Sex-specific growth dynamics in protogynous hermaphrodites

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Abstract

Sexual size dimorphism is a characteristic of many animal species but the ways in which size differences between males and females originate in sex changing species is little understood. The present study investigated growth mechanisms underlying sexual size dimorphism in protogynous hermaphrodites, using tropical wrasses as a focal study group. The aims were to: 1) identify a relationship between the strength of the social organization within a group and the timing of growth divergence between sex changing and non sex-changing individuals; and 2) to identify changes in behaviour and space use associated with sex change within a group. It was predicted that where individuals were under strict social control, sexual size dimorphism would come about by a growth spurt associated with sex change. It was also predicted that in more loosely controlled social groups, prior growth history would play a greater role in the development of sexual size dimorphism.

The strength of social systems for four species of wrasse (family: Labridae) was quantified by behavioural observations and compared to growth trajectories of sex-changing and non-sex-changing females. The wrasse species used in this study were: *Coris batuensis, Stethojulis strigiventer, Halichoeres miniatus,* and *Cirrhilabrus exquisitus*. Behavioural observations were made throughout a male removal experiment to observe changes in behaviour within a group during the process of sex change. Growth patterns were estimated through analysis of otolith microstructures using daily increment width as a proxy for daily somatic growth. Comparisons were made of social systems and growth patterns both across species and within a species to determine if social influences on growth were species specific or specific to the social system.

Check marks in the otoliths at the time of sex reversal were identified in three of the four species, with the mark occurring only in male fish. A check mark was experimentally validated in *H. miniatus* as occurring within the time period of sex change. When social groups were experimentally manipulated to induce sex change by removing the male, the dominant female changed sex, completing gonadal changes in less than 44 days. An increase in otolith accretion rate, or a growth spurt, was also found in sex-changing fish and this corresponded to an increase in somatic growth rate.

The strength of the social systems of the study populations was found to influence the growth patterns of component individuals. Both across species and within species, size differences between males and females were found to be due to growth spurts around the time of sex change in social groups with strict social control. In contrast, prior growth history was found to be more important in the expression of sexual size dimorphism in groups with looser social organisation. In three of the species an initially larger increment size was found in the females that changed to male suggesting a larger otolith size at hatching in females that eventually change sex. Growth spurts were found to be important for the size dimorphism in three out of four species. Results suggest that the social system may play a major role in influencing the growth associated mechanism underlying the sexual size dimorphism in sex changing fishes, and that at times, pre-larval factors may be influencing which individuals end up becoming the dominant males in the system.

Chapter 1: General introduction

Polygynous mating systems are common in many animal groups. These systems, where males mate with multiple females, are a result of the higher breeding potential in males compared to females which provides the opportunity for mating with multiple partners. Large males are able to exclude smaller males from the breeding population through direct defence of females or harems (female defence polygyny) or by defending access to resources to which females are attracted (resource defence polygyny) (Emlen and Oring 1977). Variations in social structures place different energy requirements on males and females within the group resulting in variations in individual resource allocation (Reavis and Grober 1999). Different life history strategies have developed to maximize individual fitness and these systems result in variation in the allocation of resources between individuals within the group (Keller and Reeve 1994), however the effects of this variation on individual development are little understood.

The majority of reef fish display polygynous mating systems and one common strategy that has evolved is protogynous hermaphroditism, changing sex from female to male. Because large males have greater reproductive success than small males due to competition for spawning partners (Wade and Shuster 2004), it is to the benefit of an individual to first mature as a female in order have some reproductive success while at small sizes (Ghiselin 1969, Warner 1984). Once the opportunity arises, such as a position as dominant male or territory holder becomes open, the female can change to male provided a large enough size is reached at which the female could successfully compete and increase reproductive potential as a male (Warner 1988). This results in strong sexual size dimorphism with males

being the largest individuals in a population. Larger body size not only provides an advantage in intrasexual competition but also prevents subordinates from reaching a size where they could challenge the dominant position and potentially change sex (Werner and Gilliam 1984).

The opportunity to change sex in protogynous fish is primarily mediated by the social system of the group (Robertson 1972, Warner et al. 1975, Shapiro 1987, Ross 1990, Munday et al. 2006a). In most cases the largest female in the group will change sex and assume the dominant position with the loss of the dominant male. Occasionally, however, a smaller female will change sex if the conditions allow for a potential increase in reproductive success (Ross 1990, Lutnesky 1994). Female defence polygyny is usually associated with harems, which are strictly organized groups with distinct dominance hierarchies. Growth in subordinate individuals is often suppressed through interactions with dominant individuals, either male or female (Robertson 1972). This also results in suppression of sex change in females until the dominant male is removed and the growth suppression is relaxed. In systems displaying resource defence polygyny there is less opportunity for growth suppression because of female mate selection. Females are able to move between male territories in order to select a spawning partner. Because of the lack of site attachment and the mobility of individuals, females are less subject to growth suppression by dominant males and are able to avoid larger females.

Previous studies have found that males in a given population tend to be larger than similarly aged females despite the fact that they may have both first matured as female (Cowen 1990, Ferreira 1993, Mackie 2003). The sexual size dimorphism resulting from the polygynous systems and protogynous sex change must manifest at some stage of development. Males end up as the larger sex for a

given age group but this could be a result of varying growth rates at several points in life. One hypothesis is variable growth during the early stages of development. Faster growth during the larval and juvenile stage in fish that will eventually change to male would give those individuals a larger initial size that would carry over to later stages (Walker et al 2007). A second hypothesis is increased growth after first sexual maturity (Adams and Williams 2001, Munday et al. 2004). Eventual sex changers could have a faster growth rate than similarly aged conspecifics during the female stage and therefore be in a position to change sex earlier. A third hypothesis is an accelerated growth rate later in life, namely at, or shortly after, the time of sex reversal (Ross 1987, Garratt et al.1993, Choat et al.1996, Walker and McCormick 2004). This would allow the new males to quickly gain and maintain a dominant male position in the event of the loss of the original dominant male. These three ideas are not mutually exclusive, however. The cumulative effect of variable growth at different life history stages may ultimately result in males being the largest individuals.

Accelerated growth at the time of sex change has been documented in several species (Ross 1987, Buston 2003, Walker and McCormick 2004). This gives the changing female a size advantage over the other females in the group and allows the changing fish the opportunity to defend a harem from transient males. This growth pattern has been identified in haremic species where there exists some degree of growth suppression in subordinate fish by dominant individuals (Robertson 1972, Ross 1990, Munday et al. 2006a). There has been little information collected on growth influences surrounding the other two hypotheses, however there is evidence suggesting that in less strict systems earlier growth plays a large role in which females eventually change to male (Adams and Williams 2001,

Mackie 2003, Munday et al 2004). The variable growth or the point of growth divergence in females and eventual males may be a result of the social system in which the individual develops. In the strict systems there may be a sex specific growth divergence later in life, near the time of sex change while in the more relaxed systems female and eventual male growth might show differences from earlier in life.

The proximate causes behind what drives differences in growth between males and females have been little studied to date. The social system of a group has been shown to influence the patterns of sex change within the group (Shapiro 1987, Ross 1990, Munday et al 2006a). The influences of the social system may have a more direct effect on the individuals within the group by driving the growth patterns as well. By determining the influences on social systems and male and female interactions for a species or group, the reproductive activity and individual development within the group may be predicted. Similarly, determining how the social system influences growth and the timing of sex change I may be able to predict how an individual will grow and develop given the system in which they exist and based on the social and environmental conditions to which the group is subjected.

The present study attempted to gain a broader understanding of some of the mechanisms behind sexual size dimorphism and sex change. The project was divided into three parts attempting to investigate different aspects of the relationship between social systems, environment, and individual growth and sex change. Wrasses (Family Labridae) were chosen as the subjects of the study because of the variety of social structures found within the family and the plasticity of individual behaviours and life history strategies. Wrasses have also been used previously in

studies related to social behaviour and sex change (e.g. Robertson 1972, Warner et al. 1975, Ross et al. 1983). Their diversity and abundance throughout reef ecosystems and the large array of niches filled also made the family a good candidate as a potentially widely applicable group in the area of reproductive behaviour in reef fish.

The first chapter in this study tested the relationship between ontogenetic growth patterns and social systems in sex changing wrasses from the Great Barrier Reef (GBR). Four species were studied from a variety of social structures attempting to cover a range from loose to strictly organized systems. Lifetime growth curves and daily growth rates were compared between individuals that changed to male and those that remained as females to see when sexual size dimorphism took place. The growth trends were compared to the general social structure of the species in an attempt to find a between species pattern for the relationship between social structure and sex-specific growth as it pertains to sex change.

In the second chapter I performed an experimental investigation of growth and behavioural changes associated with sex change in the wrasse *H. miniatus*. Using a population found at Lizard Island on the GBR, I looked at how the removal of the dominant male and the resulting sex change affects growth and behavioural changes within a strictly haremic social system. Growth affects were quantified in an attempt to determine if and when there was a divergence in growth associated with sex change. Also in this section, the occurrence of a visible discontinuity, or check, in the otoliths of sex changing fish was identified marking the time of sex reversal.

The third chapter entailed a comparative analysis of sex-related growth and social systems of the wrasse *Halichoeres miniatus* on the GBR. First, the social structures for the species were observed at two different sites on the GBR to look for differences in physical environment, behaviour and habitat use. Cumulative and daily growth curves for males and females were constructed and compared and then evaluated as related to the social structure for each location. In order to demonstrate the differences in how social systems are related to sex-specific growth both across species and within species, the first chapter provided an interspecific comparison of the social system and sex-specific growth relationship, while this section gave an intraspecific comparison of the same relationship.

Many species of reef fish are sequential hermaphrodites and display many different patterns of growth and development with respect to sex-specific growth and sex change. Within the wrasse family there is a wide range of social systems, from loosely organized groups to tightly haremic dominance hierarchies, most or all of which are also found throughout other fish families. Using this group of fish I attempt to explain the timing and reasons for sex change and identify some of the driving factors behind hermaphroditism. Wrasses are a key element to reef ecosystems due to their abundance and pervasiveness throughout reef habitats. More than 270 Species of wrasse are found throughout the Great Barrier Reef (Randall et al 1997). Their presence on reefs affects the ecology of many fish species and other organisms. Not only do wrasses serve as predators and prey, they also are often necessary to maintain healthy fish populations by functioning as cleaners and filling other symbiotic roles.

Changes in density may affect the social structure of a population which in turn may affect the number of large individuals in a population. This could have

substantial implications for how certain fisheries are managed. Variation in social structure over geographic areas could indicate variations in territory size and resource use for different populations which would require different parameters for establishing marine protected areas. Using wrasses as a model for understanding the basic theories of social dynamics could also serve as one source for identifying patterns in other species. Because of the large number of fish families containing sequential hermaphrodites, including many of commercial importance, it is essential to understand mechanisms behind growth and sex change and the social impacts on population dynamics in order to effectively manage fisheries and marine protected areas.

