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Phenotypic plasticity across natural- and sexual-selection gradients in a reef fish

Thesis submitted by
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This James Cook University thesis contains some collaborative work with Dr Ashley Frisch (Great Barrier Reef Marine Park Authority) and Mr Loic Thibaut (James Cook University). I conceived the projects, and was responsible for experimental design, data collection, model selection and analysis, interpretation and the synthesis of results into a form suitable for publication. Dr Ashley Frisch carried out radio-immune assays to measure hormone concentration in experimental fish. This data is presented in Chapter 2. Mr. Loic Thibaut helped with programming the analytical- and Monte Carlo-models presented in Chapter 5.

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Abstract

Theory suggests that the social conditions in which an individual lives can have profound influence over which set of life history and behavioural traits are best suited for maximising expected lifetime fecundity. Variation in social system type within species is therefore hypothesised to be a major driver of the evolution of trait plasticity mechanisms. This is because those individuals that can optimise their phenotype in response to current social-based selection pressures will have an advantage over those individuals who cannot. This thesis examines the ecological and evolutionary significance of phenotypic plasticity in response to social-based selection gradients using the polygynous sex-changing reef fish *Parapercis cylindrica* (family Pinguipedidae). Using a combination of theoretical, discriptive and experimental procedures I tested the general hypotheses that: 1) social factors have played a significant role in the evolution of phenotypic plasticity, and that; 2) this plasticity manifests fundamental relationships between social system type, individual phenotypic expression, and the functioning and productivity of particular social groups. I address these general hypotheses by examining four aspects of *P. cylindrica's* evolutionary ecology: 1) social stability and conflict resolution tactics; 2) sexual selection and the sex-based modification of growth and body size; 3) sex change and the adjustment of sensory organelles, and 4) The relationships between socially facilitated life history optimisation and density dependent per capita fecundity.

Conflict over reproduction is expected in polygynous societies, both among the females who share a particular male's resources, and between males and females. The first aim of this thesis was to determine the factors promoting the stability of polygynous social groups, by examining how reproduction is apportioned among female group members and the tactics individuals employ to resolve conflict over reproductive shares. Specifically, I tested the hypotheses that: 1) polygynous males promote social stability by allocating limited sperm to larger, more dominant females, thereby minimizing intersexual conflict with the most productive sexual partners; 2) dominant females enforce disproportionate sperm allocation through the threat of subordinate-female eviction; and that 3) subordinate-females cooperate with dominant females by down-regulating gamete production in response to threatening displays. I also tested the alternative hypotheses that: 4) dominant females directly control

reproduction in subordinate females, but 5) promote social stability by conceding some reproduction to subordinates as an incentive to remain peaceful in the group. Results supported hypotheses 1, 2 and 3. In addition, once a dominant female was removed from experimental groups, subordinate females were found to rapidly increased egg production. Evidence suggests that male reproductive allocation (i.e. sperm allocation), dominant-female enforcement (via the threat of attack and possible eviction), and subordinate-female cooperation (via reproductive down regulation) act together to promote social stability in *P. cylindrica*. This suite of conflict resolution tactics can be explained by the high reproductive rewards associated with the prospect of becoming a large dominant male (via sex change), and the advantage of maintaining a large polygynous group once the sexual transition has been made. The study illustrates that social stability and the partitioning of reproductive shares may frequently involve three-party negotiations between social group members.

With an increase in the degree of polygyny, males must contend with greater levels of competition for the haremic male role. Essentially, the strength of sexual selection for large male body size increases with haremic group size. The second aim of this thesis was to test the hypotheses that: 1) the magnitude of sexual size dimorphism (SSD) increases with haremic group size, driving a pattern of positive sexual size allometry among social groups (i.e. Rensch's rule), and; 2) Individual adjust growth rate during sex change from female to male in response to the potential for polygyny. Larger males were found to have larger harems with larger females, and the magnitude of SSD increased with harem size. In addition, experimental fishes were found to increase growth rate in response to polygyny potential during sex change. Thus, evidence suggests that sexual selection for male body size is the ultimate cause of variation in growth rate, and variation in growth rate is the proximate cause of sexual size allometry. The study provides compelling evidence in support of the sexual selection hypothesis for Rensch's rule, and highlights the importance of growth modification in the shaping of morphological patterns in nature.

The growth and morphology of the organelles associated with the detection of sound and movement (i.e. otoliths) are highly conservative in fishes and finely tuned to specific habitat and life-style requirements. However, changes in behaviour and use of space typically occur coincident with sexual transition. This means that the optimal configuration of the sensory apparatus may also change, driving a selection pressure for the readjustment of sensory organelles (i.e. otoliths). The third aim of this thesis was to

test the hypothesis that otolith growth and morphology is modified during sex change. Manipulative experiments illustrated that otolith discontinuities are formed during the course of sex change, characterised by a shift in the primary growth axis and a shift in microstructural density. In addition, the larger the harem the sex-changer inherited and defended, the greater the shift in otolith growth and morphology. These data suggest that sex-changing individuals may adjust their sensory apparatus so as to optimise life history in accordance with their new reproductive mode. However, the confirmation of this functional hypothesis will require further studies using an eco-physiological approach. Nonetheless, the discovery of sex-change associated otolith discontinuities represents a novel and powerful tool in fish and fisheries science. Since otolith growth is characterised by the accretion of daily bands, sex-change associated otolith signatures provide a means to detect the precise age at sex change. This will allow the life history of sex-changing fishes to be examined at a resolution not previously possible.

Positive density dependence (i.e. the Allee effect; AE) often has important implications for the dynamics and conservation of populations. The final aim of this thesis was to examine the interplay between density dependent sex ratio adjustment and the AE. Specifically, using an analytical model I show that an AE is expected whenever one sex is more fecund than the other and sex ratio bias towards the least fecund sex increases with density. I then illustrate the robustness of this pattern, using Monte Carlo simulations, against a range of body size-fecundity relationships and sex allocation strategies. Finally, I test the model using the sex-changing polygynous reef fish *Parapercis cylindrica*; positive density dependence in the strength of sexual selection for male size is evidenced as the causal mechanism driving local sex ratio adjustment, hence the AE. Model application may extend to invertebrates, reptiles, birds and mammals, in addition to 70+ reef fishes. I suggest that protected areas may often outperform harvest quotas as a conservation tool, since the latter promotes population fragmentation, reduced polygyny, a balancing of the sex ratio and hence up to a 50% decline in per capita fecundity, while the former maximises polygyny and source-sink potential.

Taken together, the results of this thesis showcase the extent to which individuals modify their traits in response to social-based natural- and sexual-selection pressures, and the fundamental role that phenotypic plasticity plays in driving ecological patterns and processes.

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Chapter 1

Introduction

1.1 The ecology and evolution of phenotypic plasticity

Knowledge on the proximate details of phenotypic plasticity is key to evolutionary theories of phenotypic variation and origins of diversity (Scheiner 1993; Gross 1996; Badyaev 2002; Tomkins 2005). The identification of selection gradients that trigger a plastic response provide insight into why patterns of diversity evolve in the first instance, and how they are maintained (Bradshaw 1965; Fricke 1980a, Warner1980b; 1991; Stearns and Koella 1982; Scheiner 1993; Gross 1996; Kohda et al. 2008). Proximately, phenotypic plasticity involves a coordinated, environmentally induced shift in molecular- cellular- neurological- and physiological-processes, resulting in altered form, function, or behaviour. Ultimately, the evolution of phenotypic plasticity allows individuals to optimise life history in the context of their immediate physical, ecological and social environment (Gause 1942; Bradshaw 1965; Gross 1996; Scheiner 1993; Warner 1980b, 1991; Blackenhorn 2000; Badyaev 2002; Bonduransky 2006). Hence, selection for individual trait plasticity should be particularly strong when progeny are dispersed over a variable environment, but have little control over their current location (Gause 1942; Bradshaw 1965; Warner 1991; Scheiner 1993). This natural selection pressure is, of course, frequently observed in Nature. Here, I examine the ecological and evolutionary significance of phenotypic plasticity using tropical reef fish as models. Specifically, I examine how individuals optimise life history and behaviour in relation to social-based selection pressures and the implications of these plastic responses to group dynamics and productivity, and the evolution of divergent sex-specific life history strategies.

For a number of reasons, tropical reef fishes are an ideal and important taxanomic group for investigating phenotypic plasticity and its role in ecology and evolution. First, most reef fish life histories include a dispersive pelagic larval phase, making local genetic adaptation of populations a potentially slow process (Victor 1984; Avis and Shapiro 1986; Warner 1991; Leis and McCormick 2002). Second, individuals frequently settle in an environment that is very different from the natal state. Habitat patch size, temperature- and water chemistry-regime, substrate type, vertical relief and depth, food availability, predator- and competitor-assemblage, and the number and density of conspecifics can all vary within the dispersive range of larvae (Leis and McCormick 2002; Jones et al. 2009). In addition, many reef fishes are restricted to the local population or reproductive group into which they settle; migration at a later stage to a more appropriate environment is often limited to those habitats available in the immediate area. Hence, reef fish cohorts will be frequently distributed across a number of physical, ecological and social gradients. These attributes should place strong selective pressure on the evolution of flexible morphological, physiological and behavioural traits (Bradshaw 1965; Warner 1991; Scheiner 1993; Blackenhorn 2000; Baydaev 2002). Finally, individuals from a broad range of fish taxa start life as one sex and later change sex to function as the other (sequential hermaphroditism; for a recent reviews see Munday et al. 2006 and Sadovy de Mitcheson and Liu 2008; also Ghiselin 1969; Warner 1975, 1988a,b; Shapiro 1988; Devlin and Nagahama 2002; appendix table A1.1). This means that females and males carry the same genes, providing a unique opportunity to examine how the sexes achieve divergent phenotypes and behaviour through the modification of shared developmental programs; an important and frequently neglected aspect of animal ecology and evolution (Badyaev 2002).

Empirical and theoretical evidence suggests that spatial and temporal variation in mating and social system dynamics can be a major driver of the evolution of phenotypic plasticity mechanisms within species, and patterns of phenotypic variation among individuals, groups and populations (Warner and Hoffman 1980a; Warner 1984b; Warner 1991; Shapiro 1988; Gross 1996; Buston 2003; Forsgren et al. 2004). The social conditions into which an individual settles can dramatically influence which set of life history strategies are best suited for maximising expected lifetime fecundity (Hamilton 1964; Wilson 1975; Warner 1975, 1991; Emlen and Oring 1977; Gross 1996; Shuster and Wade 2003). In addition, the net benefits of particular traits frequently vary through time within societies. The potential advantage of a particular trait is influenced by how other individuals are optimising their own life history strategy (Maynard Smith 1974, 1982), the level of dominance the individual possesses in relation to competing social group members (Parker 1974; Maynard Smith and Parker 1976), and the level of relatedness between competitors (Hamilton 1964); all of these factors can vary both among and within societies throughout the lifetime of individuals.

Sociality represents a major transition in the evolution of animals (Maynard Smith and Szathmary 1995). The study of variation in trait expression remains central to understanding how and why societies evolve and remain stable through time (Wilson 1975; Johnstone 2000; Buston 2003; Buston and Cant 2003; Wong et al 2007; Buston and Zink 2009), and why different behaviours and life history strategies occur within and between society types (Warner 1975, 1991; Wilson 1975; Warner and Hoffman 1980a,b; Buston 2003; Walker and McCormick 2004; Walker et al. 2007). The study of life history optimisation in response to social system type can also shed light on how the productivity of populations varies in response to socio-demographic factors. The pervasiveness of animal societies, coupled with the sophisticated social faculties that are frequently observed in individuals (such as communication and negotiation mechanisms, conflict-associated learning, and cooperative feeding, defence and breeding; Wilson 1975; Alcock 1993; Bradbury and Vehrencamp 1998; Grosenick et al. 2007), strongly suggests that: 1) social factors have played an important role the evolution of animal cognition, and that; 2) individuals can often increase their expected lifetime reproductive success by forming- or remaining in- cooperative social groups (Allee 1931; Wilson 1975; Stephens and Sutherland 1999; Stephens et al. 1999). For many species we may therefore expect robust and fundamental associations between the environmental determinants of group formation (such as population density and habitat quality), subsequent group size and the overall per capita productivity of populations (Stephens and Sutherland 1999). Indeed, increases in components of per capita fitness with initial increases in population density and group size are observed frequently in nature. These positive, density dependent processes can substantially influence the way populations and communities behave through time and space and therefore, drastically alter the way that populations and communities should be preserved and managed (Myers et al. 1995; Groom 1998; Courchamp et al. 1999; Stephens and Sutherland 1999; Hutchings 2000; DeRoose and Persson 2002; Morris 2002).

1.2 Aims and thesis outline

The extent to which tropical reef fishes modify their physiology, morphology and behaviour in response to local selection pressures, and the implications of this plasticity to the functioning of social groups, populations, and communities remains unclear (Petersen and Warner 2002). The central objective of my thesis is, therefore, to begin to address this paucity of information. Using the tropical sex-changing reef fish *Parapercis cylindrica* as the model species, I test the general hypotheses that: 1) sociol factors have played a significant role in the evolution of phenotypic plasticity, and that; 2) this plasticity drives fundamental relationships between social system type, individual phenotypic expression, and the functioning and productivity of particular social groups. I address these general hypotheses by examine four aspects of *P. cylindrica*'s evolutionary ecology using a combination of theoretical, descriptive and experimental procedures: 1) social stability and the evolution of conflict resolution tactics; 2) sexual selection and the sex-based modification of growth and body size; 3) sex change and the adjustment of sensory organelles, and 4) The relationships between socially facilitated life history optimisation and density dependent per capita fecundity.

In chapter 2, I examine how and why large polygynous social groups of *P. cylindrica* remain stable through time, given the inevitable conflict over reproductive shares between social group members. Specifically, I test the hypothesis that female reproductive skew and social group stability is determined by the combined effects of male reproductive allocation, dominant-female enforcement and subordinate female cooperation. I discuss the likely ultimate factors driving individual group choice and conflict resolution tactics within the context of protogynous sex change and positive density dependence.

In Chapter 3 I examine whether individuals adjust growth and body size in response to variation in social system. Specifically, I test the hypotheses that: 1) sexual selection (i.e. harem size and associated male reproductive success) is the ultimate cause of positive sexual size allometry among social groups, and that; 2) this allometry is driven by the adjustment of somatic growth during sex change from female to male. I discuss how phenotypic plasticity in reef fishes can be used to test general evolutionary theories of morphological diversity, and the important role that individual growth- and body size-modification plays in the shaping of morphological patterns in Nature.

Chapter 4 examines the interaction between sex-change ontogeny and otolith (ear stone) growth. Specifically, I test the hypothesis that otolith growth and morphology is adjusted during the course of sex change, and that the magnitude of this change is coincident with polygyny potential and the male's use of space. I discuss the potential applications of sex-change associated otolith discontinuities to fish and fisheries science, and the potential ultimate drivers of sex-specific divergent otolith growth.

In chapter 5, I explore the relationships between population density, social group composition, operational sex ratio and density-dependent per capita fecundity. Specifically, using current life history theory I develop analytical and Monte Carlo models with which to examine the nature of density dependent per capita fecundity under a range of sex allocation strategies and female body size-fecundity relationships. I then test the assumptions and prediction of the model by addressing the hypotheses that 1) individual *P. cylindrica* adjusts sexual differentiation strategy in response to sexual selection for large male body size, resulting in 2) an initially positive relationship between population density and per capita fecundity (i.e. an Allee effect). I discuss the likely occurrence of sex-change associated fecundity Allee effects in tropical fishes, and some of the potential implications of the Allee effect to fish ecology, conservation and management.

Each chapter represents a stand-alone scientific article suitable for publication (appendix 7). Each chapter has, however, been designed to be part of a broader, coherent body of work which examines the ecological and evolutionary significance of phenotypic plasticity in response to social system variation in reef fishes. Additional research associated with the core themes of this thesis, and undertaken within the context of the project objectives has been included in an appendix of ancillary publications (appendix 8).

1.3 The model species

Parapercis cylindrica (fig 1.1) is one of nine species from the family Pinguipedidae (common name: sand perch) found throughout the Indo-Pacific region (Stroud 1982; Randall et al. 1997). *P. cylindrica* is a short-lived reef fish, reaching a maximum longevity of 1.5 years, and primarily occupies macro algae and coral rubble habitat within sheltered tropical waters (Stroud 1982; Randall et al. 1997; Walker and McCormick 2004). Each female defends a permanent all-purpose territory in which it feeds, spawns, and seeks shelter, and males form harems by defending up to ten females (Stroud 1982). The male pelagic-pair spawns with each ripe female in his harem at sunset each day and spawning activity peaks around the new and full moon (Stroud 1982).

A strong size-based dominance hierarchy exits within each harem, and the male represents the top breeding status. Males are derived exclusively from females (sequential monandric protogyny; appendix fig, A1.1; Walker & McCormick 2004, see also Warner 1975; Munday et al. 2006), and a dominant female will typically change sex following the death of the dominant male or following a female recruitment pulse (Stroud 1982; Walker and McCormick 2004; Frisch et al. 2007). Males can be distinguished from females by the presence of black pigmentation on the jaws and branciostegal rays (Stroud 1982; Walker and McCormick 2004; fig. 1.1).

Figure 1.1 *Parapercis cylindrica*. Female front, male back

For several reasons, *P. cylindrica* is an ideal model species for plasticity studies. First, social and mating system has been shown to vary considerably over extremely small spatial scales (Walker unpublished data; appendix fig. A1.2), and information on reproductive biology and age-based growth suggest that sex allocation strategy, growth and body size are strongly influenced by socio-demographic factors (Walker and McCormick 2004). Importantly, studies to date suggest that these attributes may be common in reef fishes (appendix table A1.1), such that knowledge on the proximate and ultimate drivers of variable trait expression in *P. cylindrica* may provide insight into the evolutionary ecology of reef fishes in general. Second, *P. cylindrica* readily forms social groups, and remain reproductively active when placed in aquaria (Walker personal observations). These attributes make *P. cylindrica* an ideal species for laboratory-based experimentation. Finally, otolith growth in *P. cylindrica* follows an endogenous, diurnal rhythm, resulting in the formation of daily otolithic bands which can be used to reconstruct individual age-based life histories (Walker and McCormick 2004; also Pannella 1971; Campana and Nelson 1985; Gauldie and Nelson 1988).

Chapter 2

Male reproductive allocation, dominant-female enforcement and subordinate-female cooperation act together to promote social stability in a haremic sex-changing reef fish

Abstract

Many animals form stable, relatively peaceful societies despite the uneven apportionment of reproduction among social group members. This phenomenon raises the fundamental questions of how and why genetically selfish individuals resolve conflict over reproductive shares. Here, I examine how conflict is resolved in polygynous societies of the sperm-limited sex-changing reef fish *Parapercis cylindrica*. Specifically, I test the hypotheses that 1) polygynous males promote social stability by allocating limited sperm to larger, more dominant females, thereby minimizing intersexual conflict with the most prized sexual partners, but that 2) dominant females enforce disproportionate female reproductive success by punishing subordinate fecundity maximisers, and that 3) subordinate-females cooperate with dominant females by down-regulating reproduction in response to social interactions with dominant females. Additionally, I test the alternative hypotheses that 4) dominant females directly control reproduction in subordinate females, but 5) promote social stability by conceding some reproduction to subordinates as an incentive to remain peaceful in the group. Results support hypotheses 1, 2 and 3, suggesting that male-reproductive allocation (i.e. sperm allocation), dominant-female enforcement (via punishment), and subordinate-female cooperation (via reproductive down-regulation) act together to promote social stability in *P. cylindrica*. This study supports the novel view that social stability and the partitioning of reproduction in animals societies may frequently involve three-party negotiations between social group members.

2.2 Introduction

Within any given society, natural variability in individual competitive ability renders some order in potential- if not actual- dominance. Thus, at least one individual, for at least some of the time, is likely to have disproportionate bargaining power over the distribution of reproduction, or resources prerequisite to reproduction, among social group members (Parker 1974; Maynard Smith and Parker 1976). Indeed, a vast number of social animals display some level of reproductive skew among individuals of adjacent dominance rank (Vehrencamp 1983; Keller and Reeve 1994; Buston and Zink 2009). Understanding socialiality therefore lies in determining the strategies dominants employ to optimise their bargaining power, how subordinates optimise their own life history strategy in response to the behaviour of dominants (Maynard Smith and Parker 1976), and subsequently, how and why dominants and subordinates resolve conflict over reproductive shares (Hamilton; 1964; Vehrencamp 1983; Ratnieks and Reeve 1992; Frank 1995, 2003; Buston et al. 2007; Wong et al. 2007; Buston and Zink 2009).

Within the sex that reproductive skew is manifest, a dominant can theoretically utilise its greater bargaining power whilst promoting social stability in a number of ways. First, the dominant can opt to directly control reproduction (or resources prerequisite to reproduction), but to concede some reproduction to the subordinate as an incentive to remain peaceful in the group. The pattern of reproductive apportionment (or skew) in a stable society should then reflect the amount of reproduction the dominant must concede to keep the subordinate from entering into combat over reproductive shares (the inside option; Buston and Zink 2009), or dissolving the group (i.e. via forcible eviction or departure; the outside option; Buson and Zink 2009) (also see Keller and Reeve 1994; Johnstone 2000; Cant et al. 2006). Alternatively, dominants may opt to apportion reproduction indirectly to subordinates through the threat of punishment, whereby the dominant regulates ‘breaches in contract’ (i.e. the negotiated settlement over reproductive shares), rather than the reproductive behaviour of all individuals at all times (Gaston 1978; Hamilton and Taborsky 2005; Johnstone and Cant 1999; Johnstone 2000; Henrich et al. 2006; Wong et al. 2007). Reproductive skew in a stable society should then, primarily reflect the level of reproductive restraint required by the subordinate to keep the dominant from entering into combat over reproductive shares, or dissolving the group (i.e. via forcible eviction or departure) (Johnstone 2000; Wong et al. 2007).

A third, less well explored possibility is that one sex plays a role in allocating reproduction and resolving conflict among individuals of the other (Cant and Reeve 2002). For example, in permanently haremic fishes and other external fertilizers, males frequently economise sperm production so as to maximize energy-allocation towards somatic growth, territory-border patrol and intrasexual competition (Warner and Robertson 1978; Nakatsura and Kramer 1982; Shapiro et al. 1994; Marconato et al. 1995, 1997; Warner et al 1995). Males may preferentially allocate limited sperm, and/or resources prerequisite for reproduction to larger females, thereby ameliorating intrasexual competition among female group members (by satisfying dominant females which are most of capable of dissolving the group) and minimising intersexual conflict with the most prized sexual partners (Lutnesky and Kosaki 1994). Perhaps more likely, however, is the evolution of male reproductive allocation coupled with dominant-female enforcement; since selection would favour cheating males who allocate resources equally among females, thereby maximising the genetic diversity of young and circumventing the cost of diminishing returns (e.g. in external fertilizers where fertilization rate (FR) per spermatozoa declines as FR reaches 100%; Warner et al. 1995; Marconato and Shapiro 1996). Importantly, under these conditions it would also be advantageous for females to regulate gamete production rate according to the amount of sperm likely to be allocated, so as not to waste valuable energy for further growth and reproduction.

If individuals are to optimise reproduction, two things are necessary: (1) reliable indicators pertaining to intersexual reproductive allocation and the potential for intrasexual conflict; and (2) the accurate transduction of social stimuli to reproductive metabolism (Fox et al. 1997). These prerequisite life history characteristics appear to be widespread among animals; communication-mechanisms (Bradbury and Vehrencamp 1998), conflict- and rank- associated cognition (Grosenick et al. 2007), and (thus) the evolution of honest signalling (Bradbury and Vehrencamp 1998; Tibbetts and Dale 2004), have been identified in primates (Ferris et al. 2005; Henrich et al. 2007), birds (Schweble 1988; Goodson 2005), fishes (Munday et al. 2006; McCormick 2006, Grosenick et al. 2007), reptiles and amphibians (Moore and Millar 1984), crustaceans (Becker et al. 2005) and insects (Tibbetts and Dale 2004). In addition, changes in social setting have been shown to immediately affect neural activity within brain regions that concentrate steroid hormones (Goodson et al. 2005), and activate the release of corticosteroid hormones into circulation through stimulation of the hypothalamic-pituitary-adrenal axis (Wendelaar Bonga 1997; Mommsen et al. 1999; Young 2006). Corticosteroids are directly involved in altering gene expression by binding to intracellular glucocorticoid receptors leading to dimerization, nuclear entry, and transcriptional activation or repression of genes bearing consensus elements in their upstream promoter regions (Meisfeld et al. 1986). Corticosteroids, particularly cortisol, elevate metabolism and free-energy levels to sustain the flight or fight response, and are implicated in the regulation of somatic growth and reproductive activity (Rivier and Rivest 1991; Young 2006; Fox et al. 2007).

