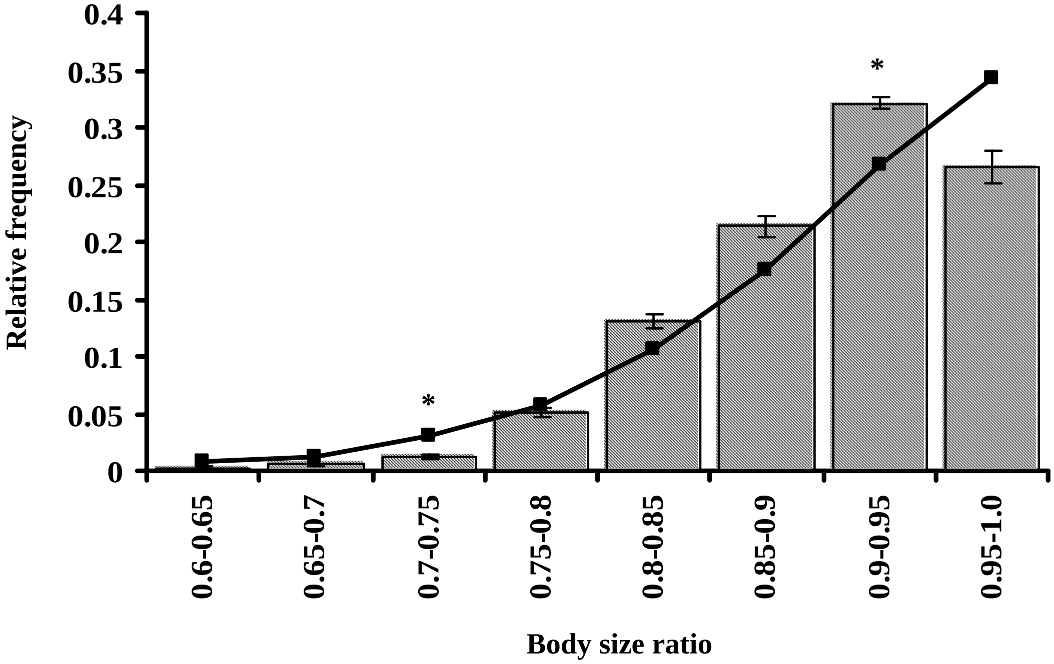


Figure 4.2a. Frequency distributions of body size ratios between group members of adjacent rank. The graph illustrates the observed relative frequency distribution (mean ± S.D.) of ratios between individuals of adjacent rank after correction for measurement error (striped bars), and the expected relative frequency distribution of ratios generated under a null model by a Monte Carlo procedure (solid line). Asterisks (*) indicate for which particular size ratio categories were the observed and expected frequency distributions significantly different at $\alpha = 0.05$.

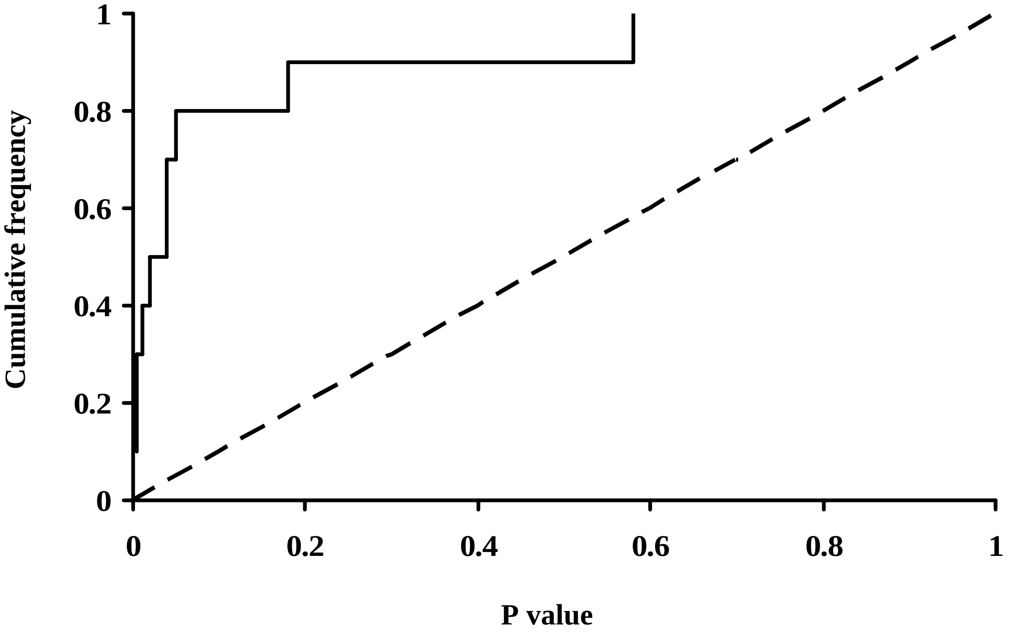


Figure 4.2b: Cumulative frequency distributions of Kolmogorov-Smirnov P-values. The observed distribution of p-values generated from the comparison of 10 observed and 10 expected size ratio frequency distributions (solid line) was compared to a uniform cumulative frequency distribution of p-values (dashed line).

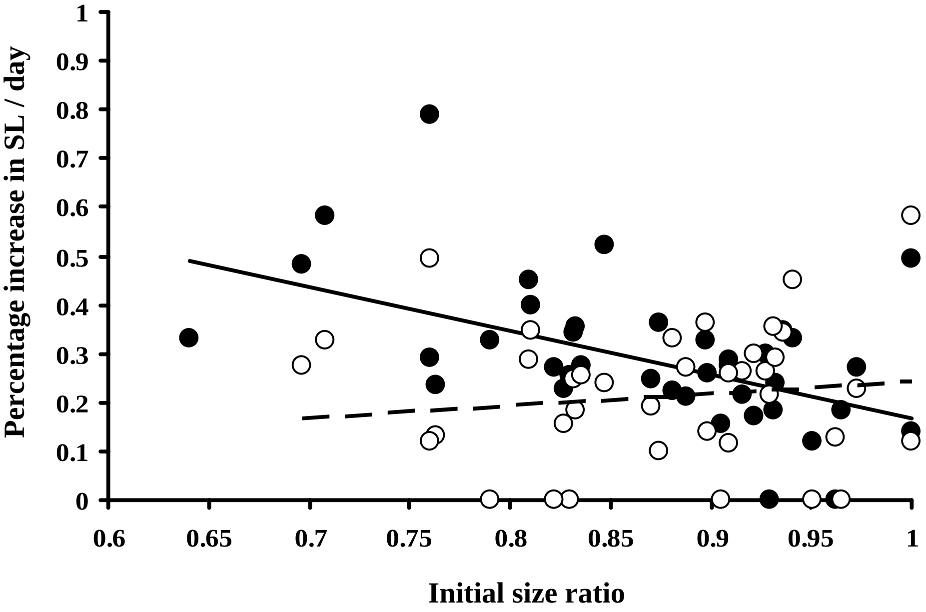


Figure 4.3: Percentage increase in standard length of subordinates per day (filled circles) and dominants (open circles) in relation to the initial size ratio between themselves and their immediate dominant or subordinate respectively. Growth rates are square root transformed. Regression lines show the relationship between initial ratio and subordinate (y = 1.0602 – 0.8911x) (solid line) and dominant (y = -0.00878 + 0.2534x) (dashed line) growth rates estimated by a linear mixed effects model. The point at which the two lines intersect represents the initial size ratio at which the percentage growth rate of subordinates is equal to that of dominants.

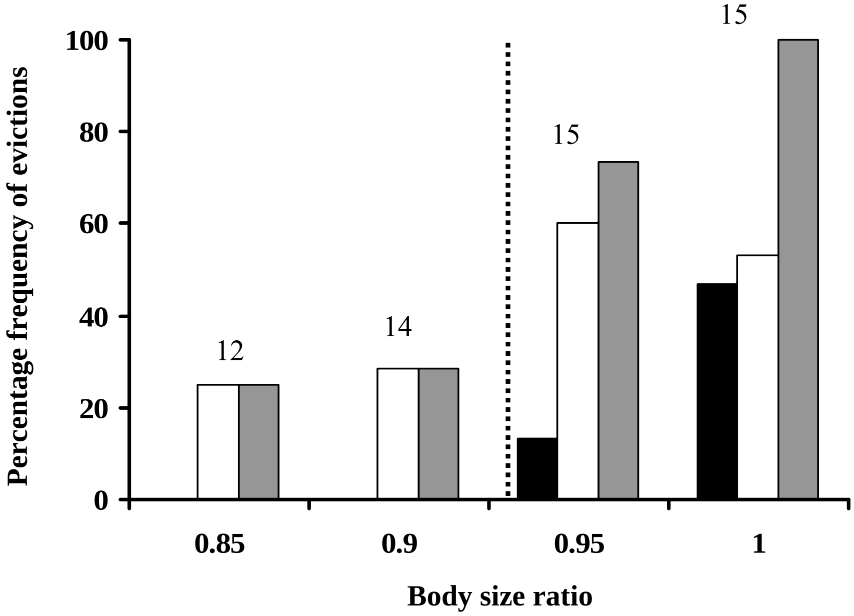


Figure 4.4: Percentage frequency of dominant (black bars), subordinate (white bars) and any evictions (grey bars) occurring in relation to the size ratio between contestants in staged contests. Numbers above bars indicate number of replicate trials per ratio category. Vertical dotted line indicates trials occurring above and below the threshold size ratio (approximately 0.93).

4.5. DISCUSSION

Conflict over rank within social queues raises the perplexing evolutionary problem of how queues can be stable, given that any subordinate that managed to increase its rank by contesting would gain a selective advantage over a subordinate that waited its turn (Wiley & Rabenold, 1984). Pay-offs from queue-jumping would be particularly high, and thus conflict particularly intense, in societies such as *P. xanthosomus* where subordinates gain no direct or indirect reproductive benefits whilst they queue (chapter 2) and face the prospect of dying before they reach the top ranked position (Wong, *pers. obs.*). In this study, I found that there was a predominance of size ratios between 0.9-0.95 in natural groups of *P. xanthosomus* and that subordinate growth was regulated in a way that size differences between individuals converged onto a ratio of 0.93 through time. Staged contests demonstrated that subordinates are rarely evicted at size-ratios below 0.93, but are frequently evicted if they exceed this size ratio. These results suggest that conflict over rank is being resolved through an interplay of dominant punishment by eviction and subordinate cooperation by growth regulation such that stable and well-defined size differences are constantly maintained between individuals over time.

Clearly, conflict resolution through the joint effects of dominant punishment and subordinate cooperation promote the stability of *P. xanthosomus* societies through time. In the presence of punishment and cooperation, subordinate *P. xanthosomus* would grow to approach ratios of 0.93 relative to their immediate dominant. At or below this size ratio, queues are relatively stable given the low frequency of subordinate evictions and the absence of dominant evictions at ratios less than 0.95. In the absence of punishment and cooperation however, subordinates would grow to approach the size of their immediate dominant, since growth in fishes is usually asymptotic and small fish grow more rapidly than large fish (Calder, 1984).

Consequently, this would lead to increasing size similarity between subordinates and their immediate dominants, and given the increased frequency of subordinate and dominant evictions at ratios of 0.95 and above, would lead to the breakdown of societal stability.

The threat of punishment has increasingly been invoked as a key factor promoting the evolution of cooperative and altruistic behaviour amongst non-relatives in human societies (e.g. Fehr & Gachter, 2002; Gardner & West, 2004; Henrich *et al.,* 2006). However, there are relatively few convincing demonstrations of the link between punishment and cooperation in animal societies (e.g. Bshary & Grutter, 2005). In addition, a complete understanding of punishment and cooperation in animal societies would require explanation of how punishment initially evolved in the population (Cant & Johnstone, 2006) and how it co-evolves with cooperation (Lehmann & Keller, 2006). Recently, Cant & Johnstone (2006) suggested that the initial evolution of punishment can be facilitated if its function is ‘self-serving’ i.e. if it provides immediate fitness benefits to the punisher regardless of the response of the opponent. Eviction in *P. xanthosomus* appears to bear the hallmarks of self-serving punishment, since a dominant that evicts a large subordinate is spared from eviction itself. Self-serving punishment is predicted to co-evolve with cooperation if: 1) the costs of being punished are sufficiently high, 2) the costs of punishing are smaller than the fitness gained from punishing, and 3) non-cooperative counterstrategies against punishment cannot be developed (Lehmann & Keller, 2006). All 3 conditions appear satisfied in *P. xanthosomus*: 1) an evicted subordinate has an extremely low probability of moving to another coral owing to intense predation during movement (Lassig, 1981) and a low and unpredictable availability of alternative coral colonies (Munday, 2002), 2) the costs to dominants of evicting subordinates (e.g. due to injury or energy expenditure) are low in relation to the benefits accrued from ensuring they themselves are not evicted (Buston, 2004a; Buston & Cant, 2006), and 3) the costs of retaliation against dominants are high because the physical confines of a coral colony, high rates of mortality outside colonies (Lassig, 1981), and the size and thus competitive differences between individuals (current chapter) ensure that dominants can usually maintain complete control over their subordinates.

Growth regulation by subordinates can be viewed as a form of cooperation since it serves to enhance the fitness of dominants by minimising threats to their rank, and enhance the fitness of subordinates by allowing continued membership within the queue. Such peaceful cooperation, whereby subordinates offset the costs of their presence in exchange for group membership (Kokko *et al.,* 2002; Buston & Balshine, In Review), contrasts with more typical forms of helpful cooperation in which subordinates provide benefits to dominants above and beyond being alone in exchange for group membership (Gaston, 1978; Mulder & Langmore, 1993; Balshine-Earn *et al.,* 1998; Buston & Balshine, In Review). Subordinates therefore need not ‘pay-to-stay’ by providing help to dominants (Gaston, 1978), but can instead ‘pay-to-stay’ within the group by ensuring they remain small and un-threatening (current chapter). Consequently, there appears to be continuum in the concept of cooperation, ranging from helpful to peaceful (Buston & Balshine, In Review). Establishing the conditions under which helpful versus peaceful cooperation should evolve is still in its infancy. Additional benefits from helping e.g. kin selected benefits may predispose subordinates to do more than just offset the costs they inflict (Buston & Balshine, In Review). In the majority of fishes, including *P. xanthosomus*, the dispersive larval phase means that kin selected benefits rarely apply (Sale, 1991), which may in turn reduce the incentives for helpful cooperation.