Chapter 2: Proximate mechanisms responsible for expression of sexual size-dimorphism in four sex-changing fishes

2.1 Abstract

In protogynous (female to male) sex-changing species, males tend to be larger than females of the same age. This indicates that females that change sex to male grow faster than other females at some point in their life. I used the record of individual growth and life-history transitions present in the microstructures of fish otoliths (ear bones) to examine the life-history stage at which growth divergences between males and females first became apparent in four species of sex-changing fish. Two species with a haremic social organisation, *Coris batuensis* and *Stethojulis strigiventer*, exhibited a divergence in the size of males and females around the time of sex change. Social regulation of growth rates among females prior to sex change and a growth-spurt at the time of sex change appear to control the development of sexual size-dimorphism in these species. In contrast, two species with less rigid social organisations, *Halichoeres miniatus* and *Cirrhilabrus exquisitus*, exhibited growth divergences between males and females long-before the average age of sex change. In these two species, females that changed sex to male grew faster than other females shortly after hatching and appeared to maintain this size advantage throughout their early life history. These results demonstrate that the social environment experienced by individuals can have a significant effect on the expression of sexual-size dimorphism and that in some sex-changing species the likelihood that an individual will change sex, or not, can be determined by its relative size during early life.

2.2 Introduction

Sexual size-dimorphism (SSD) is expected to occur in species where variation in size-dependent reproductive success differs between the sexes (Darwin 1859, Stamps 1993, Kueller and Reeve 1994). Males are usually the larger sex, which is frequently explained by the advantage of large body size during competition for mates in polygynous mating systems (Emlen and Oring 1977, Wade & Shuster 2004). There are however, a variety of societal types in which polygyny is manifest. Males may defend several females either permanently or temporarily during the breeding season (harems), or females may choose males based on some measure of their individual quality or the quality of resource they provide (leks and resource defence polygyny). Such differences in social structure, and the associated differences in interaction rates within and between sexes, can alter the growth- and mortality- schedules of individuals (Hofmann and Fernald 2000, Buston 2003, Forrester et al 2006). It is likely, therefore, that the mechanisms responsible for the expression of SSD will differ between societal types.

Many tropical reef fishes display highly labile sexual ontogenies which frequently involve a change in sexual function (Munday et al. 2006a). Protogynous (female to male) sex change is usually associated with polygnous mating systems, where a few large males monopolise most of the breeding opportunities with females. Sex change is favoured in polygynous mating systems because small males tend to have lower reproductive success than females, as a result of being excluded from breeding by larger males, and large males tend to have higher reproductive

success than females, as a result of breeding with multiple females. Consequently, individuals can maximise their lifetime reproductive success by first breeding as a female and then changing sex to male when they reach a larger size (size-advantage model of sex change, Warner et al 1975).

Sex allocation theory predicts that a female should change sex to male when her reproductive output is expected to increase by doing so (Charnov 1982). This may occur on the death of the dominant male, or when the female reaches a size large enough to compete with existing males for a number of females of greater reproductive value than herself (Warner 1984, Lutnesky 1994, Munday et al. 2006a). Not all females change sex, and since males are generally larger than females of the same age (e.g. Cowen 1990, Choat et al 1996, Mackie 2003), there must be variation in growth between sex changing and non-sex changing females at some point in life. At different stages in life, eventual males may have a faster growth rate than those that will remain as female (Fig. 1). A larger size at hatching or a faster growth rate before sexual maturation would give certain individuals an initial size advantage that could carry over to later stages, providing the initially larger females with the opportunity to change to male sooner (Fig. 1a). Similarly, a faster growth rate in particular females after sexual maturation would put those fish in a position to change sex sooner than slower growing individuals of the same age group (Fig. 1b). In other cases, eventual sex changers may have a similar growth rate as other females until the time of sex change and then undergo an acceleration in growth in conjunction with sex change or after they become male (Fig. 1c).

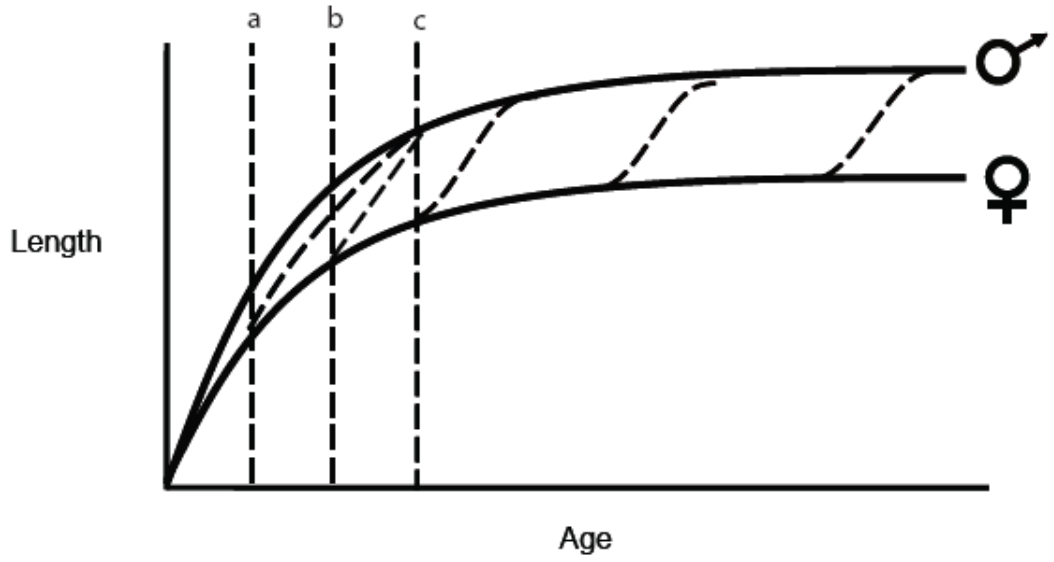

Figure 1: Hypothetical male and female growth curve plot showing potential timing of sex-specific growth divergence at (a) settlement, (b) after female maturation, and (c) at or after the time of sex change (from Walker and McCormick 2004).

The strength of the social unit experienced by individuals is likely to be a key element in determining how and when size differences between males and females are established in sex-changing species. In polygynous societies where females form permanent all purpose territories and males defend a group of females (e.g. harems), size-based dominance hierarchies among group members can result in subordinate growth being constrained by dominants (Forrester 1991, Booth 1995, Walker & McCormick 2007, Wong et al. 2007). Social regulation of subordinate growth can occur as a direct result of competition for food, or through increased activity budgets and physiological stress incurred as a result of interactions with dominants (Koebele 1985). With the loss of the dominant male, growth suppression is relaxed and the dominant female is able to maximise somatic growth and change sex, and remaining subordinate females move up the dominance hierarchy (Robertson 1972, Nakazono et al.1985, Hattori 1991).

In polygynous societies where females are highly mobile (e.g. resource defence polygyny), dominance hierarchies may form briefly during periods of breeding, but most of the time dominants have little or no social control over subordinates (Robertson and Hoffman 1977, Shapiro 1983, Cowen 1990). With less opportunity for dominants to suppress growth in subordinates, the early growth history of females may play a greater role in determining which individuals become the largest members of the population and thus have the highest probability of changing sex to male when appropriate circumstances are presented (Adams and Williams 2001, Munday et al. 2004).

Since individuals begin life as female and change sex to male at variable sizes and ages, it is difficult to ascertain when and how SSD develops in most protogynous sex-changing fishes. Microstructural increments (growth rings) within fish otoliths can be used to reconstruct age-based growth histories (Campana 2001) and could provide a valuable tool for assessing the development of SSD in sex-changing fishes. Many fish deposit otolith increments on a daily cycle (Campana and Thorrold 2001) and increment width is usually associated with somatic growth in short-lived species (Hare and Cowen 1995, Shafer 2000), therefore, otoliths retain a record of past growth performance. In addition, recent studies have illustrated that, in some species, optical discontinuities, or 'check marks' are deposited at the time of sex change (Walker & McCormick 2004, unpublished data, Walker et al. 2007). Combining estimates of growth trajectories with the occurrence of sex-change check marks enables the temporal relationship between sex-specific growth divergence and sex change to be explored. These data also enable comparisons to be made between sex-specific growth histories and the social system exhibited by different species.

I used otolith microstructure to estimate male and female growth histories for four short-lived protogynous wrasses (family Labridae); *Halichoeres miniatus, Stethojulis strigiventer, Coris batuensis,* and *Cirrhilabrus exquisitus*. In addition, the social system exhibited by each species was described, with emphasis on determining the hierarchical strength of each society. Sex-specific growth trajectories were then related to social system characteristics. Establishment of these relationships was aided by the use of check marks in the otoliths to identify the timing of sex-change. The primary objective was to test the hypothesis that the hierarchical strength within the social unit experienced by individuals affects the temporal relationship between SSD and sex change from female to male. I predicted that strict hierarchical societies will impose considerable constraints on subordinate female growth and, consequently, SSD develops at the time of sex change through a relaxation in growth inhibition following the loss of the dominant male. In contrast, I predicted that loosely organised social groups with low hierarchical strength will accommodate greater variation in subordinate growth and that variation in growth rates prior to sex change will have a much stronger influence on the development of SSD in these species.

2.3 Methods

2.3.1 Study species and collection

I compared social organisation and sex-specific growth histories using the otoliths of four common Indo-Pacific wrasses; *Coris batuensis, Stethojulis strigiventer, Halichoeres miniatus,* and *Cirrhilabrus exquisitus*. These species were

selected based on their high abundance, short life-span which aids in the construction of reliable growth trajectories using otolith increment analysis, a monandric life-history where all males are derived by sex change from adult females, and because they exhibit a range of social structures from haremic to non-haremic groups. All species are sexually dimorphic in body colouration and further descriptions of each species are available in Randall et al. (1997).

Specimens were collected from Orpheus Island (18°37'S 146°29'E) during May and June 2005, except for *C. exquisitus* which was collected from Kimbe Bay, Papua New Guinea **(**5°5'S 150°1'E**).** I attempted to collect a representative size range for each species using a combination of barrier nets, hand nets, a dilute solution of clove-oil anaesthetic and hand spears. Fish were euthanized in an ice bath, their standard length (SL) and weight recorded, and their otoliths and gonads removed for further analysis. Otoliths were cleaned of endolymph tissue and stored dry. Gonads were removed and stored in formaldehyde-acetic acid-calcium chloride (FAAC). The sex of each fish was determined first by its colour phase and then macroscopic examination of the gonads. Males of all four species are more brightly coloured than females. The drab female colouration is called the initial phase and the bright male colouration is called the terminal phase. Ovaries were identified by a rough surface texture indicating the presence of developed eggs, and testes were identified by a smooth surface and cream colouration when viewed through a dissecting microscope. To confirm macroscopic categorisation of sex, gonads were then embedded in paraffin wax blocks, transversely sectioned at 5 µm, stained with Mayer's Haematoxylin and Young's Eosin-Erythrosin and viewed through a high-power light microscope (Winsor 1994).

2.3.2 Social behaviour

Qualitative observations of social interactions were made to establish the social systems commonly displayed in three of the species, *H. miniatus, S. strigiventer,* and *C. batuensis*. Quantitative behavioural data were then collected on one social group for each species. Social structure was described based on the site attachment and percent of territory overlap of individuals (the species with higher territory overlap within the same sex were considered less haremic than those with a low percent of overlap), and the aggressiveness and amount of interactions (physical contact, chases, or displays). A focal observation was made on the male and the five largest females in the group and then on four neighbouring males for each species except for *C. batuensis* because only two males could be found in the area, each observation period lasting 15 minutes. MANOVA test on the behavioural interactions was used to determine differences in interaction rates between the species. Territory maps were constructed by marking the subjects' position over a 30 m x 30 m reference grid (made with nylon line) every minute. In order to determine the intensity of aggressiveness in inter- and intra-sexual behaviours and the reaction to the addition of a new member to the social group, one male and one female of each of the three species was captured and placed in a clear plastic bag and observed for five minutes after securing to the substrate within an established social group. Because social systems potentially change with variations in environmental conditions, these observations were solely used to gain an idea of the social system of each species in the area. Due to the more remote location of *C. exquisitus*, information on the social system had to be gathered by simple field observations and from existing reports of similar species in the literature.