Previous studies on the haremic reef fish *Parapercis cylindrica* suggest that male fecundity becomes limited at the upper end of the haremic-group size range (Stroud 1982). Sperm economy is highly likely in this species, due to the strong energetic trade-off between growth, female defence and sperm production (Stroud 1982; Walker and McCormick 2004, 2009b, Chapter 3). Stroud (1982) illustrated that individual female group members are most aggressive towards the next most dominant female within the group, and that dominant females are more tolerant towards reproductive non-competitors (i.e. non-reproducing females) compared to reproductive competitors (ripe females). Together, these studies suggest conflict over reproduction both between the sexes and among female group members. Here, I investigate how reproduction is apportioned among the females of large haremic groups. Specifically, I ask: (*i*) do males allocate sperm disproportionately among females; *(ii)* do dominant females control subordinate reproduction by limiting access to males; (*iii*) do dominant females punish subordinate females who maximise reproduction in the form of increased aggression and attack; (*iv*) do subordinate females regulate reproduction in response to behavioural stimulus from dominant females and/or males; (*v*) does cortisol concentration correlate with dominance status and reproductive activity consistent with the proximate hypothesis that reproductive regulation is driven by a behaviourally mediated neuroendocrinal mechanism?

2.3 Materials and Methods

(a) Study species

P. cylindrica is a short-lived reef fish that occupies macro-algae and coral-rubble habitat within shallow tropical waters. Each female defends a permanent all-purpose territory in which it feeds, spawns, and seeks shelter, and males form harems by defending up to ten neighbouring females. While occasionally a neighbouring haremic male may steal or ambush a pair-spawn, bachelor and roaming males are absent from populations. A strong dominance hierarchy exists within each harem where dominance status is directly related to relative body size (Stroud 1982). Males are derived exclusively from females (sequential monandric protogynous hermaphroditism) and represent the top breeding status (Stroud 1982; Frisch et al. 2007). Pelagic pair-spawning takes place every day for several days around each new and full moon throughout the year, (Stroud 1982, Walker unpublished data). The male sequentially pair-spawns in the water column with ripe females in his harem during sunset, and there is no parental care.

(b) Egg production

All field data were collected during September 2004, September-October 2005, and May 2006 within the lagoon of Lizard Island, Great Barrier Reef, Australia (14º 40.9'S, 145º 26.8' E). To establish the size-based female gamete production rate throughout the lunar cycle, at least 10 females were collected each afternoon throughout the sampling periods from randomly selected populations within the hour prior to spawning. These individuals were anesthetized with clove oil, measured (mm TL) and their eggs stripped and stored in 70% ethanol for counting in the laboratory (herein referred to as stripping data).

(c) Inter- and intra-sexual behaviour and spawning dynamics: do dominant females control reproduction in subordinate females

The rank-specific behaviour and spawning dynamics of haremic social group members were examined in the wild to determine rank-specific fecundity, male-behavioural interaction rates with females of different rank, and to examine whether dominant females control reproduction in subordinate females. At the beginning of each sampling period, all individuals from harems within a 2500m^2 area were captured using clove oil anaesthetic and a hand net, measured (mm total length; TL), visually sexed, and tagged using a subcutaneous tattoo for individual recognition (Northwest Marine Inc.). A reference grid at 5x5m resolution was placed over the habitat to aid in the tracking and relocation of individuals. Individuals were observed during 15 min intervals throughout the day up until the time of spawning (17:00-18:00) to establish harem membership (the male territory in which the female resides), and inter- and intra-sexual interaction rates.

Interaction categories were: non-violent (lateral display, head bobbing); violent (attack) (see Stroud 1982 for complete behavioural repertoire). Data were collected for a total of 21 males (harems) and 117 females.

During the spawning period a focal harem from the tagged population was observed to examine whether dominant females restricted or interfered with subordinate female spawning, and to record the number of pair-spawns, spawning participants, and the time of spawns. Differences in spawning frequency among polygynous females (n = 24) as a function of lunar phase and dominance rank were analysed using two-way ANOVA.

To determine whether reduced spawning frequency in polygynous subordinates was due to body size or dominance rank, the spawning frequency of experimental monogamous pairs were examined. During the 2005 and 2006 sampling periods, 10 patch reefs were created from a generous supply or coral rubble and macro-algae habitat and populated with a single male and female. The complete female size range was used and the male was always the largest individual. Differences in the size-based monthly spawning frequencies of monogamous and polygynous females were examined for statistical significance using ANOVA with body size entered as a covariate.

(d) Do males differentially allocate sperm to females of adjacent dominance rank?

Consecutive pair-spawn clouds from the focal harem were collected in separate 50 l plastic bags to determine fertilisation rate (FR). Spawn clouds were captured 10 s following gamete release and incubated *in situ* for approximately 2 hr with constant air supply and running sea water (Shapiro et al. 1994; Marconato et al. 1995, 1997; Warner et al. 1995). The water sample was then filtered to isolate eggs which were scored as fertilised or unfertilised based on the presence or absence of cell division respectively. Linear regression was used to determine the relationship between the number of daily spawns and mean FR (spawn n = 70, day n = 11), and the relationship between FR and dominance rank.

(d) Do dominant females punish subordinate females who maximise egg production?

To establish whether dominant females punish subordinate females who maximise egg production, I examined the rate of aggressive behaviour by dominants towards subordinates at varying fecundity levels. For each replicate observation (*n* = 19) a subordinate female was placed in a clear plastic bag and the bag then placed adjacent to the territory of a larger, dominant female. I counted the number of attack attempts (bites to the bag) during a 5 min period from first approach. Pilot studies illustrated that dominants do not respond to an empty bag. Following observation the dominant was captured and its size recorded (mm TL). Both the length (mm TL) and maximum width (mm) of stimulus subordinate females were recorded. Each individual's fecundity index was then calculated by dividing the width by the total length (see fig. 2.3.1). To avoid the confounding effect of previous feeding history on a subordinate's fecundity index, regurgitation was induced prior to taking measurements using a 10ppt clove oil solution. The effects of subordinate size (mm TL), subordinate fecundity index, and subordinate-to-dominant size ratio (subordinate TL mm/dominant TL mm) on the number of attacks by the dominant (during 5 min) were examined using multiple regression.

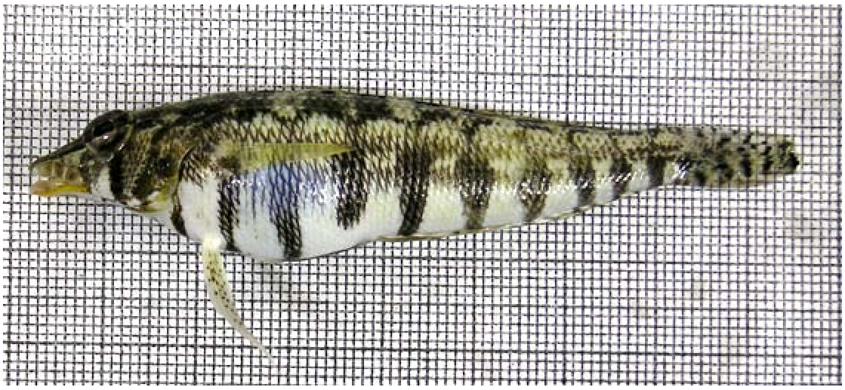

Figure 2.3.1. Example of a ripe female- as indicated by the swollen abdominal region- use in the bag experiment to examine the effect of subordinate female ripeness on dominant female aggression.

(e) Do polygynous females regulate reproduction in response to dominance status?

Experiments in 100 l aquaria were carried out at Lizard Island Research Station. Experimental fish were collected from wild populations within the Lizard Island lagoon and placed in aquaria within the hour. In all tanks an equal amount of coral rubble and macro-algae habitat per individual was provided, and each tank was supplied with running sea water and a constant air supply. All individuals were fed to satiation twice daily with shrimp and a commercial supplement.

Experiment 1 was designed to determine: whether males stimulate gonad development; the effect of dominance rank on egg production rate; the rate of increase in egg production associated with an increase in dominance status. Thirty-five microcosms were created: ten monogamous pairs (one male and one female), twenty bigamous groups (one male and two females), and five solitary females. When present, the male was always the largest individual. In ten of the bigamous groups the smaller subordinate female was physically separated from the larger dominant female and male, and in the other ten bigamous groups the larger dominant female was physically separated from the male and smaller subordinate female; the ventilated glass dividers allowed for visual- and olfactory- communication but prevented physical contact and spawning. Each afternoon the water supply was turned off so that spawned eggs could be siphoned from the tank and counted. In five of the bigamous groups where the dominant female was physically separated from the male and subordinate female, the dominant female was removed 15 days following the commencement of the experiment to create monogamous pairs. This was done to detect any change in reproductive activity associated with an increase in dominance status. Thirty days following the commencement of the experiment, all remaining individuals were sacrificed with an overdose of anesthetic (30 ppt of clove oil) and the body (mm TL) and gonad (g) measured. Egg production data was standardized for body size, and then analyzed in respect to dominance status and time (before and after dominant female-removal) using repeated-measures ANOVA. Differences in gonad size between solitary females, monogamous females, and bigamous females were analyzed using one-way ANOVA.

(f) Does female reproductive activity correlate with cortisol concentration?

Experiment 2 examined the effect of dominance status on cortisol concentration and gonad development. Twenty-eight microcosms were created: eighteen monogamous pairs (one male and one female) and 10 polygamous groups (1 male and 5 females). The entire female size-range was used, and the male was always the largest individual. Microcosms were maintained for 30 d (as above), after which time individuals were sacrificed (as above). The body (mmTL) and gonad (g) were then measured, and the bodies frozen at -80^{o} C until hormone assay. The relative effects of body size and dominance rank on gonad development (gonad weight) were analyzed using multiple regression. Difference in cortisol concentration as a function of dominance rank was analyzed using one-way ANOVA.

(g) Hormone assays

Whole-body concentration of cortisol was measured for all individuals from tank experiment 2. First, each fish was cut into small pieces (~0.5 cm^3) and homogenized in ethyl acetate (3 $ml.g^{-1}$ bodyweight) using a mortar and pestle. Next, the homogenate was transferred to a 100 ml beaker and mixed vigorously for 1 min. The mixture was allowed to settle for ~10 s, after which 2 ml of extract was transferred to an eppendorf tube for centrifugation at 3000×*g* for 5 min. Aliquots of supernatant (200 µl each) were transferred to duplicate polycarbonate vials for evaporation to dryness (~14 hr). Cortisol concentration was then measured by radio-immunoassay using the procedure described by Frisch and Anderson (2005). Extraction efficiency (mean recovery of $[^3H]$-labeled steroid that was added to triplicate whole fish before homogenization) was 65%, and assay values were adjusted accordingly. Assay specificity was verified by confirming parallelism in the binding curves of serially-diluted extracts and steroid standards. The minimum detectable cortisol concentration was 0.07 $ng.g^{-1}$.

2.4 Results

(a) Female size-based gamete production and rank-based spawning dynamics

In the wild, female egg production (from stripped eggs) was found to be linearly related to total length (No. eggs = 37.334(TL mm) – 2053, adjusted $r^2 = 0.26$, $F_{1,122} = 44.2$, $p < 0.01$; fig. 2.4.1*a*). Variance in egg production was further explained by incorporating tide-height during the spawning period (18:00) on the day of collection into the model (multiple regression; adjusted $r^2 = 0.5$, $F_{2,567} = 98.03$, $p < 0.001$); both egg production rates and spawning activity were maximized during the new and full moon when tide height was greatest during the spawning period (18:00-19:00).

The spawning frequency of polygynous females in the wild statistically differed between the new and full moon spawning peaks (ANOVA: $F_{1,32} = 180.2$, $p < 0.01$) and between dominance rank (AVOVA: $F_{7,31} = 29.65$, $p < 0.01$; fig. 2.4.1). Spawning frequency was greater during the full moon spawning peak compared to the new moon spawning peak, and larger, higher ranking females spawned more than lower ranking females (polygynous female monthly spawning frequency = 0.3284(mm TL) - 18.453, $r^2 = 0.8276$; fig. 2.4.1*b*). The time of day that polygynous females spawn with the male was also found to dependent on dominance rank (spawning time (min from 18:00) = -

3.6(female dominance rank) + 28.6, $r^2 = 0.48$, $F_{1,152} = 139.25$, $p < 0.001$); on average, the lowest ranking females spawned first and the dominant females spawned last.

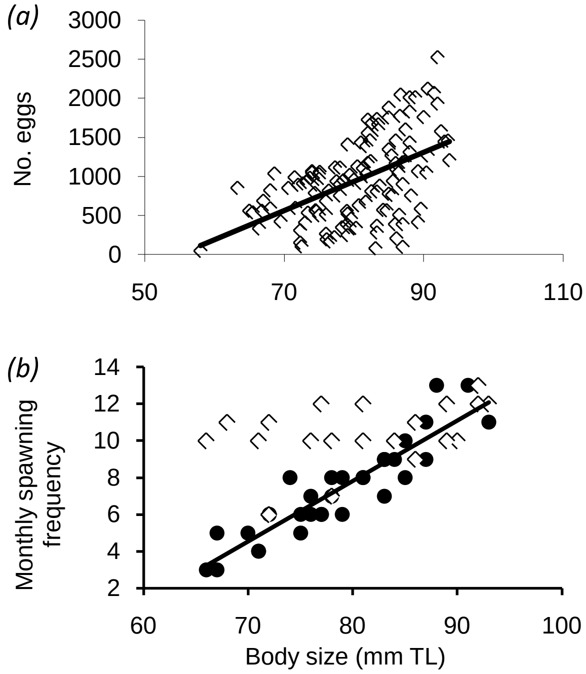

Figure 2.4.1 (*a*) Relationship between female body size and egg production (from the stripping data). (*b*) Size-based monthly spawning frequency of monogamous females (white triangle) and polygynous females (black circle).

Unlike polygynous females, no relationship was found between body size and monthly spawning frequency among experimental monogamous females in the wild (linear regression: $F_{1,18} = 3.1$, $p = 0.09$; fig. 2.4.1*b*). Monogamous females spawned statistically more than equal-sized subordinate polygynous females (ANOVA: $F_{1,48} = 26.5$, $p < 0.001$; fig. 2.4.1), and on average displayed a similar monthly spawning frequency to dominant polygynous females (fig. 2.4.1*b*).

(b) Male-to-female time allocation, fertilisation rates and sperm allocation

In the wild, the level of interactions between males (male n = 21) and the females within their harems (female n = 117) was found to statistically differ in respect to time of day (ANOVA: $F_{1,306} = 722.8$, $p < 0.001$) and female dominance rank (ANOVA: $F_{6,306} = 34.78$, $p < 0.001$, fig. 2.4.2*a,b*). Males interacted more with females leading up to the spawning period (17:00-18:00 hr; 3.29 ± 0.13 interactions per 15 min), compared to during the day (8:00-16:00 hr; 0.7 ± 0.05 interactions per 15 min), and allocated more time to females of higher-dominance rank (i.e. larger females) (fig. 2.4.2*a,b*). In addition, the disproportionate interaction time among female dominance ranks was more pronounced during the day compared to the pre-spawning period (ANOVA: $F_{6,308} = 10.77$, $p < 0.001$; fig. 2.4.2*b*).

Spawn clouds were collected from polygynous pair-spawns to determine sperm number, egg number and fertilisation rate. However, due to poor light conditions it was impossible to observe whether entire spawn clouds were captured during collection. Hence, only fertilisation rate was calculated. The mean fertilisation rate (FR) of pair-spawns predictably declined with the total number of consecutive spawns within an afternoon (FR = -0.33(No. spawns) + 98.136, $r^2 = 0.3$, $F_{1,12} = 5.18$, $p = 0.042$; fig. 2.4.2), suggesting that males suffer from sperm depletion. However, the range of FR (88-98%) and decline associated with number of spawns ($\beta = -0.33$) were low. In addition, FR was found to decline with descending dominance rank (FR = -0.292(dominance rank) + 97.126; $r^2 = 0.3$, $F_{1,68} = 3.9$, $p = 0.05$). From the pooled data, the mean fertilization rate was 96.1 ± 0.31 %.

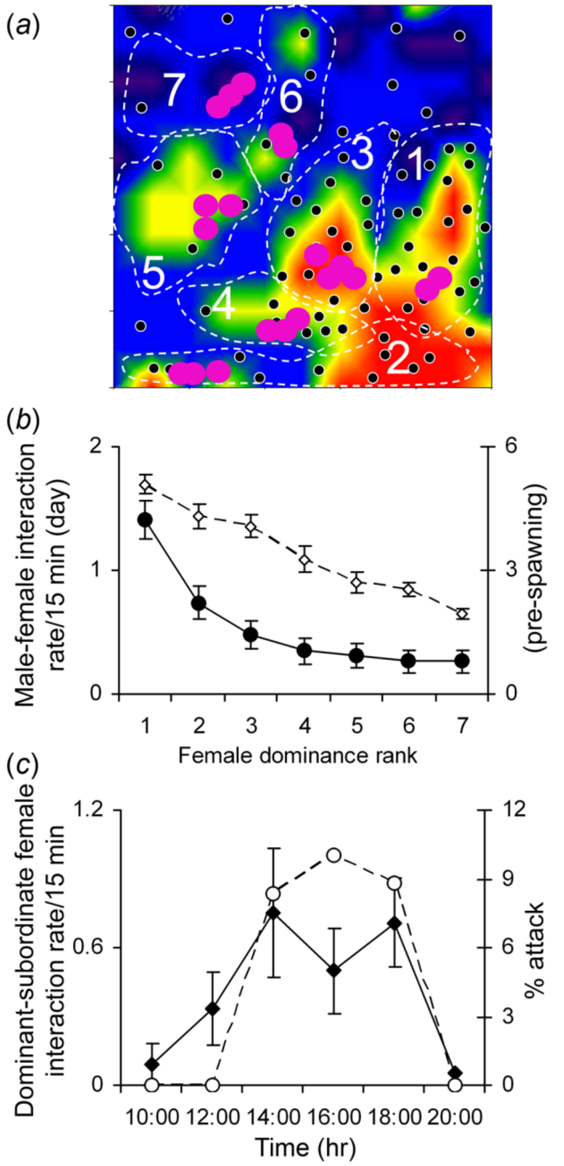

Figure 2.4.2 (*a*) A representative harem in *P. cylindrica* illustrating disproportionate male- allocation time between females (black dots = male location at 1min intervals; three15 min observation periods), rank-based female territory size (white broken lines with rank number), and pelagic pair-spawning sites within each female territory (pink dots). Composite map is a 25m^2 plot. Contours = substrate elevation from blue (0 cm) to red (40 cm). (*b*) Differences in male-behavioral interaction rate among female-dominance ranks during the day (9:00-16:00; unbroken line) and during the pre-spawning period (17:00-18:00; broken line). (*c*) Dominant-to-subordinate behavioral-interaction rate among females in respect to time of day (unbroken line), and the percentage of interactions by dominants that resulted in physical attacks (broken line). *x* = time-range maximum. Spawning occurs between 18:00 and 19:00 Data presented as the arithmetic mean ± S.E.

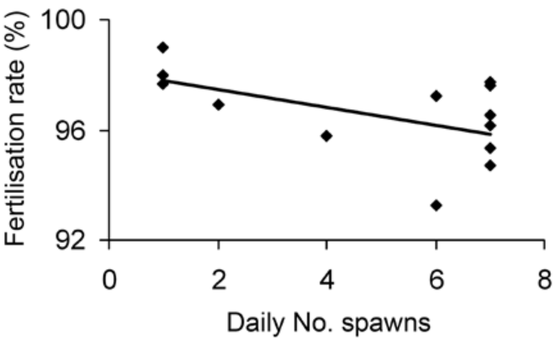

Figure 2.4.3 Relationship between number of daily pair-spawns and mean fertilization rate (± S.E.).

(d) Do dominant females control reproduction in subordinates?

A total of 66 dominant-female to subordinate-female antagonistic interactions from 186 15 min observations were recorded among females in the wild. However, only a small fraction of these constituted physical attacks (7.7%).The majority of interactions between females involved head-bobbing and lateral displays (92.3%; fig 2.4.3*c*). Dominant-female to subordinate-female interaction rate significantly increased from morning to afternoon, and then sharply declined at the commencement of the spawning period ($F_{1,5}$ = 4.75, $p < 0.001$; fig 2.4.3*c*). At the commencement of the spawning period, males became extremely mobile, sequentially visiting each female within the harem. By contrast, females moved little, and waited for the male to approach (fig. 2.4.3*a*). Once the male approached, the female performed ‘s-bend’ displays, arching her abdominal region so it was in clear view of the male. In addition, each female was observed to spawn at the same site within her territory each afternoon (fig. 2.4.3*a*). Thus, dominant females become more aggressive leading up to the spawning period, but do not actively prevent subordinate females from spawning with the male.

(e) Do dominants females punish subordinate females for maximising egg production?

Attack rate by dominant females towards subordinate females during the day was experimentally found increase with both a reduction in the size difference between the dominant female and subordinate female and with an increase in subordinate fecundity index (multiple regression: whole model r^2 = 0.69, $F_{3,15}$ = 11.04, $p > 0.01$; size ratio partial correlation coefficient = 0.55, p = 0.021, fecundity index partial correlation coefficient = 0.57, p = 0.018). Attack rate by dominant females towards subordinate females was also positively related to subordinate total length, however the independent effect of subordinate TL was non-significant (subordinate TL partial correlation coefficient = 0.12, $p > 0.5$). Subordinate fecundity index was found to explain 31.5% of the residual variance in dominant female attack rate once the effects of dominant-to-subordinate size ratio and subordinate TL were extracted from the model (residual attack rate = 68.655(subordinate fecundity index) -13.799, $r^2 = 0.315$, $F_{1,17} = 7.82$, p = 0.012; fig.2.4.4).

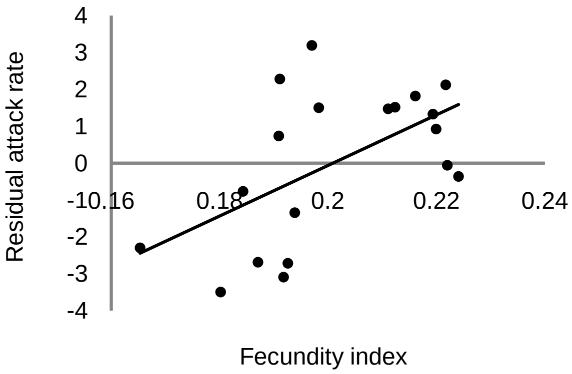

Figure 2.4.4. Relationship between subordinate female fecundity index and residual dominant female attack rate towards subordinate females (from the reduced subordinate size (mm TL) + size ratio model).

(f) Do polygynous females regulate reproduction in response to dominance status?

Interaction with the dominant females did not affect egg viability in subordinate females; a fertilization rate of ≥99% was achieved for all spawns in aquaria in both the female-dominant and female-subordinate treatments. However, interaction with the dominant female did affect reproductive output in the subordinate female; throughout the first 15 d of experiment 1, monogamous females spawned the greatest number of eggs (per unit of body size), followed by dominant bigamous females, and then subordinate bigamous females (repeated-measures ANOVA; $F_{2,12} = 5.2$, p = 0.024; fig. 2.4.5*a*). Since dominant and subordinate females were separated by a glass divider and both fed to satiation, these data illustrate that female egg production is proximately responsive to intrasexual social interaction and perceived dominance rank.

During the second 15 d experimental period following the removal of dominant females, subordinate females increased egg production to a rate greater than that of monogamous females and dominant bigamous females (repeated-measures ANOVA; $F_{2,12} = 5.2$, $p = 0.024$; fig. 2.4.5*a*). Differences in gonad size between female dominance rank, and the change in gonad size with an increase in dominance status, co-varied with egg production data (fig. 2.4.5*b*). Results from experiment 1 further illustrate that a visual- and/or olfactory-cue from a male is required to stimulate female gonad development and egg production; at the end of the experiment solitary females had a significantly lower mean gonad weight per body size compared to monogamous females, dominant bigamous females and bigamous subordinate females (ANOVA; $F_{4,40} = 15.51$, $p<0.001$; fig. 2.4.5*b*).

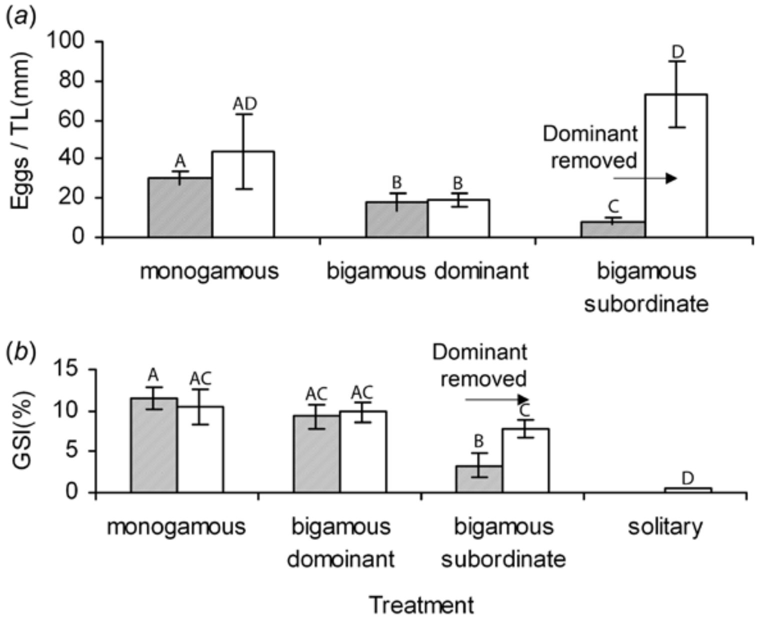

Figure 2.4.5. Differences in egg production (*a*) and gonado-somatic index (GSI) (*b*) as a function of social status (grey = experimental time period 1; before dominant-removal; white = experimental time period 2; after dominant-removal). Data presented as arithmetic mean ± SE. Letters denote *post hoc* significant differences (Tukey's HSD)

(g) Is female reproductive skew reflected in cortisol concentration?