This study provides a strong indication that a threshold size ratio of 0.93 is being maintained between individuals of adjacent rank within *P. xanthosmous* social groups. Nevertheless, there is still variation in the actual size ratios observed between individuals in natural groups. For example, there are some instances where the body-size ratio between individuals of adjacent rank exceeds 0.93 (Figure 4.2a). Variation around the size ratio could occur due to errors in size estimation by dominants or subordinates (Dall *et al.,* 2005). Alternatively, size ratio variation may occur in response to differential food acquisition which causes variation in subordinate growth rates (chapter 5). Finally, size ratio variation may be adaptive. For example, if dominants experience higher costs of evicting subordinates in some circumstances compared to others (e.g. as a result of the particular architecture or location of the coral colony inhabited), dominants may benefit from increasing the value of the threshold ratio (i.e. allowing their immediate subordinate to become more similar in size to themselves before evicting it), such that they only evict their immediate subordinate when the costs of being challenged by their immediate subordinate outweigh those from evicting their subordinate. Adaptive variation around the threshold size ratio could also occur in relation to an individual's expectation of future direct fitness (Cant *et al.,* 2006; Field *et al.,* 2006). A high ranked subordinate is likely to have greater expectations of future direct benefits than a lower ranked subordinate, since it is next in line to inherit a top ranked breeding position and has a greater probability of surviving to breed (Cant & Field, 2001; Field *et al.,* 2006). Therefore, high ranked subordinates stand to lose more by way of future fitness from being queue-jumped than lower ranked subordinates, and thus experience greater pay-offs from enforcing the threshold size ratio to ensure that their position is not usurped by those below them in the queue.

It could be also argued that the patterns of subordinate growth rate observed in this study do not reflect the actions of subordinates regulating their own growth in response to the threat of punishment, but rather other regulatory factors such as stress imposed on subordinates due to regular harassment or aggression by dominants (i.e. 'top-down' social control: Abbott & Dill, 1989; Booth, 1995; Gilmour *et al.,* 2005). However, results from this study advocate a 'bottom up' mechanism of social control (Buston, 2003a, 2004a) whereby dominants coerce subordinates into regulating their own growth by threatening them with eviction if they fail to comply. Furthermore, experiments in which subordinates were supplied excess food resulted in the reduction in food intake by subordinates even when food was readily available to subordinates and their immediate dominant was not interfering with their feeding (chapter 5). A voluntary reduction in food intake has been reported in subordinates of other species of fish (e.g. Yamagishi *et al.,* 1984; Jobling, 1985; Koebele, 1985) and supports the bottom-up viewpoint that subordinates have the capacity to regulate their own growth.

In addition to social mechanisms of subordinate growth regulation, subordinate growth regulation and the maintenance of well-defined size differences between group members of adjacent rank could occur as a simple result of resource competition (Metcalfe, 1986). Within a hierarchy, dominant individuals usually acquire a larger share of available food resources leaving ever decreasing amounts to more subordinate group members lower in the hierarchy (e.g. Coates, 1980; Forrester, 1991; Webster & Hixon, 2000). As a result, any given subordinate is incapable of acquiring sufficient food to grow to approach the size of its immediate dominant. Although such asymmetries in food acquisition as a result of competitive exclusion by dominants have the potential to promote the regulation of subordinate growth rates and the maintenance of size-based hierarchies, the results of this study point strongly towards the role of social mechanisms, particularly when subordinates reach the threshold size ratio. If food competition were the sole factor promoting subordinate growth regulation, then size ratios between group members of adjacent rank would not be expected to converge predictably onto a body-size ratio lying between the prevalent ratios of 0.9 - 0.95 in the population (i.e. the threshold size ratio of approximately 0.93). Instead, ratios may be expected to remain roughly constant over time or fluctuate in response to random differences in the relative competitive ability of individuals. However, specific experiments designed to investigate the relative influence of social regulation versus food competition would be required to assess this alternative hypothesis, and to provide a more complete picture of the mechanisms resolving conflict and stabilising size-based hierarchies in *P. xanthosomus.*

CHAPTER 5

THE EFFECTS OF FOOD LIMITATION AND SOCIAL REGULATION ON THE STABILITY OF SIZE-BASED QUEUES IN A CORAL-DWELLING FISH

5.1. ABSTRACT

Size-based queues are a familiar trademark of many animal societies, yet a complete picture of the mechanisms stabilising such societies given individual conflict over rank is lacking. Here I investigated whether size-based queues are being maintained in social fishes because: 1) subordinates are competitively excluded from limiting food resources by dominants, and as a result, they never grow large enough to challenge their dominants in rank, and/or 2) subordinate growth is socially suppressed by dominants so they do not grow large enough to challenge dominants. I tested the influences of these mechanisms in the coral-dwelling goby, *Paragobiodon xanthosomus* (Gobiidae), a social coral-reef fish exhibiting well-defined size differences between group members of adjacent rank. Supplemental feeding of subordinates (ranks 3-5) having removed and not removed the dominant breeding female revealed that both feeding and social suppression had positive and additive effects on subordinate growth rate. In a second experiment where only the rank 4 subordinates were fed, the rank 4 subordinates either restrained their own growth despite the presence of additional food, or grew and were subsequently evicted by their immediate dominant. These results support the joint role of food competition and social suppression in the regulation of subordinate growth rates, the maintenance of size differences between individuals of adjacent rank over time and hence the

resolution of conflict over rank. However, once a subordinate reaches a threshold size ratio relative to its immediate dominant, social processes are likely to represent the primary mechanism for the maintenance of stability within *P. xanthosomus* queues.

5.2. INTRODUCTION

Dominance hierarchies are a familiar feature of many animal societies (e.g. birds: Baker *et al.,* 1981; mammals: Fournier & Festa-Bianchet, 1995; fish: Frey & Miller, 1972; reptiles: Schuett, 1997; invertebrates: Reinhard & Rowell, 2005). In general, they are viewed as a means of mitigating conflict between group members over the division of limiting resources such as food, shelter and mates, since stable hierarchical relationships allow for the partitioning of those resources on the basis of social rank rather than on the outcome of repeated fighting (Alcock, 1993). As a result, dominance hierarchies are generally viewed as beneficial to all group members (Alcock, 1993).

In many cases however, the benefits of hierarchy formation are rarely evenly distributed between group members. Higher ranked individuals typically monopolise a disproportionate share of resources at the expense of lower ranked individuals (Appleby, 1980; Craig *et al.,* 1982; Ekman & Askenmo, 1984; Ranta & Lindstrom, 1992; Webster & Hixon, 2000; Stahl *et al.,* 2001), and/or have higher probabilities of inheriting dominant breeding status within the group (Poston, 1997; Field *et al.,* 1999; Buston, 2004a). These asymmetries often translate into enhanced growth, survival and reproductive output of higher ranked individuals (Stamps, 1984; Haley *et al.,* 1994; Ellis, 1995; Poston, 1997; Faulkes & Bennet, 2001; Griffin *et al.,* 2003; Heinze & Obersadt, 2003). As a result, dominance hierarchies set the stage for new conflict in the form of competition over rank, since any individual would stand to benefit from employing strategies that serve to elevate its rank at the expense of other group members (Wiley & Rabenold, 1984). Conflict over rank therefore poses problems for the stability of hierarchical societies, and begs the question of what mechanisms are involved in resolving this conflict so that social order is maintained over time.

In many fish societies, individuals form stable size-based hierarchies that function as queues for the inheritance of breeding status (Balshine-Earn *et al.,* 1998; Buston, 2003a; Hamilton *et al.,* 2006; chapter 4). Within size-based queues, relative body size is the primary determinant of competitive ability, dominance rank and hence position in the queue (Forrester, 1991; Webster & Hixon, 2000; Buston, 2003a, Hamilton *et al.,* 2006). The intensity of competitive interactions appears to increase between individuals as they become more similar in size (Rowland, 1989; Jones & McCormick 2002; Hamilton *et al.,* 2006). In some of these species, size differences between group members of adjacent rank are well-defined, and as a result, reversals of rank between individuals are uncommon (Mitchell, 2003; Buston, 2004a). Mechanisms resolving conflict over rank are likely to reflect the mechanisms promoting the regulation of subordinate growth rates over time so that sufficient size differences are maintained between group members (Buston, 2004a; chapter 4). In the absence of mechanisms regulating growth, any subordinate within a queue would have the potential to grow un-checked and reach a size where it could successfully challenge its immediate dominant, jump the queue and de-stabilise the society.

Subordinate growth regulation within a size-based hierarchy is typically viewed as a consequence of disproportional access to food (Metcalfe, 1986). This hypothesis assumes that food is limited, and that dominants obtain a disproportionate share of available food leaving ever decreasing amounts to their lower ranked subordinates (Metcalfe, 1986). As a result, any given subordinate within a size-based queue would never acquire sufficient food to elevate its growth and approach a size where it could challenge its immediate dominant and overtake it in rank. Thus, by ensuring the 'regulation' of subordinate growth rates, disproportional access to limiting food resources as a result of competitive exclusion by dominants would ensure the maintenance of size differences, social rank and queue stability over time (Figure 5.1).

Support for the food limitation hypothesis comes from various studies demonstrating elevated growth rates of group members in response to supplemental feeding, suggesting that food is in limiting supply (Jones, 1986; Forrester, 1990; Jones & McCormick 2002). In addition, behavioural observations have shown that dominants often acquire a disproportionate amount of food, or higher quality food (Coates, 1980; Metcalfe, 1986; Forrester, 1991; Ryer & Olla, 1996; Webster & Hixon, 2000; Maclean & Metcalfe, 2001; Wittig & Boesch, 2003), which can result in higher growth rates relative to subordinate individuals (Koebele, 1985; Metcalfe, 1986; Jones, 1987; Maclean & Metcalfe, 2001). However, other studies have found differential growth rates between dominants and subordinates even when all fish gain equal and unlimited access to food (Yamagishi *et al.,* 1974; Li & Brocksen, 1977; Jobling, 1985; Abbot & Dill, 1989). This indicates that disproportional food acquisition may not be the sole explanation for the regulation of individual growth rates and the maintenance of size differences within social hierarchies.

Alternatively, subordinate growth rates within size-based queues could be regulated as a result of social suppression (Figure 5.1). This perspective usually invokes a 'top-down' mechanism, where agonistic interactions by dominants suppress the growth of subordinates either because dominant aggression directly inhibits growth as a result of stress imposed on subordinates, or because energy expended from fleeing attacks diverts resources away from growth (Brown 1946; Ochi 1986; Hattori 1991; Booth, 1995; Olsen & Ringo, 1999; Gilmour *et al.,* 2005) (Figure 5.1). According to this hypothesis, dominants should suppress the growth of their immediate subordinates so that they remain at a size where they are incapable of challenging then over rank (Buston, 2004a).

More recently, a ‘bottom-up’ mechanism of social regulation has been proposed whereby queue stability is maintained because subordinates restrict their own growth in response to the threat of punishment, in the form of forcible eviction, by dominants (Buston, 2004a, Heg *et al.,* 2004a; Buston & Cant, 2006; chapter 4) (Figure 5.1). According to this hypothesis, dominants will evict subordinates that grow to a size where they can challenge them in rank (Buston, 2004a; chapter 4). Consequently, subordinates suppress their own growth so they avoid breaching a threshold size ratio relative to their immediate dominant, and as a result, never grow large enough to overtake their immediate dominant in rank (Buston, 2004a; chapter 4). According to this perspective on social regulation of growth, subordinates should suppress their growth by reducing their food intake even when it becomes available to them (Yamagishi *et al.,* 1974; Jobling, 1985; Koebele, 1985; Buston & Cant, 2006). Clearly, either or both top-down and bottom-up processes of social regulation could be involved in the maintenance of size differences mechanisms group members, the resolution of conflict over rank, and hence in the stability of size-based queues over time (Figure 5.1).

Although the effects of food limitation and social processes on the regulation of individual growth rates have received considerable attention, a complete picture of growth regulation and hierarchical stability is still lacking because most studies have considered only one mechanism. Here I experimentally test the effects of both food limitation and social regulation on the growth rates of subordinates, the maintenance of size differences between individuals and the stability of size-based queues for the coral-dwelling goby, *Paragobiodon xanthosomus* (Gobiidae). Individuals of this species are obligately associated with one species of host coral, *Seriatophora hystrix* (Pocilloporidae). Within a coral colony, individuals are organised into size-based hierarchies that function as queues to inherit breeding status (chapters 2 & 4). Previously, I showed that social regulation appears to play an important role in maintaining size differences between adjacent ranked individuals and promoting the stability of size-based queues in this species (chapter 4). Here I experimentally test whether disproportionate food acquisition and/or social regulation control subordinate growth rates and the maintenance of size differences between individuals of adjacent rank. First, I conducted a manipulative experiment in which the non-breeding, subordinate group members within natural social groups were either unfed or fed in both the presence and absence of the dominant, breeding female. Feeding was designed to assess the effects of food limitation on subordinate growth rates, and removal of the dominant female to assess the effects of social suppression by dominants on subordinate growth rates. I then conducted another experiment in which only the rank 4 subordinate was fed. This experiment provided further resolution of the importance of food limitation versus social regulation on the maintenance of size differences between individuals, and enabled me to assess if social regulation of growth was achieved by top-down suppression by dominants or bottom-up self-regulation by subordinates.