2.3.3 Validation of otolith increment periodicity

Otolith increments of small reef-fishes are usually deposited on a daily cycle (Hernaman et al 2000, Campana and Thorrold 2001). Daily increment periodicity was validated for three of the four study species (*S. strigiventer, C. batuensis,* and *H. miniatus*) by inducing a check-mark in the otolith and then holding fish in aquaria for a known period. Seventeen to 22 individuals of each species were collected from the wild and transferred to aerated, darkened, aquaria without food for 17-24 hours. This procedure produced a distinct check in otolith increment deposition. Fish were then transferred to aquaria, six to ten individuals per aquaria, with natural lighting, supplied with a constant flow of fresh seawater, and fed a diet of prawns twice a day and fish flakes once a day. Fish were sacrificed after a further 35-37 days and their otoliths removed, cleaned, stored in the dark, and sectioned and mounted following the procedures of Wilson and McCormick (1997). The number of growth increments present in the otoliths after the check-mark was compared to the number of days that fish were kept in captivity.

2.3.4 Age and growth estimates

The otolith microstructure was analysed in order to estimate age and growth history for each individual. Otoliths were sectioned by first fixing the otolith to the edge of a slide with Crystalbond^{TM} resin, the distal end of the nucleus off the edge of the slide. The overhanging end of the otolith was ground flat using 1200-grit wet/dry sand paper. The rostral end was then repositioned so the ground side was flush with the slide and again set in Crystalbond^{TM}. This end was also ground using 1200-grit sand paper and then polished using 9 μm to 3 μm-grade lapping film until a transverse section of the otolith through the nucleus remained showing growth

rings from the nucleus to the margin. Digital images of otolith sections were taken at 400x magnification and otolith increments were measured along the maximum otolith radius using the spatial analysis program Optimus 6.5. Age was estimated by the taking the average of two counts of the growth increments along the maximum otolith radius.

A close correlation between somatic growth and the width of daily otolith increments has been observed for a wide range of fishes (Hare and Cowen 1995, Campana and Thorrold 2001) including *H. miniatus*, one of the species examined here (Chapter 3). Therefore, otolith increment widths were used as a proxy for daily growth. First, I compared the size at age for males and females using ANCOVA to determine if males were larger than similarly aged females. Cumulative otolith-increment plots were then constructed for each species to estimate the timing of size divergence between males and females at a population level. The width of daily otolith increments were also compared throughout life for females that changed sex to male and those that had not changed sex to investigate the ontogenetic stage at which growth of would-be males increased compared to females that did not change sex. Statistical comparisons of otolith increment widths were made between would-be males and females at representative developmental stages in early growth history using t-tests. The first comparison was at hatching using the second daily increment and then for early life history using the half way point between hatching and settlement using the settlement mark as reference (Wilson and McCormick 1997). This allowed for an estimate of daily growth within the larval stage before changes in growth patterns associated with settlement occurred. The third comparison was made at the half way point between the settlement mark and the average age of sex change. The average age at sex change was estimated from sex-change check marks

in the otoliths. The age-frequency distribution of males in the population was used to estimate the average age of sex change for *S. strigiventer*, because sex-change checks could not be confirmed in this species. Growth later in life, after sex-change, was compared on the 25th day after the average of check mark occurrence (estimated age of sex change used for *S. strigiventer*).

Sex-change check marks were identified by examining the otolith section near the estimated time of sex change for each species based on the age-frequency distributions of males and females. The occurrence of a check with sex reversal has been validated for one of the species, *H. miniatus* (Chapter 3), and has also been observed in two species of tropical reef fishes (Family Pinguipedidae, Walker and McCormick 2004, Walker et al 2007). Check marks were considered to be associated with sex change if: (1) their appearance was similar to validated sex-change check marks, (2) they occurred only in males, and (3) they occurred at an age consistent with the presence of males in the population.

To determine if females exhibited a growth spurt at the time of sex change, I compared increment widths for males 20 days before and 20 days after the putative sex-change check mark. Two separate analyses were conducted. First I used ANOVA to compare otolith increment widths of males 20 days after the check mark with otolith increment widths of non-sex changed females of the same age. The average age of check occurrence in males was used as the comparison age for females. I then used repeated-measures ANOVA to compare the otolith increment widths for males and females for the period 20 days before the check mark and 20 days after the check mark. Homogeneity of variance was tested using residual analysis for all statistics.

2.4 Results

2.4.1 Social structures

The territory size and frequency of agonistic interactions within the group varied between species. MANOVA test on the interaction rates showed differences between the species ($F_{(12, 46)} = 4.8712$, $P < 0.001$). *C. batuensis* males had the largest territory size of the three species (Fig. 2). Males were observed to travel between three to nine female territories within their territory and there was no overlap between male territories. Females were site attached with territories entirely contained within a single male territory (Fig 3a). Female territories overlapped by 2.5% in the focal group examined. Males were aggressive towards unfamiliar males. The introduction of another male to the area resulted in continuous attacks by the resident male while the introduction of a female was tolerated with only displays from resident females. Because of the site attachment of females, the low percentage of overlap between female territories, and the aggressiveness of males toward other males with no overlap in territories, the social structure for this species at Orpheus Island was determined to be strictly haremic.

S. strigiventer had the smallest territory sizes of the three species (Fig. 2) indicating that individuals were site attached. Males defended territories which overlapped 12.5% with other male territories and encompassed two to six females which appeared to be shared by more than one male (Fig. 3b). Female territories overlapped 6.4% with other females and females also moved between more than one male territory. *S. strigiventer* also had the highest intra- and inter-sexual aggression of the three species. The introduction of new fish to the group showed both males and females to be highly aggressive towards new adults. The territory

male attacked the bag containing another male continuously for the entire observation period and a large resident female did the same towards the bag containing another female. Both males and females showed the highest number of chases per minute and also the highest overall interactions compared to the other species (Fig. 4a, b). The highly aggressive nature of individuals within the focal group as well as the relatively low percentage of male territory overlap (compared to *H. miniatus*) indicate that *S. strigiventer* has a strictly organised social structure however, most likely not to the degree of *C. batuensis*.

Territory sizes for males and females in *H. miniatus* were larger than for *S. strigiventer* (Fig. 2), however males of *H. miniatus* had the largest amount of overlap in territories with other males (27.7%, Fig. 3c). Individuals were site attached and females moved through several male territories while overlapping other female territories by 5.3% (Fig. 3c). Males attacked an introduced male continuously throughout the observation period and females occasionally displayed to the new male. An introduced female was displayed to and occasionally attacked by other females. The interaction rates of female *H. miniatus* were generally higher than for *C. batuensis* females but lower than *S. strigiventer* females (Fig. 4a, b). *H. miniatus* was determined to display a loosely haremic social structure because of the less aggressive interactions within the group, the higher percent of male territory overlap compared to the other two species and the movement of females within the group. Although no information was found for *H. miniatus* in the literature, a similar species which is found in the same area, *H. melanurus*, has been demonstrated to show similar behavioural patterns and has been determined to be loosely haremic (Colin and Bell 1991, Karino et al. 2000).

C. exquisitus is a planktivorous species that forms mixed male and female schools with no obvious delineation of territories (Munday and McCormick pers obs). Other species with similar life histories show loosely organized social structures where females move between male spatial territories (Robertson and Hoffman 1977, Shapiro 1986).

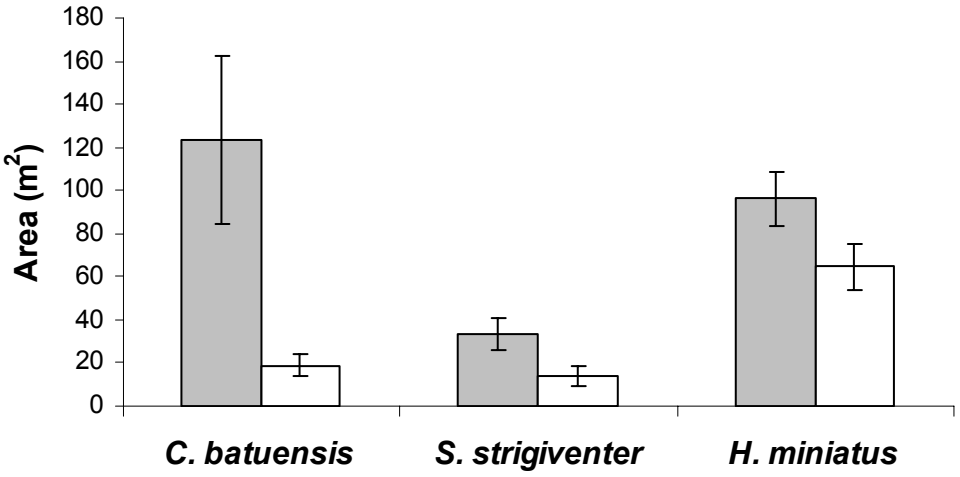

Figure 2: Average male (gray) and female (white) territory size (+/- SE) for three species of wrasse from Orpheus Island, GBR (*C. batuensis*: male n=2, female n=5; *S. strigiventer*: male n=5, female n=5; *H. miniatus*: male n=5, female n=5).