Cortisol concentration was found to statistically differ between males and females of different social rank (ANOVA: $F_{4,50} = 5.1$, $p = 0.002$; fig. 2.4.6). Cortisol concentration was higher in females compared to males, and among females was lowest in monogamous individuals, mid-range in polygamous dominants, and highest in polygamous subordinates (fig. 2.4.6). No relationship was found between cortisol concentration and female body size (linear regression; $F_{1,46} = 3.4$, $p = 0.07$). From the same experimental female fish, female gonad weight was found to be related to both body size and dominance rank (multiple regression: $F_{2,45} = 33.6$, $p < 0.001$). Gonad size increased with body size (partial correlation coefficient = 0.47, $p < 0.001$), however for any given body size gonad size decreased with descending dominance rank (i.e. from dominance rank 1 to 5; partial correlation coefficient = -0.33, $p = 0.02$).

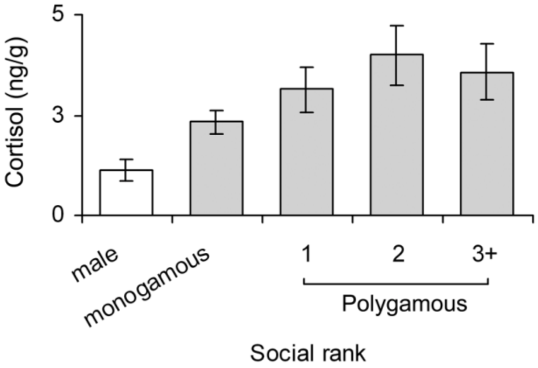

Figure 2.4.6 Whole-body cortisol concentration as a function of dominance rank after 30 d (white = male, grey = female). Data presented at arithmetic mean ± SE.

2.5 Discussion

A fundamental question in the study of social evolution is how social groups remain stable through time in the face of conflict over reproduction (Keller and Reeve 1994; Johnstone 2000; Young et al. 2006). Results for the reef fish *Parapercis cylindrica* suggest that social stability may be owed to the combined effects of male reproductive allocation, dominant-female enforcement, and subordinate-female cooperation.

Reproductive skew: patterns and proximate factors

In *P. cylindrica,* monogamous females spawned more per month than similar-sized polygynous females. In addition, reproduction among polygynous females was disproportionate in respect to dominance status, with larger dominant females displaying a greater spawning frequency than smaller subordinate females. Experimental data illustrate that female reproductive skew (FRS) is proximately driven by behaviorally mediated individual plasticity in reproductive activity; visual and/or olfactory interaction with a dominant female triggers reproductive down-regulation in subordinate females, while removal of the dominant female triggers up-regulation in subordinate females. The relationship between social interaction and reproductive rate is likely to be mediated through the stress hormone cortisol, the dominant corticosteroid in fishes (Carragher et al. 1989; Fox et al. 1997; Frisch and Anderson 2000; Barton 2002). In *P. cylindrica,* cortisol concentration and the rate of egg production covaried with dominance rank. Studies to date suggest that antagonistic interaction with dominants stimulate cortisol production through activation of the hypothalamic-pituitary-interrenal axis. In turn, cortisol may disrupt oocyte development by down-regulating the pituitary's sensitivity to gonadotropin-releasing hormone and by altering the stimulatory effects of gonadotropins on gonadal steroid production (Rivier and Rivest 1991). It is noteworthy, however, that whole-body cortisol concentration does not prevent subordinate *P. cylindrica* females from changing sex to function as male (Frisch et al. 2007). Thus, it seems that cortisol regulates reproductive rate and gamete production, but not reproductive tactic *per se*. This evolutionary decoupling between reproductive tactic and reproductive activity is likely to be extremely advantageous for individuals who must act quickly to secure reproductive dominance, and/or take advantage of alternative reproductive tactic-potentials when the opportunity arises (Walker and McCormick, 2009b).

Equally important, is the novel finding that male behavioural interactions are required to stimulate female gonad development and the production of eggs, and that male to female interaction rate in the wild positively correlates with female dominance rank, body size, and ultimately, fecundity. While the effect of male interaction on female-hormone synthesis and subsequent oogenesis was outside the scope of the present study, experimental data clearly illustrates that the male stimulates reproduction in females. It is therefore likely that females regulate reproduction in response to the male-versus-dominant female interaction ratio.

Reproductive skew and sociality: ultimate factors

Sperm economy is a common reproductive tactic among haremic fishes and is thought to be associated with the strong energetic trade-off between sperm production, growth and intrasexual competition (Warner and Robertson 1978; Nakatsura and Kramer 1982; Marconato et al. 1995, 1997; Warner et al. 1995). While sperm economy can increase conflict among polygynous group members (Warner et al. 1995), I suggested that this conflict could be minimised if males allocate more sperm to larger, more dominant females to the detriment of subordinate females. Results supported the hypothesis: mean fertilisation rate was found to predictably decrease with the number of daily spawns (illustrating sperm limitation); yet dominant females achieved significantly higher FR despite the fact that they produce more eggs and spawn later in the day. I also suggested that dominant females are likely to enforce disproportionate female reproductive success, since the male may be able to increase fecundity by cheating. This hypothesis was also supported by results: evidence suggests that dominant females utilise a punishment strategy in the form of increased aggression and attack in response increased egg production by subordinates. Finally, I suggested that in the event of male sperm allocation and/or dominant female punishment, subordinate females should down regulate reproduction to avoid the waste of valuable energy and the prospect of costly punishment. Consistent with this hypothesis, subordinate females were found to regulate reproduction in response to both male (subordinate female up-regulation) and dominant female (subordinate female down-regulation) behavioural interactions. All together, the results of this study suggest a transactional framework for conflict resolution in *P. cylindrica*, whereby males, dominant females and subordinate females negotiate the terms of group membership.

So, why do subordinates remain with the polygynous group, and why do dominates tolerate subordinates at all? In the case of *P. cylindrica* and monandric sequential hermaphroditic fishes in general (Thresher 1982; Warner 1984; Munday 2004), the stability of polygynyous societies must be assessed in the context of sex-change and resultant lifetime fecundity. Selection should favour females which optimise the trade-off between reproductive losses associated with group membership, and future reproductive success following an increase in female dominance rank and finally a change in sexual function from female to male. While subordinates can potentially reduce the dominant female's fecundity in the present, they also equate to the dominant female's future potential reproductive success (following sex change). The same argument holds for subordinates. However, for such a mechanism to be in place there must be some follow-on effect from being associated with the polygynous group as a subordinate (as opposed to opting for monogamy) and eventually taking over a productive harem (i.e. the dominance hierarchy must also act as a social queue) Previous studies strongly suggest that this is the case (Walker and McCormick 2009b); when male *P. cylindrica* were removed from social groups in the wild, dominant females changed sex and subsequently took over the harem from which they were associated and remaining females moved up the dominance hierarchy. Dominant sex-changing females from small polygynous social groups recreated small harems and monogamous pairs, while dominant sex-changing females from large polygynous social groups recreated large harems. Hence, reproductive dominance-rank inheritance (via sex change) is likely to be the principle mechanism driving tolerance among dominant females, and group choice among subordinates (Shapiro and Boulon 1987; Walker and McCormick 2009b).

Reproductive down-regulation (or failure) in subordinates due to aggression by the dominant is often interpreted as an extreme form of dominant control, which may serve as a backup strategy for dominants during lapses in subordinate reproductive restraint (e.g. Young et al. 2006). This is to say that the physiological manifestation of stress in the subordinate is an advantageous trait for the dominant. This kind of interpretation likely follows from the historic emphasis on 'social-inhibition' of trait expression by the dominant, as opposed to socially driven plasticity in the subordinate, and the tendency to view the stress-response as a deleterious physiological condition (e.g. Metcalfe 1986; Carragher 1989; Young et al. 2006). However, the adaptive significance of stress-responses must be interpreted in relation to the present- and future- fitness consequences for the individual displaying it (Mayr 1962; Tinbergen 1964; Sherman 1988). Therefore, stress-induced reproductive down-regulation (or failure) is best interpreted as an extreme form of subordinate restraint which acts to resolve conflict over resources with the dominant, allowing the subordinate to remain in the group, and increase reproduction later.

Typically, reproductive skew is conceptualised, and empirically examined using a two-party transactional framework (but see Cant and Reeve 2002), such that the observed pattern of skew is assumed to reflect interactions solely between individuals of the same sex. However, this approach is at odds with observations in a number of species. Several studies, for example, have illustrated that females exert control over the distribution of paternity (Davies 1992; Eberhard 1998; Whittingham and Dunn 1998; Cant 2000). In the present study, we found evidence to suggest that males exert at least some control over the distribution of maternity; by preferentially allocating sperm among larger, more dominant females. Taken together, these studies suggest that the inclusion of three-party interactions into the existing two-party transactional framework (Buston and Zink 2009) may provide valuable insight into the evolution and ecology of animal societies.

Chapter 3

Sexual selection explains sex-specific growth plasticity and positive allometry for sexual size dimorphism in a reef fish.

Abstract

Rensch (1950) noted that in clades where males are the larger sex, sexual size dimorphism (SSD) tends to be more pronounced in larger species. This fundamental allometric relationship is now known as 'Rensch's rule'. While most researchers attribute Rensch's rule to sexual selection for male size, experimental evidence is lacking. Here, I suggest that ultimate hypotheses for Rensch's rule should also apply to groups of individuals, and that individual trait plasticity can be used to test those hypotheses experimentally. Specifically, I show that in the sex-changing fish *Parapercis cylindrica*, larger males have larger harems with larger females, and that SSD increases with harem size. Thus, sexual selection for male body size is the ultimate cause of sexual size allometry. In addition, I experimentally illustrate a positive relationship between polygyny-potential and individual growth rate during sex change from female to male. Thus, sexual selection is the ultimate cause of variation in growth rate, and variation in growth rate is the proximate cause of sexual size allometry. Taken together, these results provide compelling evidence in support of the sexual selection hypothesis for Rensch's rule, and highlight the potential importance of individual growth modification in the shaping of morphological patterns in Nature.

3.2 Introduction

When species are compared within a clade, sexual size dimorphism (SSD) is often found to vary with body size, a pattern initially observed by Rensch (1950). This allometry is detected as $\beta \neq 1$, where β is the slope of a model II regression of log(male size) on log(female size) (Fairbairn 1997; 2005). Most frequently, β exceeds 1 (i.e. positive size allometry), representing an increases in SSD with size when males are the larger sex, and a decrease in SSD with size when females are the larger sex, a trend formalized as 'Rensch's rule' (*sensu* Fairbairn 1997) (primates; Clutton-Brock et al. 1977, turtles; Berry and Shine 1980, water striders; Anderson 1997; Fairbairn 2005, mites; Colwell 2000, salmonid fishes; Young 2005, birds; Payne 1984; Colwell 2000; Székely et al. 2004; Raihani et al. 2006; Dale et al. 2007). The conformation of Rensch's rule in a broad range of taxa highlights the need to examine the evolutionary processes behind it (Fairbairn 1997). This remains an important research focus (Fairbairn 1997; Dale et al. 2007).

In comparative studies of SSD, it is generally assumed that adult female- and male-body size is genetically fixed, such that Rensch's rule reflects co-evolution between absolute female- and male-body size coupled with greater evolutionary divergence in absolute male body size (Fairbairn 1997; Teder and Tammaru 2005; Dale et al. 2007). However, recent studies have documented a pattern positive size allometry among genetically similar subpopulations (e.g. Pyron et al. 2007; Fernández-Montraveta and Moya-laraño 2007; Lengkeek et al. 2008), suggesting that 1) Rensch's rule can also represent an environmentally induced sex-specific phenotypic response, such that 2) comparative studies of SSD among populations and species may be confounded by intrapopulational processes (Teder and Tammaru 2005; Fairbairn 2005; Young 2005), but that 3) we can use body size plasticity to test evolutionary theories pertaining to broader morphological patterns (e.g. Rensch's rule) (Warner 1991; Buston and Cant 2006; Kohda 2008).

Absolute interspecific patterns in trait expression represent the genetic response of different species to selection through evolutionary time. Similarly, trait plasticity represents a functional adaptive response to local environmental conditions (Brashaw 1965; Warner 1991). Hence, irrespective of the taxonomic scale of inference or the details of proximate causality, ultimate hypotheses (i.e. those pertaining to adaptive significance) for positive size allometry are general (Mayr 1961; Tinbergen 1964; Sherman 1988; Warner 1991), and can be grouped into three broad categories (*sensu*

Dale et al. 2007): (i) sex-specific constraints, (ii) natural selection, and (iii) sexual selection.

(i) The sex-specific constraints hypothesis argues that positive allometry is the product of each sex responding differently to a similar selection pressure (Clutton-Brock et al. 1977; Webster 1992; Fairbairn 1997). At the population and species level, positive allometry can be proximately manifest, for example, if there are different amounts of genetic variation in males and females and the sex with more additive genetic variation for body size has a stronger evolutionary response to selection (Leutenegger & Cheverud 1982). Similarly, a natural selection gradient (e.g. temperature) among subpopulations may trigger a correlated, but divergent phenotypic response in females and males (Fairbairn 2005; Teder and Tammaru 2005). A pattern of positive size allometry is ultimately expected when optimal female body size varies less than optimal male body size in relation to the selection gradient (Fairbairn 2005; Teder and Tammaru 2005).

(ii) Natural selection, such as intersexual resource competition (Clutton-Brock et al. 1977; Payne 1984; Webster 1992; Fairbairn 1997). For example, if increased body size is associated with a reduction in the amount of interspecific competition, then larger species may become more size dimorphic as the sexes genetically diverge into different ecological niches (MacArthur 1972, Shine 1982). Similarly, reduced interspecific competition with greater subpopulation mean body size may trigger a plastic response in sex-specific growth and body size so the sexes may utilise different niches (Kohda et al. 2008).

(iii) The sexual selection hypotheses states that size allometry is ultimately due to greater size-dependent reproductive success in one sex compared to the other (Smith 1977; Payne 1984; Webster 1992; Fairbairn 1997), where the strength of sexual selection acting on the targeted sex drives the overall magnitude of SSD (Shuster and Wade 2003). Among populations and species, a pattern of size allometry is expected as the non-targeted sex displays a correlated, but weaker evolutionary response to selection on the targeted sex. A pattern of positive allometry consistent with Rensch’s rule is expected in taxa where sexual selection is stronger for males (the typical scenario; Shuster and Wade 2003), irrespective of whether large- or small-male size is sexually selected (Székely et al. 2004; Dale et al 2007). In contrast, in taxa where sexual selection is stronger for female size, negative allometry is expected. Similarly, if mean body size increases with group size and the level of polygyny among subpopulations, we may ultimately expect a pattern of positive size allometry as males allocate more energy to rapid somatic growth in response to the increasing benefit of large body size in securing and maintaining reproductive dominance (Grosenick et al. 2007).

Knowledge on the details of trait plasticity is key to evolutionary theories of phenotypic variation and origins of diversity (Scheiner 1993; Gross 1996; Baydaev 2002; Tompkins 2005; Bonduriansky 2007), while the identification of selection gradients that trigger a plastic response provide insight into why patterns of diversity evolve in the instance, and how they are maintained (Gause 1942; Bradshaw 1965; Fricke 1980, Warner and Hoffman 1980; 1991; Scheiner 1993; Gross 1996). It is therefore of general interest to determine whether the hypothesised ultimate drivers (such as sexual selection) of broad macroevolutionary patterns (such as Rensch’s rule) drive similar morphological patterns among groups of flexible individuals.

For several reasons, tropical reef fishes are an important and ideal model system for investigating individual trait plasticity and subpopulation patterns of SSD. First, most reef fish life histories include a dispersive pelagic larval phase, making local genetic adaptation of populations a potentially slow process (Warner 1991; Leis and McCormick 2002). Second, individuals frequently settle in an environment that is very different from the natal state. Habitat patch size, temperature- and water chemistry-regime, substrate type, vertical relief and depth, food availability, predator- and competitor-assemblage, and the number and density of conspecifics can all vary within the dispersive range of larvae (Leis and McCormick 2002). In addition, many reef fishes are restricted to the local population or reproductive group into which they settle; migration at a later stage to a more appropriate environment is often limited to those habitats available in the immediate area. Hence, reef fish cohorts will be frequently distributed across a number of physical-, ecological- and social-gradients post settlement. These attributes should place strong selective pressure on the evolution of flexible morphological-, physiological- and behavioural-traits (Warner 1991; Scheiner 1993; Blackenhorn 2000; Baydaev 2002) including growth and body size (Ross 1987; Buston 2003). Finally, individuals from a broad range of fish taxa start life as one sex and later change sex to function as the other (sequential hermaphroditism; Warner 1975, 1988; Shapiro 1988; Munday et al 2006; appendix table A1.1). This means that females and males carry the same genes, providing a unique opportunity to examine the role that shared developmental-modifiers play in SSD patterns and processes; an important and frequently neglected aspect of SSD evolution (Badyaev 2002).

In this study I conduct an intrapopulation test on the ultimate, sexual selection hypothesis for positive size allometry, and the proximate, differential plasticity hypothesis for positive size allometry, using the sex-changing polygynous reef fish *Parapercis cylindrica* (family Pinguipedidae). Like many haremic sex-changing fishes (Table A1) individuals form hierarchical social groups (Walker and McCormick 2004, 2009). Dominance rank is based on relative body size and the male represents the top breeding status. The dominant female within the harem will change sex to function as male following the death of the dominant male, or following a female recruitment pulse (Frisch et al. 2007, see also Warner 1975, 1988; Shapiro 1988; Munday et al 2006). In a previous study I found evidence to suggest that *P. cylindrica* individuals accelerate somatic growth during sex change to facilitate reproductive dominance (Walker and McCormick 2004). Here, I use a null model approach to test for positive allometry among social groups in the wild (monogamous pairs and harems), and examine whether the magnitude of SSD increases with the level of polygyny. In addition, using manipulative experiments in the wild and in laboratory microcosms, I test the hypothesis that polygyny potential (the number of resident females) determines the magnitude of growth acceleration during sex change.

3.3 Methods

(a)Model species

P. cylindrica is a short-lived fish that occupies macro-algae and coral-rubble habitat within sheltered tropical waters (Randall 1997). Each female defends a permanent all-purpose territory in which it feeds, spawns, and seeks shelter, and males form harems by defending up to 10 neighbouring females (Stroud 1982). Mating- and social-system type ranges from isolated monogamous groups in low density areas to contiguous harems of up to 10 females per male in high density areas (Walker unpublished data). A strict, size-based dominance hierarch exists within each harem, and the male is always the largest individual within the group. Males are derived exclusively from females (sequential monandric protogyny) and can be distinguished from females by the presence of black pigmentation on the jaws and branchiostegal rays (Stroud 1982). The largest, dominant female of a polygynous group will change sex within 14-33 days following male removal (Walker and McCormick 2004, 2009a; Frisch et al. 2007).

Reproduction entails the male and female simultaneously releasing gametes into the water column (Stroud 1982; Thresher 1984). Spawning occurs within the hour prior to sunset, and a haremic male can sequentially pair-spawn with up to ten females during that time (Stroud 1982; Chapter 2). Reproduction occurs throughout the year; with peaks in gamete production and spawning activity occurring around the new- and full-moon (Chapter 2).

Harem size can be used as an accurate proxy for male reproductive success; Stroud (1982) illustrated that females spawn with the male whose territory they reside in 98% of the time (spawn observation n = 465, male n = 20), and while infrequently a neighbouring haremic male may steal or join in on a spawning bout, roaming bachelor males are absent from populations. Data on individual spawning frequencies, size-based egg production rates and fertilisation rates, illustrates that male reproductive output is primarily driven by haremic-group size, but starts to asymptote at larger group sizes (Stroud 1982; Chapter 2).

(b) The ultimate, sexual selection hypothesis for Rensch's rule.

The ultimate, sexual selection hypothesis for Rensch's rule was tested among 55 reproductive social groups (monogamous pairs and harems) within the lagoon of Lizard Island, Great Barrier Reef, Australia (14º 40.9'S, 145º 26.8' E). Social group composition was first determined by observing the coloration and behaviour of individual fish (232) and by the mapping of their territories. One 15min observation was made on each individual. Females (n = 177) were assigned to the male (n = 55) whose territory encompassed the particular female. Pilot studies revealed that a 15-minute observation time gives an accurate estimate of individual territory location (see appendix fig. A3.1). Following the determination of social group composition, all individuals were collected with clove oil aesthetic and a hand net, and measured with callipers (mm total length; TL). The largest female within each group was assigned as the dominant female, and SSD was defined as the difference between log(male body size mm TL) and log(dominant female body size mm TL).

To test for positive allometry among social groups (Rensch's rule), and to explore the relationship between polygyny (social group size) and SSD (the sexual selection hypothesis for Rensch's rule) a random distribution of body sizes expected under a null model was constructed using a Monte Carlo procedure (Manly 1997). This procedure was necessary because at least some level of SSD is expected when males are derived from females. In addition, when there is only one male per group, and the male is ubiquitously the largest individual within each group, both group size and the shape of the body size distribution intrinsically affect the expected overall magnitude of SSD, and the expected linear regression slope between log(dominant female body size) and log(male body size) when groups of individuals are formed randomly (appendix fig. A3.2). The Monte Carlo procedure involved the random selection of individuals from the pool of 232 individuals and combining them into groups based on the exact distribution of group sizes found in our sample. The randomly selected individuals allocated to each group were then ranked according to relative body size, where rank 1 = male and rank 2 = dominant female. I then calculated the expected intercept and slope between log(dominant female body size mm TL) and log(male body size mm TL) (i.e. the expected pattern of sexual size allometry) using reduced major axis regression (RMA; Fairbairn 1997) and the expected intercept and slope between social group size and SSD (i.e. the expected pattern between polygyny and SSD) using ordinary least squares regression (OLS). This whole procedure was iterated 10000 times, generating 2 final expected intercept- and expected slope-probability distributions. (appendix fig. A3.2). The best-fit RMA regression slope for observed log(dominant female body size mm TL) versus observed log(male body size mm TL), and the best-fit OLS regression slope for observed harem size versus observed SSD, were then assigned statistical significance based on their probability of occurrence, according to their corresponding null model slope-distribution. This null model design was appropriate since it excludes the factors of interest (the relationships between polygyny and dominant female- and male-body size) while retaining the protogyny sex change rule and all other factors of the sampling design (i.e. group- and body-size distributions) (Manly 1997; Buston and Cant 2006).

(c) The proximate, growth plasticity hypothesis for Rensch's rule: field experiment

The growth plasticity hypothesis for Rensch's rule was tested in both the wild and in laboratory microcosms by removing the male from social groups to induce sex change in the dominant female. It was predicted that growth during sex change would be positively related to the potential for polygyny (the number of females the sex-changer encounters during sex change).

In the wild, a population displaying natural variation in social group size was found, and a reference grid at 5 x 5 m resolution was laid over the substrate. All individuals (n = 53) were captured and measured (as above), visually sexed (based of colouration), and tagged with a subcutaneous elastomer tattoo for individual recognition using a 27-gage hypodermic needle (Northwest Marine Inc.). Individuals were held until full recovery from anaesthetization and then returned to the site of capture, and all individuals were observed to remain within their original location. Pilot studies in aquaria revealed a 0% mortality rate over 30 d associated with tattooing (n = 30). Three 15-minute observations were made on all individuals to determine territory location and harem membership (as above). Once individuals and social groups were mapped (in relation to the reference grid) the male was removed from each reproductive social group (n = 11) to induce sex change. Immediately following male removal (at ~ 1 h, ~ 2 h, and ~ 3h) and then each day for 30 d, 15-minute observations were made on remaining females to track changes in colouration and behaviour. After 30 d all individuals were euthanized to determine growth and the gonads fixed in formalin-acetic acid-calcium chloride solution. The final sex of each individual was initially determined by colour patterns and behaviour (Stroud 1982; Frisch et al. 2007), and was later confirmed by gonad histology, based on the presence of characteristic sex cells (Patiño and Takashima 1995; Frisch et al. 2007). Analysis of variance was used to test for a statistically different standardized growth (log(final size mm TL) - log(initial size mm TL)) between sex-changers (n = 12) and non-sex changing females of adjacent dominance rank (i.e. the next largest female within each group), with log(initial size) and polygyny potential entered as covariates. Multiple regression and residual analysis was then used to examine the independent effects of log(initial size mm TL) and polygyny potential on standardized growth among sex-changers and non-sex changing dominant females.