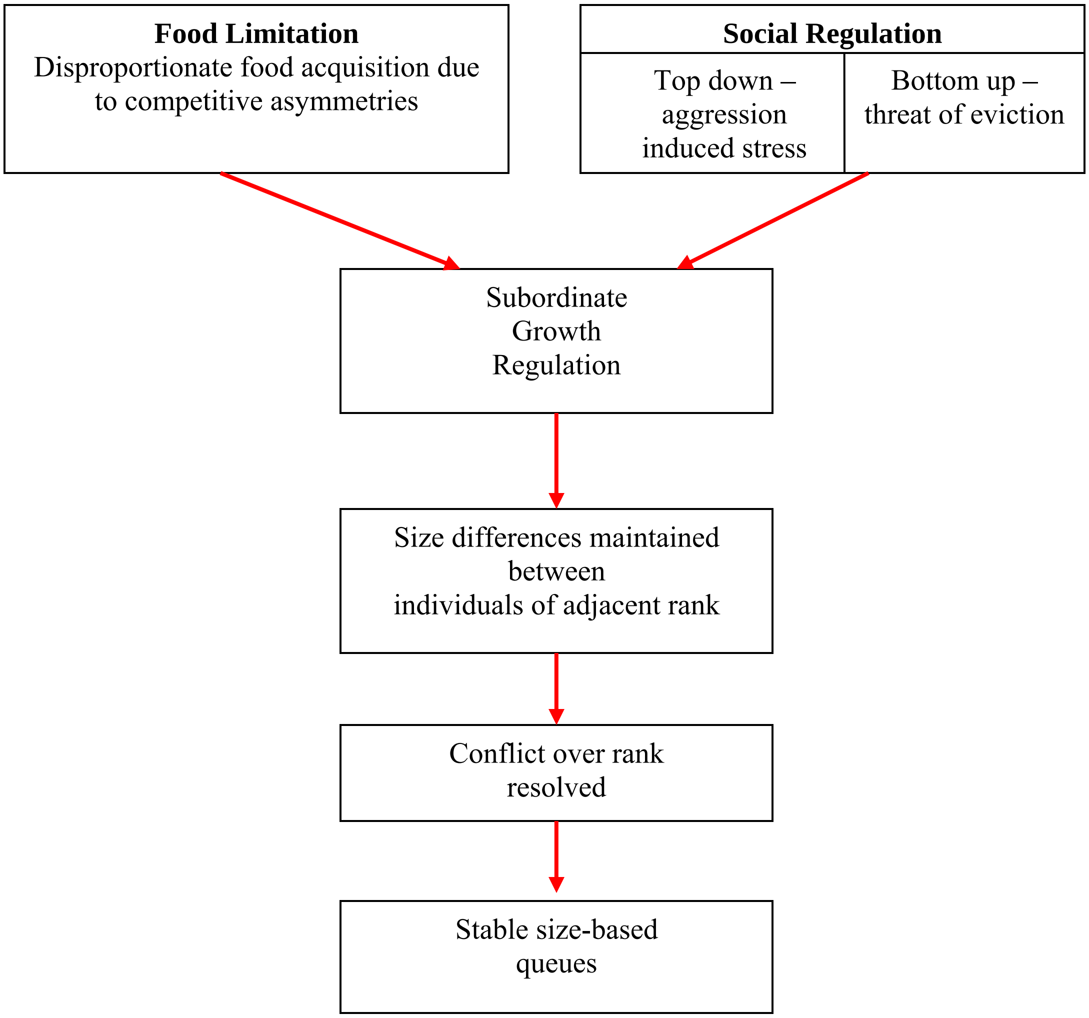


Figure 5.1: Flow diagram illustrating the two potential mechanisms promoting the resolution of conflict over rank between group members and hence the stability of size-based queues over time.

5.3. METHODS

Study site and species

The study was conducted at Lizard Island (14° 40'S, 145° 28'E) on the northern Great Barrier Reef, Australia between February 2005 – November 2005. Field observations and experiments were conducted in the Lizard Island lagoon and aquarium observations and experiments were carried out at Lizard Island Research Station. Social groups of *P. xanthosomus* comprise one large mature male and female (termed rank M and F) that represent the monogamous breeding pair (chapter 2). In addition, groups consist of several smaller, immature females that are organised into a size-based dominance hierarchy (chapter 2). The largest immature female in the group is termed the rank 3, the second largest the rank 4 and so on down the hierarchy (chapter 2). The size-based hierarchy acts as a queue for breeding, with the largest immature female acquiring breeding status in the absence of the mature female (chapter 4). With the exception of the breeding male and female, size ratios between group members of adjacent rank are regulated so that threshold ratios of approximately 0.93 are maintained over time (chapter 4).

Food versus social effects on subordinate growth rate

To determine the relative effects of food limitation versus social regulation on subordinate growth rates, I set-up four experimental treatments in the laboratory: 1) Subordinates Unfed + Dominant Female Present, 2) Subordinates Fed + Dominant Female Present, 3) Subordinates Unfed + Dominant Female Removed and 4) Subordinates Fed + Dominant Female Removed. These treatments allowed me to distinguish whether food limitation, social regulation by the dominant female or both these factors influence subordinate growth rates. Specifically, if subordinate growth rates are regulated solely in response to disproportionate acquisition of food, I predicted that: a) growth rates of subordinates in treatment 1 should exhibit a low, baseline level (Figure 5.2a), b) growth rates of subordinates in treatment 2 should be greater than that in treatment 1, because subordinates are being fed to satiation (Figure 5.2a), c) growth rates of subordinates in treatment 3 should be equal to that of treatment 1, because removal of the dominant female should have no effect on subordinate growth rates (Figure 5.2a), and d) growth rates of subordinates in treatment 4 should be greater than treatments 1 and 3 because subordinates are now being fed to satiation, and equal to that of treatment 2 because removal of the dominant female has no impact on subordinate growth rates (Figure 5.2a). Therefore, there should be no significant statistical interaction between feeding and dominant removal (Figure 5.2a).

Conversely, if subordinate growth rates are regulated solely in response to social regulation by dominants, I predicted that: a) growth rates of subordinates in treatment 1 should exhibit a low, baseline level (Figure 5.2b), b) growth rates of subordinates 2 should be equal to that of treatment 1, because feeding subordinates to satiation should have no effect on growth when the dominant female is present (Figure 5.2b), c) growth rates of subordinates in treatment 3 should be greater than that of treatments 1 and 2, because the dominant female has been removed (Figure 5.2b), and d) growth rates of subordinates treatment 4 should be greater than that of treatment 3, and thus also treatments 1 and 2, because removal of the dominant female subsequently facilitates an additional effect of feeding (Figure 5.2b). Therefore, there should be a significant statistical interaction between feeding and dominant removal (Figure 5.2b).

Finally, if subordinate growth rates are regulated in response to both food limitation and social regulation, I predicted that: a) growth rates of subordinates in treatment 1 should exhibit a low, baseline level (Figure 5.2c), b) growth rates of subordinates in treatment 2 should be greater than treatment 1 given an effect of feeding (Figure 5.2c), c) growth rates of subordinates in treatment 3 should be greater than treatment 1, and approximately equal to treatment 2 (assuming that the magnitude of food versus social effects on subordinate growth rate is approximately equal), given an effect of social regulation (Figure 5.2c), and d) growth rates of subordinates treatment 4 should be greater than all other treatments given the combined effects of both food and social processes (Figure 5.2c).

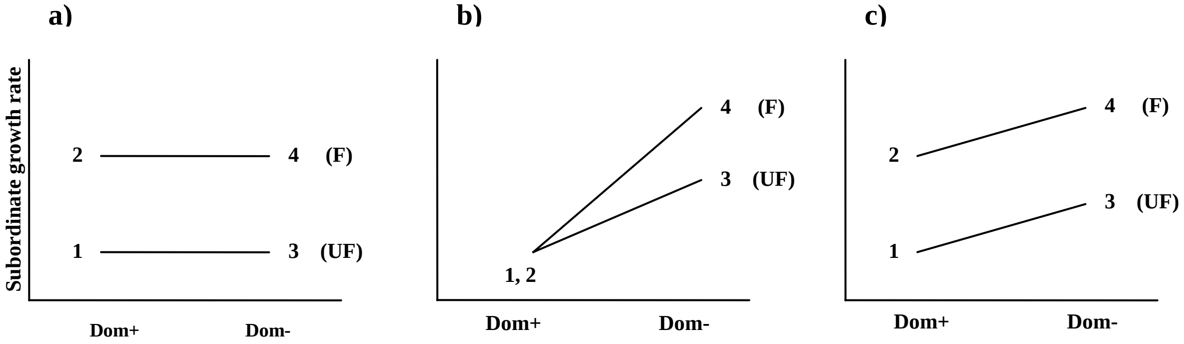


Figure 5.2: Specific predictions relating to the effects of each experimental treatment (1, 2, 3, 4) on the growth rates of subordinates if **a)** food limitation, **b)** social regulation, or **c)** both mechanisms influence subordinate growth rates. UF = subordinates unfed, F = subordinates fed, Dom + = dominant female present, Dom - = dominant female absent.

Twenty-eight colonies of *S. hystrix*, each containing 5 resident gobies (i.e. 2 dominant breeders and 3 subordinate non-breeders), were collected haphazardly from the reef. Corals and their gobies were transferred to the laboratory and placed in separate aquaria supplied with fresh running seawater. Gobies were removed from their coral by inverting the coral over a bucket of water and allowing the gobies to fall out. Gobies were then temporarily anaesthetised with clove oil solution (Munday & Wilson, 1997). The body size of each group member was measured (standard length (SL) ± 0.1mm) using calipers. Length was used as an estimate of size rather than weight, because length is a measure of skeletal growth whereas changes in weight may reflect fluctuations in energy reserves. Sex was determined by inspecting the shape of the genital papillae – males have a long, conical papilla and females have a short, blunt papilla (Lassig, 1977). Each fish was also tagged by injecting a small spot of fluorescent elastomer (Northwest Technologies Inc.) just under the skin in the dorsal musculature. These tags have high retention rates with no adverse effects on growth or survival (Malone *et al.,* 1999). Gobies were then immediately placed back onto their original coral where they recovered fully within 5 minutes.

Seven groups were randomly assigned to each of 4 experimental treatments. In treatments 3 and 4 where the dominant female was removed, this was achieved when group members were measured and sexed. Only the dominant female (and not the dominant male) was removed because she is the primary aggressor within a social group (chapter 2). Removal of the male in addition to the dominant female would also result in the rank 3 subordinate female changing sex to male, confounding the growth responses of subordinates with the energetic cost of sex change (Lassig, 1976).

To assess the influence of food and social factors on group stability, initial body-size ratios between group members of adjacent rank was calculated to enable comparisons of the size ratios at the start versus end of the experiment under each treatment. Size ratios were calculated as: SL rank N+1 / SL rank N, with rank N+1 being the immediate subordinate of rank N (chapter 2). For rank 3 subordinates, the size ratio between themselves and the breeding female were calculated, even if the breeding female was larger than the breeding male, since it was previously shown that conflict only occurs between the breeding female and the rank 3 as opposed to the breeding male and the rank 3 (chapter 2). Size ratios between the breeding male and female (where present) were excluded since they are of effectively equivalent rank and are not engaged in conflict over rank (chapter 4).

Subordinates (ranks 3-5) in the Fed treatments were fed using high nutrient fish pellets (INVE NRD, size 5/8) twice daily. A small pinch of fish pellets was placed into a 5ml syringe, filled with seawater, and the plunger replaced. Each subordinate was then hand-fed by dropping one pellet at a time near the target fish. Within a few days of feeding in this way, the target fish would dart out of the coral and catching the falling pellet in its mouth. Pellets were dropped so that they fell to the aquarium floor if the subordinate missed the pellet, ensuring it would not be consumed by dominants. Feeding of specific subordinates was facilitated because subordinates are usually found on the outer edges of the coral, and because they typically reside in spatially distinct 'sub-territories' within the coral colony (Wong, *pers. obs.*). At each feeding period, subordinates were fed until they had ceased feeding and were judged to be satiated. All fish including dominants had access to any natural food that entered the aquarium in the constant flow of fresh, unfiltered seawater.

After 21 days, all fish were removed from their corals, anaesthetised and body size re-measured to calculate growth rates. Growth rates were expressed as a percentage increase in body size per day to control for differences in absolute body size. Final body-size ratios between group members of adjacent rank were then calculated as previously described. For rank 3 subordinates, the body-size ratio between themselves and the removed female were calculated for treatments where the breeding female had been removed. Gobies were replaced back onto their original coral colonies and returned to their point of collection.

A 2-way ANCOVA was used to analyse the effects of feeding and dominant removal on the growth rate of subordinates, whilst controlling for any possible effect of the initial ratio (covariate) between subordinates and dominants. Growth rate data was checked for normality, homogeneity of variances, linearity, similarity in covariate means, and homogeneity of slopes (i.e. no interaction between the main effects and the covariate), as assumed by the ANCOVA (Quinn & Keough, 2002). Paired t-tests were used to compare the initial versus final body-size ratios between group members of adjacent rank for each treatment.

Food versus social effects on the maintenance of threshold size ratios

To determine the relative effects of food limitation versus social regulation on the maintenance of threshold size ratios between individuals, I used a supplemental feeding experiment in which only the rank 4 subordinate was fed. If threshold size ratios are maintained because of disproportional food acquisition, then supplemental feeding of the rank 4 to satiation should result in the rank 4 growing such that it breaches the threshold size ratio between itself and its immediate dominant and is subsequently evicted or evicts it dominant from the group. Alternatively, if threshold size ratios are maintained because of social suppression of subordinate growth by dominant group members, then feeding the rank 4 to satiation should not result in growth of the rank 4 beyond the threshold size ratio. Finally, if threshold size ratios are maintained by subordinates regulating their own size in relation to the size of their immediate dominant, subordinates should decline additional food and cease growing when they reach the threshold size ratio.

Fourteen coral colonies each containing 4 resident gobies were collected from the reef and transferred to the laboratory where they were placed individually into aquaria supplied with fresh running seawater. Resident gobies were removed from their coral colonies, anaesthetised, measured, sexed and tagged as previously described. The dominant, breeding pair from each group was returned to their original coral colony in the aquaria. The remaining fish from all groups were sorted to create 14 pairs of fish that differed in size by a ratio of 0.92. Previous work revealed that the value of the threshold size ratio for *P. xanthosomus* is approximately 0.93 (chapter 4), thus pairs were manufactured so that when the experiment commenced the ratio between each rank 3 and 4 was just below the threshold. The new pairs of fish were always immature females. Each of the 14 manufactured subordinate pairs was introduced into one of the 14 coral colonies already containing the breeding male and female. To control for any effects of prior residency, no fish was placed back into its original coral. In 7 of the groups, the rank 4 subordinate was fed 3 times a day for 21 days using the syringe method described previously ('fed' groups). In the remaining 7 groups, the rank 4 subordinate was not fed ('control' groups) and only had access to any natural food arriving in the supply of unfiltered seawater. All groups were monitored daily and the occurrences of any eviction recorded (chapter 4). On completion of the experiment, each group member from all 14 groups was re-measured in order to determine final size ratios. Corals and their resident gobies were then returned to their original collection site. Statistical tests could not be applied due to small sample sizes in this experiment.

5.3. RESULTS

Food versus social effects on subordinate growth rate

Subordinate growth rates (percent change in SL / day) did not differ in relation to the rank of the subordinate for each of the 4 treatments (2-way ANCOVA: Rank, df = 2, F = 1.65, *p* = 0.2), therefore growth rates of subordinates ranked 3-5 were combined in each treatment for all subsequent analyses. Growth rates of the dominant male and female (where present) did not differ between the 4 treatments (ANOVA, df = 3, F = 0.66, *p* = 0.58). Both supplemental feeding and dominant removal had significant positive effects on subordinate growth rate (Figure 5.3) (2-way ANCOVA: Feeding, df = 1, F = 9.54, *p* = 0.003; Dominant removal, df = 1, F = 8.26, *p* = 0.005) having controlled for the effects of initial size ratio (covariate; df = 1, F = 12.8, $p < 0.001$). There was no significant interaction between the effects of feeding and dominant removal (Figure 5.3) (df = 1, F = 0.17, *p* = 0.68).