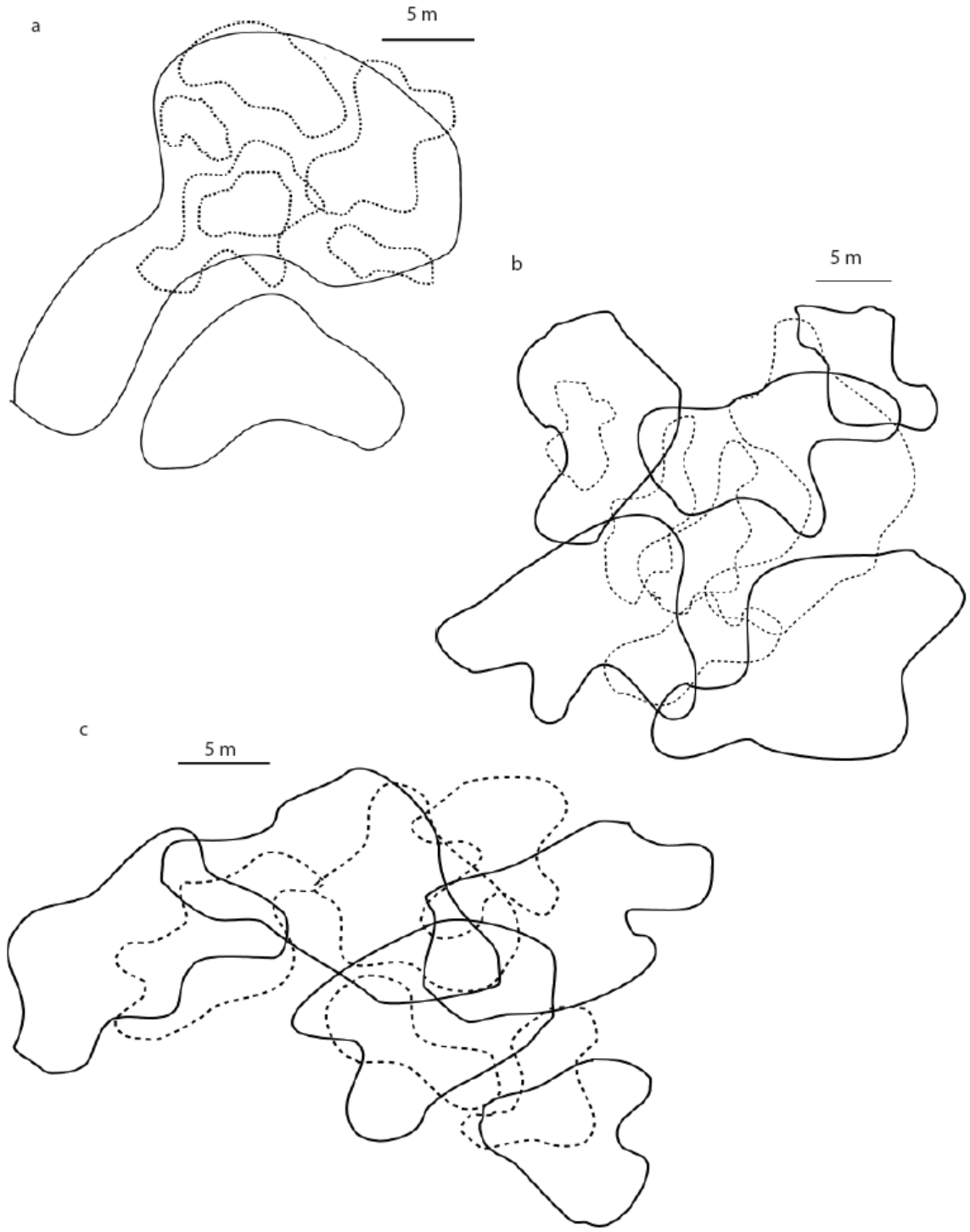

Figure 3: Relative size and area estimates of territory maps for representative social groups of (a) *C. batuensis*, (b) *S. strigiventer*, and (c) *H. miniatus* from Orpheus Island, GBR. Solid lines show males and dashed show females.

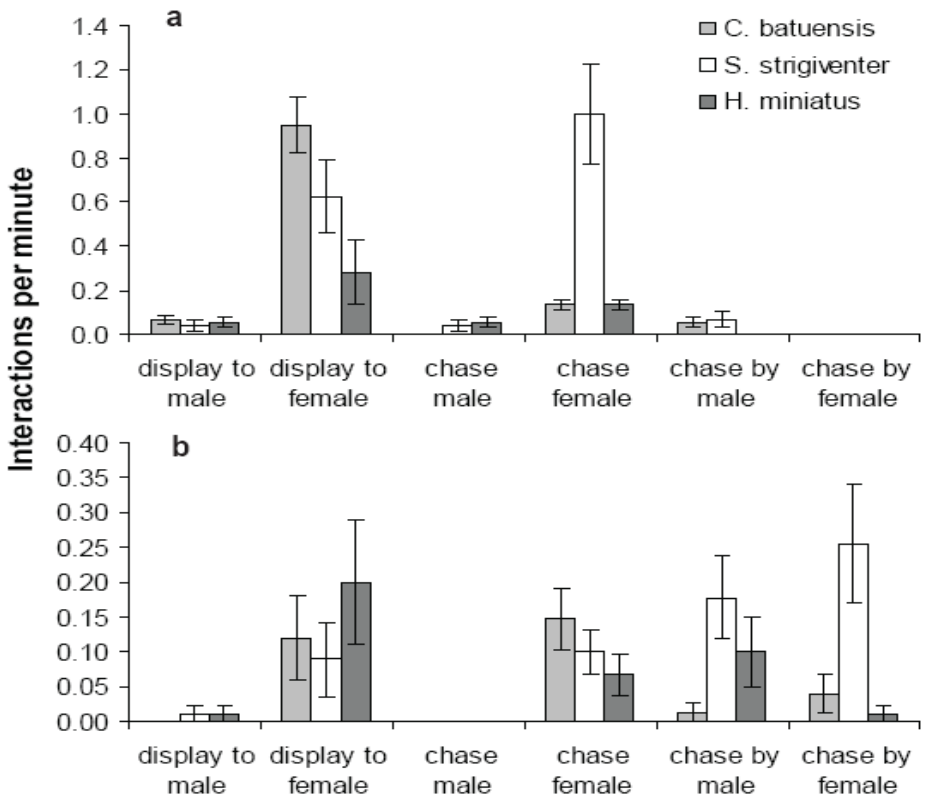

Figure 4: Mean interaction rates (+/- SE) for (a) males (n=5) and (b) females within a male territory (n=5) of three species of wrasse from Orpheus Island, GBR.

2.4.2 Size and age

The size- and age-frequency distributions of each species were characteristic of protogynous sex changing fishes, with smaller size- and age-classes dominated by females and larger size- and age-classes dominated by males (Fig. 5). On average, males were larger and older than females in all species (Table 1). There was more overlap in the age distributions of males and females than overlap in the size distributions of males and females, indicating that size is more closely associated with the timing of sex change than is age (Table 1, Fig 5). No initial-phase (drab colouration) males were found in the collections and no males were present in the smaller size classes, indicating that the populations are likely to be monandric, where all males are derived through sex change by adult females. Males

of all species were larger (16.8% to 34.3%) for their age than similarly aged females (ANCOVA: *C. batuensis* $F_{(1, 68)}$=33.835, P<0.01; *H. miniatus* $F_{(1, 68)}$=38.222, P<0.01; *S. strigiventer* $F_{(1, 73)}$=19.969, P<0.01; *C. exquisitus* $F_{(1, 52)}$=86.885, P<0.01, Fig. 6) which suggests that females that change sex to male have grown faster than other females at some stage in their life.

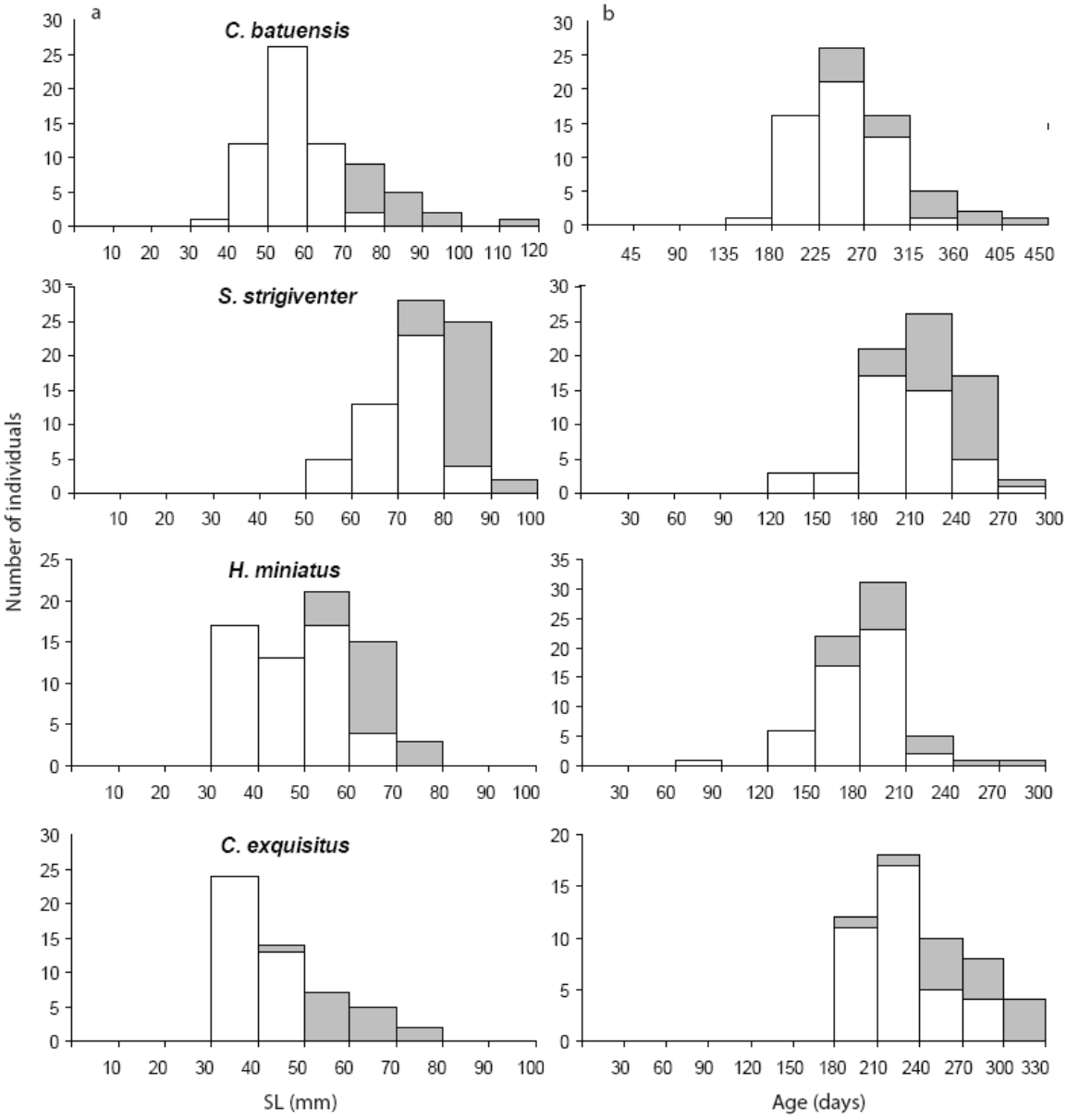

Figure 5: Size (a) and age (b) distribution for three species of wrasse from Orpheus Island, GBR (*C. batuensis, S. strigiventer, H. miniatus*) and one from Kimbe Bay, PNG (*C. exquisitus*). Females are shown in white and males in gray.