(d) The proximate, growth plasticity hypothesis for Rensch's rule: laboratory experiment

Laboratory experiments were carried out at Lizard Island Research Station to isolate the effect of polygyny potential (number of females) on growth during sex change. Experimental fish within each aquarium were collected from a different social group and site within the Lizard Island lagoon, such that they had never interacted before the experiment. A total of 20 social groups were created in individual 100-150 l microcosms containing equal quantities of rubble and algae habitat per individual; ten containing one male and four females (at 85, 80, 75 and 70 ± 0.2 mm TL); the 'polygyny' treatment, and ten containing one male and two females (at 85 and 80 ± 0.2 mm TL); the 'monogamy' treatment (following male removal). The male was always the largest individual. After 5 days acclimation the male was removed from 5 of the 10 social groups at random from the polygyny and monogamy treatments to induce sex change. The other 5 social groups in each treatment served as controls for male removal. At 900h and 1500h each day, individuals were fed to satiation with brine shrimp and commercial fish food. Observations were carried out to ensure that all individuals were feeding (which was found to be the case). Thirty days following male removal all individuals were euthanized to determine growth and the gonads fixed for histological processing. The final sex of individuals was determined by colour patterns and gonad histology (as above).

Two-way ANOVA was used to test for statistically different standardized growth (log(final size)-log(initial size)) as a function of treatment (monogamy versus polygyny), reproductive state (sex changer versus control non-sex changing female (the largest female from social groups where the male was not removed)), and the interaction between treatment and reproductive state.

3.4 Results

(a) The ultimate, sexual selection hypothesis for Rensch's rule.

Parapercis cylindrica was found to display a pattern of positive allometry for SSD concordant with Rensch's rule. The observed best-fit slope from the log(dominant female size) versus log(male body size) RMA regression was statistically greater than that predicted by the protogynous sex change rule and sampling regime alone (i.e. the null model for positive allometry; $\beta_{(\mathrm{RMA\ null})} = 1.10$, $y0_{(\mathrm{RMA\ null})} = -0.15$; appendix fig.

A3.2) (observed best-fit RMA model; log(male body size mm TL) = 1.31 x log(dominant female body size mm TL) - 0.53, $r^2 = 0.71$, Tukey's t-test; $t_{(\beta \text{RMA observed})} = 2.15$, df = 53, $p = 0.018$; fig. 3.4.1*a*), representing greater-than-expected variability in male size and an increase in SSD with dominant-female size. In addition, the observed relationship between the level of polygyny and SSD was found to support the ultimate, sexual selection hypothesis for Rensch's rule. The magnitude of SSD was frequently less than that predicted by the null regression model among monogamous pairs, and increasingly greater than that predicted by the null model with an increase in haremic-group size (null model for SSD; $\beta_{(\text{OLS null})} = -0.003$, $y0_{(\text{OLS null})} = 0.06$; appendix fig A3.2) (residual SSD = 0.008(harem size) - 0.02, $r^2 = 0.4$, $F_{1,53} = 35.32$, $p < 0.01$; fig. 3.4.1*b*).

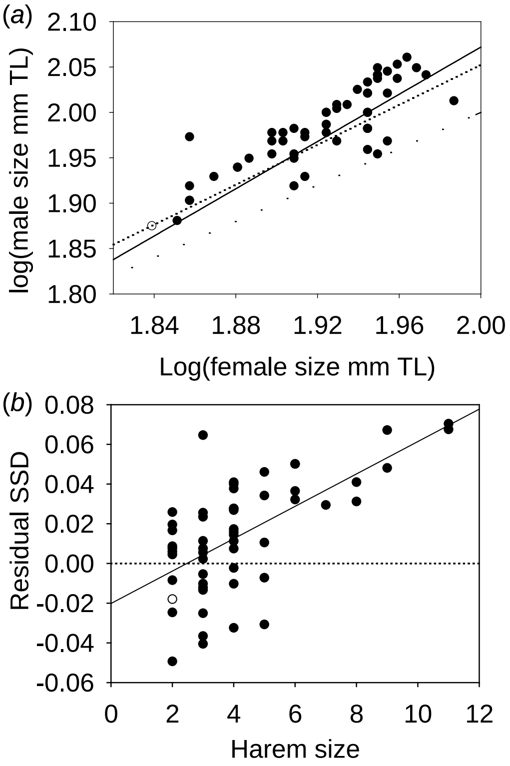

Figure 3.4.1 (*a*) Relationship between standardized dominant female and male body size among social groups illustrating positive sexual size allometry. Solid line, best-fit RMA linear regression model, dotted line, Monte Carlo null regression model (i.e. that predicted by the protogynous sex change rule and sampling regime; see appendix figure A4.2); dashed line; isometric line (x = y). (*b*) Relationship between the level of polygyny (harem size including one male) and residual SSD from the reduced null model (observed SSD – expected SSD from the Monte Carlo null regression model; dotted line).

b) The proximate, growth plasticity hypothesis for Rensch's rule

Results from both the wild and laboratory experiments were found to support the proximate, growth plasticity hypothesis for Rensch's rule. In the wild, a total of 12 females from 11 male-removed social groups were observed to take on the male-behavioural mode and subsequently change sex. At the end of the experimental period (30 d) these 12 individuals all had gonads containing proliferating testicular tissue, either developing or fully developed peripheral sperm sinuses, and only remnant degenerating ovarian tissue. By contrast, all individuals that maintained female coloration and behaviour had gonads containing both mature- and developing-oocytes, indicating active oogenisis. The largest dominant female was the one to change sex and take over the social group from which they originated in all but one case. In the exception, the two largest females changed sex and the harem was split between them.

Standardized growth (log(final size)-log(initial size)) during the 30 d experimental period was found to be statistically greater for sex-changing individuals compared to non-sex changing females (the next largest female within each harem) for any given log(initial size mm TL) or level of polygyny-potential (ANOVA; $F_{2,19} = 16.56$, $p < 0.001$; fig. 3.4.2), confirming that individuals accelerate growth during sex change to become the largest members of the population. Moreover, while log(initial size mm TL) was a strong predictor of growth among non-sex changed females (standardized growth = -3.43 * log (initial size mm TL) + 0.67, $r^2 = 0.54$, $F_{1,9} = 10.67$, $p = 0.009$; fig. 3.4.2*a*), this was not the case among sex-changed individuals ($F_{1,10} = 4.57$, $p = 0.06$; fig. 3.4.2*a*). Incorporating polygyny potential into the regression model increased the amount of variance explained (adjusted $r^2 = 0.66$, $F_{2,9} = 12.06$, $p = 0.003$), and illustrated female density to have a greater effect on standardized growth during sex change (partial correlation coefficient = 0.8, $p = 0.004$) compared to log(initial size) (partial correlation coefficient = -0.7, $p = 0.03$) (fig. 3.4.2*b*). Polygyny potential did not help to explain residual standardized growth in non-sex changing females (fig. 3.4.2*b*). Laboratory results support field results; only the dominant female from each male-removed social group changed sex, and these sex-changed individuals displayed greater standardized growth than control non-sex changed females of equal initial size (i.e. dominant females from social groups where the male was not removed; fig. 3.4.2*c*). In addition, there was a significant interaction between sex-changer verses non sex-changer and treatment ($F_{1,16} = 7.1$, $p = 0.016$); monogamous sex-changers displayed only slightly greater growth compared to monogamous control non sex-changing females, while polygynous sex-changers displayed more than twice the growth of polygynous control non sex-changing females (fig. 3.4.2*c*).

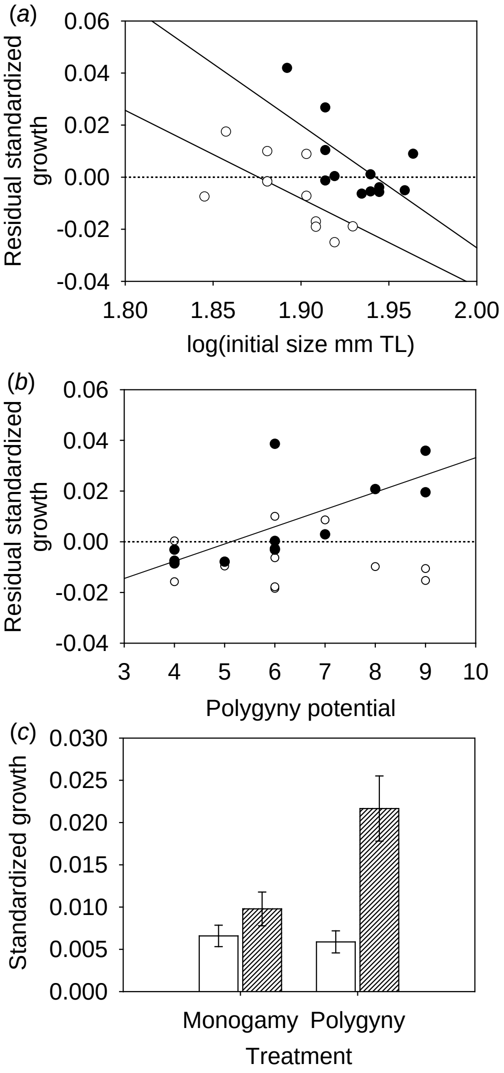

Figure 3.4.2 (a) Relationship between log(initial size) and residual standardized growth (from the reduced polygyny-potential model) in non-sex-changing females (white dots) and sex-changed individuals (black dots) during the 30 day experiment in the wild. (b) Relationship between polygyny-potential and residual standardized growth (from the reduced log(initial size) model) among non-sex-changing females (white dots) and sex-changed individuals (black dots) in the wild. (c) Differences in standardized growth (+s.e.) between control non-sex-changing females (white) and sex-changed individuals (shaded) with respect to treatment during the 30-day laboratory experiment. All initial sizes = 85 ± 0.2 mm TL.

3.5 Discussion

In the wild, *Parapercis cylindrica* displayed a pattern of positive allometry for SSD concordant with Rensch's rule, illustrating greater phenotypic variation in male size compared to dominant-female size. In addition, SSD was found to predictably increase with the level of polygyny, consistent with the ultimate, sexual selection hypothesis for positive size allometry. Manipulative experiments illustrated that polygyny potential (the number of resident females) affects the magnitude of growth acceleration during sex change from female to male, and is this sex-specific differential growth plasticity that proximately drives variation in SSD among social groups. Since the number of resident females reflects the potential advantage of changing sex, the number of competitors for the haremic-male role, and the cost of maintaining dominance, there is more incentive for a highly polygynous sex-changer to invest in relatively larger body size, both to aid in combat and as an honest signal of dominance, thereby reducing the probability of contest (Grosenick et al. 2007).

Dominant-female body size positively co-varied with polygyny and SSD. Ultimately, female *P. cylindrica* also benefit from relatively larger body size due to an increase in egg production capacity (i.e. fecundity selection; Head 1995; Walker et al. unpublished manuscript). The fact that dominant female body size varies less than male body size suggests the presence of sex- and/or rank-specific growth- and body size-strategies. First, females experience a stronger energetic trade-off between growth and gamete production compared to males (Walker and McCormick 2004). Second, there is potential for conflict over dominance rank via sex change; if a dominant female was to approach a size equal to that of the male (or a new dominant sex-changing individual), then the male/sex-changing individual may be forced to attack and evict that female from the social group so as to maintain the top breeding status (Johnstone 2000; Buston 2003; Wong et al. 2007). By remaining smaller than the haremic male, the dominant female avoids the prospect of punishment, and may inherit a productive harem in the future (i.e. via sex change, as illustrated in the present study).

Studies to date on SSD patterns and processes have largely focused on adult sex-specific morphology at the population and species level, where it is generally assumed that adult female- and male-body size is genetically fixed, such that Rensch's rule reflect co-evolution between absolute adult female- and male-body size coupled with greater evolutionary divergence in absolute adult male body size (Fairbairn 1997; Dale et al. 2007). The present study illustrates, however, that Rensch's rule may also be manifest through the evolution of sex-specific developmental modifiers (Badyaev 2002), and the phenotypic expression of relative body size in response to an ultimate sex-specific selection gradient. Clearly, the potential for adaptive sex-specific differential growth plasticity to explain patterns of SSD will diminish when inference is made at higher taxonomic levels. Nonetheless, the present results illustrate that such inferences may not simply reflect sex-specific genetic divergence, or patterns of absolute body size. Yet, irrespective of the taxonomic level of inference, or the details of proximate causation, Rensch's rule remains general in respect to ultimate causal factors. Using birds as the focal taxa, Dale et al. (2007) recently provided the strongest evidence to date that Rensch's rule among species is the product of sexual selection processes. Here, I complement Dale and co-workers' findings by providing the first experimental evidence that a sexual selection gradient among social groups drives growth- and body size-plasticity, and a pattern of positive allometry for SSD. The present study highlights both the usefulness of phenotypic plasticity for testing evolutionary theories (Warner 1991), and the role that individual growth modification can play in the shaping of morphological patterns in nature (see also Buston 2003; Fairbairn 2005; Pyron et al. 2007; Kohda 2008; Lengkeek et al. 2008).

Theory suggests that traits targeted by sexual selection (such as male body size) should evolve heightened condition dependence; a form of developmental plasticity that links the degree of trait expression to the quantity of metabolic resources available to the individual, optimizing the trade-off between viability and reproduction (*sensu* Bonduriansky 2007) (McAlpine 1979; Andersson 1982; Nur and Hasson 1984; Rowe and Houle 1996). Indeed, several studies on polygynous species illustrate that males display more pronounces changes in growth rate and body size in response to food supply compared to females, subsequently driving a pattern of positive allometry for SSD (e.g. Badyaev 2002; Bonduriansky 2007; Fernández-Montraveta and Moya-laraño 2007). In the present study, however, I illustrate that males (i.e. sex changing individuals) regulate growth rate and body size in response to the strength of sexual selection itself, even when food is unlimited. Essentially, I illustrate that growth and body size is conservative, but finely tuned to ultimate selection pressures. These results suggest that there are costs associated with either rapid growth rate or large body size independent of metabolic viability. For example, it is possible that rapid growth during sex change reduces longevity, but that reduced longevity is traded-off with the higher reproductive success associated with attaining larger body size in more polygynous societies. I suggest that the modification of sexually selected traits (e.g. growth and body size) in response to social conditions, rather than- or in addition to- current food availability, may be common when individuals are able to accurately and continuously perceive their social setting and status, when there are potential costs associated with the expression of the targeted trait (independent of metabolic viability), and when individuals must act quickly to secure dominance should the opportunity arise (e.g. when a dominant male dies in a polygynous size hierarchy) (see also Buston 2003; Wong et al. 2008).

This study experimentally illustrated SSD to be the product of accelerated growth during sex change in a protogynous fish that forms permanent, size-based dominance hierarchies (see also Ross 1987; Walker et al. 2007; Munday et al. in press). In contrast, female and male growth has been found to diverge well before the mean age at sex change in protogynous polygynous fishes which form loosely organized harems (e.g. parrotfishes *Scarus frenatus* and *Chlorurus sordidus*, Munday et al. 2004), or species whose social interactions are largely confined to brief spawning periods (e.g. coral trout *Plectropomas maculates*, Adams and Williams 2001). It appears that the absence of social constraints on subordinate growth permits high variability in growth to be expressed early in life. Those individuals that achieve relatively larger body size during the juvenile and female phase go on to become large polygynous males via sex change, while those individuals who experience relatively poor growth during the juvenile and female phase tend to remain female, presumably to avoid reproductive exclusion by larger males (Warner 1975, 1988). Nonetheless, it appears that these sex-changers still employ accelerated growth during sex change to achieve SSD, albeit to a lesser degree compared to hierarchical sex-changers (Ryen 2008). The shift from sex change-associated growth divergence, to pre-sex change growth divergence has even been illustrated between populations of the same species (i.e. the wrasse *Halecoeres miniatus*; Ryen 2008). Alternation between these two pathways for SSD was found to be related to the strength of the dominance hierarchy operating within each population. With an increase in the strength of the dominance hierarchy, individuals relied more on sex-change associated growth acceleration to achieve SSD (Ryen 2008).

Taken together, studies to date on the temporal and ontogenetic relationships between sex change and SSD suggest that while rapid juvenile and female growth may be advantageous within a loosely organized social system (due to the increased chance of becoming a large dominant male; Adams and Williams 2001) such a strategy may be selected against within a strict, hierarchically organised social group (Buston 2003; Walker and McCormick 2004; Walker et al 2007; Wong et al. 2007). Moreover, due to their larval dispersive phase, and the highly patchy nature of the marine environment, individuals frequently encounter a social environment that is very different from the natal state. Hence, in sequentially hermaphroditic animals (annelids, molluscs, crustaceans, fish), including most polygynous reef fishes (Thresher 1984; Munday et al. 2006; appendix table A1.1), selection for individual growth- and body size-plasticity is likely to be stronger than selection for any one particular growth tactic.

Because males are derived from females, sequentially protogynous reef fishes provide a unique opportunity to explore the ways in which sexes achieve divergent phenotypes through the modification of shared developmental programs; an important and frequently neglected aspect of morphological evolution (Badyaev 2002). In addition, reef fishes display extraordinary flexibility in the expression of behavioural, physiological and morphological traits, and show unprecedented diversity in mating- and social-system types (Thresher 1984; Shapiro 1991; Warner 1991; Munday et al. 2006). These attributes mean that the proximate and ultimate drivers of SSD are likely to vary considerably among social groups, populations and species, making reef fishes ideal model systems for testing theories pertaining to morphological evolution and diversity (Warner 1991). While the present study highlighted sexual selection to be the driver of positive size allometry among groups of *P. cylindrica*, natural selection for SSD appears to be more important in other species (e.g. Kohda et al. 2008). As poikilotherms, moreover, fishes are extremely sensitive to prevailing physical environmental factors, particularly temperature (Atkinson 1994). Fish are therefore likely to frequently experience growth and body size trade-offs in relation to conflicting environmental selection pressures (Young 2005; Pyron et al. 2007; Lengkeek et al. 2008). While it is unlikely that physical factors played a significant role in determining the patterns of SSD reported here (due the extremely small spatial scale of the study), plasticity studies at larger spatial scales should incorporate physical variables in the analysis to get at the true relationships between ultimate selection pressures and patterns of SSD.

Further research is required on the temporal and ontogenetic relationships between sex change and SSD in fishes, and the mechanisms driving variance in SSD among social groups, populations and species. Such studies will enhance our understanding of morphological evolution, and enable predictions of how species with labile sexual differentiation strategies and indeterminate growth respond to natural- and anthropogenic-induced changes in population density and social organization.

Chapter 4

Fish ears are sensitive to sex change

Abstract

Many reef fishes change sex during their life. The testing of life history theory and effective fisheries management therefore relies on our ability to detect when this fundamental transition occurs. This study experimentally illustrates the potential to glean such information from the otolithic bodies of the inner-ear apparatus in the sex-changing fish *Parapercis cylindrica*. It will now be possible to reconstruct the complete, often complex life history of hermaphroditic individuals; from hatching through to terminal-reproductive status. The validation of sex-change associated otolith growth also illustrates the potential for sex-specific sensory divergence. It is possible that sex-changing fishes alter otolith composition, and thus sensory-range specificity, to optimize life history in accordance with their new reproductive mode.

4.2 Introduction

A vast number of animals, including most tropical reef fishes, change sex during their life (Munday et al. 2006; Sadovy de Mitcheson and Lie 2008). The testing of life history theory and effective fisheries management therefore relies on understanding why and when this fundamental transition occurs (Buston et al. 2004; Alonzo and Mangel 2005). A major impediment to gaining such knowledge stems from an inability to detect the age at sex-change in individuals without the continuous monitoring of populations.

In teleost fishes however, the proportion of mineral to organic material utilized during otolith (ear stone) growth fluctuates under an endogenous rhythm, producing a sequence of daily bipartite increments that can be used to infer an age-based history of growth (Pannella 1971; Campana and Neilson 1985; Gauldie and Nelson 1988; Thorrold and Hare 2003). In addition, precise changes in otolith accretion are known to occur during metamorphosis from the larval- to demersal-stage in reef-settling taxa (Wilson and McCormick 1997). The resultant, optically apparent discontinuous zone which forms in the otolith lends itself as an age-specific settlement-signature. Like metamorphosis, sex-change involves rapid changes in morphology, physiology and behaviour. It is therefore possible that otolith accretion also varies during sex-change, resulting in the formation of a sexual-transition marker. This would make it possible to reconstruct the complete life history of hermaphroditic individuals; from hatching through to terminal-reproductive status.

Here, I explore daily otolith accretion during sex-change from female to male in the haremic reef fish *Parapercis cylindrica.* By removing males from bigamous- and polygynous-social groups to induce sex-change in dominant females, it was possible to compare otolith growth between sex-changers and control non-sex changing females.

4.3 Materials and Methods

(*a*) *Study species*

The sharpnose sandperch *Parapercis cylindrica* (Family Pinguipedidae) is a common inhabitant of coral reefs throughout the Indo-Pacific (Randall et al. 1997). Females defend permanent all-purpose territories and males defend up to 10 females. All individuals mature first as female, and later change sex to function as male (monandric protogynous hermaphroditism) (Walker and McCormick 2004). A strong dominance hierarchy exists within each harem, and sex change is socially mediated (Frisch et al. 2007). Sagittal-otolith increment-periodicity has been validated as daily, and increments can be optically discerned throughout the fish's lifespan (<500 d.) (Walker and McCormick 2004). Otolith-somatic growth displays isometric proportionality, and the effect of somatic growth rate on otolith-somatic scaling is negligible (Walker and McCormick 2004).

(*b*) *Experimental regime*

Social groups were manipulated by removing the male to induce sex-change in the dominant female. This enabled a comparison of otolith growth before and after sex-change induction (male removal) and between sex-changing individuals and non-sex changing females.

To isolate the effect of female density on otolith accretion during sex change, removal experiments were conducted in the laboratory at Lizard Island Research Station. Individuals were collected from the lagoon of Lizard Island, Great Barrier Reef (14° 40.9'S, 145° 26.8' E). Social groups were created in individual 100-150 l microcosms containing equal quantities of rubble and algae habitat per individual: 10 polygynous social groups containing 1 male and 4 females (at 85, 80, 75 and 70 ± 0.2 mm TL); and 10 bigamous social groups (monogamous social groups after male-removal) containing 1 male and 2 females (at 85 and 80 ± 0.2 mm TL). The male was always the largest individual. After 5 d acclimation the male was removed from 5 of the 10 bigamous- and polygynous-social groups to induce sex-change. The other 5 social groups in each treatment served as controls for male removal. At 900h and 1500h each day, individuals were fed to satiation with brine shrimp and commercial fish food. Thirty d following male removal all individuals were euthanized and measured (mm TL). The sagittal otoliths were removed and stored dry, and the gonads removed and fixed in a formalin-acetic acid-calcium chloride solution (FAAC).

To determine the natural relationship between female density otolith growth during sex change, removal experiments were carried out in the wild. A population displaying a density gradient was mapped over a 2500m^2 area in the Lizard Island lagoon. All individuals (n = 53) were captured with clove oil anesthetic and a hand net, sexed (based on coloration), measured with calipers (± 0.1mm T.L.) and tagged with a subcutaneous tattoo for individual recognition. Three 15 min observations were made on each individual and their territory mapped (in relation to the reference grid) to determine social group membership and female density (the number of females within a $100m^2$ area of the sex-changing fish). The male from each harem was then removed to induce sex change. Thirty d following male removal all individuals were collected, measured (mm TL) and their sagittal otoliths and gonads removed and preserved as above.

The gonads of all individuals from the field and laboratory experiments were transversely sectioned at five-microns and stained with Myer's Haematoxylin and Young's Eosin-Erythrosin. These sections were examined under a compound microscope and individuals were categorized as females or sex-changed individuals based on the presence of characteristic sex cells (Patiño and Takashima 1995).

(*c*) *Otolith diagnosis and analysis*

Sagittal otoliths were processed to produce a transverse section perpendicular to the distal-rostral plane, such that daily increments could be observed from the nucleus to the outer margin of the otolith (following the methods of Wilson & McCormick 1997). Calibrated digital images of the otolith sections were then taken at 400x magnification using a high power microscope. Increment width profiles from the otolith margin to 10 d before male removal were measured along the maximum otolith radius for all individuals from the field and laboratory experiments using the spatial analysis program Optimus. In otoliths where a discontinuity was observed within the experimental period (<30 d), the day of formation was recorded by back-calculation from the otolith margin.

Parametric assumptions were explored prior to statistical inference using residual analysis, and when performing repeated-measures analysis of variance, the assumption of compound symmetry was tested using Mauchly's test (Zar 1999). For all analyses the alpha value was set at 0.05, and all descriptive statistics are presented as the arithmetic mean ± standard error.

Differences in daily otolith growth between treatments from the laboratory and field experiments were analyzed with repeated-measures analysis of variance. For the laboratory experiment this entailed a comparison of daily otolith growth before and after sex change induction, and between control non-sex changed dominant females (initial size = 85 ± 0.2 mm TL; n = 10), sex-changed monogamous individuals (initial size = 85 ± 0.2 mm TL; n = 5) and dominant sex-changed polygynous individuals (initial size = 85 ± 0.2 mm TL; n = 5). The field experiment entailed a comparison of daily otolith growth before and after sex change induction, and between sex-changed individuals (12) and non-sex changed females. Only non-sex changed females larger than 80mm TL were included in the analysis so as to rule out size- and age-effects (n = 7).