By the end of the experiment, the body-size ratios between group members of adjacent rank had increased (i.e. the size difference became smaller) in groups where subordinates were fed compared to groups where subordinates were unfed. For subordinates that were unfed in the presence of the female, there was no significant difference between the mean initial body-size ratio (mean ± S.E. = 0.854 ± 0.014) and mean final body-size ratio (0.852 ± 0.015) between group members of adjacent rank (Figure 5.4a) (Paired t-test, $t_{16} = 0.52$, *p* = 0.61). This indicates that size differences between group members of adjacent rank did not change over time. In contrast, when subordinates were fed in the presence of the female, the mean final body-size ratio (0.881 ± 0.011) between group members of adjacent rank was significantly larger than the mean initial body-size ratio (0.864 ± 0.014) (Figure 5.4b) ($t_{17} = -2.28$, *p* = 0.036), indicating that size differences between group members of adjacent rank became smaller over time. In addition, the mean final body-size ratio between rank 3 subordinates and breeding females in this treatment (0.858 ± 0.019) was significantly larger than the mean initial body-size ratio between them (0.833 ± 0.019) (Paired t-test: $t_6 = -3.61$, p = 0.011). This resulted from significantly higher growth rates of the rank 3 subordinate (0.156 ± 0.03) compared to the breeding female (0.001 ± 0.02) in this treatment ($t_6 = -4.2$, $p = 0.006$).

For groups where subordinates were unfed in the absence of the breeding female, there was no significant difference between the mean initial body-size ratio (0.826 ± 0.016) and mean final body-size ratio (0.849 ± 0.017) between group members of adjacent rank (Paired t-test, $t_{16} = -1.42$, $p = 0.17$) despite a trend towards larger final mean ratio (Figure 5.4c). However, when subordinates were fed in the absence of the female, the mean final body-size ratio (0.869 ± 0.016) between group members of adjacent rank was significantly larger than the mean initial body-size ratio (0.828 ± 0.019) (Figure 5.4d) ($t_{20} = -2.79$, $p = 0.01$), indicating that the size difference between group members of adjacent rank become smaller over time.

Food versus social effects on threshold size ratios

Two distinct responses were observed in the seven groups where only the rank 4 subordinate was fed. In four of the fed groups, none of the rank 4 fish were evicted from the group by the end of the experimental period. These rank 4 fish did not grow despite supplemental feeding. Consequently, the body size ratio between the rank 4 fish and the rank 3 fish at the end of the experiment was similar to that at the start of the experiment (Figure 5.5). The average growth rate (percent change in SL / 21 days) (mean ± S.E. = -0.005 ± 0.184) of these rank 4 fish was many times higher than the growth rate of rank 4 fish in the unfed controls (mean ± S.E. = -1.776 ± 0.587), indicating that supplemental feeding had a positive effect on growth. In 2 of these 4 Fed groups, the rank 4 subordinate showed clear signs of a reduction in food intake as the experiment progressed. These individuals ceased feeding after 8-10 days and continued to ignore the majority of food pellets dropped in their direct vicinity even when the rank 3 was not interrupting their feeding, suggesting a voluntary reduction in food intake.

In the remaining three fed groups, the fed rank 4 subordinates were evicted from their groups by the end of the experimental period. The average growth rate of these rank 4 fish (percent change in SL / 21 days) (mean ± S.E. = 3.612 ± 1.768) was approximately 4.6 times greater than that of the unfed rank 3 fish (0.777 ± 0.284). As a result, the size ratio between the fed rank 4 and the unfed rank 3 increased from an average of 0.922 to 0.948 by the end of the experiment (Figure 5.5). Although evictions themselves were not observed and thus the identity of the 'evicter' could not be confirmed, I previously demonstrated that rank 4 subordinates were always evicted by their immediate dominant i.e. the rank 3 as opposed to any other group member (chapter 4).

In all 7 control (unfed) groups, none of the rank 4 subordinates were evicted by the end of the experimental period. These rank 4 subordinates exhibited negative growth rates over the 21 day period (percent change in SL / 21 days) (mean ± S.E. = -1.776 ± 0.587) i.e. they decreased in body size. This resulted in a marked reduction in the size ratio between the ranks 3 and 4, from an average of 0.919 to 0.874 (Figure 5.5). This pattern was unexpected given previous evidence that threshold size ratios of approximately 0.93 are maintained over time (chapter 4), but may be related to the restricted food availability in this treatment.

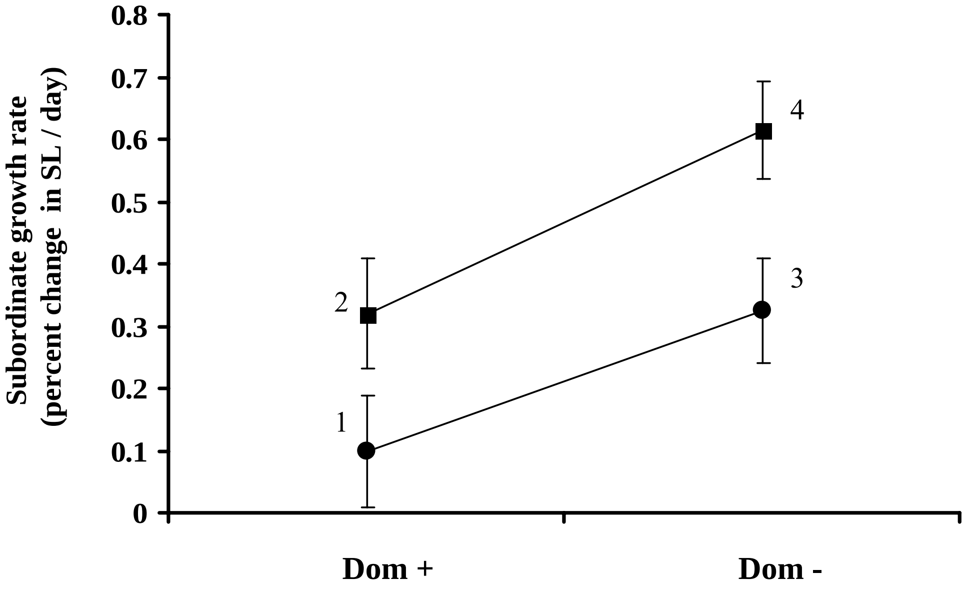


Figure 5.3: Growth rates (percent change in SL / day) of subordinates that were unfed (circles) and fed (squares) in the presence (dom +) and absence (dom -) of the dominant, breeding female**.** Least square means ± S.E. are shown. Numbers represent treatment numbers.

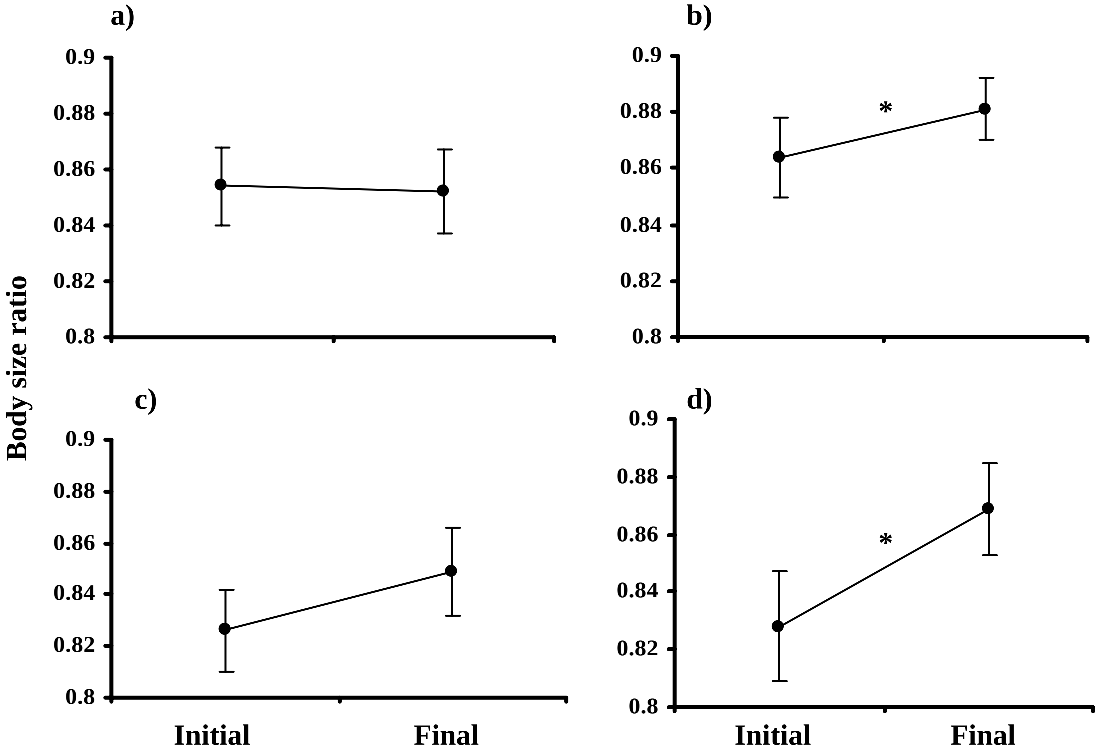


Figure 5.4: Mean ± S.E. body-size ratios at the start (initial) and end (final) of the supplemental feeding and female removal experiment. **a)** Treatment 1 where subordinates (ranks 3-5) were unfed in the presence of the breeding female, **b)** treatment 2 where subordinates were fed in the presence of the breeding female, **c)** treatment 3 where subordinates were unfed in the absence of the breeding female and **d)** treatment 4 where subordinates were fed in the absence of the breeding female. Asterisks (*) indicate a significant difference at $\alpha = 0.05$.

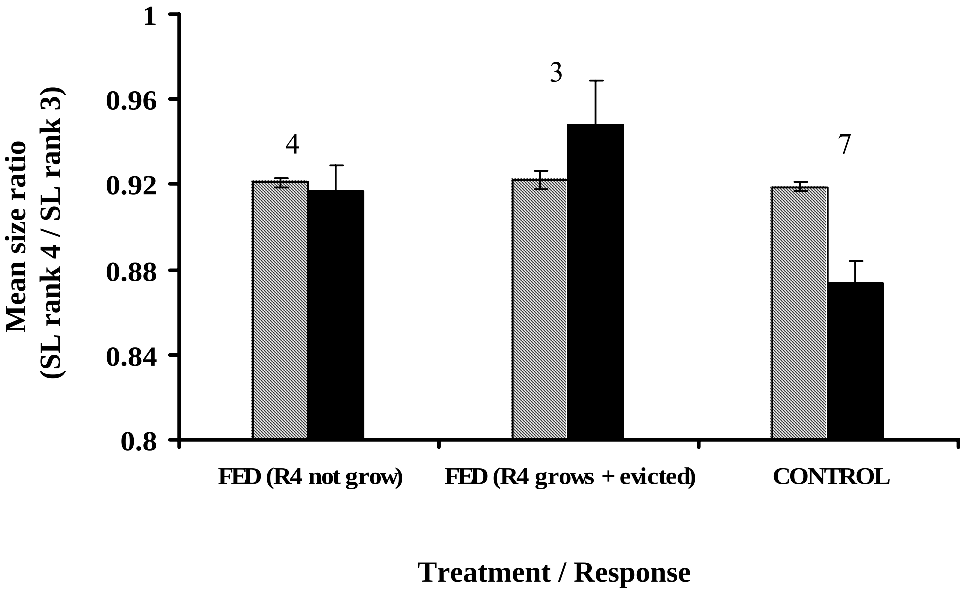


Figure 5.5: Mean size ratios ± S.E between ranks 3 and 4 at the start (striped bars) and end (filled bars) of 21 days supplemental feeding of rank 4 fish. Fed groups are those in which the rank 4 was fed to satiation throughout the experimental period. No group members were fed in control groups. Numbers above the bars show the number of replicate groups per result.

5.5. DISCUSSION

Identifying the factors involved in the regulation of subordinate growth rates provides the key to understanding the structure and stability of size-based dominance hierarchies. In the first experiment, removal of the breeding female resulted in an acceleration of subordinate growth rates, demonstrating that the presence of dominant group members is important for the regulation of subordinate growth rates and the maintenance of size differences within the hierarchy. Supplemental feeding of subordinates resulted in a similar increase in subordinate growth rates within the hierarchy, even when the breeding female was present. This demonstrates that food is a limiting resource for subordinate growth, and therefore, that the size of subordinates is not just constrained by the presence of the breeding female. Given that there was no statistical interaction between these factors, these results support the joint and additive roles of social regulation and food limitation in the regulation of subordinate growth rates and the maintenance of size differences within size-based queues in *P. xanthosomus*.

To my knowledge, this is the first experimental demonstration of the effects of both disproportional food acquisition and social regulation on the growth rate of subordinates using naturally established social hierarchies of reef fishes. Other studies have examined the influence of just one mechanism, primarily social regulation, whilst controlling for any effects of disproportional food acquisition (Yamagishi *et al.,* 1974; Jobling, 1983, 1985; Abbott & Dill, 1989), thus the relative influences of the two processes could not be determined. The most comparable study was conducted by Koebele (1985), who tested the relative effects of both disproportional food acquisition and social effects (namely stress and activity differences) on the growth rates of the cichlid, *Tilapia zillii*. In contrast to the current study, there was no evidence for social effects on individual growth rates or size variation within groups, supporting the role of disproportional food acquisition alone in the reinforcement of subordinate-dominant size differences within the hierarchy. The differences between this and the current study may reflect differences in the benefits of social regulation between the two species. In *P. xanthosomus*, social hierarchies function as queues for the inheritance of dominant breeding status, with individuals gaining no current reproductive success while they queue (chapter 4). Therefore, dominant individuals would benefit from continually suppressing the growth of their immediate subordinates in order to maintain their position in the queue. In addition, subordinates would also benefit from controlling their own growth relative to their immediate dominant because they stand to be evicted from the queue if they grow too large (chapter 4). In contrast, *T. zillii* does not form stable social groups or queues to inherit breeding status under natural conditions (Fryer & Iles, 1972) therefore it seems unlikely that individuals would be selected to develop the ability to socially suppress the growth of other individuals.