Table 1: Numbers, age ranges, and size ranges for males and females of the four species of wrasse (means are below range).

	n		size (mm)		age (days)	
Species	**m**	**f**	**m**	**f**	**m**	**f**
C. batuensis	15	63	71.4 - 118.1	37.6 - 76.5	245 - 442	175 - 324
			84.5	56.7	315	244
S. strigiventer	28	45	75.5 - 93.0	52.5 - 85.1	187 - 277	126 - 292
			85.6	71.1	233	207
H. miniatus	18	51	55.7 - 77.2	36.9 - 62.8	158 - 296	86 - 240
			65.6	48.4	200	178
C. exquisitus	15	44	49.7 - 74.1	32.4 - 51.7	206 - 330	184 - 284
			60.8	40	274	229

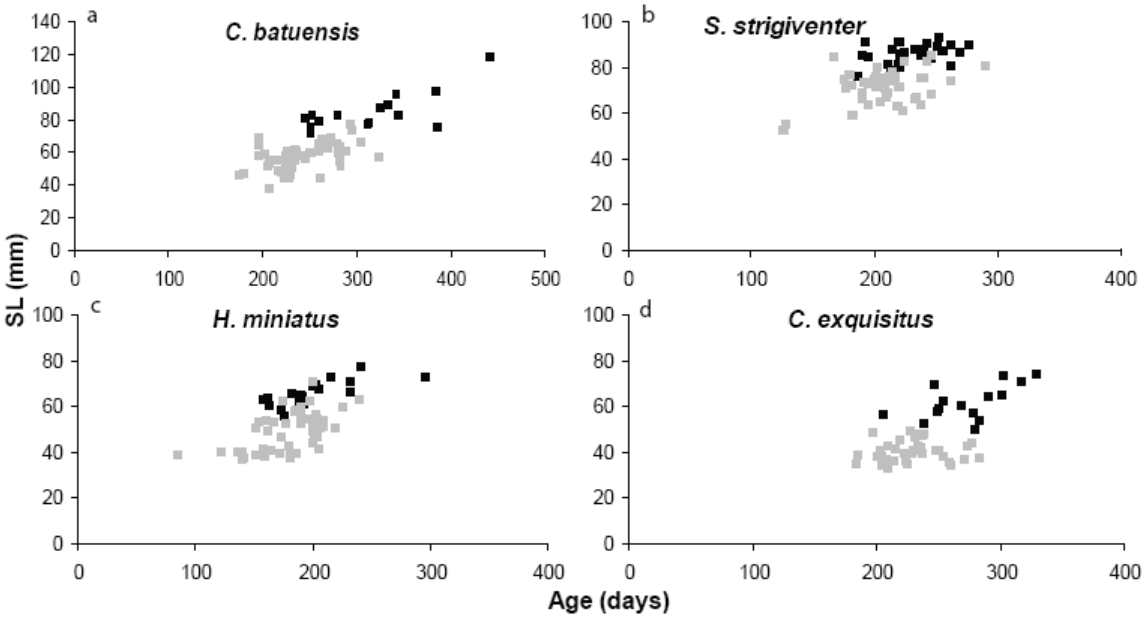

Figure 6: Size-at-age scatter-plots for three species of wrasse from Orpheus Island, GBR (*C. batuensis, S. strigiventer, H. miniatus*) and one from Kimbe Bay, PNG (*C. exquisitus*). Females are shown in gray and males in black.

2.4.3 Daily increment validation

Increments in the three species, *C. batuensis, S. strigiventer*, and *H. miniatus*, were found to accrue on a daily basis. Otoliths of all individuals exhibited an abrupt change in increment deposition at the correct number of days (+/- 4days)

from the margin, marking when the fish were placed in the darkened aquaria for 17-24 hours.

2.4.4 Sex-specific growth

Otolith increment plots were used to estimate the approximate timings of growth divergences between males and females. Estimates of average lifetime growth using otolith increment plots revealed differences in the cumulative otolith growth trajectories of males and females for three out of four species (Fig. 7). *C. batuensis* exhibited similar linearly increasing trajectories for both the males and females throughout life. For *S. strigiventer,* male and female cumulative increment plots followed the same trajectory until approximately 210 days, roughly the time of sex change, when the female curve began to asymptote while the male curve continued to increase linearly. In contrast, male and female otolith increment trajectories diverged much earlier in *H. miniatus* and *C. exquisitus*; around 35 days for *C. exquisitus* and 50 days for *H. miniatus*. In both species, *H. miniatus* and *C. exquisitus*, the female curve had an asymptotic slope while the male curve continued to increase linearly (Fig. 7).

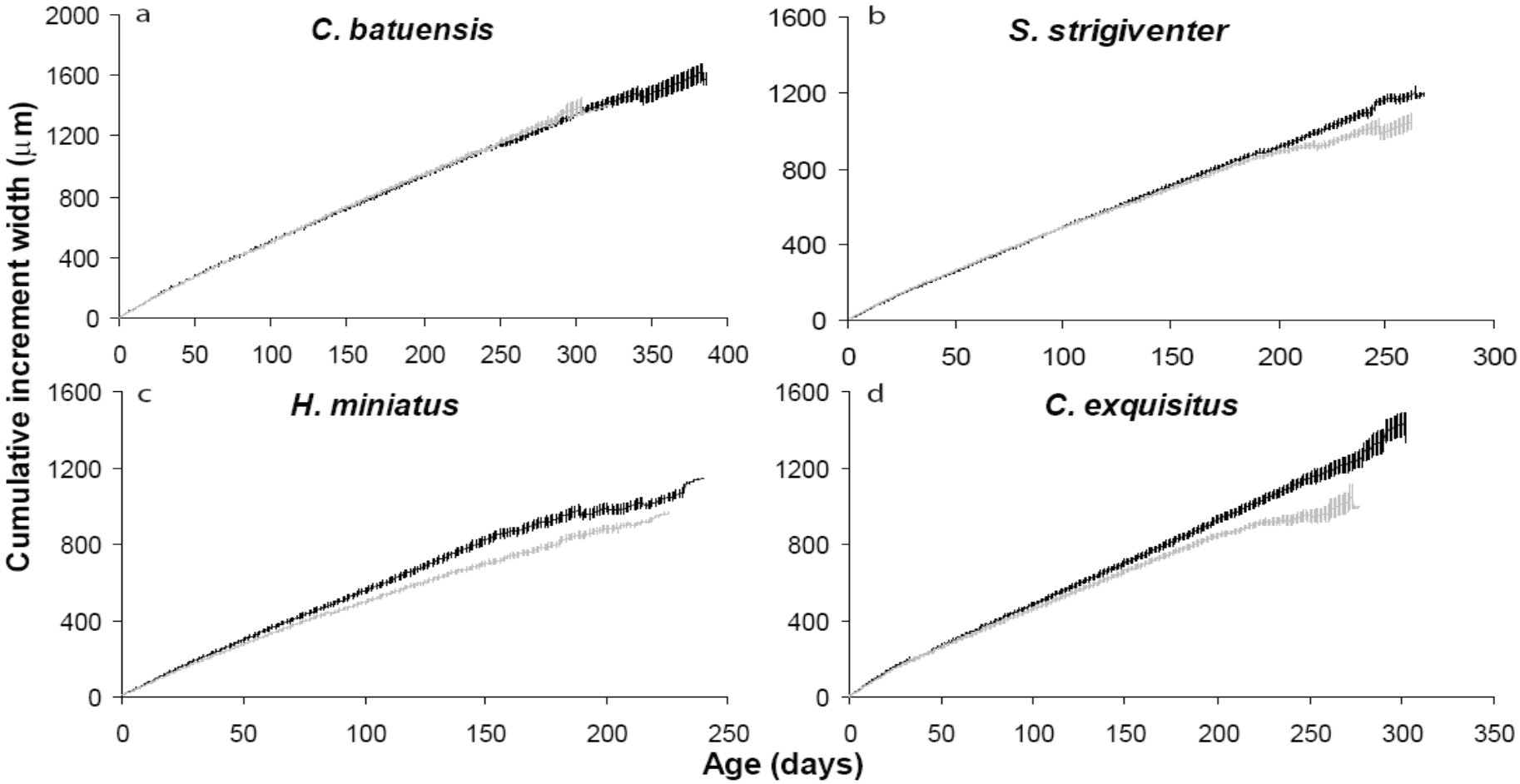

Figure 7: Average cumulative otolith increment widths (+/- SE) for males (black) and females (gray) of three species of wrasse from Orpheus Island, GBR (*C. batuensis, S. strigiventer, H. miniatus*) and one from Kimbe Bay, PNG (*C. exquisitus*).

Examination of daily increment plots enabled the timing of growth divergences to be explored in more detail (Fig. 8). *C. batuensis* showed similar male and female daily growth until near 260 days when the average female increment widths dropped and the male widths remained relatively constant. Statistical comparisons revealed no differences in otolith increment widths between males and females at any life stage (Table 2). *S. strigiventer* showed initial differences in increment widths but similar average daily increment widths afterwards until about 210 days when the males exhibited an increase in increment width. A similar increase was not evident in female increment-width profiles. Statistical comparisons confirmed initial differences in otolith widths of would-be males and females at hatching, but then similar otolith growth at later stages, until after the time of sex change (Table 2). The daily increment plots for *H. miniatus* and *C. exquisitus* were consistently lower for the females than males throughout their life (Fig. 8c, d). In both species, females that changed sex to male exhibited larger daily increment widths than other females at hatching and settlement and then again after female maturation and male maturation (Table 2). Male increment widths for *C. exquisitus* were larger than females at all later stages (Table 2). In all species, the higher variance in the increment widths in the last few days was likely due to lower numbers of older individuals sampled.

Table 2: Results for daily increment t-test comparisons at different life stages for four species of wrasse.

	t-value	df	p
C. batuensis			
hatching	-0.432	66	0.667
pre-settlement	-0.285	66	0.777
early female	0.939	66	0.351
pre-male	1.502	61	0.138
post male	1.539	66	0.129
S. strigiventer			
hatching	2.410	71	**0.019**
pre-settlement	1.689	71	0.096
early female	-0.454	71	0.651
pre-male	-1.282	52	0.206
post male	-2.082	44	**0.043**
H. miniatus			
hatching	-2.467	50	**0.017**
pre-settlement	-2.026	50	**0.048**
early female	-1.373	50	0.176
pre-male	-2.025	50	**0.048**
post male	-2.201	50	**0.032**
C. exquisitus			
hatching	-2.678	66	**0.010**
pre-settlement	-2.010	66	**0.049**
early female	-2.600	65	**0.012**
pre-male	-2.844	65	**0.006**
post male	-3.834	59	**<0.001**