For fishes from the laboratory experiment, one-way ANOVA was used to compare the time-lag between sex-change induction and signature formation between monogamous sex-changers and polygynous sex-changers. For experimental fish from the wild, regression analysis was used to explore the predictive power of female density on: (1) the time of check mark formation; (2) somatic growth during sex change and; (3) otolith growth during sex change.

4.4 Results and Discussion

All non-sex changed individuals displayed full female function and deposited regular, continuous otolith-increments during the 30 d experiment (fig. 4.4.1b,*c*). By contrast, all sex-changed individuals deposited an optically dense discontinuous zone between 6 and 20 d following male removal, followed by a rapid increase in increment width and a change in the primary growth axis (fig. 4.4.1*b,d,e*). Increment width profiles were found to statistically differ between sex-changers and non-sex changers in both the wild (RM ANOVA; $F_{1,17} = 122.73$, $p < 0.01$; fig. 4.4.1*b*) and laboratory (fig. 4.4.1*b*). Interestingly, female density further influenced otolith accretion among initially equal-sized sex-changed fishes in the laboratory: polygynous sex-changers deposited statistically wider daily increments following the discontinuous zone compared to monogamous sex-changers (RM ANOVA; $F_{1,8} = 113.69$, $p = 0.032$; fig. 4.4.1*b*), and mean time-lag between sex-change induction and signature-formation was statistically shorter among polygynous sex-changers (10.4 ± 1.7 d) compared to monogamous sex-changers (17.8 ± 2.4 d; 1-way ANOVA; $F_{1,9} = 7.5$, $p = 0.025$). Similar patterns were observed among sex-changed fishes in the wild (table 1). The greater otolith growth of polygynous sex-changers compared to monogamous sex-changers paralleled a faster gonad-transition rate and greater somatic growth during sex change (fig. 4.4.1*c,d*, table 4.4.1). Thus, the otolith signature not only acts as a temporal marker of sex change, but also provides information on the magnitude of masculinisation during sex change.

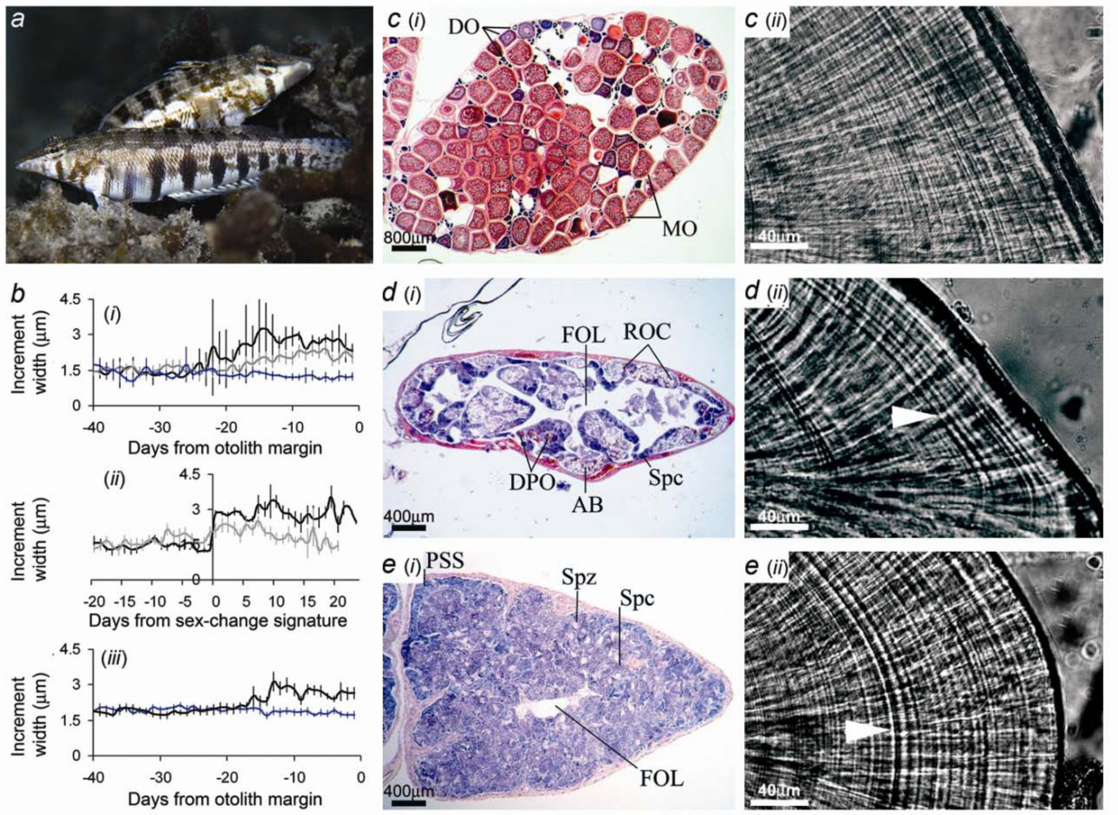

Figure 4.4.1 (*a*) *Parapercis cylindrica*; female front, male back. (*b*(i)–(iii)) Mean otolith increment-width profiles ± s.e. for experimental fishes. (*b*(i)) Laboratory fish. (*b*(ii)) Laboratory fish with 'day' centered to zero on the sex-change signature. (*b*(iii)) Fish from the wild. Blue, Non-sex changing control females; grey, monogamous sex changers; black, polygynous sex changers. All increment profiles are significantly different ($p < 0.05$) according to the repeated measures ANOVA. (*c*–*e*); photomicrographs of the (g), gonads; and (o), otoliths from experimental fishes in the laboratory. All initial sizes = 85 mm TL. (*c*(i)) Control non-sex changing female displaying active oogenesis and (ii) regular otolith increments. (*d*(i)) Monogamous sex changer displaying partial gonad-transition and (ii) otolith-increment discontinuity (arrow). (*e*(i)) Polygynous sex-changer displaying complete gonad-transition and (ii) otolith-increment discontinuity (arrow). DO, developing oocytes; MO, mature oocytes; DPO, degenerating previtelogenic oocytes; ROC, remnant oocyte constituents; AB, atretic body; FOL, former ovarian lumen; Spc, spermatocytes; Spz, spermatozoa and PSS, peripheral sperm sinus.

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Table 4.4.1 Statistical results for the experiment in the wild. (Regression analysis on the relationship between female density and somatic growth during sex change, otolith growth during sex change, the time lag between sex-change induction and signature formation.)

Predictor	Dependant	n	Model	r^2	$F_{\text{d.f.}}$	*p*-value
Female Density (100m^2)	Otolith growth (µm)	12	y = 5.3063(x) + 37.303	0.37	$F_{1,10} = 6.92$	0.03
	Somatic growth (m TL)	12	y = 1.6205(x) - 4.0741	0.56	$F_{1,10} = 13.89$	0.01
	Time of signature formation (days since sex-change induction)	12	y = -0.8649(x) + 22.486	0.44	$F_{1,10} = 8.56$	0.02

The discovery of otolith settlement-signatures precipitated major advances in population biology and ecology (Thorrold and Hare 2003). Similarly, sex-change signatures could greatly facilitate theoretical and applied science. To date, biologists and fisheries managers have relied on indirect proxies of the mean age at sex-change when performing life history analysis (e.g. the age or size at which the female- and male-frequency distributions intersect). Unfortunately, such measures overshadow the labile nature of sex-change induction, hence its role in population dynamics and life history evolution. Sex-change signatures, coupled with gonad histology, make it possible to explore variance in the size and age at sex change, and individual growth history from hatching through to terminal-reproductive status. This will aid in determining the proximate and ultimate factors which drive sexual form and function, reduce error in individual- and population-fecundity estimates, and provide a means to detect sex-change associated migration. Presently, such studies will be confined to 'model species' in which daily otolithic patterns are optically discernable throughout the individual's lifetime (i.e. short lived species). It is possible however, that the change in otolith growth during sex change is accompanied by a change in otolith chemical composition (see Therrold and Hare 2003). Thus, the profiling of otolith microchemistry may facilitate the detection of sex-change signatures in longer lived species with optically compact daily increments.

The potential importance of sex-change linked otolith accretion goes beyond its role as a methodological tool in ecology. In individual fishes, otoliths are involved in spatial orientation and the detection of sound (Thorrold and Hare 2003). Consequently, otolith development is expected to be directional and highly conservative. Our study therefore suggests that the readjustment of sensory organelles (i.e. otoliths) and ultimately, sensory range specificity, may be an important component of sex inversion. Variation in otolith growth among sex-changers under different social setting is consistent with this idea. Highly polygynous males contend with greater topographic complexity compared to females and monogamous males, and engage in more antagonistic interactions and physical combat (i.e. associated with harem defence) (Walker & McCormick, unpublished manuscript). Thus, the magnitude of change in otolith accretion during sex-change may reflect the extent of otolith adjustment necessary to optimize sensory performance in light of current social and environmental pressures. Investigation of this hypothesis, using an eco-physiological approach (e.g. Wright et al. 2005), will be a focus of future research.

Chapter 5

Density dependent sex ratio adjustment and the Allee effect: A model and a test using a sex-changing fish

Abstract

Positive density dependence (i.e. the Allee effect; AE) often has important implications for the dynamics and conservation of populations. Here, I show that density dependent sex ratio adjustment in response to sexual selection may be a common AE mechanism. Specifically, using an analytical model we show that an AE is expected whenever one sex is more fecund than the other and sex ratio bias towards the least fecund sex increases with density. I illustrate the robustness of this pattern, using Monte Carlo simulations, against a range of body size-fecundity relationships and sex allocation strategies. Finally, I test the model using the sex-changing polygynous reef fish *Parapercis cylindrica*; positive density dependence in the strength of sexual selection for male size is evidenced as the causal mechanism driving local sex ratio adjustment, hence the AE. Model application may extend to invertebrates, reptiles, birds and mammals, in addition to 70+ reef fishes. I suggest that protected areas may often outperform harvest quotas as a conservation tool, since the latter promotes population fragmentation, reduced polygyny, a balancing of the sex ratio and hence up to a 50% decline in per capita fecundity, while the former maximises polygyny and source-sink potential.

5.2 Introduction

An Allee effect occurs when some component of per capita fitness deteriorates as population density or size decreases towards zero, resulting in a positive density dependent relationship (Allee 1931; Stephens et al. 1999). A number of mechanisms have been proposed to drive the Allee effect, such as reduced predator vigilance, reduced care for young, or reduced foraging efficiency at low population sizes or densities (Sæther et al. 1996; Courchamp et al. 1999; Stephens and Sutherland 1999). However, the reduced probability of finding mates in small, sexually reproducing populations is thought to be the most common mechanism (Ehrlich and Roughgarden 1987; Levitan et al. 1992; Groom 1998; Kindvall et al. 1998; Wells et al. 1998; Berec 2001; Davis et al. 2004). An Allee effect in some component of fitness can result in a decline in per capita population growth rate at small population density or size (i.e. a demographic Allee effect; also referred to as depensation and inverse density dependence; Stephens et al. 1999), and, if population growth rate becomes negative, there will be a critical population density or size below which the population slides to extinction (Courchamp et al. 1999). Hence, unlike negative density dependent processes which tend to stabilise population dynamics, Allee effects tend to drive erratic population behaviour, whereby populations either boom (when above the critical density threshold) or bust (when below the critical population density threshold). Consequently, knowledge of potential Allee effect mechanisms is a key component to understanding the dynamics of groups, populations and communities, and prerequisite to strategic conservation efforts (Myers et al. 1995; Leirmann et al. 1997; Courchamp et al. 1999, 2008; Stephens and Sutherland 1999; Hutchings 2000; De Roos and Persson 2002; Morris 2002).

One potentially wide spread Allee effect mechanism which is yet to receive attention is density dependent sex ratio adjustment. Sex ratio adjustment in response to local selection pressures is a common feature of many plant and animal populations and represents some of the strongest evidence in support of Darwin's theory of Natural selection (Hamilton 1969; Trivers and Willard 1973; Werren 1980; Charnov 1982; West et al. 2000 Wade et al. 2003; Reese et al. 2008). An initially positive relationship between population density and per capita fecundity (*PCF*) should be expected whenever one sex can produce more offspring than the other and sex ratio bias towards the least fecund sex increases with population density (see "The model"). This is because as density increases, the reproductive capacity of the most fecund sex is better exploited and therefore, more offspring per individual can be produced. Importantly, however, unlike Allee effects described to date which place emphasis on density dependent individual female fitness, the sex ratio based Allee effect involves a density dependent increase in individual fitness in the most fecund sex only.

In sexual reproducing organisms males are typically more fecund than females. Indeed, polygynous mating systems are frequent in nature, wherein a few dominant males monopolise the reproductive efforts of several females. In such species, any mechanism which selects for a positively density dependent reduction in the proportion of male mating group members from an equal sex ratio can potentially drive a fecundity Allee effect (see " The model"). Theoretical and empirical evidence suggests that sexual selection for large or high quality males within polygynous groups may provide such a mechanism. For example, for separate-sex species (gonochores) it has been argued that under strong sexual selection for male size or quality, females should be selected to produce fewer sons as density increases, resources become limited and maternal condition declines (Trivers and Willard 1973; see also Wade et al. 2003). This is because females in poor condition produce poor quality sons, and poor quality sons will have little prospect of realising their fecundity potential in the presence of strong sexual selection (Trivers and Willard 1973). For hermaphrodites, moreover, it has been shown that if large individuals can monopolise the male reproductive role, then there is a clear advantage to allocating most or all reproductive effort to female function when small, and later allocating most or all reproductive effort to male function when a competitive size is reached (see the size advantage hypothesis for protogynous hermaphroditism; SAH, Ghiselin 1969; Warner 1975; Munday et al. 2006). It follows that with a positively density dependent increase in the strength of sexual selection for large male size, there should be an increase female function among smaller individuals (Warner 1975; 1984a,b; Lutnesky 1994; Walker and McCormick 2009b).

While protogynous hermaphroditism (sex change from female to male) is not an essential proximate feature of density dependent sex ratio adjustment and the Allee effect (see ‘The model’), sex-changers are, for a number of reasons, an important and ideal model system for examining it. First, the vast number of sex-changers (1000's) coupled with their taxonomic extent (annelids, molluscs, crustaceans and fish) warrants attention. Protogynous sex change is in fact the norm for a number of ecologically and economically important reef fish families, including Labridae, Scaridae, Serranidae, Pomacanthidae, Pomacentridae and Pinguipididae (Thresher 1984; Sadovy de

Mitcheson and Liu 2008). Second, sex change induction typically involves a strong environmental component, whereby local social or demographic conditions influence how many individuals change sex, which individuals change sex, and the timing of their sexual transition (Robertson 1972; Shapiro 1984; Ross 1990; Lutnesky 1994; Walker & McCormick 2009b). Finally, many sex-changers are site attached and ecologically or energetically restricted in their use of space, such that population density directly influence the potential for polygynous mating groups to form and the degree of polygyny (e.g. hawkfishes; Donaldson 1989, 1990, angelfishes; Moyer 1990, butterflyfishes; Hourigan 1989, groupers; Shpigel and Fishelson 1991, triggerfishes; Fricke 1980; Ishihara and Kuwamura 1996). Together, these points suggest that local density dependent sex ratio adjustment in response to sexual selection may provide a mechanistic explanation for depensation in many marine populations and communities (see Paddack et al. 2009).

Here, I develop analytical and Monte Carlo models with which to examine the nature of density dependent per capita fecundity under a range of sex allocation strategies and body size-fecundity relationships. I then test the model empirically using the polygynous sex-changing reef fish *Parapercis cylindrica*. I discuss the likely extent of Allee effects associated with density dependent sex ratio adjustment among tropical reef fishes, and more generally the implications of sex ratio adjustment in response to sexual selection for the dynamics, conservation and management of polygynous species.

5.3 The model

Consider a patchy environment which supports varying group sizes (*GS*) and, let's say that the male is more fecund than the female. A fecundity Allee effect will occur whenever sex ratio bias (from 1:1) towards the least fecund sex (in this case the female) increases with *GS.* Most simply, if we assume that there is only ever one male per group, that all females have equal fecundity (*FF)* and that all their eggs are fertilized, the initial increase in per capita fecundity (*PCF*) with *GS* may be expressed as:

$$PCF = FF\left[\frac{(GS-1)}{GS}\right]$$

or;

$$PCF = FF\left(1-\frac{1}{GS}\right)$$

Essentially, $PCF = \frac{1}{2}FF$ when $GS = 2$ (monogamy), and approaches FF hyperbolically with a further increase in GS (i.e. with an increase in polygyny). However, PCF will cease to increase with GS once the male fertility threshold ($M_{\max}$) is reached. After which point, not all the eggs from all females can be fertilised (due to sperm depletion) and therefore PCF will decrease with GS hyperbolically:

$$PCF = \frac{M_{\max}}{GS}$$

The complete representation for PCF in response to GS is thus:

$$PCF = \min\left[\frac{M_{\max}\ , FF(GS-1)}{GS}\right] \qquad (1)$$

Simply, PCF at GS is determined by the limiting factor; either male fecundity or the number of females in the group (fig. 5.3.1). This analytical model (AM) relies on the assumption that all individuals are equivalent; FF is constant and the male is randomly chosen from equivalent individuals. While these assumptions are unlikely to be met in nature, the AM will be useful for examining the amount of variation in PCF as a function of GS due to the sex ratio effect only.

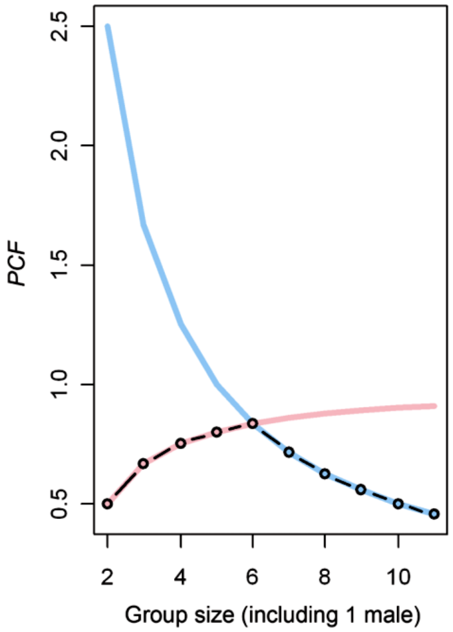

Figure 5.3.1 Graphical representation of the analytical model components where the female:male potential fecundity ratio = 1:5. Red line = female per capita fecundity component, blue line = male fecundity-depletion component (in response to the number of females), black trajectory = the solved analytical model trajectory for PCF (equation 1; see 'The model').

The female body size-fecundity relationship and the details of the size-based sex allocation strategy influence the shape and magnitude of the *PCF* vs *GS* relationship, yet the pattern of initially increasing *PCF* with *GS* is robust. To illustrate this point we employed a set of Monte Carlo models (MC) which were programmed in R. For each MC model, using the same body size distribution, we specified a particular female body size-fecundity relationship (FF_{b}) and sex allocation strategy (SA) combination. FF_{b} categories were: positively linear (FF_{bL}; fig. 5.3.2(*a*i)); a power curve where mean female fecundity for 100 randomly drawn individuals is similar to the mean female fecundity of 100 randomly drawn individuals with body size-fecundity relationship $FF_{\mathrm{bL,}}$ ($FF_{\mathrm{bP(1)}}$; fig. 5.3.2 (*b*i)); a power curve where maximum female fecundity for 100 randomly drawn individuals is similar to the maximum female fecundity of 100 randomly drawn individuals with body size-fecundity relationship $FF_{\mathrm{bL,}}$ ($FF_{\mathrm{bP(2)}}$; fig. 5.3.2 (*c*i)). The sex allocation strategies were: male is the largest group member; male is the smallest group member; male is randomly allocated. For each FF_{b} and SA combination, the Monte Carlo procedure involved the random selection of individuals from the same body size distribution, allocating them among a group size distribution, and then assigning one group member as the male (based on the particular SA strategy). The fecundity of remaining female group members were then assigned their fecundity value based on each female's body size and the particular FF_{b} relationship. *PCF* for each group was then calculated for a range of male fecundity values. This whole procedure was all repeated for each FF_{b} and SA combination 1000 times and the average *PCF* vs *GS* profiles plotted (fig. 5.3.2).

The MC model predicts the difference in *PCF* as a function of *GS* to be greatest when the male is the largest group member and FF_{b} is positively linear (fig 5.3.2, 5.3.3*a*). However, *PCF* for any given *GS* is predicted to be greater when the male is the smallest group member, intermediate when the male is chosen randomly and lowest when the male is the largest group member (fig. 5.3.2, 5.3.3*b*). In addition, for any given SA, a shift from a linear FF_{b} (FF_{bL}) to the power curve $FF_{\mathrm{bP(1)}}$ and finally to the power curve $FF_{\mathrm{bP(2)}}$ tends to reduce maximum *PCF* and variation in *PFC* (fig. 5.3.2, 5.3.3*a*).

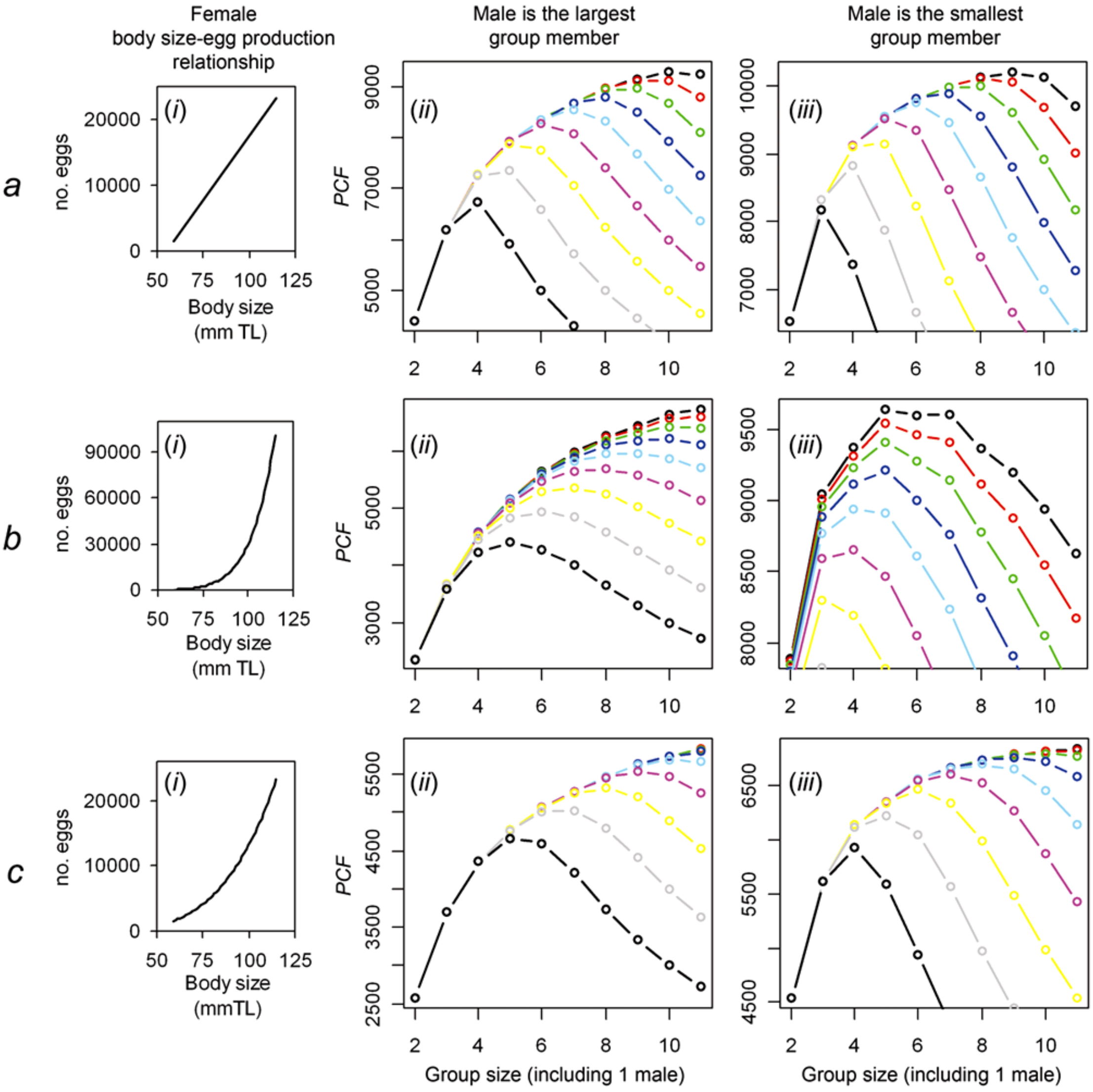

Figure 5.3.2 The Monte Carlo model; predicted relationship between *GS* and *PCF* for a range of female body size-egg production relationships (*a*(*i*)-*c*(*i*)), size-based sex-allocation strategies (*ii-iii*), and maximum male fecundities (coloured lines). Each line colour represents the same male fecundity value across panels. All simulations were run using the same body size distribution.