The additive effect of supplemental feeding on subordinate growth rate demonstrates that food is a limiting resource for *P. xanthosomus* societies. Dominant individuals in size-based hierarchies often gain disproportional access to food and other essential resources at the expense of subordinates (e.g. Webster & Hixon, 2000; Whiteman & Côté, 2004b). Although relative food acquisition of dominants and subordinates was not quantified here, other studies that have assessed the natural foraging behaviour of site-attached, plankton feeding fish have demonstrated that dominant group members are capable of defending prime feeding areas and acquire more food and/or higher quality food than subordinate group members (Coates, 1980; Forrester, 1991; Webster & Hixon, 2000). In *P. xanthosomus*, the larger the group member, the lower the probability it will be the target of aggression from other group members within the coral (chapter 2). This would allow individual mobility and hence foraging capacity to increase with increasing dominance, making it likely that differential food acquisition in relation to rank could occur in *P. xanthosomus* (Wong, *pers. obs.*).

In the first experiment, supplemental feeding of subordinates in both the presence and absence of the breeding female resulted in a significant increase in the body-size ratios between group members of adjacent rank i.e. individuals became more similar in size to each other over time. In addition, the size ratio between rank 3 subordinates and breeding females in groups where subordinates were fed increased significantly over time as a result of enhanced growth rates of the rank 3. In contrast, size ratios did not significantly increase over time in groups where subordinates were unfed. These results support the interpretation that subordinates are food limited and suggests that additional food enables subordinates to grow more rapidly so that they approach the threshold size ratio (chapter 4) with respect to their immediate dominant as quickly as possible. Furthermore, the increase in size ratios as a result of supplemental feeding suggests that size ratios may vary among social groups in relation to food availability. Groups subject to high levels of food, for example if their host coral is located in an area of high current velocity (Mcfarland & Levin, 2002), would be predicted to exhibit larger ratios between group members of adjacent rank compared to groups receiving less food, because subordinates would able to grow up to the threshold size ratio more quickly when food is abundant. This is consistent with the observation that size ratios between individuals of adjacent rank show variation around the threshold (chapter 4). Since subordinate growth rates are affected by food availability, both within- or between-group variation in food availability could contribute to variation in the observed size ratios. Analysis of body-size ratio variation in relation to feeding patterns would therefore be an interesting area of future research.

Within natural social groups, subordinates converge onto threshold size ratios of approximately 0.93 relative to their immediate dominant, and any subordinate that breaches the threshold stands a high probability of being evicted from the group (chapter 4). Combined with the fact that removal of the dominant female resulted in a significant increase in subordinate growth rates in the first experiment, these results provide strong support for the importance of socially-mediated mechanisms in the regulation of subordinate growth rates and thus the maintenance of size differences within size-based queues. Social regulation of subordinate growth has typically been viewed solely as a product of ‘top down’ processes whereby stress inflicted by dominants or increased energetic expenditure as a result of aggressive interactions with dominants results in subordinate growth suppression (Brown 1946; Ochi 1986; Hattori 1991; Booth, 1995; Olsen & Ringo, 1999; Gilmour *et al.,* 2005). Alternatively, recent studies in social fishes have emphasised a ‘bottom up’ perspective whereby subordinates restrain their own growth to avoid inflicting costs on dominants and thus coming into conflict with them (Buston, 2003a, 2004a; Heg *et al.,* 2004a; Buston & Cant, 2006; chapter 4). In support of the bottom-up perspective, the second experiment of the current study indicated that subordinates are capable of restraining their own growth. In 2 of the 4 Fed groups in which the rank 4 subordinates barely grew, they showed clear signs of a reduction in food intake as the experiment progressed. Following high rates of feeding for the first 8-10 days, these subordinates suddenly ceased feeding and continued to ignore the majority of food pellets dropped in their direct vicinity even when the rank 3 was not interrupting their feeding, suggesting they have voluntarily reduced their food intake. Voluntary reductions in food intake have also been reported for other fish species (Yamagishi *et al.,* 1974; Jobling, 1985; Koebele, 1985), indicating that subordinates may have greater control over the regulation of their own growth than is generally expected. Combined with the prevalence of threshold size ratios in natural populations that are maintained via subordinate growth regulation (chapter 4) and the occurrence of subordinate eviction when in breach of the threshold ratio (current study and chapter 4), the demonstration of reduced food intake in the second experiment provides further support for the role of the threat of eviction in promoting the self-regulation of subordinate growth and body size (Buston, 2004a).

In the second experiment, the reduction in the size ratios between ranks 3 and 4 in the control groups was surprising given that threshold size ratios of approximately 0.93 are maintained between individuals of adjacent rank over time (chapter 4). However, the fact that ratios of 0.93 were not maintained suggests that additional factors were influencing the value of the size ratio actually expressed at the time of the experiment. It is possible that unfamiliarity between the manufactured rank 3 and 4 individuals in the experiment resulted in elevated levels of social instability between group members causing the suppression of subordinate growth rates to a level below that observed in stable groups. Support for this comes from the observations that interactions between unacquainted individuals are often more aggressive than between acquainted individuals (e.g. Issa *et al.,* 1999; Broom, 2002). Once dominance relationships are fully established, ratios between the ranks 3 and 4 would be expected to increase over time and approach the threshold size ratio, as is observed in natural populations of *P. xanthosomus* (chapter 4), although further experiments are needed to test this hypothesis. Additionally, the reduction in size ratios between rank 3 and 4 fish may have occurred because the rank 4 subordinates in the unfed (control) treatments experienced severe food deprivation during the experiment. Under natural conditions, a coral colony would be exposed to a significantly larger volume of water and its associated planktonic food compared to that under aquarium conditions. Planktonic food sources may therefore have been unnaturally low in the current experiment resulting in the negative growth rates of rank 4 subordinates and hence the reduction in size ratios. No reduction in size ratios was observed in the fed groups, presumably because supplemental feeding of the rank 4's was sufficient to compensate for any suppression of subordinate growth rates as a result of unfamiliarity or low food availability.

The second experiment also provided an insight into the relative effects of food limitation and social regulation on the maintenance of hierarchy stability. This experiment was designed so that the initial body-size ratio between a rank 4 subordinate and its immediate dominant was just below the threshold, enabling testing of the relative effects of food and social processes on queue stability. If food limitation is responsible for stability, supplemental feeding was predicted to have resulted in the growth of subordinates over the threshold and the subsequent breakdown of group stability as evidenced by eviction of either the rank 3 or the rank 4 individuals. If social regulation is responsible for stability, the growth of the rank 4 was predicted to have been suppressed in the presence of the rank 3 despite supplemental feeding such that size ratios between them do not breach the threshold. In 4 of 7 groups where the rank 4 subordinate received supplemental food, the rank 4 did not grow over the threshold size ratio, suggesting that social suppression of growth became the primary force maintaining queue stability once the subordinate approached the threshold size ratio in these groups. In the remaining 3 groups however, the rank 4 subordinates grew over the threshold size ratio and were subsequently evicted by the rank 3's, which is consistent with food limitation rather than social suppression of growth in promoting stability in these groups.

Although it appears that food limitation might have influenced stability in these last 3 groups, it is difficult to imagine how limited access to food can be the primary and sole cause of the maintenance of threshold size ratios observed between individuals of adjacent rank over time (chapter 4). Thus, it is more likely that both food and social processes were involved in the regulation of threshold size ratios within these groups, but that the social component of subordinate growth regulation was for some reason diluted in the second experiment. One possible reason for the diluted effect of social suppression could relate to an experimentally-induced difficulty in suppressing subordinate growth given that the rank 4's were being fed pellets of extremely high nutrient content (http://www.inve.com/fish). It is unlikely that natural food resources of *P. xanthosomus* are of such high nutritional value, thus the elevation in growth rates as a result of consuming food of high quality may have exceeded the growth suppressive capacities of either dominants or subordinates. Another potential reason for the lack of growth suppression could relate to differences in the rates of interaction between ranks 3 and 4 (Sneddon *et al.,* 2005). Reduced rates of interaction e.g. due to spatial separation within the coral may have reduced the levels of aggression as well as the perceived threat of eviction, hence reducing the effectiveness of social processes promoting growth suppression. Alternatively, the rank 4 subordinates in these groups may have grown despite the presence of social suppression in order to challenge the rank 3's over rank. Previously, I demonstrated that a subordinate that grows over the threshold size ratio has a small possibility of winning the contest and evicting its dominant despite an overwhelming probability of being evicted from the group itself (chapter 4). Consequently, it may pay some subordinates to evade social suppression and challenge their immediate dominants over rank, depending on the pay-offs they receive from queuing peacefully versus contesting (Buston, 2004a; Cant *et al.,* 2006).

In conclusion, this study demonstrates that both food limitation and social processes are involved in the regulation of subordinate growth rates and thus the maintenance of size differences within size-based queues in *P. xanthosomus*. As a result, both food limitation and social regulatory mechanisms help to promote the resolution of conflict over rank. However, once subordinates reach the threshold size ratio, social mechanisms of growth regulation are likely to exert a greater influence on the maintenance of threshold size ratios and hence the stability of size-based queues, with food limitation playing a secondary role. Social regulation can potentially be both top-down and bottom-up – however the occurrence of reduced food intake by subordinates in conjunction with previous evidence (chapter 4) provides stronger support for the role of bottom-up self-restraint mechanisms of subordinate growth regulation in response to the threat of eviction. Conflict over rank in *P. xanthosomus* hierarchies is thereby resolved as a result of both the threat of punishment by dominants and constraints imposed on subordinates as a result of competitive exclusion and disproportional access to food.

CHAPTER 6

GENERAL DISCUSSION

A comprehensive insight into the evolution and maintenance of animal societies requires that three fundamental questions in behavioural ecology be addressed: 1) what ecological factors promote the evolution of mating systems? 2) what ecological factors promote the evolution of group-living? and 3) why are there conflicts of interest between group members and how are they resolved? I investigated these questions for a coral-dwelling reef fish, *Paragobiodon xanthosomus* (Gobiidae), using a combination of behavioural experiments and observations in the laboratory and field which were designed to test key hypotheses regarding the evolution and maintenance of reproductive and social behaviour. I show that the evolution and maintenance of monogamy, in conjunction with various ecological factors, underpins the evolution and maintenance of social groups, and that monogamy within social groups provides fuel for conflict over reproduction which in turn has a powerful influence on the structure and stability of the society. This thesis therefore provides a comprehensive investigation into the formation, structure and stability of an animal society, demonstrating the mechanisms responsible for the occurrence of monogamy, group-living and conflict resolution.

In *P. xanthosomus,* monogamy occurred within the context of social groups in which additional females are immediately available to males. Therefore, the classical hypothesis that monogamy evolves in response to constraints on the ability of males to monopolise females due to a widespread and uniform dispersion of limiting resources (Emlen & Oring, 1977) does not apply to *P. xanthosomus*. Instead, monogamy in *P. xanthosomus* is likely to have evolved and be maintained in response to female competition over limiting food resources and paternal egg care, which selects for the suppression of subordinate reproduction by dominant females. The occurrence of monogamy as a result of female competition over limiting food resources is consistent with that found in other social animals (e.g. Woodroffe & MacDonald, 1995; Clutton-Brock *et al.,* 1998). To my knowledge however, this study provides the first experimental demonstration of the occurrence of paternal care constraints in a monogamous fish, lending empirical support to this hypothesis (Kuwamura *et al.,* 1993; Whiteman & Côté, 2004a). The demonstration of paternal care constraints in *P. xanthosomus* is also particularly intriguing since in other species of goby with paternal egg care, males are clearly capable of caring for multiple clutches and hence mating polygynously (e.g. Lindstrom & Seppa, 1996; Karino & Arai, 2006). This contrast raises the question of why males of some species appear to suffer higher costs of paternal care than others. To answer this question, quantitative analyses into the potential ecological, social and biological factors underlying the variation in costs of paternal care between monogamous and polygynous gobies would be required. Future research in this area would therefore be important for providing a more complete picture of the role of paternal care constraints in the evolution of mating systems in general.

Various mechanisms for the suppression of subordinate reproduction by dominant females have been proposed, including the physical exclusion of subordinate group members from the male (Sandell, 1998; Kokita, 2002), or the direct suppression of the reproductive behaviour or physiology of other females within the group (Faulkes & Bennett, 2001; Young *et al.,* 2006). Here I showed that reproductive suppression by females in *P. xanthosomus* occurs via a novel mechanism that has yet to be invoked for other monogamous species. Specifically, dominant, breeding females employ the threat of eviction to ensure that subordinate females suppress their own size and reproductive status. The suppression of subordinate size and reproductive status is beneficial to breeding females, since large and mature subordinates pose the greatest threats as resource competitors. In contrast to the traditional viewpoint that the size and reproductive status of subordinates is directly determined by dominants (Faulkes & Bennett, 2001; Young *et al.,* 2006), this mechanism emphasises that dominant control over subordinates is indirect, and that subordinates play a more active role in determining their own size and reproductive status within the group. I expect that the threat of eviction has the potential to serve as a powerful suppressive mechanism in any species where the costs of evicting for dominants are low relative to the costs of being evicted for subordinates. This would apply to species in which dispersal between groups is energetically costly or risky, where the presence of subordinates does not provide significant benefits to dominants because they are un-helpful or un-related to dominants, and when the balance of power between individuals in the group is biased towards dominants, for example due to their greater size, age or experience (Beekman *et al.,* 2003). Future studies in a range of different taxa exhibiting these general characteristics are likely to provide more examples of the threat of eviction and its role in shaping the reproductive behaviour of individuals.

The occurrence of monogamy within the context of social groups generates a conspicuous inequality in the reproductive benefits gained from group-living for dominant breeders compared subordinate non-breeders. To determine why non-breeding subordinates tolerate group-living, I applied the theory of cooperative breeding (Brown, 1974) to identify the ecological factors that may tip the pay-offs to non-breeding subordinates in favour group-living as opposed to dispersing to breed elsewhere. This investigation was particularly important since two of the key mechanisms typically invoked to explain group-living in other taxa, namely kin selection (Hamilton, 1964) and benefits from helping (Emlen, 1991) can not apply to *P. xanthosomus* since subordinates are un-related to dominant breeders and do not help within the group. I found that ecological constraints on dispersal due to high costs of movement between coral colonies favour group-living by non-breeding subordinates. In addition, benefits accrued from eventually inheriting the high quality territory on which the subordinate currently resides also promotes group-living. This investigation therefore demonstrates that ecological constraints on dispersal as well as benefits of remaining in the current group can promote the tolerance of group-living by subordinates in light of asymmetries in the division of reproduction and the absence of kin selection and cooperative breeding.