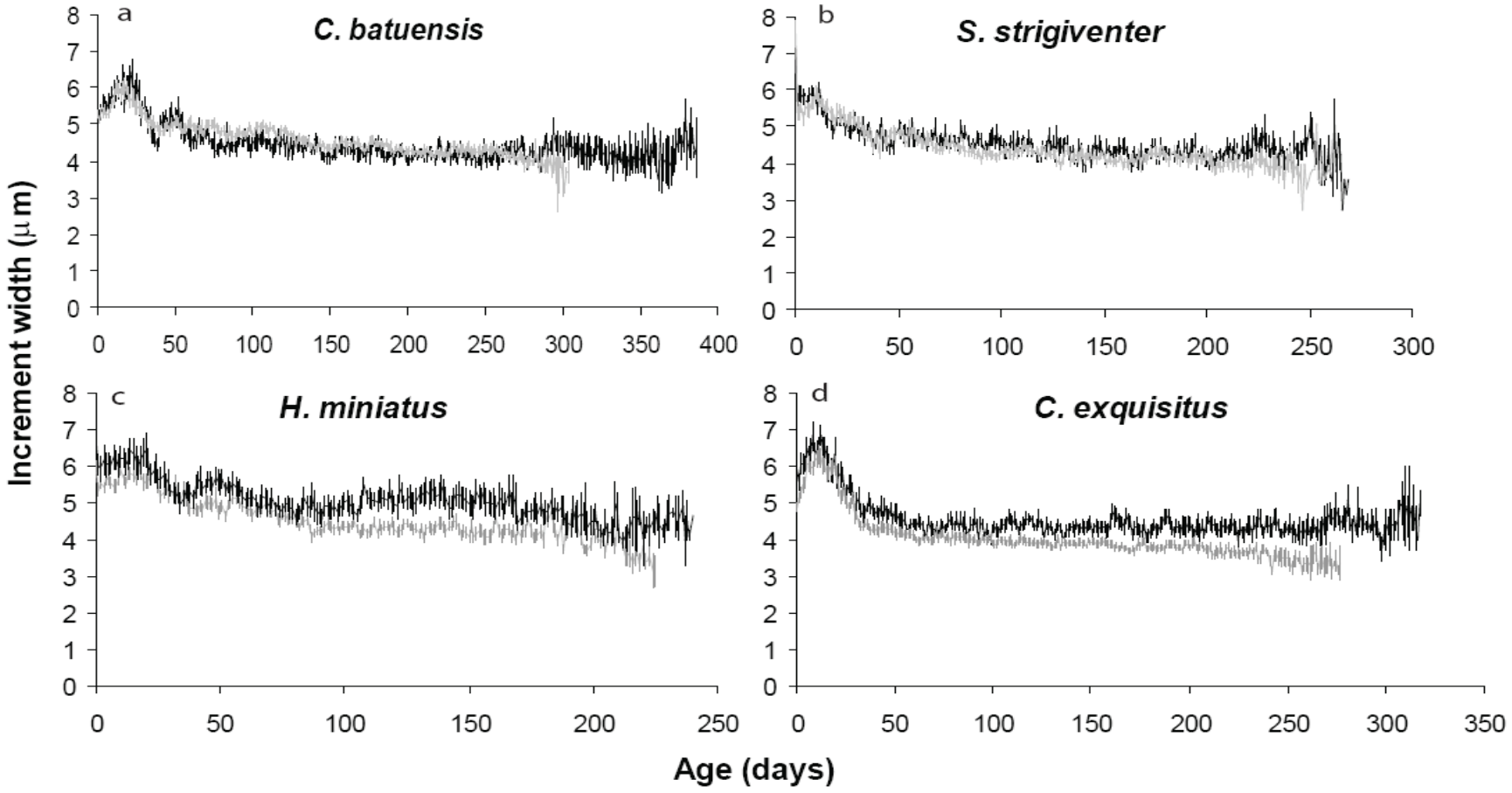

Figure 8: Average daily increment widths (+/- SE) for males (black) and females (gray) of three species of wrasse from Orpheus Island, GBR (*C. Batuensis, S. strigiventer, H. miniatus*) and one from Kimbe Bay, PNG (*C. exquisitus*).

2.4.5 Sex-change check mark

Check marks in the form of an optical discontinuity in the otolith microstructure were identified near the average age of sex change in males for *C. batuensis*, *H. miniatus* and *C. exquisitus*. Females did not have a similar otolith check mark within the age range of sex change. There were too many checks in the otoliths of *S. strigiventer* to determine which one might be associated with sex change. In all three species where a distinctive check mark was detected, the average otolith increment widths of males increased significantly after the check-mark (*C. batuensis*: $F_{(1, 38)} = 13.51$, $P < 0.001$; *H. miniatus*: $F_{(1, 38)} = 54.231$, $P < 0.001$; *C. exquisitus*: $F_{(1, 38)} = 42.497$, $P < 0.001$), indicating a period of increased growth after the check mark which was not exhibited by those individuals that remained female. In *C. batuensis* the male and female increment averages were not significantly different before the check mark ($F_{(1, 38)} = 3.208$, $P = 0.090$) indicating similar growth rates, but were significantly different afterwards ($F_{(1, 38)} = 13.793$, $P < 0.001$). In the other two species, *H. miniatus* and *C. exquisitus*, the would-be males had a higher average daily increment width for both 20 days before ($F_{(1, 38)} = 126.60$, $P< 0.001$; $F_{(1, 38)} = 78.806$, $P < 0.001$) and after ($F_{(1, 38)} = 334.31$, $P < 0.001$; $F_{(1, 38)} = 430.63$, $P < 0.001$) the check compared to the females that did not change sex.

The check-centred plots estimating male growth 20 days before and after sex change revealed an increase in the daily increment width in association with the time of the check mark (Fig. 9). In both *C. batuensis* and *H. miniatus* there was a sharp increase in the increment width (20.6% and 29.8%) from the day before the check to the day after (Fig. 9). Repeated measures ANOVA confirmed a significant increase in growth after the check compared to before (*C. batuensis*: $F_{(14, 25)} = 6.7522$, $p<0.001$; *H. miniatus*: $F_{(18, 21)} =$

5.8937, p<0.001; *C. exquisitus*: $F_{(14, 25)}$ = 15.344, p<0.001). There was no significant change in growth found for females from before to after the average age of male check mark occurrence.

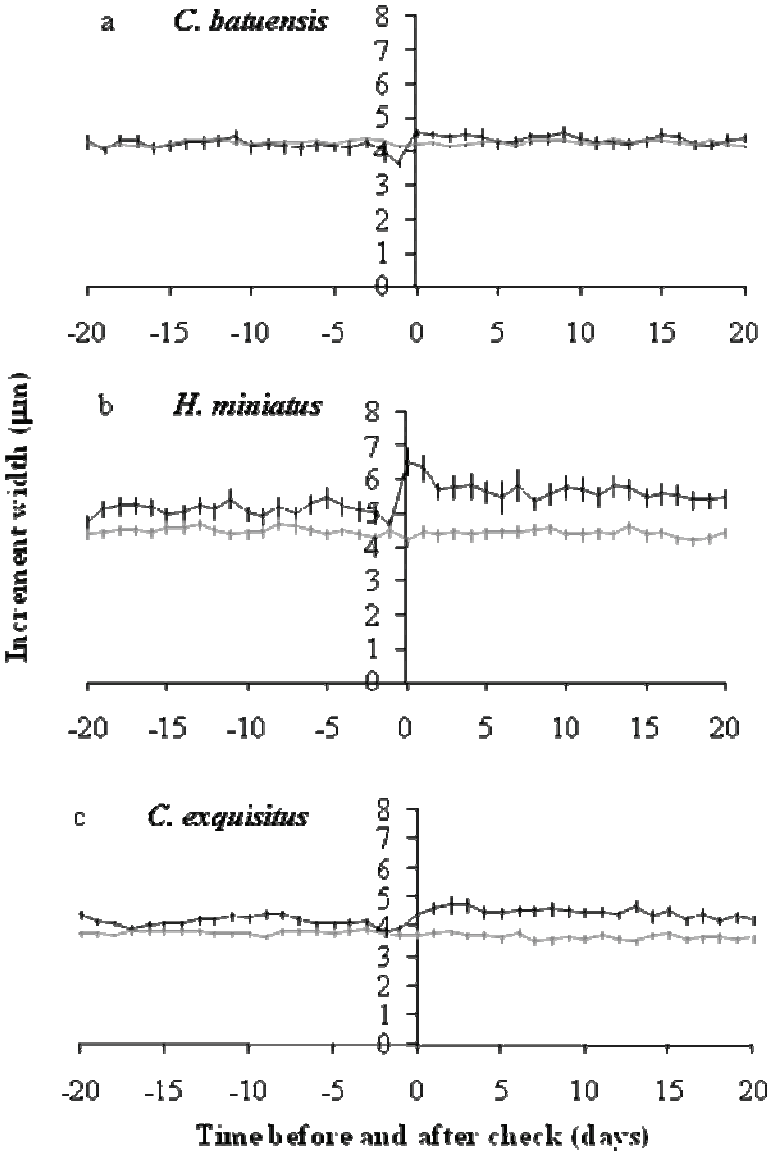

Figure 9: Average otolith increment widths (+/- SE) for 20 days before and 20 days after the identified check associated with sex change in males of three species of wrasse from

Orpheus Island, GBR. The average age of check mark occurrence was used for females (females = grey, males = black).

2.5 Discussion

The present study examined the proximate causes of sexual size dimorphism in four species of protogynous hermaphrodites. All four species had population demographics characteristic of protogynous species; however, sex-based growth trajectories estimated from the otoliths differed between the species. The variation in sex-specific growth patterns were related to the intensity and type of social system displayed by each species, indicating that the social system has a strong influence on how sexual size dimorphism is expressed in these species. Furthermore, males of each species exhibited a check mark in the otoliths, which provided a unique individual estimate of the time of sex change. Growth near the time of check formation increased in all changed individuals but not in those that remained as female.

Each of the four species had a different social structure, ranging from loosely organized multiple male and female groups in *C. exquisitus* to strictly organized, single male dominated harems as in *C. batuensis*. The different sex-based growth patterns estimated from the otoliths were associated with the strictness of social organisation exhibited by the species. In the more loosely organised social systems of *H. miniatus* and *C. exquisitus*, clear differences in the growth trajectories of would-be males and females were evident long before the age of sex change, although there was also growth acceleration by males at the time of sex change. In the stricter haremic systems of *C.*

batuensis and *S. strigiventer*, would-be males and females had similar growth trajectories until the timing of sex change, at which time males exhibited rapid growth acceleration. These results are consistent with the hypothesis that in haremic systems growth of subordinate females is usually suppressed by the presence of dominant breeders, whereas in less haremic or non-haremic systems there is less opportunity for growth suppression of subordinate individuals.

Previous studies have found that subordinate growth can be regulated by the presence of dominants in social systems where strong dominance hierarchies prevail (Robertson 1972, Hattori 1991, Buston 2003, Wong et al. 2007). The social regulation of growth in these societies is expected to result in similar growth trajectories among individuals within a social group. Furthermore, in haremic sex-changers, the timing of sex change is usually controlled by the presence of the dominant male, such that the dominant female changes sex following the loss of the male (Robertson 1972, Lutnesky 1994, Black et al. 2005). There are likely to have fewer social constraints on individual growth where looser social organisations prevail. In this case, variations in growth at earlier life stages would have a greater chance of influencing which individuals become the largest individuals in the population and potentially the first to change sex (Cowen 1990, Mackie 2003, Munday et al. 2006a). Consequently, it seems that the expression of sexual size dimorphism will be mostly controlled by the social dominance hierarchy in haremic sex-changing species, whereas individual growth histories can have a much greater influence on the expression of sexual size dimorphism in species with less rigidly controlled social organisation.

It has been recently demonstrated that sexual size dimorphism in the haremic sand perch, *Parapercis cylindrica* is caused by accelerated growth in males at the time of sex change (Walker and McCormick 2004, Walker et al. 2007). Similar growth spurts at sex change have been identified in other species including *Thalassoma duperrey* (Ross 1987) and *Amphiprion percula* (Buston 2003). The present study found an increase in growth late in life in sex changed individuals for the more haremic species, *S. strigiventer* and *C. batuensis*. In the two other species which exhibited divergent growth trajectories early in life, an increase in growth was also associated with the time of sex reversal indicating that variable growth at one stage does not exclude the possibility of variable growth at later stages.

Growth patterns in the more haremic social systems studied here showed an increase in growth around the time of sex change. At Orpheus Island, *C. batuensis* is a strictly haremic species. Males and females showed similar growth curves until the time of sex change when females that changed to males showed an increase in growth compared to other females. Provided that in haremic systems growth and sex change are suppressed by the interactions with a dominant male (Robertson 1972, Victor 1987, Black et al. 2005, Chapter 3), having a higher growth rate throughout development would not necessarily be an advantage for or be evident in individuals that change sex. Accelerated growth rate in conjunction with sex reversal would provide the new male with the size necessary to maintain a dominant position and keep smaller females in the harem (Nakashima et al. 2000).