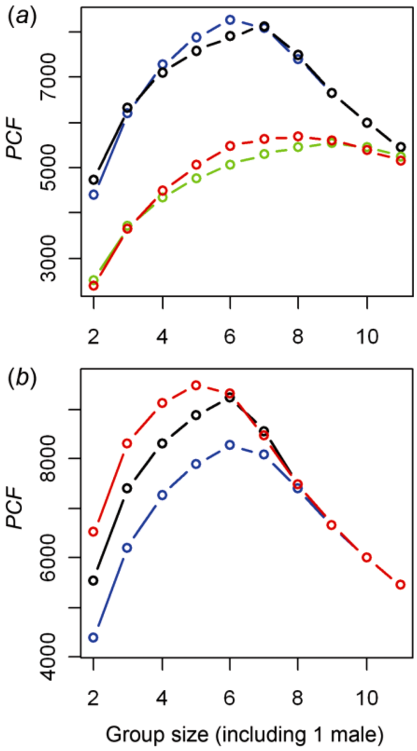

Figure 5.3.3 The Monte Carlo model with analytical model comparison. (*a*) Differences in the predicted relationship between *GS* and *PCF* for a range of female body size-fecundity relationships (FF_{b}) using the same body size distribution and with sex allocation strategy (males is the largest group member) and male fecundity held constant (60000 eggs). Black; the analytical model (where female fecundity = FF_{AM}), blue; Monte Carlo model with linear female body size-fecundity relationship FF_{bL} (i.e. panel *a*(*i*) in figure 5.3.2), red; Monte Carlo model with the power-curve female body size-fecundity relationship $FF_{\mathrm{bP(1)}}$ (i.e. *a*(*ii*) in figure 5.3.2), green; Monte Carlo model with the power-curve female body size-fecundity relationship $FF_{\mathrm{bP(2)}}$ (i.e. *a*(*iii*) in figure 5.3.2). (*b*) Effect of sex-allocation strategy on predicted *PCF* in relation to *GS* with male fecundity (60000 eggs) and the female body size-fecundity relationship (FF_{bL}) held constant. Blue; male is the largest group member, red; male is the smallest group member, black; male group member is randomly assigned in relation to body size rank. All simulations were run using the same body size distribution.

5.4 A test of the model using a polygynous sex-changing fish

We used data on the haremic reef fish *Parapercis cylindrica* (family Pinguipedidae) collected within the lagoon of Lizard Island, Great Barrier Reef (14º 40.9’S, 145º 26.8’ E) to test the predictions of the size advantage model for protogynous sex change, and to examine whether the analytical model (which is insensitive to the protogyny sex-change rule) or the Monte Carlo model (which incorporates the protogynous sex change rule) best explains the relationship between *GS* and *PCF*. *P. cylindrica* is a short-lived fish that occupies macro-algae and coral-rubble habitat within sheltered tropical waters (Randall, 1997). Each female defends a permanent all-purpose territory in which it feeds, spawns, and seeks shelter, and males form harems by defending several neighbouring females (Stroud 1982; Walker and McCormick 2009). The male pelagic-spawns with each ripe female in his harem during sunset, and spawning activity peaks around the new and full moon (Stroud 1982). Males are derived exclusively from females (sequential monandric protogyny) and can be identified by coloration (Stroud 1982; Walker and McCormick 2004; Walker and McCormick 2009*b*).

Social group composition was first determined by observing the coloration and behaviour of individual fish in the wild (n = 232) and by the mapping of their territories. This was carried out at six sites within the lagoon of Lizard Island. Prior to observations, a 30m x 50m reference grid at $5m^2$ resolution was laid over the substrate to aid in the tracking of individuals and to estimate subpopulation density. One 15min observation was made on each individual. A 15 min observation time has been shown to give an accurate estimate of individual territory location and size (Walker and McCormick 2009*b*). Females (n = 177) were assigned to the male (n = 55) whose territory encompassed the particular female. Following the determination of group composition, all individuals were collected with clove oil anaesthetic and a hand net, and measured with callipers (mm total length; TL).

To establish the size-based egg production rate, 123 females were collected from randomly located social groups within the Lizard Island lagoon. All individuals were collected within the hour prior to spawning. These individuals were anesthetized with clove oil, measured (mm TL) and their eggs stripped and stored in 70% ethanol for counting in the laboratory. The relationship between body size and daily egg production was analysed using ordinary least squares (OLS) regression.

To determine the maximum female monthly spawning frequency, 20 experimental patch reefs were created in the lagoon of Lizard Island using a generous quantity of coral rubble and macroaglae habitat. Each patch reef was recruited with an adult female and male, with the entire female size distribution being used among the 20 patches. Experimental fishes were captured randomly from several adjacent subpopulations and experimental pairs were created such that each individual had never interacted before the experiment. Following an acclimation period (5-7 d) experimental pairs were observed each day to determine monthly spawning frequency. The relationship between female body size and monthly spawning frequency was analysed using OLS regression.

Monthly spawning frequencies and fertilisation rates were investigated among a subset of natural social groups within the lagoon of Lizard Island (n = 7). At the beginning of each sampling period, all individuals from social groups were captured using clove oil anaesthetic and a hand net, measured (mm TL), visually sexed, and tagged using a subcutaneous tattoo for individual recognition (Northwest Marine Inc.). A reference grid at 5 x 5m resolution was placed over the substrate to aid in the tracking and relocation of individuals. Each afternoon the male was continuously observed to record the number of spawns, and the spawning participants. The relationship between group size and descending mean female spawning frequency (FSF) was analysed using the hyperbolic regression function: $FSF = (ab) / (a + x)$, where x = group size (including the male) and *a* and *b* are constants.

Consecutive pair-spawn clouds from the tracked subset of social groups (n = 7) were collected in separate 50l plastic bags to determine fertilisation rate. Spawn clouds were captured 10 s following gamete release and incubated *in situ* for approximately 2 hours with constant air supply and an external flowing sea water bath (Shapiro et al 1994, Marconato et al 1995, 1997, Warner et al 1995). The water sample was then filtered to isolate eggs, and eggs scored as fertilised or unfertilised based on the presence or absence of cell division respectively.

Group size-dependent per capita fecundity (*PCF*) (at the monthly scale) among the broader distribution of social groups in the wild (n = 55 social groups) was estimated by: 1) correcting individual female body sizes for daily egg production using the body size by egg production function; 2) multiplying daily egg production by group size-specific female monthly spawning frequency; 3) adding the individual monthly fecundity estimates for females within each group; 4) dividing the total monthly fecundity for each group by total group size (including the male). *PCF* was not corrected for fertilisation rate (FR) due to the low level of variance in observed FR with consecutive pair-spawn number (FR = -0.33(No. spawns) + 98.136, $r^2 = 0.3$, $F_{1,12} = 5.18$, $p = 0.042$; mean FR range; 96.1-98%).

I parametised the Monte Carlo model by randomly selecting individuals from the pool of 232 individuals found among natural social groups at Lizard Island, and combining them into groups based on the observed group-size range (2-11). I then assigned the largest individual within each group 'male', and corrected the body size of remaining individuals (females) for monthly fecundity using the body size (TL mm) versus daily egg production relationship observed at Lizard Island (No. eggs d^{-1} = 37.334(TL mm) – 2053, $r^2 = 0.26$, $F_{1,122} = 44.2$, $p < 0.01$) and the monthly spawning frequency of experimental monogamous females observed at Lizard Island (i.e. the assumed maximum female spawning rate; 10.42 ± 3.8, female n = 20). I then divided the sum of monthly fecundity for females within each group by total group size (including the male), and then calculated expected *PCF* in relation to *GS* for a range $M_{\max}$ values (which defines the peak in *PCF*, and the point preceding hyperbolic decline in *PCF*). This whole procedure was repeated 10000 times for each value of $M_{\max}$. Finally, I fitted the observed *GS* vs. *PCF* data to the Monte Carlo model by finding the value of $M_{\max}$ that minimised the sum of squares (*SS*) using numerical optimisation (function 'optimise()' in R).

To parametize the analytical model, I took the average female body size found in the Lizard Island sample and corrected it for female monthly fecundity (*FF*) using the same egg production- and monthly spawning frequency-functions used in the Monte Carlo model. I then calculated *PCF* in relation to *GS* (using equation 1) for a range of $M_{\max}$ values. Finally, I fitted the observed *GS* vs. *PCF* data to the analytical model by finding the value of $M_{\max}$ that minimised *SS* using numerical optimisation (as above).

5.5 Results and discussion

Among the six sites surveyed, a positive relationship was observed between subpopulation density (individuals/100m^2) and mean haremic-group size (mean group size = population density(0.5) + 1.7, $r^2 = 0.84$, $F_{1,4} = 21.69$, $p < 0.001$; fig. 5.5.1). In all subpopulations, every male (pooled n = 55) encountered and observed was found to be defending at least one female and there was only ever one male per group (i.e. males had discrete territories in which the females reside, and there were no bachelor or roaming males found). In addition, the male was always the largest haremic-group member, consistent with the size advantage model for protogynoys sex change (see also Walker and McCormick 2009*b*).

I stripped the eggs from females (n = 124) to determine daily egg production during spawning periods. Females were found to start producing mature eggs at 58 mm TL, and daily egg production during spawning periods was found to increase linearly with body size thereafter (No. eggs . d^{-1} = 37.334(TL mm) – 2053, adjusted $r^2 = 0.26$, $F_{1,122} = 44.2$, $p < 0.01$). From the observational data, experimental monogamous females were found to spawn 10.42 ± 3.8 SE times per month, and monthly spawning frequency was independent of body size (OLS regression; $F_{1,18} = 3.43$, $p = 0.08$). Mean female spawning frequency . month^{-1} (FSF) among the subset of natural social groups was found to be similar to the experimental monogamous spawning frequency up until a group size of five (i.e. four females per male) after which point FSF declined hyperbolically (FSF . $\text{month}^{-1}_{(\text{group size} \geq 4)}$ = (22.883 x 4.499) / (22.883 + group size), $r^2 = 0.8$, $F_{1,2} = 27.57$, $p = 0.034$; fig. 5.5.2*a*).

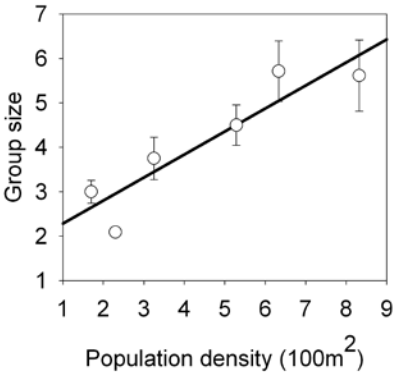

Figure 5.5.1 The relationship between subpopulation density and mean haremic-group size (including 1 male) (± S.E.).

Information on group-specific individual body sizes, daily egg production, and female spawning rate was used to calculate group-specific *PCF*. The observed *PCF*-by-group size data was best explained by the Monte Carlo model which incorporated the protogyny sex change rule. Iteration of M_{max} returned a best fit M_{max} value of 55041.48 eggs . month^{-1}. (SS = 66785949), compared to the analytical model (which is insensitive to the sex change rule) which returned a best fit M_{max} value of 55651.27 eggs . month^{-1} (SS = 70246881) (fig. 5.5.2*b*). Importantly, data confirm that the protogyny sex change rule results in lower *PCF* values at small group sizes (i.e. monogamous pairs) compared to those expected from a shift in sex ratio alone; the Allee effect in fecundity for *P. cylindrica* is the result of both a density dependent socially facilitated shift in sex ratio towards increased female function, and a corresponding increase in mean- and maximum-female size. Note, however, that the analytical model (i.e. sex ratio) explains most of the variance in *PCF* (fig. 5.5.2*b*).

The employed analytical model differs from the Monte Carlo model in that the former predicts the sex ratio effect only (based on mean female fecundity) while the latter incorporates the sex change rule and its effect on group-specific female body sizes and subsequent individual egg production. The predicted *PCF* vs *GS* relationship differs between these two models because making the largest group member 'male' has a greater effect on the female body size distribution (thus egg production) in small groups compared to large groups. While the difference in predicted group-specific *PCF* between the two models was quite low, the Monte Carlo model should nonetheless be considered in future studies. This is because the difference between the analytical *PCF* prediction and the Monte Carlo *PCF* prediction will increase when the relationship between female body size and egg production is non-linear (see 'The model').

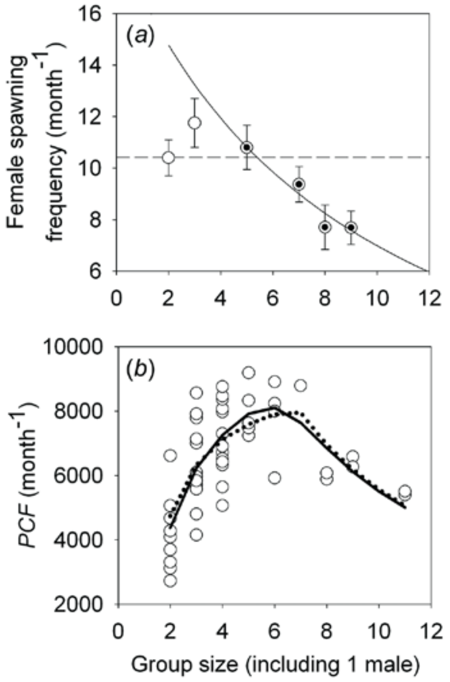

Figure 5.5.2 (*a*) Relationship between group size and mean female monthly spawning frequency (± S.E.) including the experimental monogamous pairs. (*b*) Estimated per capita fecundity with best fit analytical model trajectory (dotted line) and best fit Monte Carlo model trajectory (solid line).

In polygynous sex-changing fishes, sex ratio in local groups is frequently adjusted so that there is generally only one male, who tends to be the largest individual (Warner 1984; Shapiro 1988; Munday et al. 2006). This flexible sex determination strategy can be explained by sexual selection acting on large male body size and the subsequent differential size-based fecundity schedules of females and males (i.e. the SAH; Warner 1975; 1988a,b; Munday et al. 2006; Walker and McCormick 2009). Because groups are constrained to have a male, as *GS* decreases, mean female body size also decreases, and the overall proportion of males in the population increases. This reduces the reproductive potential of the population (by up to 50%) because fewer eggs are produced. Central to this phenomenon, is the tendency of reef fishes to develop discrete home ranges or territories (Sale 1980); either reduced population density, or increased habitat fragmentation, reduces *GS* in site attached fishes, driving a shift in mating system from polygyny to monogamy and a balancing of the sex ratio (via sex change) (often referred to as facultative monogamy; hawkfishes, Donaldson 1989, 1990; angelfishes, Moyer 1990; butterflyfishes, Hourigan 1989; groupers, Shpigel and Fishelson 1991; triggerfishes, Fricke 1980; Ishihara and Kuwamura 1996).

In essence, fragmented and low density populations contain a large number of males that would otherwise be functioning as female. At present, sperm limitation is seen as a major concern for the resilience of commercially exploited protogynous taxa (Adams et al. 2002; Alonzo and Mangel 2004, 2005). This is because fishers tend to remove the largest individuals from the population, which are typically male. However, sperm limitation may be the first of a two-stage process in the decline of exploited protogynous stocks. For example, evidence suggests that locations open to fishing often harbour protogynous fish populations with a greater proportion of small males and transitional individuals (those in the process of sex change) compared to locations closed to fishing (Adams et al. 2002). This may reflect a reduction in the potential for polygyny, reduced sex-change suppression by males due to reduced behavioral encounter rates, and subsequently, greater rates of sex-change from female to male. If males are capable of producing more offspring than females, than overcompensation in sex-change frequency can only diminish *PCF*.

It is important to emphasise, however, that the sex-ratio based Allee effect does not translate into a higher extinction risk for sex-changers compared to gonochores with fixed, equal sex ratios, in fact the contrary. Gonochores with a fixed, equal sex ratio bare the full cost of producing males irrespective of density, such that gonochore *PCF* can only be equal to sex-changer *PCF* (i.e. at low density when sex-changers are forming monogamous pairs) or less than sex-changer *PCF* (i.e. at high density when sex changers are forming polygynous groups). Thus, considering two populations, one comprised of sex changers and the other gonochores, and assuming a negligible cost of sex change, sex-changers will experience a productivity boost with an increase in density whenever overall per capita population growth rate is positive. Moreover, should negative per capita population growth rate be experienced at low population density (i.e. a critical density threshold is present), then our equivalent, hypothetical gonochore population simply cannot persist. In essence, a sex-changer population can occupy demographic niches which are unavailable to the equivalent gonochore population- so long as the sex-changer population (or a source sub-population) stays above the critical threshold density. In this way sex-changers may be more resistant to short term local declines in overall mortality rate or fecundity-whether naturally or anthropogenically induced- thereby providing greater resilience to reef fish communities in general.

Protogynous hermaphroditism is common among reef fishes and occurs in many commercially exploited taxa (Thresher 1984; Warner 1984b; Alonzo and Mangel 2004, 2005; Munday et al. 2006). In fact, protogynous hermaphroditism is the norm for a number of reef fish families (Thresher 1984; Sadovy de Mitcheson and Liu 2008). A recent review of the literature suggests that at least 70 tropical reef fishes may meet the basic prerequisites for a sex ratio based Allee effect (i.e. males are more fecund than females and female sex ratio bias increases with population density and group size) (Walker and McCormick 2009). The present study suggests that habitat fragmentation and reduced population density may have a greater impact on the productivity of these fishes and consequently, the broader tropical reef community, than currently appreciated. On the other hand, the present study suggests that the implementation of marine protected areas (i.e. areas closed to human activity and fishing; MPA's) is likely to have a greater positive effect on protogynous fish stocks and community stability than the implementation of fishing quotas. MPA's allow the preservation of high-quality, continuous habitat types and high-density populations to maximize polygyny, and subsequent productivity and source-sink potential. By contrast, fishing quotas will encourage fishers to focus on high density populations so as to maximise fishing efficiency, leading to fragmented populations, higher rates of sex-change, and reduced productivity and population resilience.

While sex ratio based Allee effects are likely to be most common in sex-changers (due to the extremity and frequency of sex ratio adjustment), sex change is not a fundamental requirement for the Allee effect to occur. So long as polygyny can be realised, density dependence in offspring sex ratio adjustment towards the least fecund sex (usually the female) will also lead to an Allee effect. A number of insects, reptiles, birds and mammals are known to adjust the sex ratio of their young in response to local environmental conditions (Hamilton 1969; Trivers and Willard 1973; Werren 1980; Charnov 1982; West 2000; Wade et al. 2003; Reese et al. 2008). The results of this study may therefore be applicable to a wide variety of taxa and population and community types. Assessment of how sex ratio based positive density dependence alters the outcome of population and community models may be a fruitful avenue for future research.

General Discussion

6.1 Summary of results: implications, applications and future research directions

Using the tropical sex-changing reef fish *Parapercis cylindrica* as the model species, I tested the general hypotheses that: 1) social factors have played a significant role in the evolution of phenotypic plasticity, and that; 2) this plasticity drives fundamental relationships between social system type, phenotypic expression, and the functioning and productivity of particular societies.

The combination of theoretical, descriptive and experimental approaches adopted in the present study highlighted social system dynamics to be a major driver of the evolution of phenotypic plasticity mechanisms, and the expression of alternative behavioural, physiological and morphological traits among individuals and social groups. These plastic responses can be grouped into three ultimate classes: conflict resolution and group stability tactics, sexual selection for male body size, and context dependent sensory optimisation.

In *P. cylindrica*, a strict dominance hierarchy exists within each harem, where the male represents the top breeding status. The males of such societies reap the highest reproductive rewards, and females within larger haremic groups disproportionately secure reproductive shares from sperm-limited males, driving a pattern of reproductive skew among female group members. A central aim of this thesis, therefore, was to determine how these more productive societies remain stable through time, given the conflict over reproductive shares inherent within them. In Chapter 2, I provided the first experimental evidence in support of the hypothesis that male reproductive allocation (i.e. disproportionate sperm allocation to larger, more dominant females), dominant female enforcement (via punishment), and subordinate female corporation (via endogenously controlled reproductive down regulation), act together to promote social stability. In addition, individual females were experimentally found to rapidly adjust reproduction following a shift in dominance rank. These results, coupled with the experimental illustration of a flexible sexual differentiation strategy (Chapters 3 and 4) highlight remarkable flexibility in both reproductive mode and reproductive rate in response to ultimate socio-demographic processes. Fundamentally, the prospect of becoming a large dominant male and taking over a productive harem, rather than group productivity *per se*, appears to be the primary ultimate factor which drives the evolution of these conflict resolution tactics and therefore, social group stability.

Reproductive rate and allocation tactic were not the only plastic responses observed in relation to social group dynamics. The comparative and experimental data presented in Chapter 4 illustrate that individual growth and body size is also flexible and that individuals modify growth and body size during sex change according to the benefit of large male body size in securing and maintaining reproductive dominance. This plastic response manifested a pattern of positive allometry for sexual size dimorphism among social groups, thus providing the first experimental evidence to date that sexual selection is responsible for this commonly observed pattern in Nature (i.e. positive sexual size allometry, also known as Rensch's rule; Walker and McCormick 2009, see also Fairbairn 1997; Dale et al 2007). Importantly, the confirmation of growth- and body size-adjustment in response to ultimate factors highlights the usefulness of reef fishes for testing more general theories pertaining to the origins and maintenance of morphological diversity, and illustrates the potential importance of individual growth modification in the shaping of morphological patterns in Nature (Walker and McCormick 2009b; see also Buston 2003; Walker et al. 2007; Munday et al. 2009).

Changes in locomotion patterns and use of space occur coincident with sexual transition. This means that optimal spatial-sensory range specificity may also differ as individuals change sex. In Chapter 4, I experimentally illustrated that otolith (ear stone) growth and morphology changes during sex-change from female to male, and that the social conditions in which an individual changes sex influences the extent of sex-specific divergent otolith growth. It is currently unclear whether this change in otolith microstructure represents a shift in sensory range specificity, or whether it simply represents a selectively neutral consequence- or perhaps even a trade-off- of the rapid shift in endocrinology and metabolism that occurs coincident with sex change (see Frisch et al. 2007). However, given the expectation of highly directional and conservative otolith growth (Thorrold and Hare 2002), it is certainly possible that sex-changers in different social environments alter otolith accretion to facilitate life history optimisation in accordance with their new reproductive mode. This hypothesis will, however, require further experimental testing using an ecophysological approach.

Irrespective of the proximate and ultimate details of sex change associated divergent otolith growth, the validation of sex change associated otolith discontinuities, or signatures, represents a novel and powerful tool in ecology and fish- and fisheries-science (Walker and McCormick 2009a). Since its initial description (Walker and McCormick 2004) otolith sex-change signatures have been documented in one additional sand perch (*Parapercis snyderi*), and in three short lived wrasses (family Labridae; Ryen 2008; Munday et al. 2009) (i.e. four out of five species examined). These signatures will enable the life histories and ecology of hermaphroditic reef fishes to be examined with a degree of resolution not previously possible; by enabling the timing of individual sex change to be discerned in relation to other key life history attributes, such as previous age-based growth, the timing of settlement, and the timing of female maturation. Sex change associated otolith signature will also provide a means to detect the relative timing of sex change among social group members, and, coupled with otolith microchemistry techniques, may enable the detection of sex change associated migration and niche shifts (see Thorrold and Hare 2002).

Finally, in Chapter 5 I developed analytical and Monte Carlo models with which to examine the mechanistic relationships between social system, sexual differentiation strategy and density dependent per capita fecundity (*PCF*). Specifically, I established the likelihood of positive density dependent per capita fecundity (*PCF*) under a range of sex allocation strategies and body size-fecundity relationships. I illustrated that an Allee effect is expected whenever one sex can produced more offspring than the other and sex ratio bias towards the least fecund sex increases with population density and mating group size. Moreover, the Allee effect is greatest when the least fecund sex displays a linear body size-fecundity relationship and the most fecund sex is the largest mating-group member. Importantly, these conditions appear to be met in many animals, including at least seventy common tropical reef fishes. I used the polygynous sex-changing reef fish *Parapercis cylindrica* as an example, and to test the model. Data on *P. cylindrica* suggested that density dependent sexual selection for large male body size is the ultimate causal mechanism underlying local size-based sex ratio adjustment, and thus the Allee effect. Given the pervasiveness of site attachment, polygynous mating systems and socially facilitated hermaphroditism in reef fishes, Allee effects associated with sex ratio adjustment in response to sexual selection are likely to be particularly frequent in- but not limited to- these taxa. I suggested that marine protected areas may therefore often outperform fishing quotas as a conservation tool, since the former maximises polygyny potential and source-sink potential, while the latter promotes population fragmentation, the formation of monogamous pairs and a balancing of the sex ratio, and hence up to a 50% decline in *PCF*. Evidence suggests this previously overlooked, density dependent mechanism is likely to be widespread among tropical fishes (Chapter 5; appendix table A1), and may help to explain the apparent lack of recovery of many exploited tropical reef fish populations and communities (Paddock et al. 2009). The quantitative assessment of how this Allee effect alters the outcome of alternative fisheries management- and fish conservation-strategies will be an important focus of future research.