In contrast, levels of saturation in alternative coral colonies had no effect on subordinate dispersal. This is likely to result from weak selection on subordinates to disperse in relation to changes in saturation levels because of the generally high levels of saturation in the environment. Additionally, there was no effect of social factors such as the social rank of subordinates on subordinate dispersal decisions. This is also likely to be due to weak selection on subordinates to respond to changing social conditions, since the low mobility of individuals would constrain their ability to accurately assess social conditions within alternative groups. Recent theoretical studies have strongly promoted social rank as being a key determinant of subordinate grouping and dispersal decisions (Field *et al.,* 1999; Buston, 2002; Kokko & Ekman, 2002; Mitchell, 2005). Although these studies are important for enhancing our general understanding of the optimal strategies of subordinates, the results of the current study emphasise that these theoretical advances may often have limited power to predict the actual behaviour of subordinates since the strength of selection on subordinates to respond adaptively to potentially fitness-maximising opportunities may be weak owing to constraints imposed by the species' ecology over evolutionary time. Clearly, the potential for such evolutionary constraints to occur needs to be considered when assessing the behaviour of individuals in an attempt to understand of the evolution and maintenance of animal societies.

The occurrence of group-living in *P. xanthosomus* provides an interesting contrast to the social organisation of other related monogamous coral-dwelling gobies that do not form groups despite similarities in their ecology (e.g. *Gobiodon histrio*, Munday *et al.,* 1998; *Paragobiodon melanosomus*, Thompson *et al.,* In Press). In non-group forming species, the breeding partners are the sole occupants of the coral colony irrespective of the size of the coral colony (Munday *et al.,* 1998; Thompson *et al.,* In Press). Since the formation of social groups hinges on the pay-offs from group-living received by both residents and potential immigrants (Higashi & Yamamura, 1993), interspecific differences in social organisation amongst coral-dwelling gobies are likely reflect interspecific differences in the pay-offs from group-living experienced by both the dominant breeders (residents) and non-breeding subordinates (potential immigrants). From the breeder's perspective, group-living may be favoured if the presence of non-breeding subordinates is beneficial to them. This would lead to the prediction that dominant breeders in *P. xanthosomus* gain more from the presence of subordinates than breeders in non-group forming species. Although subordinate *P. xanthosomus* do not provide benefits to breeders in terms of helpful assistance, their presence may be beneficial if, for example, they increase breeder survival by reducing their risk of predation through enhanced vigilance or a dilution of risk (Krause & Ruxton 2002). In support of this, survival rates of *P. xanthosomus* appear to be lower than survival rates of *G. histrio*, since *P. xanthosomus* rarely live for more than 1 year (Wong *et al.,* unpub. data) in comparison with a maximum of 5-6 years for *G. histrio* (Munday, *pers. comm.*). Consequently, the benefits to breeders from tolerating subordinates as a means of reducing their own predation risks may be greater for *P. xanthosomus* than for non-group forming species. Additionally, subordinate group members have been shown to act as food finders (Clifton, 1991), thus breeders in *P. xanthosomus* may benefit the presence of non-breeding subordinates if subordinates serve to enhance their foraging efficiency. In support of this, in feeding experiments where only the rank 4 subordinate was being fed, more dominant group members learnt to recognise the feeding ‘darts’ of the rank 4 and would quickly approach and displace it from the feeding location. Differences in coral architecture may be a potential factor underlying differences in the foraging efficiency of breeders in non-group forming versus group forming species, and hence the benefits to breeders of tolerating subordinates as a means of improving foraging efficiency, although further investigation into this explanation is required. Finally, the benefits to dominant breeders of tolerating subordinates for group forming species may not infact be greater than for non-group forming species, but breeders in group forming species may experience higher costs from preventing subordinates from settling in corals compared to breeders in non-group forming species. This might occur if on average, the size of coral colonies is larger for group forming versus non-group forming species, since a larger coral colony would increase the costs incurred by breeders from patrolling the coral colony and evicting any non-breeders that attempted to settle (Thompson *et al.,* In Press).

From the subordinate’s perspective, group-living may be favoured in some but not other species if there are differences in the levels of habitat saturation experienced by individuals of different species in the environment. According to theory, non-group forming species should be subject to lower levels of habitat saturation enabling subordinates to disperse to breed independently elsewhere (Selander, 1964). However, this is unlikely to be the case given that the majority of coral colonies appear to be occupied in non-group forming species (Hobbs & Munday, 2004), as also observed for *P. xanthosomus* (Wong, *pers. obs.*). Alternatively, subordinates in the non-group forming species may experience lower costs of dispersal between coral colonies enabling subordinates to disperse to breed elsewhere (Emlen, 1982). This is a possibility for *G. histrio* which has been shown to produce skin toxins that act as deterrents to predators, in contrast to *P. xanthosomus* which lacks skin toxins (Schubert *et al.,* 2003). Finally, subordinates in the non-group forming species may experience a lower variation in the size and thus quality of corals in their environment compared to *P. xanthosomus.* This would select against group-living since the differences in coral quality would be insufficient to compensate subordinates for missed reproductive opportunities elsewhere (Stacey & Ligon, 1987). In support of this, the range of coral sizes in the non-group forming *P. melanosomus* is considerably less than for *P. xanthosomus* (Thompson *et al.,* In Press). Clearly, further research into the factors underlying the differences in the social organisation of these closely related species would be important for providing rigorous tests of current hypotheses for the evolution of group-living, and thus for providing a greater understanding of the evolution and maintenance of social groups in general.

Although the evolution and maintenance of social groups suggests that the benefits of group-living outweigh the costs for both breeders and non-breeders, this does not necessarily imply that group members are living harmoniously with each other. Within groups of *P. xanthosomus*, individuals are organised into size-based dominance hierarchies that function as queues to inherit breeding status. As a result, individuals are engaged in intense conflict over rank as each individual would benefit from elevating its rank within the queue at the expense of others (Wiley & Rabenold, 1984). The existence of social groups in which reproductive benefits are unequally distributed as a result of monogamy is therefore responsible for the occurrence of conflict within groups, which needs to be resolved for societies to be stable over time. Surprisingly, experimental investigations into the mechanisms resolving conflict over rank within animal societies are lacking despite theoretical references to this issue (Wiley & Rabenold, 1984; Cant *et al.,* 2006; Mesterton-Gibbons *et al.,* 2006). In this thesis, I demonstrated that the resolution of conflict over rank in *P. xanthosomus* occurs via a novel pathway involving two key strategies employed by dominants and subordinates: 1) the threat of punishment imposed by dominants on subordinates in the form of eviction from the group, and 2) cooperation by subordinates in response to the threat of eviction by dominants in the form of growth and size regulation within the group. The combined effect of these two processes promotes the maintenance of threshold size ratios between individuals of adjacent rank within the queue. The maintenance of these threshold size ratios minimises the occurrence of actual conflict and thus resolves conflict over rank. As a result, the mechanisms promoting conflict resolution play a fundamental role in determining both the structure and stability of *P. xanthosomus* societies.

This investigation provides one of the few experimental demonstrations of the link between punishment and cooperation in an animal society (e.g. Bshary & Grutter, 2005). Such studies are presumably few owing to the difficulty in demonstrating all key aspects that define both punishment and cooperation. To convincingly invoke punishment, it must be shown that: 1) punishment occurs following defection, 2) punishment imposes a cost on the punisher (i.e. dominant), 3) punishment imposes a higher cost on the punished (i.e. subordinate), 4) punishment and/or the threat of punishment leads to a reduction in the probability that the punished individual will defect again in the future, and 5) punishment is self-serving in that it provides immediate fitness benefits to the punisher regardless of the response of the punished (Clutton-Brock & Parker, 1995; Cant & Johnstone, 2006). For *P. xanthosomus*, I demonstrated that dominants punish subordinates since: 1) dominants evict subordinates significantly more often when subordinates defect by breaching the threshold size ratio (chapter 4), 2) punishment is likely to entail energetic costs to dominants since it involves repeated chases and displays throughout the coral (chapter 4), 3) an evicted subordinates suffers higher costs since it faces high risks of mortality outside groups (Lassig, 1981; Wong, *pers. obs.*), 4) subordinates regulated their growth to remain below the threshold size ratio suggesting they are attempting to avoid defecting and being punished (chapter 4), and 5) a dominant that evicts a subordinate that has breached the threshold gains immediate fitness benefits since it is spared from eviction itself (chapter 4).

To convincingly invoke cooperation by subordinates, it must be shown that the cooperative behaviour enhances the fitness of both the dominant and subordinate (Krebs & Davies, 1993; Buston & Balshine, *In Review*). In light of an underlying competitive relationship between dominants and subordinates, subordinate growth regulation in *P. xanthosomus* can be viewed as cooperative since it serves to enhance the fitness of dominants by minimising threats to their rank, and enhance the fitness of subordinates by ensuring their continued membership within the group. Once again, these results indicate that subordinates play a direct role in the regulation of their own growth and size, as suggested in the analysis of female mate guarding via the suppression of subordinate size and reproductive status. Growth regulation constitutes an atypical form of cooperative behaviour, since subordinates are actively offsetting the costs they would otherwise inflict on dominants (Kokko *et al.,* 2002) as opposed to more usual method of providing fitness benefits to dominants, for example through the provision of help (e.g. Mulder & Langmore, 1993; Balshine-Earn *et al.,* 1998). This therefore suggests that there is a continuum underlying the forms of cooperative behaviour occurring in response to punishment, ranging from helpful (Gaston, 1978) to peaceful (Buston, 2004b; Buston & Balshine, *In Review*), as exemplified by *P. xanthosomus*. I expect that future studies will reveal increasing occurrences of peaceful cooperation in queues including those that are not necessarily size-based (e.g. Poston, 1997; Field *et al.,* 1999; East & Hofer, 2001). In such queues, peaceful cooperation is more likely to manifest itself as the self-regulation of subordinate behaviour or body weight, as opposed to body size as may be prevalent in fishes (Buston, 2004a; Heg *et al.,* 2004; current study). Studies investigating the occurrences of peaceful cooperation in other species would require the experimental manipulation of the costs imposed on dominants by subordinates, by manipulating the extent to which the potentially peaceful cooperative behaviour is expressed by subordinates. Subsequently, the behavioural responses of both parties to the varying costs by imposed by subordinates would need to be measured, as demonstrated in the current study. Such investigations are likely to reveal a greater diversity of cooperative behaviours than is currently known, and increase our understanding of the mechanisms promoting conflict resolution and social stability within both human and animal societies.

Aside from the role of punishment, conflict resolution is also predicted to occur as a result of constraints imposed on subordinates such that they simply lack the ability to behave selfishly (Ratnieks *et al.,* 2006). This situation might arise if dominants competitively excluded subordinates from food resources which subsequently prevented subordinates from successfully challenging dominants over rank. In this thesis, I demonstrated that subordinate growth rates were elevated when they received supplemental food even when the breeding female was present, suggesting that food limitation plays an important role in the maintenance of size differences between group members of adjacent rank irrespective of the social conditions within the group. Feeding also resulted in a general decrease in the size differences between individuals within a group, and in some cases, the accelerated growth of subordinates such that they breached the threshold size ratio and were consequently evicted. Food-related constraints on subordinates therefore play an important role in promoting the regulation of subordinate growth rates, the maintenance of well-defined size differences between group members of adjacent rank, and hence the resolution of conflict over rank in *P. xanthosomus*. Both punishment and cooperation, and constraints in the form of food limitation, thereby interact to determine both the structure and stability of *P. xanthosomus* societies over time.

To conclude, this thesis describes investigations into the ecological and social factors underlying the evolution of monogamy, group-living and the resolution of conflict over reproduction in *P. xanthosomus* societies. Analysis of these three integrally related issues has enabled me to: 1) conduct quantitative applications and tests of key theoretical concepts in behavioural ecology, 2) to provide empirical evidence in support of recent hypotheses, and 3) to propose new hypotheses relating to the evolution of reproductive and social behaviour. In so doing, I have provided a comprehensive picture of both the mechanisms underlying the formation, structure and stability *P. xanthosomus* societies, and contributed to a greater understanding of the processes governing the evolution and maintenance of animal societies in general.

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Habitat patch size, facultative monogamy and sex change in a coral-dwelling fish, *Caracanthus unipinna*

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Synopsis

We investigated the inter-relationships between coral colony size, social group size, mating system, and patterns of sex allocation in the pygmy coral croucher, *Caracanthus unipinna* (Caracanthidae), an obligate coral-dwelling fish. Histological examination of the gonads from all individuals in social groups revealed that the predominant mating system was harem polygyny. However, both group size and mating system co-varied with coral colony size, with pair forming and monogamy occurring on small corals and group forming and harem polygyny on large corals. This species therefore displays mating system plasticity in response to varying habitat patch size. Within-group sexual size dimorphism and individual gonad structure indicate that *C. unipinna* is also likely to be a protogynous hermaphrodite. These social and reproductive features of *C. unipinna* contrast with some other coral-dwelling fishes, which display a lack of social and mating system plasticity in response to habitat patch size, and either bi-directional or protandrous sex change. Possible reasons for this dichotomy include differences in spawning mode, parental care and levels of intrasexual aggression.

Introduction

Habitat characteristics can have profound effects on the evolution of social and reproductive behaviours (Crook & Gartlan 1966, Jarman 1974, Shapiro 1991, Warner 1991). For example, the spatial and temporal distribution of habitat may be a key determinant of animal dispersion, which in turn may influence their optimal social and reproductive strategies (Emlen & Oring 1977, Davies & Lundberg 1984, Brotherton & Manser 1997). In addition, habitat patch size and quality may influence social group size by affecting individual pay-offs involved in group formation or dispersal (Stacey & Ligon 1987, Komdeur 1992, Covas et al. 2003). Since social group size and structure can predetermine the resulting mating system (Crook & Gartlan 1966, Zimen 1976), habitat patch size can therefore play an important role in the evolution of social and mating systems and their variation within and between species (Trumbo & Eggert 1994, Pribil & Searcy 2001).

Coral reef fish exhibit a tremendous diversity of social and mating systems (Thresher 1984, Barlow 1986) and this variation provides an excellent opportunity to investigate the effects of habitat patch size on reproductive behaviours. The propensity for co-variation between habitat patch size and reproductive behaviour would be greatest in habitat-specialist fishes due to a number of ecological and behavioural characteristics shared by these species. Most rely upon a specific habitat that is discrete, isolated, unpredictably distributed and potentially limiting (Fricke & Fricke 1977, Lassig 1977, Fricke 1980). In addition, individuals themselves are generally small-bodied, experience high predation pressure away from their habitat and are strongly site attached with limited mobility (Munday & Jones 1998). As a result, habitat-specialist reef fish are expected to exhibit particular adaptations that maximise their reproductive opportunities in response to these ecological restrictions.