S. strigiventer appeared to display a female defence system at the present site. Individuals formed overlapping harems where males defended a group of females from

other males. Male and female growth curves of *S. strigiventer* show similar patterns until approximately the time of sex change, and individual otolith analysis showed a growth divergence after the time of sex change. This pattern suggests that growth of females is socially regulated until the time of sex change. An initially larger size at hatching was found in the individuals that changed to male, which may indicate a parental influence on which individuals eventually change sex, however this pattern was not maintained beyond the first few days post-hatching.

Larger size at hatching was also found in males of *H. miniatus* and, in addition, the average male and female growth curves in this species split early in development, with would-be males having larger cumulative otolith growth increments than females that did not change sex after about day 50. Genetic selection for larger size at hatching would give those individuals a larger size throughout life than conspecifics and potentially put them in a position to change sex sooner. A genetic influence on male traits such as increased aggression may also give certain individuals the opportunity to change sex before other similarly aged fish. Alternatively the superior condition of certain mothers over others due to food availability, habitat availability, reduced stress levels or other environmental factors may allow for the production of better quality offspring which again could carry over to later stages (Walker et al. 2007, Donelson et al 2008). Either way, it seems that a larger size at hatching is correlated with the probability of becoming male later in life. Male *H. miniatus* had loosely established territories between which females moved regularly, which may explain why there was more potential for size and growth patterns during early life to be more closely associated with sex change in this species than in the two species with a stricter social organisation.

H. miniatus showed high variability in the average daily male increment widths. Increases in increment sizes could indicate accelerated growth at various stages such as hatching, settlement, female maturation and sex change. The increase in increment size near the estimated time of sex change could again be representative of an increase in growth in sex changers and suppression in growth in the females. This is also supported by the asymptotic nature of the female cumulative growth plots. Individuals that change to male may be experiencing variable growth, generally faster, at major stages throughout life compared to individuals that remain as female thus placing them in a position to change earlier than similarly aged members of the same group.

C. exquisitus exhibited the most consistent divergence in growth between males and females from an early stage. Individuals that changed to male maintained a higher growth throughout life. This may be due to that fact that unlike the other three species examined, *C. exquisitus* is a planktivorous schooling species. In other planktivorous groups, mating with females is not monopolized by a dominant male; rather they form large loosely organized multi-male and female groups in which males compete for spawning rights (Shapiro 1980). Sex change is initiated by a change in the number of males in the group and the change in the interactions of the females with those males (Shapiro 1980, 1986). When a male is removed from the group, a dominant position opens and the opportunity is recognized by a large or dominant female. In *C. exquisitus*, if a single dominant male is not suppressing growth in females and sex change occurs when a male position is vacated, then faster growth at the earlier stages and continued larger size throughout life would allow those females to be in a position to change to male sooner than slower growing members of the same group. There is less opportunity

for territories to be defended in these social groups as the inhabited area is constantly shifting according to the food source (Robertson and Hoffman 1977). Males are not able to defend specific sites so instead must compete for positions within the group. This may allow for greater female selectivity in spawning partners and less suppression of growth by dominant individuals.

A second aim of this study was to try and identify a "check" in the otoliths of individuals that had changed sex, marking approximately the time of sex change. In one species, *S. strigiventer*, there were multiple checks throughout the otolith and I was unable to discern which could have been associated with the time of sex reversal. In the other three species, however, I found changes in the otolith microstructure near the estimated time of sex change, similar to check marks found in the sand perch *P. cylindrica* (Walker and McCormick 2004) and in the congeneric *P. snyderi* (Walker et al. 2007). The presence of such a mark in the otolith creates a tool by which the timing of sex change can be identified as well as changes in growth and developmental history associated with sex change.

All three species in which a check was found showed an increase in daily increment width in the males after the time of sex change. Given the short lived, fast-growing nature of these species, otolith growth may be used as a proxy for growth and the increased increment width after the check may indicate an accelerated growth rate in individuals at or shortly after the time of sex change (Shafer 2000, Munday et al. 2004). This would allow individuals changing to male to attain a larger size than the rest of the group and maintain the position of dominant male. A specific acceleration in growth should be most prominent in strictly haremic groups where aggressive interactions

determine the individuals' position in the hierarchy and growth within the group. As predicted in *C. batuensis* and *H. miniatus,* where the social system appears to be haremic to varying degrees, accelerated growth occurred during sex change. This mechanism of sexual size dimorphism has been demonstrated in two other species with haremic social systems (Walker and McCormick 2004, Walker et al. 2007).

The present study focused on only one of the environmental conditions, the social environment, to describe the proximate causes of sexual size dimorphism in four species of wrasse. Sex change has been demonstrated to be socially mediated in a large number of protogynous reef fish (Robertson 1972, Shapiro 1980, Ross et al. 1983, Warner and Swearer 1991, Munday et al. 2006a). There may also, however, be variations in these patterns based on other environmental conditions such as population density, geographic location or latitudinal variations, resource availability, and feeding habits, which could also cause differences in the social system as well as variable growth both between species as well as within the same species.

Sexual size dimorphism in sex changing species can have varying mechanisms through which males become larger than females. The present study shows the potential for differential growth throughout life between groups with different social systems. The beginning of differential growth patterns between sex-changers and non sex-changers appears to be influenced by the social environment of the group. By understanding the proximate mechanisms controlling which individuals change sex, I could potentially predict which individuals within a group will eventually change given their social environment. This study demonstrates how protogynous species with different social

organizations have different growth and development patterns and these patterns regulate when and which fish will eventually change to male.

Chapter 3: Sexual size-dimorphism, growth spurts and behaviour associated with sex change in the wrasse *Halichoeres miniatus*

3.1 Abstract

In protogynous sex-changing fishes, females are expected to compete for the opportunity to change sex following the loss of a dominant male and may exhibit growth and/or behavioural traits that help them maintain their dominant status after sex change. I used a male removal experiment to examine changes in female growth and behaviour associated with sex change in the haremic wrasse *Halichoeres miniatus* and to test if any changes in growth associated with sex change were recorded in otolith structure. Dominant females began displaying male-characteristic behaviour almost immediately after the harem male was removed. Territory size and the frequency of interactions between females increased following male removal. In contrast, feeding frequency of females decreased. The largest female(s) in each social group changed sex following male removal and exhibited a transitional growth spurt associated with sex change. Sex changers grew an average of 8.51 mm SL during the experimental period, whereas non sex-changers grew an average of 3.32 mm SL. This growth acceleration likely enables newly sex-changed males to rapidly reach a size where they can defend the harem from other males and maintain dominance over the remaining females. An optical discontinuity (check mark) was present in the otoliths of sex changed fish and otolith accretion rate increased significantly after the check mark, corresponding with the increased growth rate of sex-changing females. Wild caught males, but not females, exhibited an analogous check

mark in their otoliths and similar increases in otolith increment widths after the check. This indicates that a transitional growth spurt is a regular feature of sex-change dynamics of *H. miniatus*.

3.2 Introduction

Polygynous mating systems, where a small number of males monopolise breeding opportunities with many females, favour the evolution of large male size because large males gain a fitness advantage compared to smaller males during intrasexual competition for breeding partners (Emlen and Oring 1977, Keller and Reeve 1994, Shuster and Wade 2003). Polygynous mating systems also favour the evolution of protogynous (female to male) sex change, because the reproductive success of males in these mating systems tends to increase more steeply with size or age than does female reproductive success (size-advantage model of sex change; Ghiselin 1969, Warner 1975). Small males have very low reproductive success because they are excluded from breeding by larger males, whereas large males can have very high reproductive success if they become a harem master or breeding-territory holder. Female reproductive success tends to increase more evenly with size, therefore, an individual will benefit by first reproducing as a female and then changing sex to male when large enough to defend a group of females or a breeding site that females visit (Warner 1975, Charnov 1982).

Protogynous hermaphroditism is particularly common among reef fishes and is usually associated with a polygynous mating system (Warner 1984, Munday et al. 2006a), with males tending to be the largest individuals in the population (Sadovy and

Shapiro 1987). Having a large body size has two potential advantages for males. First, it enables them to defend a harem or breeding site from other males (Robertson 1972). Second, it helps them to maintain dominance over females and prevent them gaining a size where they could change sex and challenge the male for his position as harem master or territory holder (Robertson 1972, Sakai 1997). Sex change typically occurs following the loss of a dominant male and it is usually the largest female in a social group that changes sex to male (Shapiro 1986, Ross 1990, Lorenzi et al. 2006). Due to the high reproductive success that males can attain in polygynous mating systems, females might be expected to compete for the opportunity to change sex following the loss of the dominant male. Any mechanism that enables a large female to maintain her size advantage over other females will increase her prospects of changing sex when an opportunity arises and the likelihood she will be able to maintain her new position as the dominant male after sex change.

In species where a strict social hierarchy is enforced, such as strongly haremic species, female growth can be limited by interactions with the harem male and other females (Lorenzi et al. 2006, Wong et al. 2007). Social control of subordinate growth helps maintain a size-based dominance hierarchy where the largest, dominant female changes sex following the loss of the male (Robertson 1972, Nakazono et al. 1985, Black et al. 2005). Females in the process of sex change may also increase their growth rate at the time of sex change (Walker and McCormick 2004), a phenomenon known as a "transitional growth spurt" (Ross 1987, Choat et al. 1996). Such acceleration in growth would help ensure that the dominant position is maintained after sex change. In species that exhibit a looser social organisation there is less opportunity for female growth rate to

be limited by social interactions with males or other females. Females usually change sex when a territorial position becomes available (eg. Warner and Swearer 1991). In this case, females that grow faster before sex change could have an advantage over slower growing females of the same age because they would be in a position to change sex earlier and gain the benefits of dominant male status (Adams and Williams 2001, Munday et al. 2004). A growth spurt at sex change might still occur in these species because males compete with other males for breeding territories and a large size would help a newly territorial male defend his breeding site from other males.

Although the largest female in a social group usually changes sex when the dominant male disappears, there are instances where the largest female is not the first or only female to change sex. For example, the largest female would not benefit from sex change if her reproductive potential as a female is greater than the combined fecundity of all the smaller females in the social group (Munoz and Warner 2003). In this situation one of the smaller females might change sex following disappearance of the dominant male (Munoz and Warner 2004). Alternatively, small females may change sex in the presence of larger females and males to become bachelor or sneaker males (Moyer and Zaiser 1984, Hoffman et al. 1985, Sakai 1997). Females can benefit from such "early sex change" because it places them in a better position to take over a harem or breeding territory following the loss of a dominant male (Taborsky 1998, Munday et al 2006a).

In addition to physiological changes associated with sex reversal, there are also behavioural changes that occur. Male behaviour has been observed in females just a few minutes or hours after the removal of the dominant male (Reavis and Grober 1999, Nakashima et al. 2000, Sakai et al. 2003). This rapid transition of behaviour is most

apparent in haremic species such as *Labroides dimidiatus* and *Halichoeres melanurus* (Nakashima et al. 2000, Sakai et al. 2002). Typically, the dominant female will expand her territory to encompass the territories of other females in the group and begin exhibiting male courtship behaviour before there is any evidence of sex change in the gonads (Sakai et al. 2003). Presumably, the dominant female assumes the male role almost immediately to prevent bachelor or territorial males outside the group from usurping the position and to prevent other females from within the group ascending to the dominant position.