6.2 Formal allometric analysis and the unification of morphological evolutionary theories

Sexual size dimorphism (SSD) is one of the most variable and diverse phenotypic traits observed in animals. Fortunately, this variation is not without pattern. It was Bernhard Rensch who first noted that sexual size dimorphism (SSD) tends to vary with body size among populations and species (Rensch 1950). Specifically, he noted that SSD increases with body size in clades where males are the larger sex (the typical scenario), and by way of exception, that SSD decreases with body size in clades where females are the larger sex. This finding sparked immense interest and research effort in the study of evolutionary processes which drive common morphological patterns in Nature. Fairbairn recently advanced the field by illustrating that both patterns of SSD noted by Rensch actually represent a common evolutionary pattern: covariation in female size coupled with greater variation in male size, a trend now known as ‘Rensch’s rule’. Fairbairn illustrated this point using formal allometric regression (Fairbairn 1997; 2005): when a model II regression is performed on the log sizes of females (x axis) and males (y axis), the slope of that model indicates whether female and male body size diverges with mean size ($\beta > 1$; i.e. Rensch’s rule, or positive sex size allometry), converges with mean size ($\beta < 1$; negative sexual size allometry), or covaries in isometry ($\beta = 1$). The intercept indicates whether females are initially the smaller or larger sex.

To date, the study of sexual size allometry has been restricted almost solely to terrestrial animals (particularly birds and insects) using the mean sex-based adult body sizes of populations or species (for a review see Fairbairn 2007). In these studies, the pattern of allometry is assumed to proximately reflect genetic divergence between the sexes and among populations (or species) in response to the hypothesised ultimate driver (i.e. the evolutionary process, such as sexual selection for male body size or natural selection for SSD; Chapter 3). In this thesis, I entertained the idea that ultimate hypotheses for sexual size allometry should apply regardless of the taxonomic scale of inference, and that we can use individual phenotypic plasticity to test those hypotheses experimentally (see also Warner 1991). In essence, formal allometric analysis can assist in the unification of morphological evolutionary theories, by enabling the systematic testing of ultimate hypotheses at both macro-and micro-evolutionary scales. This is because when ultimate hypotheses are formalised using the principles of allometry, results at different taxanomic scales become directly comparable, irrespective of the proximate details of body size divergence.

However, before we can begin to generalise about the causal relationships between various selection pressures and macro- and micro-morphological patterns, we must first enhance our knowledge on the precise scale at which SSD variation is manifest within particular clades. In this regard, knowledge on the proximate details of growth and body size divergence (e.g. phenotypic plasticity vs. genetic divergence) is essential. Future research needs to balance out what has to date been an almost exclusive focus on absolute sex-specific adult body sizes (which are generally assumed to reflect genetic divergence), with more detailed studies on the proximate mechanisms underlying body size variation among individuals (i.e. which report on the relative contribution of phenotypic plasticity mechanisms). Such studies will provide valuable insight into the origins of morphological variation, and will facilitate the development of more general and realistic models of morphological evolution and the appropriate partitioning of proximate and ultimate factors (see below).

The unification of evolutionary theories of morphological diversity also requires the appropriate assignment of proximate and ultimate causal factors at the various scales of inference (Mayr 1961; Tinbergen 1964; Sherman 1988). The proximate and ultimate levels of allometric analysis are frequently mismatched in the scientific literature, which results in ambiguous conclusions and the incorrect rejection of ultimate hypotheses. For example, the phenotypic plasticity hypothesis for sexual size allometry (i.e. a proximate hypothesis) is often tested as an alternative to the sexual selection hypothesis for sexual size allometry (i.e. an ultimate hypothesis) (e.g. Fairbairn 2005; Lengkeek et al 2007). Future research on the evolution of sexual size allometry would greatly benefit from the explicit stating and testing of both proximate and ultimate causal factors, an approach which is frequently ignored despite the known dangers associated with doing so (Mayr 1961; Tinbergen 1964; Sherman 1988).

6.3 The Allee effect as the basis of sociality

Warder C. Allee brought attention to the possibility of an initially positive relationship between components of fitness and population density and group size more than 50 years ago (Allee 1931). Until recently, however, this concept was generally regarded as an intriguing but relatively unimportant aspect of population ecology (*sensu* Stephens and Sutherland 1999). The increasing realisation that the incorporation of Allee effects into models of population dynamics render quantitatively different results, together with recent interest in the implications of sociality for conservation, have shown that the consequences of the Allee effect to social and life history evolution, ecology and conservation are profound (Stephens and Sutherland 1999; Courchamp et al. 1999; De Roose and Persson 2002).

For many species, survival and/or reproduction can be severely limited at small group sizes. Animal aggregations, beyond the level expected from habitat limitations or aggregation of resources can be attributed to conspecific attraction. Such attraction, arising as a result of the net benefits of conspecific presence, is a direct product of the Allee effect (Stephens and Sutherland 1999). It has been suggested that the degree of sociality of individuals might therefore reflect the degree of severity of the Allee effect to which it is subject (Stephens and Sutherland 1999). In *P. cylindrica*, (in addition to at least 70 other territorial protogynous sex-changing haremic fishes; appendix table A1) maximum potential lifetime fecundity, and per capita fecundity, is directly related to social group size. This is because males are more fecund than females and sex ratio is adjusted such that there is generally only one male per group (Chapter 5). Thus the evolution of sociality in these animals is essentially a density dependent process, since all individual can potentially function as male and attain the dominant breeding position (Chapter 2). However, not all individuals reap the rewards of larger group size all the time, and some individuals will not reap the rewards of larger group size at all. Thus, while positive density dependence can explain the potential benefit of group formation (i.e. sociality *per se*), it cannot explain the stability of those groups through time (i.e. the evolution of social behaviours). Rather, the key to understanding social group stability (hence much of the diversity in social behaviour) lies in determining the tactics dominants employ to maximise their greater bargaining power, the tactics subordinates employ to maximize their own life history in response to the behaviour of dominants, and subsequently how- and under what conditions- conflict over reproductive shares is resolved between genetically selfish social group members (Chapter 2; Buston and Zink

2009). Within this context, the degree of reproductive skew (i.e. the degree of disproportionate allocation of reproduction) within a particular social group reflects the degree to which individuals of different social status and competitive ability are willing to negotiate, based on the net benefits of group coercion (i.e. the strength of the Allee effect). Knowledge of the interplay between density- group size-dependent individual fitness potentials (i.e. the Allee effect) and the struggle for dominance and survival within those groups (i.e. the allocation of reproductive shares), will be key to understanding the evolution of sociality and a social behaviour. The Allee effect, therefore, can be regarded not only as a suite of problems associated with rarity (due to reduced per capita productivity with reduced population density and group size), but also as a basal component of the evolution of sociality in animals (see also Stephens and Sutherland 1999).

6.4 Concluding remarks

This thesis contributes to a larger body of work which showcases reef fishes as highly labile and sophisticated creatures (e.g. Robertson 1972; Frick 1980b; Warner 1988a,b; Shapiro 1988; Buston 2003; Munday et al. 2006; Grosenick et al. 2007; Walker and Ryen 2007; Wong et al. 2007; Bshary et al. 2008). Combined, these studies illustrate that we need to be focusing more on the scale of individuals if we are to achieve a comprehensive understand of reef fish ecology and evolution. These studies also highlight just how useful individual plasticity in fishes can be for testing general evolutionary theories.

Humans rely on the marine environment for their survival, either directly or indirectly. Understanding the ecology and evolution of marine organisms empowers us to successfully manage and conserve that environment. The continued study of marine animals, and how they modify their phenotype and behaviour in response to sociol- habitat- and community-based selection pressures, will facilitate our efforts to conserve them, and will provide valuable insight into life history and social evolution in general.

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Appendix List

Tables

Figures

Thesis chapter publications

A7.1 Male reproductive allocation, dominant female enforcement and subordinate female cooperation act together to promote social stability in a haremic reef fish. In review at the time of thesis submission, Animal Behaviour.

A7.2 Walker, S.P.W. and M.I. McCormick. 2009. Sexual selection explains sex-specific growth plasticity and positive allometry for sexual size dimorphism in a reef fish. Proceedings of the Royal Society B 276: 3335-3343.

A7.3 Walker, S.P.W. and M.I. McCormick. 2009. Fish ears are sensitive to sex change. Biology Letters 5: 73-76.

A7.4 Walker, S.P.W., L. Thibaut and M.I. McCormick. 2009. Density dependent sex ratio adjustment and the Allee effect: A model and a test using a polygynous sex-changing fish. In review at the time of thesis submission, American Naturalist.

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A8.1 McCormick, M.I., C. Ryen, P. Munday, and S.P.W. Walker. 2009. Alternative growth strategies for sexual size dimorphism between two polygynous society types in a tropical reef fish. In review at the time of thesis submission, PLOS One.

A8.2 Munday, P.L., C.A. Ryen, M.I. McCormick, and S.P.W. Walker. 2009. Growth acceleration, behaviour and otolith check marks associated with sex change in the wrasse *Halichoeres miniatus*. Coral Reefs 28: 623-634.

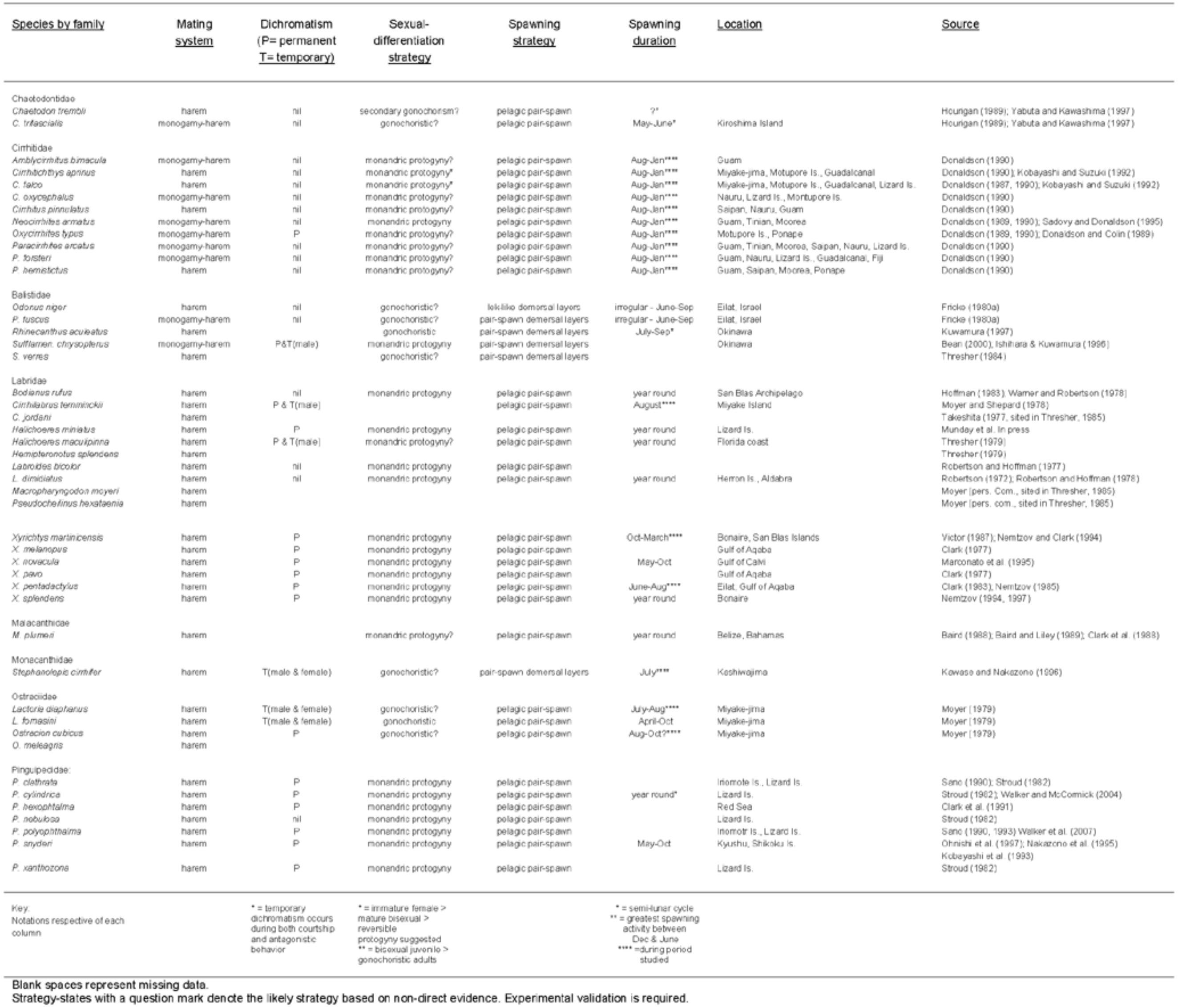
A8.3 Walker S.P.W., and C. Ryen. 2007. Opportunistic hybridization between two congeneric tropical reef fish. Coral reefs 26(3): 539.

A8.4 Frisch A, S.P.W. Walker, M.I. McCormick, and T. Solomon-Lane. 2007. Regulation of protogynous sex change by competition between corticosteroids and androgens: An experimental test using sand perch, *Parapercis cylindrica*. Hormones and Behaviour 52: 540-545.

A8.5 Walker SPW, C. Ryen, and M.I. McCormick. 2007. The temporal and ontogenetic relationships between sex change and sexual size dimorphism in a protogynous hermaphrodite, *Parapercis snyderi* Jordan & Starks 1905. Journal of Fish Biology 71: 1347-1357.

A8.6 Walker S.P.W. and M.I. McCormick. 2004. Otolith-check formation and accelerated growth associated with sex change in an annual protogynous tropical reef fish. Marine Ecology Progress Series 266: 201-212.

Appendix table A1.1 Haremic tropical reef fishes and their reproductive life histories



Species by family	Mating system	Dichromatism (P= permanent T= temporary)	Sexual-differentiation strategy	Spawning strategy	Spawning duration	Location	Source
Chaetodontidae							
Chaetodon trembli	harem	nil	secondary gonochorism?	pelagic pair-spawn	?*		Hourigan (1989); Yabuta and Kawashima (1997)
C. trifascialis	monogamy-harem	nil	gonochoristic?	pelagic pair-spawn	May-June*	Kiroshima Island	Hourigan (1989); Yabuta and Kawashima (1997)
Cirrhitidae							
Amblycirrhitus bimacula	monogamy-harem	nil	monandric protogyny?	pelagic pair-spawn	Aug-Jan****	Guam	Donaldson (1990)
Cirrhitichthys aprinus	harem	nil	monandric protogyny*	pelagic pair-spawn	Aug-Jan****	Miyake-jima, Motupore Is., Guadalcanal	Donaldson (1990); Kobayashi and Suzuki (1992)
C. falco	harem	nil	monandric protogyny*	pelagic pair-spawn	Aug-Jan****	Miyake-jima, Motupore Is., Guadalcanal, Lizard Is.	Donaldson (1987, 1990); Kobayashi and Suzuki (1992)
C. oxycephalus	monogamy-harem	nil	monandric protogyny?	pelagic pair-spawn	Aug-Jan****	Nauru, Lizard Is., Montupore Is.	Donaldson (1990)
Cirrhitus pinnulatus	harem	nil	monandric protogyny?	pelagic pair-spawn	Aug-Jan****	Saipan, Nauru, Guam	Donaldson (1990)
Neocirrhites armatus	monogamy-harem	nil	monandric protogyny	pelagic pair-spawn	Aug-Jan****	Guam, Tinian, Moorea	Donaldson (1989, 1990); Sadovy and Donaldson (1995)
Oxycirrhites typus	monogamy-harem	P	monandric protogyny?	pelagic pair-spawn	Aug-Jan****	Motupore Is., Ponape	Donaldson (1989, 1990); Donaldson and Colin (1989)
Paracirrhites arcatus	monogamy-harem	nil	monandric protogyny?	pelagic pair-spawn	Aug-Jan****	Guam, Tinian, Moorea, Saipan, Nauru, Lizard Is.	Donaldson (1990)
P. forsteri	monogamy-harem	nil	monandric protogyny?	pelagic pair-spawn	Aug-Jan****	Guam, Nauru, Lizard Is., Guadalcanal, Fiji	Donaldson (1990)
P. hemistictus	harem	nil	monandric protogyny?	pelagic pair-spawn	Aug-Jan****	Guam, Saipan, Moorea, Ponape	Donaldson (1990)
Balistidae							
Odonus niger	harem	nil	gonochoristic?	lek-like demersal layers	irregular - June-Sep	Eilat, Israel	Fricke (1980a)
P. fuscus	monogamy-harem	nil	gonochoristic?	pair-spawn demersal layers	irregular - June-Sep	Eilat, Israel	Fricke (1980a)
Rhinecanthus aculeatus	harem		gonochoristic	pair-spawn demersal layers	July-Sep*	Okinawa	Kuwamura (1997)
Sufflamen. chrysopterus	monogamy-harem	P&T(male)	monandric protogyny	pair-spawn demersal layers		Okinawa	Bean (2000); Ishihara & Kuwamura (1996)
S. verres	harem		gonochoristic?	pair-spawn demersal layers			Thresher (1984)
Labridae							
Bodianus rufus	harem	nil	monandric protogyny	pelagic pair-spawn	year round	San Blas Archipelago	Hoffman (1983); Warner and Robertson (1978)
Cirrhilabrus temminckii	harem	P & T(male)		pelagic pair-spawn	August****	Miyake Island	Moyer and Shepard (1978)
C. jordani	harem						Takeshita (1977, sited in Thresher, 1985)
Halichoeres miniatus	harem	P	monandric protogyny	pelagic pair-spawn	year round	Lizard Is.	Munday et al. In press
Halichoeres maculipinna	harem	P & T(male)	monandric protogyny?	pelagic pair-spawn	year round	Florida coast	Thresher (1979)
Hemipteronotus splendens	harem						Thresher (1979)
Labroides bicolor	harem	nil	monandric protogyny	pelagic pair-spawn			Robertson and Hoffman (1977)
L. dimidiatus	harem	nil	monandric protogyny	pelagic pair-spawn	year round	Herron Is., Aldabra	Robertson (1972); Robertson and Hoffman (1978)
Macropharyngodon moyeri	harem						Moyer (pers. Com., sited in Thresher, 1985)
Pseudocheilinus hexataenia	harem						Moyer (pers. com., sited in Thresher, 1985)
Xyrichtys martinicensis	harem	P	monandric protogyny	pelagic pair-spawn	Oct-March****	Bonaire, San Blas Islands	Victor (1987); Nemtzov and Clark (1994)
X. melanopus	harem	P	monandric protogyny	pelagic pair-spawn		Gulf of Aqaba	Clark (1977)
X. novacula	harem	P	monandric protogyny	pelagic pair-spawn	May-Oct	Gulf of Calvi	Marconato et al. (1995)
X. pavo	harem	P	monandric protogyny	pelagic pair-spawn		Gulf of Aqaba	Clark (1977)
X. pentadactylus	harem	P	monandric protogyny	pelagic pair-spawn	June-Aug****	Eilat, Gulf of Aqaba	Clark (1983); Nemtzov (1985)
X. splendens	harem	P	monandric protogyny	pelagic pair-spawn	year round	Bonaire	Nemtzov (1994, 1997)
Malacanthidae							
M. plumeri	harem		monandric protogyny?	pelagic pair-spawn	year round	Belize, Bahamas	Baird (1988); Baird and Liley (1989); Clark et al. (1988)
Monacanthidae							
Stephanolepis cirrhifer	harem	T(male & female)	gonochoristic?	pair-spawn demersal layers	July****	Koshiwajima	Kawase and Nakazono (1996)
Ostraciidae							
Lactoria diaphanus	harem	T(male & female)	gonochoristic?	pelagic pair-spawn	July-Aug****	Miyake-jima	Moyer (1979)
L. fornasini	harem	T(male & female)	gonochoristic	pelagic pair-spawn	April-Oct	Miyake-jima	Moyer (1979)
Ostracion cubicus	harem	P	gonochoristic?	pelagic pair-spawn	Aug-Oct?****	Miyake-jima	Moyer (1979)
O. meleagris	harem						
Pinguipedidae:							
P. clathrata	harem	P	monandric protogyny	pelagic pair-spawn		Iriomote Is., Lizard Is.	Sano (1990); Stroud (1982)
P. cylindrica	harem	P	monandric protogyny	pelagic pair-spawn	year round*	Lizard Is.	Stroud (1982); Walker and McCormick (2004)
P. hexophtalma	harem	P	monandric protogyny	pelagic pair-spawn		Red Sea	Clark et al. (1991)
P. nebulosa	harem	nil	monandric protogyny	pelagic pair-spawn		Lizard Is.	Stroud (1982)
P. polyophthalma	harem	P	monandric protogyny	pelagic pair-spawn		Iriomotr Is., Lizard Is.	Sano (1990, 1993) Walker et al. (2007)
P. snyderi	harem	P	monandric protogyny	pelagic pair-spawn	May-Oct	Kyushu, Shikoku Is.	Ohnishi et al. (1997); Nakazono et al. (1995) Kobayashi et al. (1993)
P. xanthozona	harem	P	monandric protogyny	pelagic pair-spawn		Lizard Is.	Stroud (1982)
Key: Notations respective of each column		* = temporary dichromatism occurs during both courtship and antagonistic behavior	* = immature female > mature bisexual > reversible protogyny suggested ** = bisexual juvenile > gonochoristic adults		* = semi-lunar cycle ** = greatest spawning activity between Dec & June **** =during period studied		

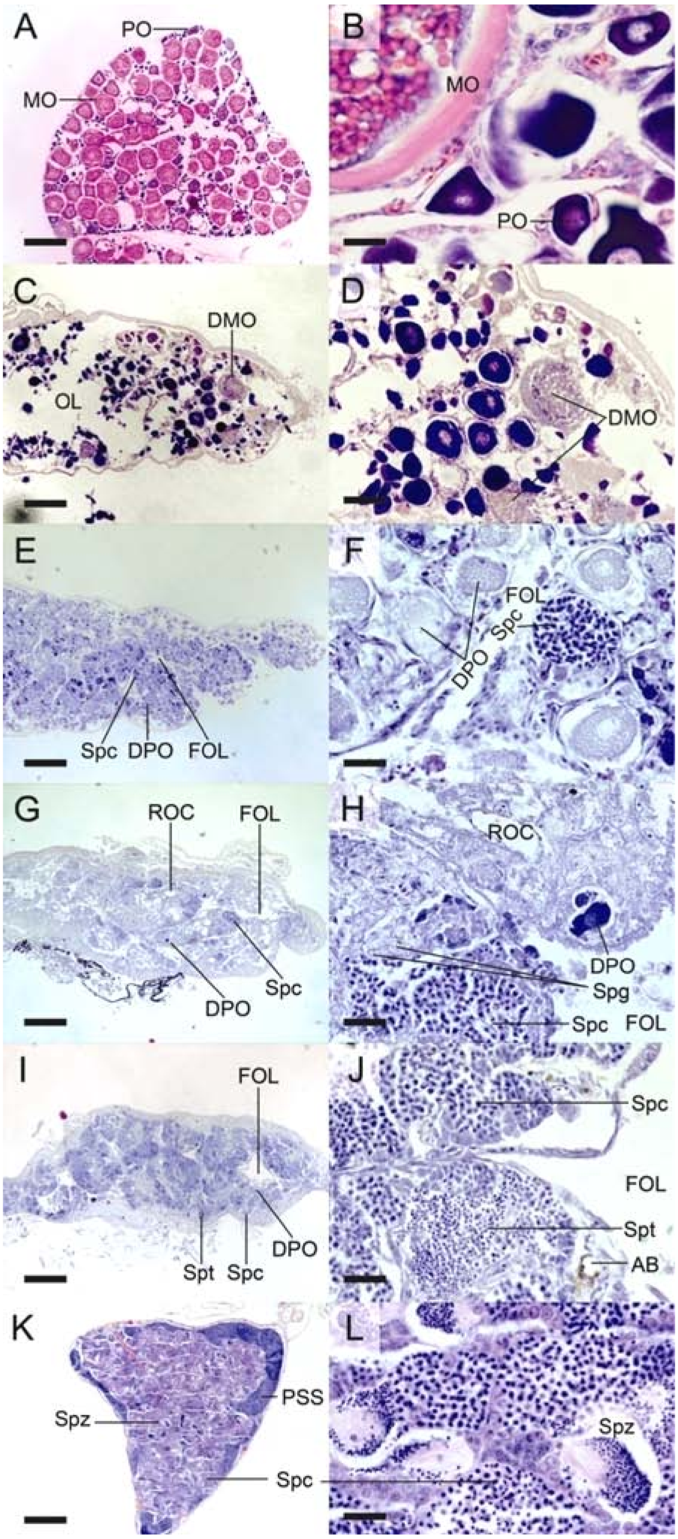
Blank spaces represent missing data.
Strategy-states with a question mark denote the likely strategy based on non-direct evidence. Experimental validation is required.