One possible adaptation is the maintenance of social and mating system plasticity in response to changes in habitat patch size. For example, group sizes and mating systems of the obligate coral-dwelling hawkfishes, *Neocirrhites armatus* and *Oxycirrhites typus* (Cirrhitidae) covary in response to coral size, with pair-forming and facultative monogamy occurring on small corals, and group-forming and harem polygyny on large corals (Donaldson 1989). A similar scenario occurs in the damselfish, *Dascyllus marginatus* (Pomacentridae), with a shift in mating system from monogamy to harem polygyny and eventually to promiscuity on increasingly large coral colonies (Fricke 1980). Individuals of these species are therefore able to respond to changes in the environmental potential for polygyny (Emlen & Oring 1977), employing a range of mating strategies that maximise reproductive success in relation to current habitat conditions.

However, other habitat-specialist reef fish lack such mating system plasticity. In some species, social group size increases with habitat patch size but the mating system remains monogamous with only the largest two individuals breeding (e.g. *Amphiprion akallopisos*, Fricke 1979, *P. echinocephalus*, *Paragobiodon xanthosomus*, Lassig 1977, Kuwamura et al. 1993). Other species are even less flexible, with both social group size and mating system being fixed to a monogamous pair irrespective of habitat size (*Gobiodon histrio*, Munday et al. 1998, *Bryaninops yongei*, Munday et al. 2002). Clearly, other factors aside from habitat size are involved in determining the expression of the mating system in these species, and could include constraints on paternal egg care (Wittenberger & Tilson 1980, Kuwamura et al. 1993) and intrasexual aggression and mate guarding (Fricke 1979, Barlow 1986, Kuwamura et al. 1993).

Hermaphroditism is another reproductive characteristic commonly exhibited by habitat-specialist fishes (e.g. *Amphiprion spp*, Fricke & Fricke 1977, Moyer & Nakazono 1978, Fricke 1979, *Caracanthus spp*, Cole 2003, *Dascyllus spp*, Shpigel & Fishelson 1986, Schwarz & Smith 1990, Cole 2002, *Gobiodon spp*, Nakashima et al. 1996, Munday et al. 1998, Cole & Hoese 2001, *Neocirrhites armatus* & *Oxycirrhites typus*, Donaldson 1989, *Paragobiodon echinocephalus*, Kuwamura et al. 1993, 1994). Despite similarities in the ecology of habitat-specialist fishes there exists remarkable inter-specific diversity in the forms of hermaphroditism observed, which seems related to their mating systems. When the mating system is a pair (i.e. obligate monogamy), two hermaphroditic patterns occur. Bi-directional sex change appears to be favoured where individuals move among habitat patches to form breeding pairs, but where there are few opportunities to find a new partner, because it enables an individual to mate with any other single individual encountered (Nakashima et al. 1996, Munday 2002). Alternately, protandry is favoured where individuals do not move among habitat patches but rely on the presence of sub-adults in the social group to reconstitute reproductive pairs, and partners maximise their gains from obligate monogamy if the larger individual is a female (Fricke & Fricke 1977, Moyer & Nakazono 1978). In larger social groups, protogynous sex change is favoured if large males are able to monopolise the mating opportunities with most of the females in the group, and therefore have much higher reproductive success than small males (Warner 1988). Where social group size alternates between pair forming and larger groups (i.e. facultative monogamy), protogynous sex change might still be expected because large males are likely to achieve greater reproductive success than large females when polygyny occurs. Consequently, it appears that habitat patch size can influence patterns of hermaphroditism through its effect on social group size and the mating system.

In this study, we investigated the inter-relationships between coral size, social organisation, mating system and patterns of sex allocation in the obligate coral-dwelling fish, *Caracanthus unipinna* (Caracanthidae). This species inhabits a wide range of corals from the families *Acroporidae*, *Pocilloporidae* and *Poritidae* (Myers 1999) and shares the suite of ecological traits exhibited by other habitat-specialists, namely occupation of discrete habitat patches, small body size, limited mobility and reduced availability of mates (Myers 1999, Cole 2003). First we examined the relationship between social group size and coral colony size in *C. unipinna*. We then used histological analysis of the gonads of all individuals from whole social groups to estimate the mating system and to determine how the mating system changes with increasing group size. Finally we used a combination of gonad structure and size-and age-frequency distributions of males and females to examine the potential for hermaphroditism in *C. unipinna*.

Materials and methods

Habitat patch size vs. social group size

We collected social groups of *C. unipinna* from Cocos Island, Indian Ocean, during November 2002. To determine the effects of coral size on social group size and composition, we collected 29 whole social groups of *C. unipinna* from coral colonies spanning the size-range inhabited by this species. We collected fish following anaesthetizing with clove oil/alcohol solution (Munday & Wilson 1997). We regarded all individuals inhabiting the same coral head as members of a social group since coral colonies were sufficiently discrete and isolated to make movement unlikely. To estimate coral size, we placed a measuring stick marked at 1 cm intervals at the edge of each coral colony and took a digital photograph of the coral and the measuring stick. A grid subdivided into 1×1 cm squares was later placed over each photo and we counted the number of squares filled by the coral. We then calculated actual coral area by scaling the 1×1 cm grid to the scale on the measuring stick.

Mating system and composition of social groups

To determine sex and reproductive status of each group member, we dissected the gonads of each fish for histological examination and fixed them in vials containing FAACC (4% formaldehyde, 5% acetic acid and 1.3% calcium chloride) for 7 days and then transferred them to 70% ethanol. We embedded gonads in paraffin wax, transversely sectioned at $5\,\mu$m, mounted onto glass slides and stained with Mayer's alum haemotoxylin and Young's eosin–erythrosin.

We estimated reproductive status of each individual by taking a transect across a random section of gonad and recording the stage of germ-cell under each mark on an eyepiece micrometer at 200x magnification. In females, we categorised germ cells into five stages based on descriptions by West (1990): (1) chromatin nucleolar, (2) perinucleolar, (3) cortical alveolar, (4) vitellogenic, and (5) ripe. We classed females with only pre-vitellogenic oocytes (i.e. stages 1, 2 and 3) as immature, and those with vitellogenic oocytes (i.e. stages 4 and 5) as mature (West 1990). In males, we categorized germ cells into two stages as described by Cole & Hoese (2001): (1) spermatocytes and (2) spermatozoa. Only males with spermatozoa were considered mature. We did not use gonia to estimate reproductive status because they appeared identical in testes and ovaries. To analyse the overall architecture of male and female gonads, we took a photograph of the whole gonad at $4\times$ magnification for each individual collected.

Pattern of sex change

To determine if *C. unipinna* was hermaphroditic we first examined the sex ratio and size- and age-frequency distributions of the sexes, both within and among social groups. We then examined gonad structure to determine if there was evidence for sex change. Protogynous species often have a female-biased sex ratio and males tend to be larger or older than females. (Sadovy & Shapiro 1987). In contrast, protandrous species often have a male-biased sex ratio and females tend to be larger or older than males (Sadovy & Shapiro 1987). In bi-directional sex changers the sex ratio can be equal and the size- and age-frequency distributions of males and females often overlap almost completely (Munday et al. 1998).

Otolith increments were used to compare the age of males and females within and among social groups. The head of each individual collected was removed and placed into a vial containing 70% ethanol. Otoliths were then removed from the otic cavity, cleaned, and allowed to dry. A thin transverse section of each otolith was prepared using standard methodology (see Choat & Axe 1996 for details). The thin section was viewed with a light microscope and the number of light–dark increments counted. The structure of the otolith increments matched that seen in many other species of reef fish where otolith increments have been validated as annual (Choat & Robertson 2002), therefore each increment was assumed to represent 1 year of age. The length and depth of the head of each fish was measured prior to removing the otoliths and head depth (HD) was used as a proxy for total length (TL) when comparing the size-frequency distribution of the sexes. A strong correlation (Pearson's correlation; $r = 0.89$, $p < 0.001$) between HD and TL for 50 randomly selected specimens demonstrated that head depth was a reliable measure of relative body size.

The gonads of sex-changed individuals may retain evidence of their previous structure, or differ in anatomical structure from the gonads of non sex-changed individuals. We inspected the gonads of all individuals to determine if there was evidence of sex change. The presence of (1) remnant oocytes in the testis, (2) an ovarian lumen in the testis and (3) sperm sinuses in the testis wall were considered to be evidence for protogynous sex change (Sadovy & Shapiro 1987).

Statistical analysis

We used Pearson's correlation coefficient to test if there was a significant relationship between head depth and total length, and coral area and group size. We used a chi-square goodness of fit test to determine if the sex ratio differed from 1:1. We used paired *t*-tests to determine if there was a significant difference between male and female body size or age within social groups.

Results

Frequency and composition of social groups

Social groups ranged in size from 1 to 6 individuals (Figure 1). Groups of 4 predominated, although singletons and groups of 2, 3 and 6 were also observed (Figure 1). Multi-female harems were the most frequently observed mating system (19 of 29 groups). These harems all consisted of one mature male and two or more mature females. The largest social group contained one mature male and five mature females. There was never more than one mature male per social group. There was no significant difference between the mean sizes of harem females when ranked according to body size and after controlling for coral size, suggesting that no size-based dominance hierarchy exists between females (ANCOVA: $F_{2,37} = 1.53$, $p = 0.231$).

Pairs generally consisted of a mature male and mature female (4 of 6 pairs) and were therefore likely to be monogamous. However, a mature female pair (1 of 6 pairs), and mature male and immature female pair (1 of 6 pairs) were also found. Singletons were relatively uncommon, comprising immature females (2 of 4 individuals), a mature female (1 of 4 individuals) or an immature bisexual (1 of 4 individuals). No single immature males were observed.

Coral size vs. group size

Coral photos were available for 19 of the 29 social groups collected. Social group size showed a significant positive correlation with coral area (Pearson's correlation: $r = 0.637$, n = 19, $p = 0.003$, Figure 2), suggesting that coral size influenced the number of individuals inhabiting each coral colony.

Evidence for sex change

The population sex ratio, size- and age-frequency distributions of males and females, and gonad

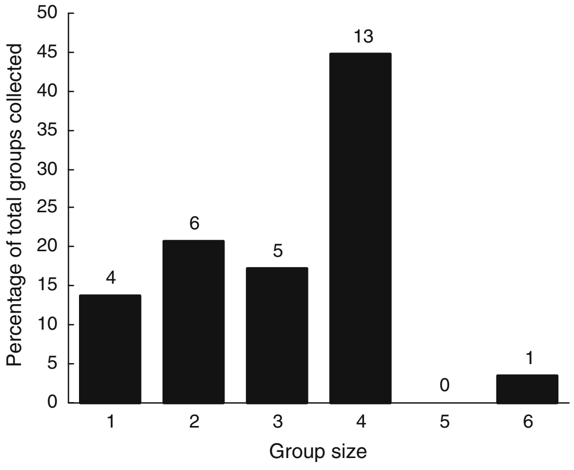

Figure 1. Percentages of social group sizes collected (n = 29 groups). Values above bars show the number of each group size collected.

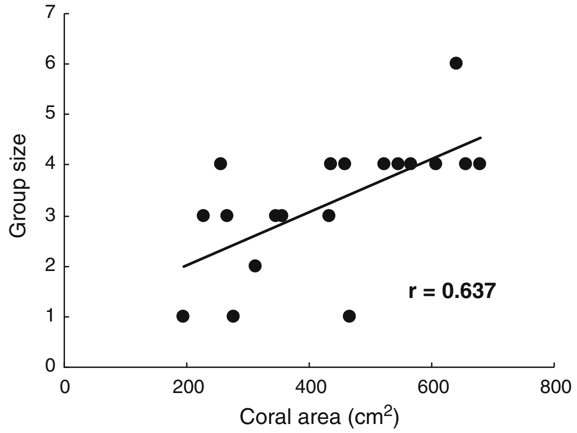

Figure 2. Relationship between coral area (cm^2) and social group size.

structure all were consistent with a protogynous mode of sexual development. The predominance of multi-female harems and an absence of singleton males resulted in a female-biased sex ratio of 1:2.7, which differed significantly from unity (Chi-squared goodness-of-fit test: $\chi^2 = 17.28$, df = 1, $p < 0.01$).

Within social groups, the male was either the same size, or larger than, the largest female in all groups containing two or more individuals (Figure 3a). Males were never smaller than females within social groups (paired *t*-test: $t = 5.3$, n = 24, $p < 0.01$), clearly demonstrating that sexual size-dimorphism occurs at the social group level. Among social groups, the size range of males and females overlapped considerably, with males ranging in size from 12 to 22 mm and females from 6 to 19 mm (Figure 3b). The largest size classes were occupied only by males and the smallest size classes only by females (Figure 3b), indicative of protogynous sex change.

Although otolith increment periodicity was not validated, the structure of otolith increments was similar to that seen in many other species with annular otolith increments (Choat & Robertson 2002). Within social groups, males were usually the same age or older than the oldest female in that group, although males were younger than the oldest female in 3 groups (Figure 4a). Even so, the mean age of males (6.0 years) was significantly greater than females (4.0 years) within social groups (Paired *t*-test: $t = 2.87$, n = 20, $p < 0.01$). Among social groups, the ages of males and females showed considerable overlap, with females ranging in age from 1 to 9 years and males from 3 to 9 years (Figure 4b). However, only females occupied the lowest age classes (Figure 4b), indicative of protogynous sex change.

The gonads of mature females (n = 57) consisted of a central mass of ovigerous tissue containing vitellogenic oocytes surrounded by a peripheral lumen, which in turn was encompassed by an ovarian wall (Figure 5a). This structure was clearly present in 50 of 57 females collected. The ovaries of the remaining females were not intact, consequently the overall architecture of these individuals was not clearly visible. The gonads of all mature males collected in this study (n = 24)

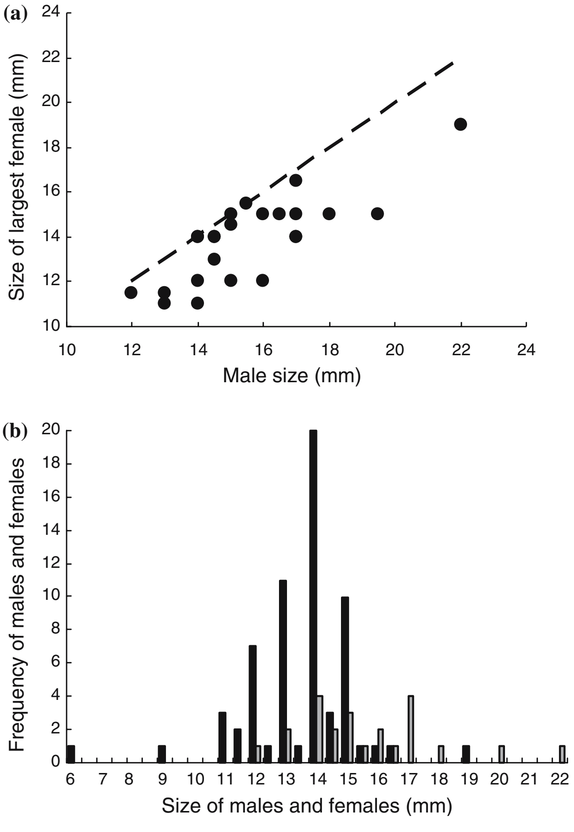

Figure 3. (a) Relationship between the size of the male and the largest female within social groups. The dashed line illustrates a 1:1 relationship between male and female size. Points below the dashed line indicate social groups where the largest female was smaller than the male. (b). Size-frequency distribution of male and female *Caracanthus unipinna* in the population sample, illustrating range, frequency and overlap in male and female body sizes. Black bars = females, grey bars = males.