Appendix table A1.1 (continued) Haremic tropical reef fishes and their reproductive life histories.

Species by family	Mating system	Dichromatism (P= permanent T= temporary)	Sexual-differentiation strategy	Spawning strategy	Spawning duration	Location	Source
Pomacanthidae							
Centropyge argi	monogamy-harem	T(male)		pelagic pair-spawn	year round	Curacao, Florida	Thresher (1982); Moyer (1990); Moyer et al. (1983)
C. bicolor	harem	T(male)	monandric protogyny	pelagic pair-spawn		Ponape, Lizard Is., Papua New Guinea	Thresher (1982), Aldenhoven (1986), Moyer (1990)
C. bispinosus	harem	nil	monandric protogyny	pelagic pair-spawn	year round*	Lizard Is., Philippines, Enewetak atoll, Guam	Thresher (1982), Moyer (1990), Moyer et al. (1983)
C. ferrugatus	monogamy-harem	P	monandric protogyny	pelagic pair-spawn	May-Nov	Miyake-jima, Okinawa	Sakai and Kohda (1997)
C.flavicauda	harem	P		pelagic pair-spawn		Miyake-jima	Moyer (1990)
C. flavissimus	harem	T(female)		pelagic pair-spawn	?*	Enewetak Atoll, Guam	Thresher (1982); Moyer (1990)
C. heraldi	harem	P		pelagic pair-spawn		Enewetak Atoll, Guam	Moyer and Nakazono (1978)
C. interruptus	monogamy-harem	P&T(male)	monandric protogyny	pelagic pair-spawn	May-Oct	Miyake-jima	Moyer and Nakazona (1978)
C. loriculus	harem	P(Size related)		pelagic pair-spawn	?*	Enewetak Atoll	Thresher (1982)
C. multicolor	harem	nil		pelagic pair-spawn		Enewetak Atoll	Thresher (1982)
C. multifasciatus	harem	nil		pelagic pair-spawn		Enewetak Atol, Philippines, Papua New Guinea	Moyer (1990)
C. multispinis	harem	P	monandric protogyny	pelagic pair-spawn		Aldabra, Eilat	Bruce (1980); Moyer (1990)
C. nox	harem	T(female)		pelagic pair-spawn		Philippines, Papua New Guinea	Thresher (1982)
C. potteri	monogamy-harem	T(male & female)	monandric protogyny	pelagic pair-spawn	year round**	Hawaii	Lutnesky (1988,1994, 1995); Lobel (1978)
C. shepardi	harem	P&T(female)	monandric protogyny	pelagic pair-spawn		Guam	Moyer (1981)
C. tibicen	harem	T&P(male & female	monandric protogyny	pelagic pair-spawn		Miyake-jima, Philippines	Thresher (1982)
C. vroilikii	harem	T(female)	monandric protogyny	pelagic pair-spawn		Miyake-jima, Okinawa, Philippines	Thresher (1982)
Chaetodontoplus mesoleucus	monogamy-harem	nil	monandric protogyny ?	pelagic pair-spawn		Philippines	Moyer (1990)
Euxiphipops sexstriatus	harem	nil		pelagic pair-spawn			J. Aldenhoven (pers.com., sited in Thresher (1982)
Holacanthus ciliaris	harem	nil		pelagic pair-spawn		Curacao, Panama	Moyer et al. (1983)
H. tricolor	harem	P(Size related)	monandric protogyny	pelagic pair-spawn	most of year	Vergin Islands, Puerto Rico, Panama	Neudecker and Lobel (1982); Moyer et al. (1983); Hourigan and Kelley (1985)
H. trimaculatus	harem	nil		pelagic pair-spawn		Guam	Shepard, cited in Moyer & Nakazona (1978)
Pygoplites diacanthus	harem	nil		pelagic pair-spawn		Enewetak Atoll, Philippines, Papua New Guinea	Thresher (1982)
P. imperator	monogamy-harem	P&T(female)		pelagic pair-spawn	year round	Enewetak Atoll	Fricke (1980a); Thresher (1982)
P. narvachus	harem	nil		pelagic pair-spawn		Philippines, Papua New Guinea	Moyer et al (1983)
P. paru	monogamy-harem	nil		pelagic pair-group spawn		Cayman Islands.	Moyer et al (1983)
P. sexstriatus	harem	nil		pelagic pair-spawn		Lizard Is., Philippines, Papua New Guinea	Moyer et al (1983)
P. zonipectus	monogamy-harem	nil		pelagic pair-spawn	year round	Sea of Cortez	Moyer et al (1983)
Scaridae							
Scarus vetula	harem-lek like	p		pelagic pair-spawn		La Parguera	Robertson and Warner (1978); Clavijo (1983)
Sparisoma radians	harem	P	monandric protogyny	pelagic pair-spawn			Robertson and Warner (1978)
Sparisoma viride	harem	P	monandric protogyny	pelagic pair-spawn		Belize, Grand Turks, Caicos Islands	Cardwell (1989); Kolts 1993)
Serranidae							
Cephalopholis argus	harem	T(male & female)*	monandric protogyny?	pelagic pair-spawn	Dec-May?	Gulf of Aqaba	Shpigel and Fishelson (1991); Thresher (1984)
C. hemistiktos	monogamy-harem	T(male & female)*	monandric protogyny?	pelagic pair-spawn	Dec-May?	Gulf of Aqaba	Shpigel and Fishelson (1991); Thresher (1984)
C. miniata	harem	T(male & female)*	monandric protogyny?	pelagic pair-spawn	Dec-May?	Gulf of Aqaba	Shpigel and Fishelson (1991); Thresher (1984)
Serranus baldwini	harem	nil	simultaneous-male	pelagic pair-spawn	year round	Panama, Belize	Peterson and Fischer (1986); Leonard (1993)
S. fasciatus	harem	nil	simultaneous-male	pelagic pair-spawn	year round	Punta Santa Inez	Peterson (1987)
Tetraodontidae							
Canthigaster valentini	harem	P	gonochoristic	pair-spawn demersal layers	year round	Lizard Is.	Gladstone (1987a, 1987b,1988); Gladstone and Westoby (1988)
C. punctatissima	harem	nil				Punta Santa Inez, Baja California Sur	Koboyashi (1986)
C rostrata	harem	P(size related?)	gonochoristic?	pair-spawn demersal layers		San Blas Islands, Panama	Sikkel (1990)
Key: Notations respective of each column		* = temporary dichromatism occurs during both courtship and antagonistic behavior	* = immature female > mature bisexual > reversible protogyny suggested ** = bisexual juvenile > gonochoristic adults		* = semi-lunar cycle ** = greatest spawning activity between Dec & June **** =during period studied		

Blank spaces represent missing data.
Strategy-states with a question mark denote the likely strategy based on non-direct evidence. Experimental validation is required.

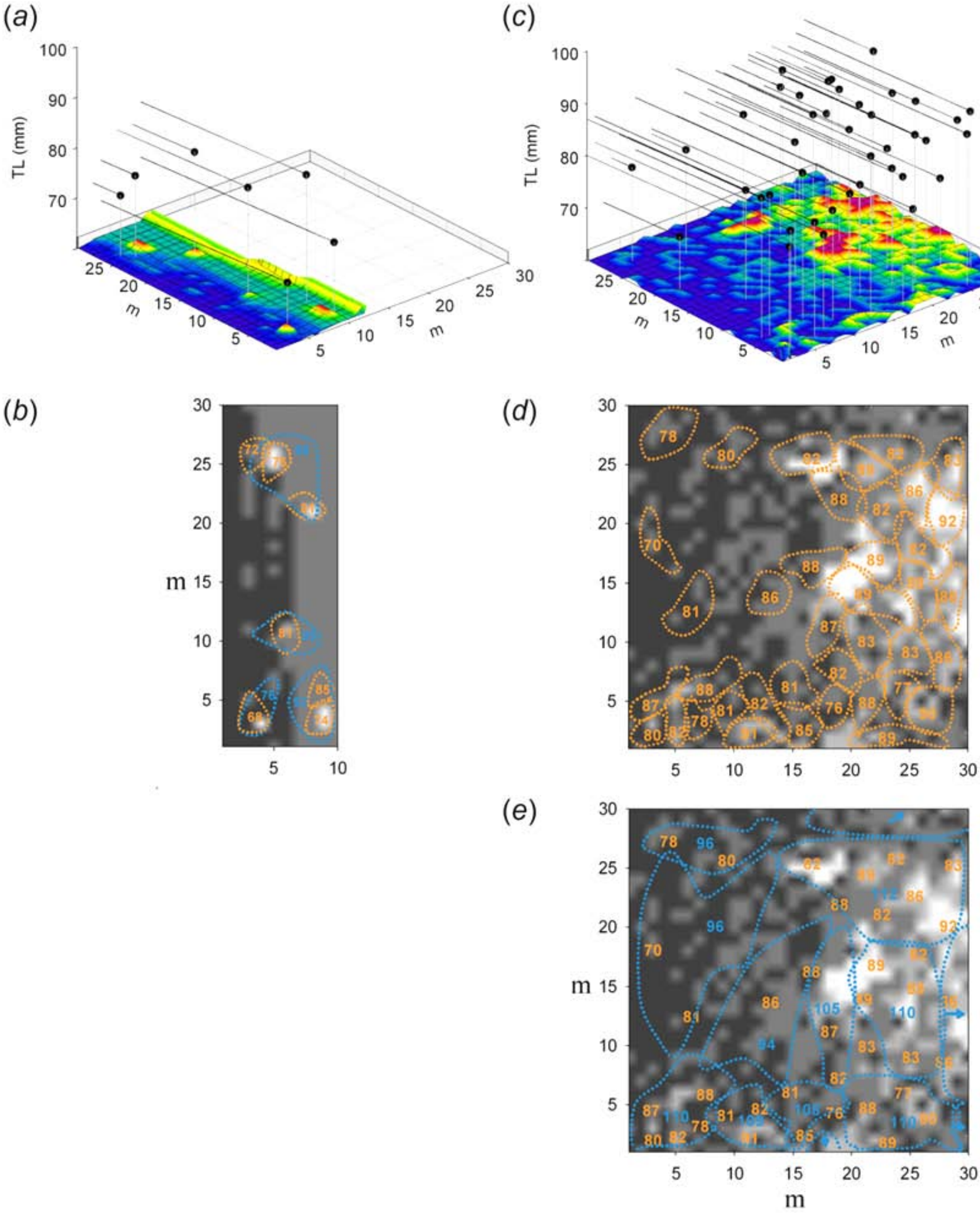
Appendix figure A1.1 Photomicrographs of gonad transverse sections illustrating the progression of sex change in *Parapercis cylindrica*



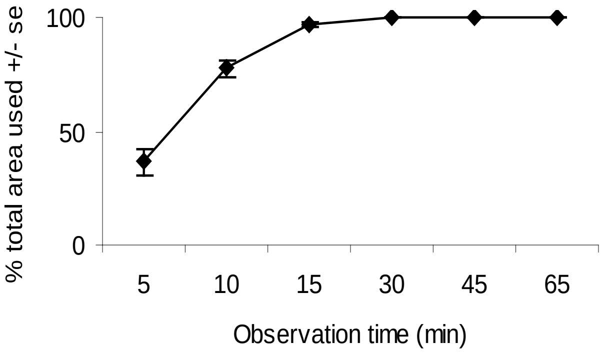
Stage 1 (A, B). In *P. cylindrica* all individuals begin life as female (monandric protogynous hermaphroditism). Pelagic pair-spawning takes place year-round, with peaks in gamete production occurring around the new and full moon. Thus the ovaries of mature females ubiquitously contain both mature (A, bar = 260μm) and developing (B, bar = 20μm) oocytes. **Stage 2 (C, D).** Sex change begins with the degeneration (atresia) of yolky mature oocytes (D, bar = 60μm), leaving a noticeable ovarian lumen (C, bar = 20μm) and fragmented lamellae. **Stage 3 (E, F).** Previtellogenic oocytes begin to degenerate after the degeneration of mature oocytes (E, bar = 200μm). At this point spermatogonia begin to proliferate adjacent to loose connective tissue and remnant oocyte cavities, and crypts of spermatocytes begin to appear (F, bar = 20μm). **Stage 4 (G, H).** At near-completion of oocyte degeneration the gonad's comprised largely of coalescing lamellae, remnant oocyte cavities and small atretic bodies (G, bar = 200μm). However spermatogenesis is more advanced. The gonad begins to fill with crypts of spermatocytes (H, bar = 20μm).

Stage 5 (I, J). Crypts of spermatocytes increase and crypts of spermatids appear, filling the gonad and reducing the size of the former ovarian lumen (I, bar = 200μm; J, bar = 20μm). At this stage a small amount of degenerating previtellogenic oocytes and remnant ovarian tissue may still be present. **Stage 6 (K, L).** The gonad is comprised entirely of testicular tissue (K, bar = 200μm). Crypts of spermatozoa are numerous (L, bar = 20μm) and the peripheral sperm sinuses are well developed and filled with mature sperm. **MO = mature oocytes, PO = previtellogenic oocytes, DMO = degenerating mature oocytes, DPO = degenerating previtellogenic oocytes, OL = ovarian lumen, FOL = former ovarian lumen, ROC = remnant oocyte cavity, Spg = spermatogonia, Spc = spermatocytes, Spt = spermatids, Spz = spermatozoa.**

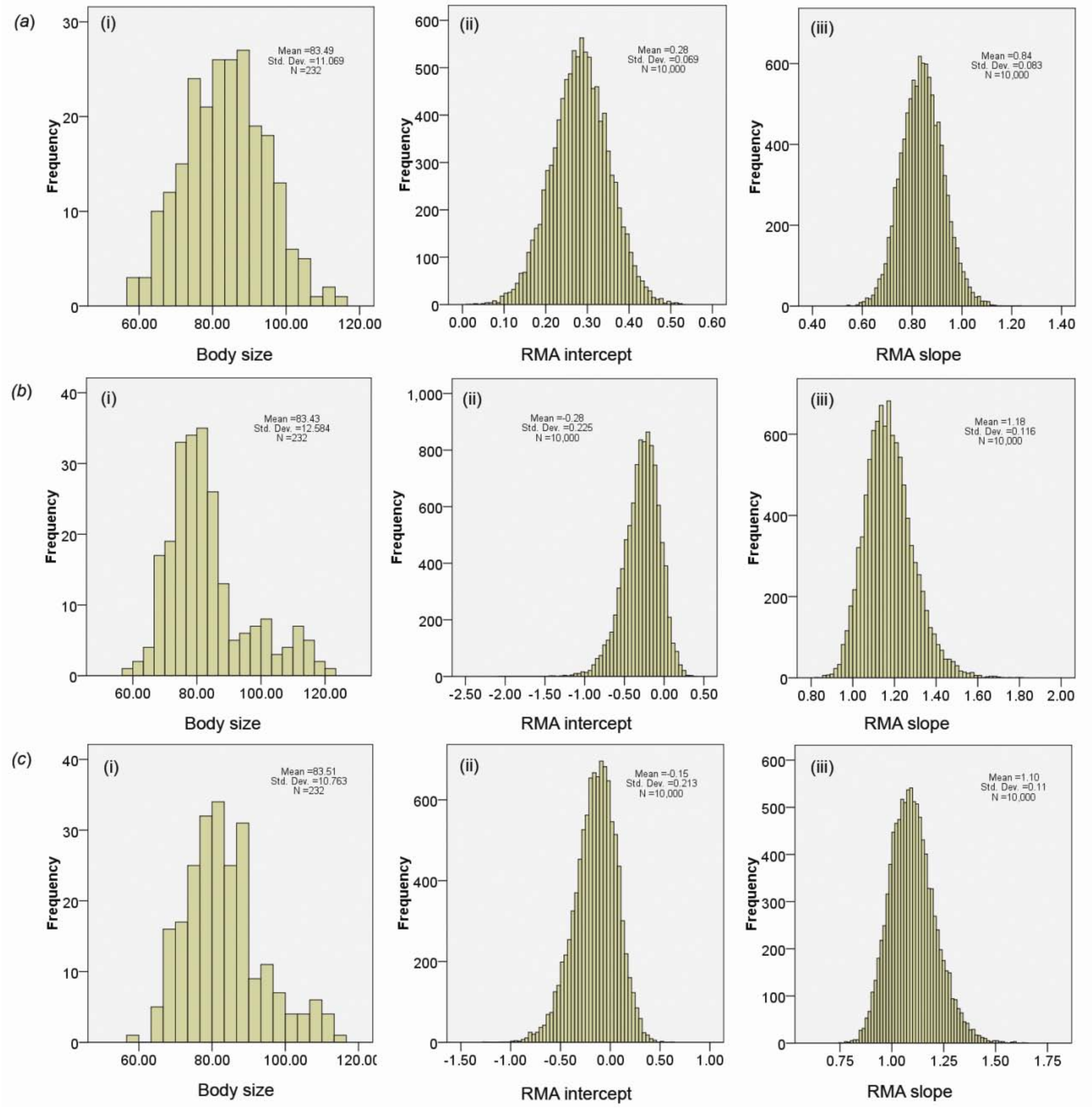
(Diagnosis based on unpublished data and Stroud 1982, Walker and McCormick 2004, Nakamura et al. 1989, and Patiño et al. 1995).



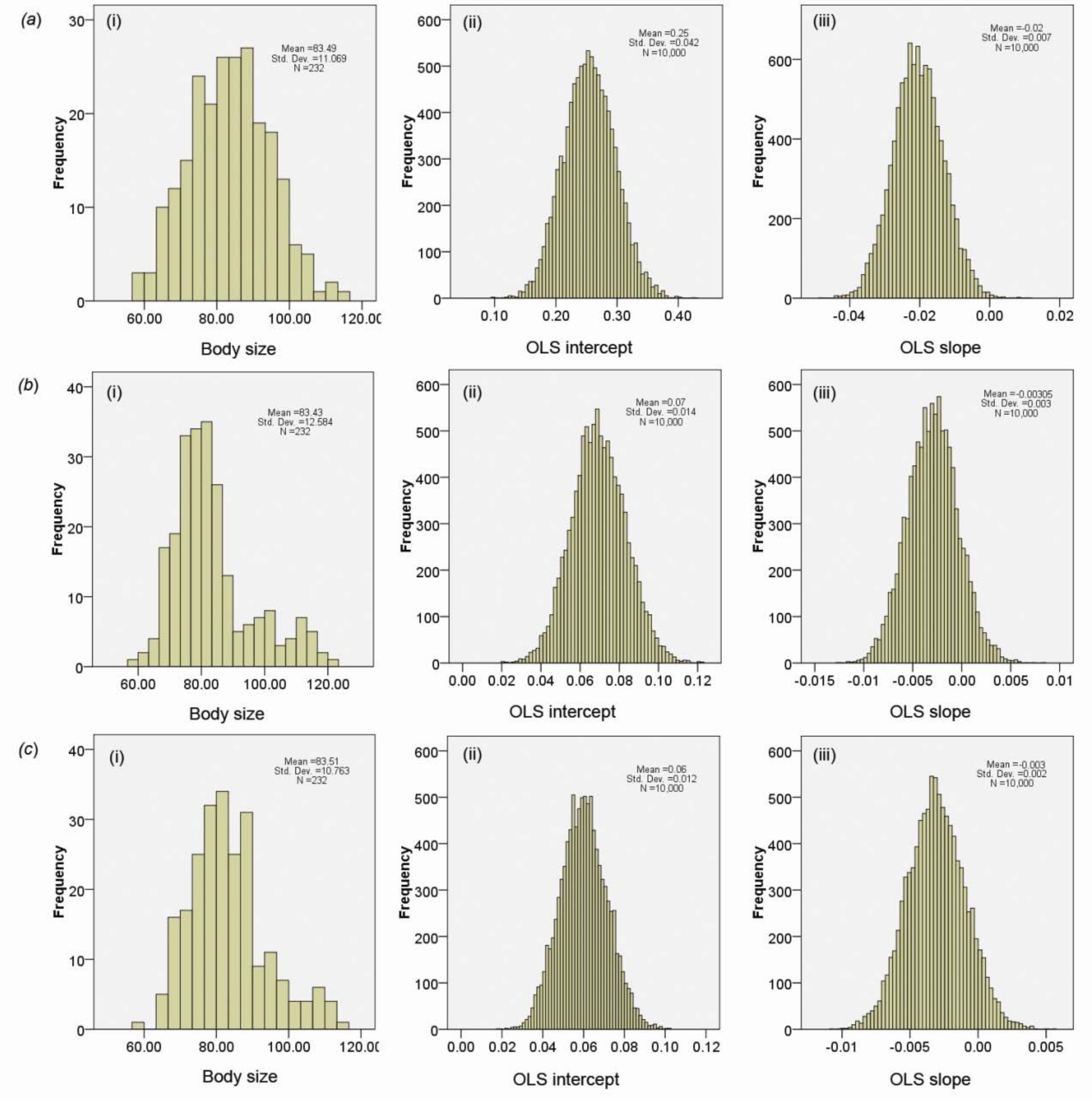
Appendix figure A1.2 A representative low density subpopulation (a-b) and a representative high density subpopulation (c-e) at Lizard Island, illustrating variation in sex-specific body sizes (mm total length), sex ratio, mating system, and habitat complexity. 3D composite maps (a,c); blue to red = substrate height from 0-40cm. 2D composite maps (b,d,e); black to white = substrate height from 0-40cm, Orange = female body size and territory border, blue = male body size and territory border (Walker unpublished data).



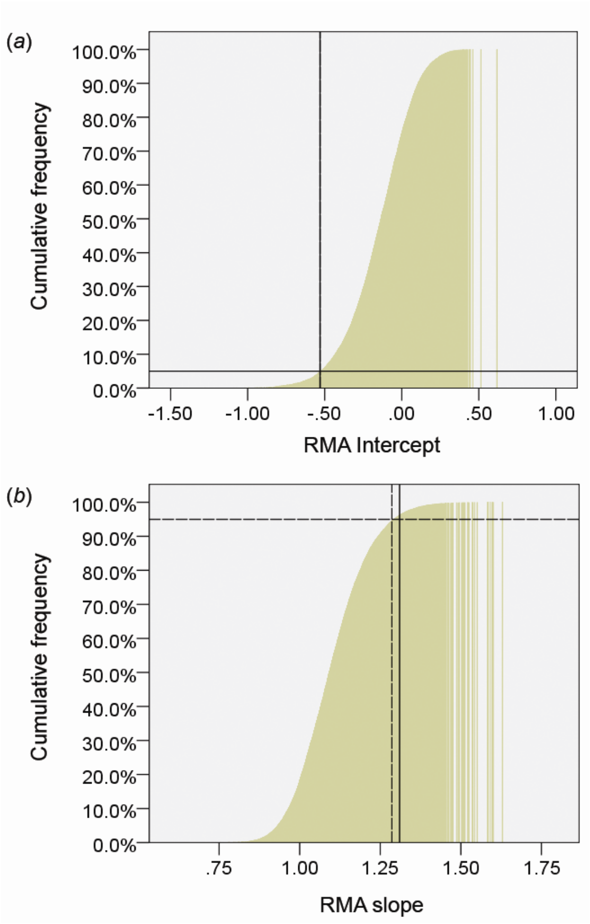
Appendix figure A3.1. The relationship between cumulative observation time and the percentage of total territory usage after 65 minutes in *P. cylindrica* (n = 10).



Appendix figure A3.2.1 Reduced major axis regression (RMA) Monte Carlo simulations. Variation in the expected intercept (ii) and slope (iii) of an RMA regression on log(dominant female size) (x) versus log(male size) (y) in response to different body size (mm TL) frequency distributions (i) under the sex change rule. The same group size distribution was used for all simulations (see methods). (*a*) A normal body size distribution, (*b*) A highly skewed body size distribution, (*c*) Actual body size distribution observed for *P. cylindrica* at Lizard Island. The sex change rule is that there is only ever one male per group, and that male is the always the largest individual within the group. Note that when the body size distribution is normal (*a*(i)), negative allometry (slope < 1) is expected (*a*(iii)). However, if the body size frequency distribution is skewed (*c*(i)), then a pattern of positive allometry (slope > 1) is expected.



Appendix figure A3.2.2 Ordinary least squares regression Monte Carlo simulations. Variation in the expected intercept (ii) and slope (iii) of an OLS regression on group size(including one male) (*x*) versus log(male size)-log(dominant female size) (i.e. sexual size dimorphism) (*y*) in response to different body size frequency distributions (i) and the sex change rule. The same group size distribution was used for all simulations (see methods). (*a*) Normal body size distribution, (*b*) Highly skewed body size distribution, (*c*) Actual body size distribution observed for *P. cylindrica* at Lizard Island. The sex change rule is that there is only ever one male per group, and that male is the always the largest individual within the group. Note that SSD is expected to decline with group size (i.e. slope < 0) when the body size distribution is normal (*a*(i)), but that the slope approaches 0, and the overall magnitude of SSD reduces, as the body size distribution becomes more skewed.



Appendix figure A3.2.3 Cumulative probability distributions for the null model RMA parameters based on the exact body- and group-size distributions observed for *P. cylindrica*. (*a*) Null model RMA intercept probability distribution; (*b*), null model RMA slope probability distribution. Solid line = observed value bin.

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