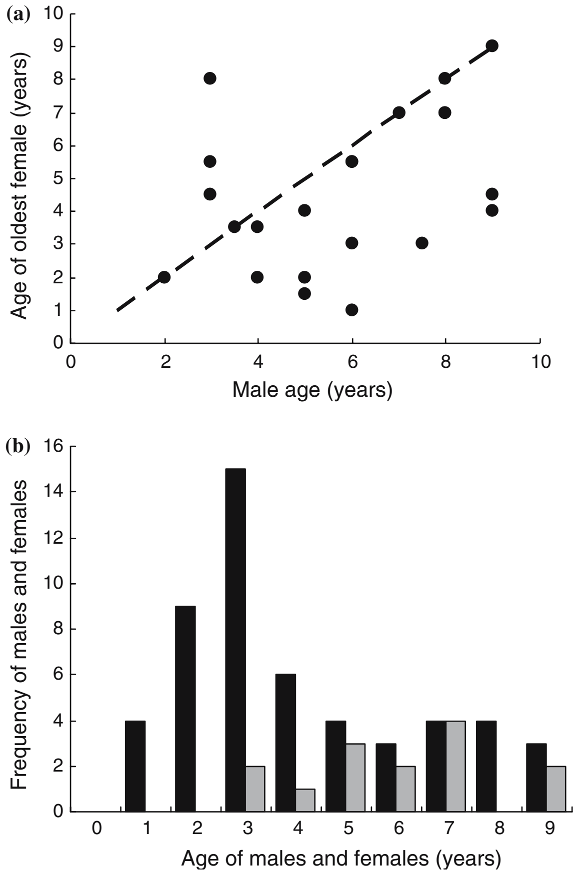

Figure 4. (a) Relationship between the age of the male and the oldest female within social groups. Points below the dashed line indicate social groups where the oldest female was younger than the male. (b). Age-frequency distribution of male and female *Caracanthus unipinna* in the population sample, illustrating the range, frequency and considerable overlap of male and female ages. Black bars = females, grey bars = males.

were entirely testicular and lacked female oocytes of any developmental stage (Figure 5b). However, overall testis structure still resembled ovarian structure in terms of the centrally located germ cells surrounded by a peripheral lumen (Figure 5b), the latter of which appeared non-functional due to an absence of sperm. The lumen was enclosed by a testis wall comprised of sperm sinuses that contained spermatozoa (Figure 5b). This structure was present in 23 of 24 males collected (the testis of the remaining male was not intact so overall architecture was not clearly visible).

Gonads of immature females contained only pre-vitellogenic oocytes (n = 7, Figure 5c), and that of the inactive bisexual contained both pre-vitellogenic oocytes and spermatocytes (n = 1, Figure 5d). There were no immature males in the sample.

Discussion

Close associations between resource availability, social group size, and mating systems are expected among habitat-specialist fishes (Fricke 1980, Donaldson 1989, Shapiro 1991). The present study provides further support for the influence of habitat patch size on both social organisation and mating system evolution. An increase in coral size was accompanied by a general increase in social group size of *Caracanthus unipinna*, indicating that spatial restrictions might limit the number of individuals residing within corals. Furthermore, the resulting mating system was influenced by social group size, with facultative monogamy occurring in small groups, and harem polygyny in larger groups.

Facultative monogamy in relation to coral size has also been reported in the coral-dwelling hawkfishes, *Neocirrhites armatus* and *Oxycirrhites typus* (Donaldson 1989). It is striking that *C. unipinna*, *N. armatus* and *O. typus* should display facultative monogamy and mating system plasticity in response to coral size, given that the mating systems of some other habitat-specialists remain fixed to an obligately monogamous pair irrespective of coral and/or group size (e.g. *Amphiprion akallopisos*, Fricke 1979, *Bryaninops yongei*, Munday et al. 2002, *Gobiodon histrio*, Munday et al. 1997, *Paragobiodon echinocephalus*, Kuwamura et al. 1993). The paternal care constraints hypothesis (Kuwamura et al. 1993) could provide a possible explanation for these differences (Kuwamura et al. 1993). Parental care in reef fish is usually conducted by males (Perrone & Zaret 1979), and could act as a constraint on the ability of males to mate with multiple females if care entails significant energetic or predation costs (Emlen & Oring 1977). In support of this hypothesis, paternal care is associated with obligate monogamy in a wide variety of reef fish (Whiteman & Côté 2004), and habitat-specialists whose mating systems remain fixed to obligate monogamy tend to be demersal spawners with paternal egg care (Fricke, 1979, Kuwamura et al. 1993, Hirose 1995, Munday et al. 1998). Conversely, *C. unipinna*, *N. armatus* and *O. typus* are pelagic spawners with no paternal

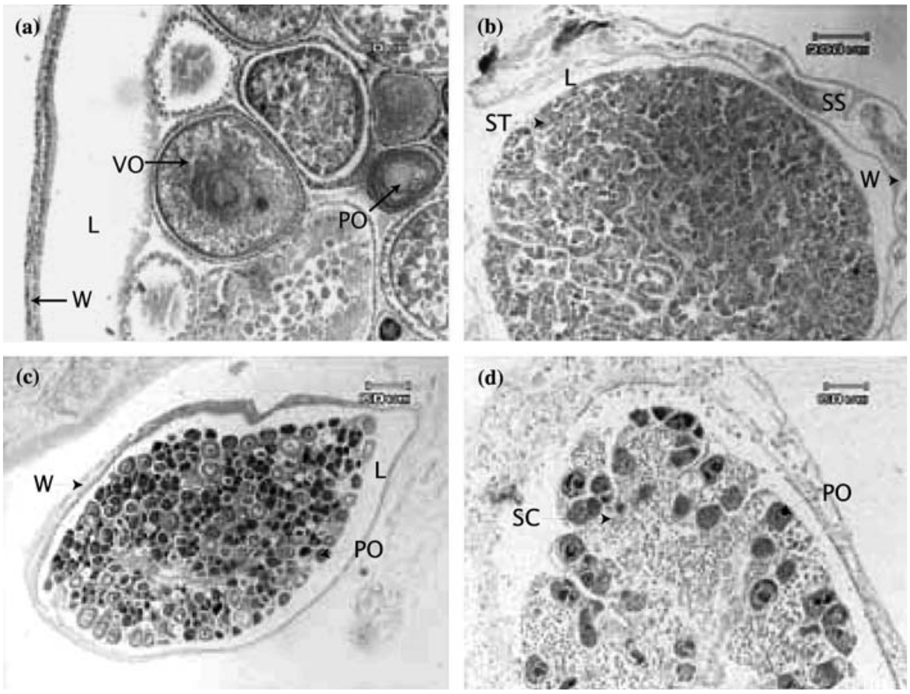

Figure 5. (a–d) Gonad morphology of *Caracanthus unipinna*: (a) Cross section of an ovarian lobe from an adult female showing the gonadal wall (W), peripheral lumen (L), pre-vitellogenic (PO) and vitellogenic oocytes (VO); (b) Cross section of a testis lobe of an adult male showing centrally located spermatogenic tissue (ST) surrounded by a peripheral lumen (L), which in turn is encompassed by the testis wall (W). The wall is partitioned into sperm sinuses (SS); (c) Ovarian structure from an immature female, showing the peripheral lumen (L) and pre-vitellogenic oocytes (PO); (d) Detail of an inactive bisexual gonad consisting of both spermatocytes (SC) and pre-vitellogenic oocytes (PO).

egg care (Donaldson 1989, Cole 2003). In such species, males are not constrained by parental care duties, raising the potential for the evolution of facultative monogamy (Emlen & Oring 1977).

Contrary to this hypothesis however, many polygynous species exhibit paternal care (Breder & Rosen 1966, Fricke 1980, Sunobe & Nakazono 1990, Lindstrom & Seppa 1996) as well as monogamous species lacking paternal care (Neudecker & Lobel 1982, Barlow 1987, Hourigan 1989) especially amongst non-habitat-specialist reef fish. These examples suggest that paternal care hypothesis should be viewed as a contributory rather than definitive factor in the evolution of facultative monogamy.

The intrasexual aggression and mate guarding hypothesis (Wittenberger & Tilson 1980) could also contribute to the dichotomy in mating system plasticity amongst habitat-specialists. In general, males are expected to exclude other males that may be rivals for their mates, and females are expected to exclude other females that might compete for the same mate or access to resources (Barlow 1986). The intensity of intrasexual aggression is likely to vary within and between species depending on the net costs and benefits of sharing mates and resources. If intrasexual aggression is intense, group sizes and mating systems are predicted to be more restricted (Reavis & Barlow 1998, Kokita 2002). Conversely, a lack of intrasexual and especially female aggression could facilitate mating system plasticity and the evolution of facultative monogamy. Given the pelagic spawning mode of *C. unipinna* (Cole 2003), intrasexual aggression and mate guarding by females may be minimal since they do not have to compete for a monopoly on male care, allowing for the development of harem polygyny in large corals. However, the occurrence of intrasexual aggression does not necessarily preclude harem formation in other reef fish (e.g. *Centropyge* spp, Moyer & Nakazono 1978, Aldenhoven 1986, *Malacanthus plumieri*, Baird, 1988), thus once again, the intrasexual aggression hypothesis should be viewed as a possible contributory factor in the evolution of facultative monogamy.

The current study provides evidence for protogynous sex change in *C. unipinna*, based on characteristics of its population structure and gonad morphology. Although there was considerable overlap in the size- and age-frequency distributions of males and females, this is likely the result of pooling the size and ages of individuals from each social unit. The resulting size- and age-frequency distributions of the population sample therefore obscure the more distinct within-group distributions, in which males were as large as or larger than females, and males were as old as or older than females with the exception of three groups where the male was younger than the oldest female. This final point does not necessarily preclude protogyny, since these three males were still larger than the highest ranking female in each social group. It is possible that body size distribution is simply a better predictor of protogynous sex change than is age. For example, these males may have initially been young females that experienced elevated growth rates relative to older female group members, and thus changed sex despite being younger.

Various aspects of gonad morphology are also indicative of protogynous hermaphroditism in *C. unipinna*. Firstly, in males, an apparently nonfunctional peripheral lumen surrounded the centrally located testicular tissue. This feature is characteristic of ovarian structure and supports protogyny (Sadovy & Shapiro 1987, Cole 2003). In addition, peripheral sperm sinuses running through the testis wall have only been reported in secondarily derived males (Bruce 1980, Hastings 1981, Sadovy & Shapiro 1987, Cole 2003). Although mature males had purely testicular gonads lacking in any remnants of earlier ovarian tissue, transition from female to male in fish is often accompanied by a complete degeneration of ovarian tissue, leaving no trace of sex change in the form of testicular oocytes (Sadovy & Shaprio 1987). Experiments involving the removal of the largest male from a large number of social groups would be useful to determine with certainty the occurrence of protogyny in *C. unipinna*.

Why in particular should protogyny prevail over other forms of sex change in *C. unipinna*? Protogyny is favoured where harems occur at sufficiently high frequency. Harems were the most commonly observed mating system in *C. unipinna*, and this selects for protogyny since a large male gains greater reproductive success than would a large female, and a small male would suffer reduced reproductive success compared to a small female due to its inferiority in male-male competition (Warner 1988). Similar patterns of sex allocation are found in other haremic reef fish, including *Centropyge spp* (Pomacanthidae) (Moyer & Nakazono 1978, Aldenhoven 1986), *Dascyllus marginatus* (Pomacentridae) (Fricke 1980), *Labroides dimidiatus* (Labriidae) (Nakashima et al. 2000), *Malacanthus plumieri* (Malacanthidae) (Baird 1988) and *Neocirrhites armatus* & *Oxycirrhites typus* (Cirrhitidae) (Donaldson 1989).

Although *C. unipinna* displays similar patterns of habitat use to that of some bi-directional sex changing species (e.g. *Gobiodon* spp, Nakashima et al. 1996, Munday et al. 1998, *Paragobiodon echinocephalus*, Kuwamura et al. 1994) i.e. small discrete habitats and limited mobility, the current study found no evidence of bi-directional sex change based on the size- and age-frequency distributions of the sexes and histology of the gonads. Bi-directional sex change is thought to be favoured in species that are pair forming and monogamous, because it enables pairing with any conspecific encountered should an individual need to move in search of a new mate (Nakashima et al. 1995, Munday 2002). *C. unipinna* however, exhibits a facultatively monogamous mating system in which harem polygyny occurs in large corals. Selection for bi-directional sex change should therefore be weak since movement to locate a new partner is rarely necessary.

Naturally occurring bi-directional sex change has been found in the polygynous species, *Trimma okinawae* (Sunobe & Nakazono 1993, Sunobe, personal communication). This species form harems with one large male and one or more smaller females, similar to the mating system of *C. unipinna*. In polygynous species, the capacity for bi-directional sex change might be favoured where there are frequent shifts in dominance within social groups, either by immigration of new individuals or by rank changes between existing group members (Sunobe & Nakazono 1993, Nakashima et al. 1995). Bi-directional sex change might be favoured under these circumstances because the relative reproductive success attained by functioning as a male or a female could change more than once in an individuals life. Frequent changes in dominance are unlikely in *C. unipinna* because movement among social groups is severely restricted by extreme habitat specialisation and the patchy distribution of corals. Furthermore, this species is relatively long-lived for a small-bodied species (Munday & Jones 1998) therefore social groups are likely to remain together for considerable periods of time. Limited mobility and long life reduce the potential for group turnover resulting in more stable dominance relationships and negating the need for bi-directional sex change.

Habitat patch size has an important influence on the social organization, mating system and pattern of sex change of *C. unipinna*, but this is by no means a universal trait amongst coral-dwelling reef fish. Further detailed research into the behaviour and ecology of these species would therefore be important for exploring the underlying causes of inter-specific variability in the social and reproductive systems of habitat-specialist reef fish.

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