Otoliths are often used as a proxy record for the growth history and life history transitions of fishes (Hare and Cowen 1995, Shafer 2000) and potentially provide a useful tool for comparing growth patterns is sex-changing fishes without the need for regular monitoring of a large number of individuals in the wild (Munday et al 2004). The width of increments deposited periodically on the otoliths are frequently correlated with somatic growth (Hare and Cowen 1995, Campana 2001) providing a record of the growth history of individual fish. Furthermore, some life history transitions are recorded as an optical discontinuity, or "check-mark", in the otolith (Brothers and McFarland 1981, Eckmann and Rey 1987). The best known of these check marks is associated with the transition from a pelagic to benthic life-style in larval fishes (Wilson and McCormick 1999). However, several recent studies have also identified a change in otoliths structure associated with sex change in protogynous fishes (Walker and McCormick 2004, Walker et al. 2007, Walker and McCormick unpublished data). Such check marks could provide an important individual record of the timing and growth patterns associated with sex change without the need for direct observations of individuals over long time frames.

We used a male removal experiment to examine changes in female growth and behaviour associated with sex change in the circle-cheeked wrasse, *Halichoeres miniatus*. Body size, territory size, and behavioural interactions of females were monitored in natural social groups before, during and after sex change to determine which females in the social hierarchy changed sex and how their growth and behaviour changed following the loss of the dominant male. Previous studies have suggested that sex changing females of some haremic species increase their growth rate to gain a dominance advantage (Ross 1990, Garratt et al. 1993). I tested for a growth spurt in sex changing *H. miniatus* by removing males from replicate patch reefs and monitoring the growth rate of females that changed sex and females that did not change sex. I also examined the otoliths of sex-change females and non sex-change females to determine if daily otolith increments record changes in somatic growth associated with sex change and to experimentally validate that sex change leaves a well-defined check mark in the otoliths of *H. miniatus*. I then searched for the presence of a sex-change check mark in the otoliths of wild caught fish and used this mark to compare the growth rates of individuals before and after sex change in the natural population.

3.3 Methods

3.3.1 Study site and species

This study was conducted at Lizard Island on the northern Great Barrier Reef (GBR, 14°40'S 145°28'E), Australia between September and November 2005. The study species, *Halichoeres miniatus*, is a small, short-lived wrasse that inhabits the shallow

macroalgal zone of reef flats throughout the Western Pacific (Randall et al. 1997). Otolith increments in this species have been previously validated as daily (Chapter 2). Individuals exhibit strong sexual dimorphism, with males being larger and more brightly coloured than females (Randall et al. 1997). The dull coloured females (initial phase) change sex to become brightly coloured males (terminal phase). No direct developing (primary) males have been recorded in the population of *H. miniatus* at Lizard Island (chapter 4); therefore, this species appears to be a monandric protogynous hermaphrodite at this site, where all males are derived by adult sex change. Social organisation of this species can vary from haremic, with males aggressively defending a territory encompassing the territories of multiple site-attached females, to a more loosely organized system where male territories overlap and females move freely between territories (see chapter 4). Harems were the only social system observed at Lizard Island.

3.3.2 Experimental induction of sex change

A male removal experiment was used to test the effect of sex change on female growth rate. Five social groups of *H. miniatus* occupying discrete rubble patches were identified. The male and three to seven of the largest females in each group were captured using hand nets and a dilute solution of clove oil anaesthetic solution (Munday and Wilson 1997). Each fish was measured using callipers (SL to nearest 0.1 mm) while underwater in a plastic bag and sexed according to its colour phase. Each fish was then uniquely tagged with an elastomer micro-tag (Northwest Marine Technologies) injected under the skin. Females were returned to the site of capture. Males were euthanized and their otoliths removed and stored dry before sectioning. Each social group was monitored

regularly to ensure no transient males entered the area and took over the harem. After 44 days all fish remaining in the social groups were recaptured, euthanized, and their length, weight, and colour phase recorded. Average growth of sex-changers and non sex-changers during the experimental period was compared using one-way ANOVA. Otoliths of all fish were removed and stored dry. Gonads were removed and examined under a dissecting microscope to estimate the sex of each fish and then preserved in formaldehyde-acetic acid-calcium chloride (FAACC) for histological analysis.

In one of the five groups, behavioural observations were made on a natural social system of *H. miniatus* to observe the effects of male removal on female behaviour. A 15m x 25m grid of nylon line was placed over the largest rubble patch. The male and the four largest females in the group were observed for three days prior to male removal to establish the social structure of the group. Similar observations were conducted for three days after male removal to determine the initial effects of male removal on the group. Behavioural observations were also made for three days immediately prior to the end of the experiment to examine the longer-term effects of male removal. Intraspecific interactions (chases, displays, physical contact, spawning) as well as feeding behaviour (strikes to the substrate) and location (plotted every 60s) of each fish within the grid area were recorded continuously for 15 minutes per individual every hour for five hours per day. Territory size for each fish was estimated by marking the furthest points travelled during the observation period. After the initial set of three-day behavioural observations taken after the male was removed, observations continued every three to five days throughout the 44 day experimental period. Observations from the first three days following male removal and the last three days following male removal were compared

to the observations from the three days before male removal to determine the short and longer-term effect that male removal had on female behaviour. Due to time constraints, only one social group was able to be monitored in this way. The other four groups were casually observed to verify that the interactions within the group were similar to those of the observed group and indicative of a haremic social structure.

Gonadal development of all sex-changed individuals was assessed by histological analysis of the fixed gonads. Gonads of all sex-changed females and an equal number of females that did not change sex were embedded in paraffin wax blocks, transversely sectioned at 5 μm, and stained with Mayer's Haematoxylin and Young's Eosin-Erythrosin (Winsor 1994). Sections were viewed by high-power light microscopy. The proportion of male and female sex-cell types was estimated across a representative section of each gonad. The cell type under each of the major increment on an eyepiece micrometer was recorded at 400x magnification across the gonad section. Females gonadal tissue was categorized into: (a) pre-vitellogenic oocytes (small, dark staining cells with a distinct nucleus), (b) developing oocytes (cortical alveoli stage), (c) vitellogenic oocytes, and (d) ripe oocytes. Male gonadal tissue was categorized into: (a) spermatogonia, (b) spermatocytes, (c) spermatids (grouped cells with tails), and (d) spermatozoa. The presence of remnant ovarian tissue (degenerating oocytes, oocyte follicles, and ovarian lumen) characteristic of female to male sex-change females was recorded.

The otoliths of all fish in the male removal experiment were examined to validate that sex-change causes a distinctive discontinuity (check mark) in the otoliths and to directly test the relationship between otolith increment width and somatic growth during

sex change. If sex change causes a distinctive check mark to be deposited in the otolith, I expected that all females that changed sex to male would exhibit a check within 44 days from the otolith edge. A similar check mark would be absent from the otoliths of females that did not change sex. Otoliths of all fish were sectioned along the nucleus following the procedures of Wilson and McCormick (1997) and viewed with a high-power light microscope. The timing of optical discontinuities in the otoliths (check marks) was estimated by counting the number of daily otolith increments between the check mark and the otolith edge.

If otolith increment width provides a reliable indicator of somatic growth, and females exhibit a somatic growth spurt during sex change, I expected that otolith increment widths would increase significantly in females that changed sex to male, but not in females that did not change sex. Daily otolith increment widths of all fish were estimated from digital images captured using a camera connected to a high-power microscope and then measured with an image analysis program (Optimus 6.5). Two statistical comparisons were made on the increment estimates. First, the width of daily otolith increments in sex-change fish was compared 20 days before and 20 days after the check mark using a repeated measures ANOVA. Increment widths following male removal were then compared between females that changed sex and females that did not change sex using one-way ANOVA in order to check for a difference in the average daily increment growth.

3.3.3 Laboratory validation of sex change

A manipulative experiment in the laboratory was used to further test for the effect of sex change on female growth and to validate the presence of a sex change check-mark in the otoliths. Six groups of *H. miniatus* were established in 50L aquaria using individuals from areas outside of the study sites. Fish were collected, measured (SL to 0.1mm), sexed, and tagged with coloured micro-tags as described above. One male and three females were placed in each of three aquaria and four females were placed in each of three other aquaria. The size range of individuals in these new social groups was varied from small to large using similar size differences between fish for each group in an attempt to resemble natural size dominance hierarchies. Fish were fed three times daily; minced prawns twice a day and flake food once a day. After 35 days all the fish were euthanized and their length, weight, and colour phase recorded, otoliths removed, and their gonads removed and examined macroscopically to determine the functional sex of each fish. Ovaries were identified by a rough surface texture indicating the presence of developed eggs. Testes were identified by a smooth surface and cream colouration. Again, histological sections were examined to verify sex reversal by looking for vestiges of ovarian tissue. Otoliths of all fish were examined for the presence of a sex-change check mark and tested for changes in daily increment width associated with a growth spurt as described above.

3.3.4 Growth and sex change in the natural population

To investigate the patterns of sex change and growth in the natural, un-manipulated population I collected male and female *H. miniatus* from various locations in

the Lizard Island lagoon using hand nets and a dilute solution of clove oil anaesthetic. The standard length (SL) and colour phase of each fish was recorded. Otoliths were removed for age estimation and analysis of otolith increment width. The sex of each fish was initially determined by macroscopic examination of the gonads under a dissecting microscope. Histological sections of gonads were examined as described above to verify sex and to compare gonadal development between manipulated and un-manipulated individuals.

Otolith microstructure was used to age males and females and to obtain estimates of daily growth rates under natural conditions. Otoliths were prepared, sectioned and viewed as described above. The age of each fish was determined by counting the number of daily increments from the nucleus to the otolith margin.

The minimum age of sex change in the population was estimated from the age frequency distribution of males and females. Within the estimated age range of sex change, otoliths of all fish were then examined for a check mark that might indicate the time of sex reversal. The age at sex change was estimated by counting the number of daily otolith increments between the check mark and the otolith edge. To determine if growth rate increased following the presumed sex-change check mark, mean daily increment sizes were compared for 20 days before and 20 days after the check mark using repeated measures ANOVA test. Average increment widths of sex changers (males) were also compared to those of non sex changers (females) during the sex change period using a one-way ANOVA. In this analysis, the width of otolith increments in females above the average age of sex change was compared to the width of otolith increments in males following the presumed sex change check mark.

3.4 Results

3.4.1 Experimental induction of sex change

Natural social groups of *H. miniatus* contained a single dominant male and three-seven large females within the male's territory (Table 1). Numerous smaller females were also observed within the territories of the larger females, but were not captured and measured. Between one and three females changed sex to male in each of the five social groups where the dominant male was removed (Table 1). In each group it was the largest female(s) that changed sex. In three of the groups (groups 2, 4 & 5) more than one female changed sex. One female from group 1 was not recaptured and may have moved from the group or died.

All sex-changed fish had a well-developed testes dominated by male tissue (Fig. 1a). Spermatozoa were present in peripheral sperm sinuses indicating that these were fully functional males. Most testes contain a remnant ovarian lumen and some contained degenerating oocytes or oocyte follicles. Gonads of all non-sex-changing females exhibited characteristic ovarian development (Fig. 1b). In groups where more than one individual changed sex to male there was no difference in the proportion of sex cell types present in the gonads of the largest males compared to smaller males (one-way ANOVA: $F_{(4,2)} = 2.654$, $P = 0.292$).

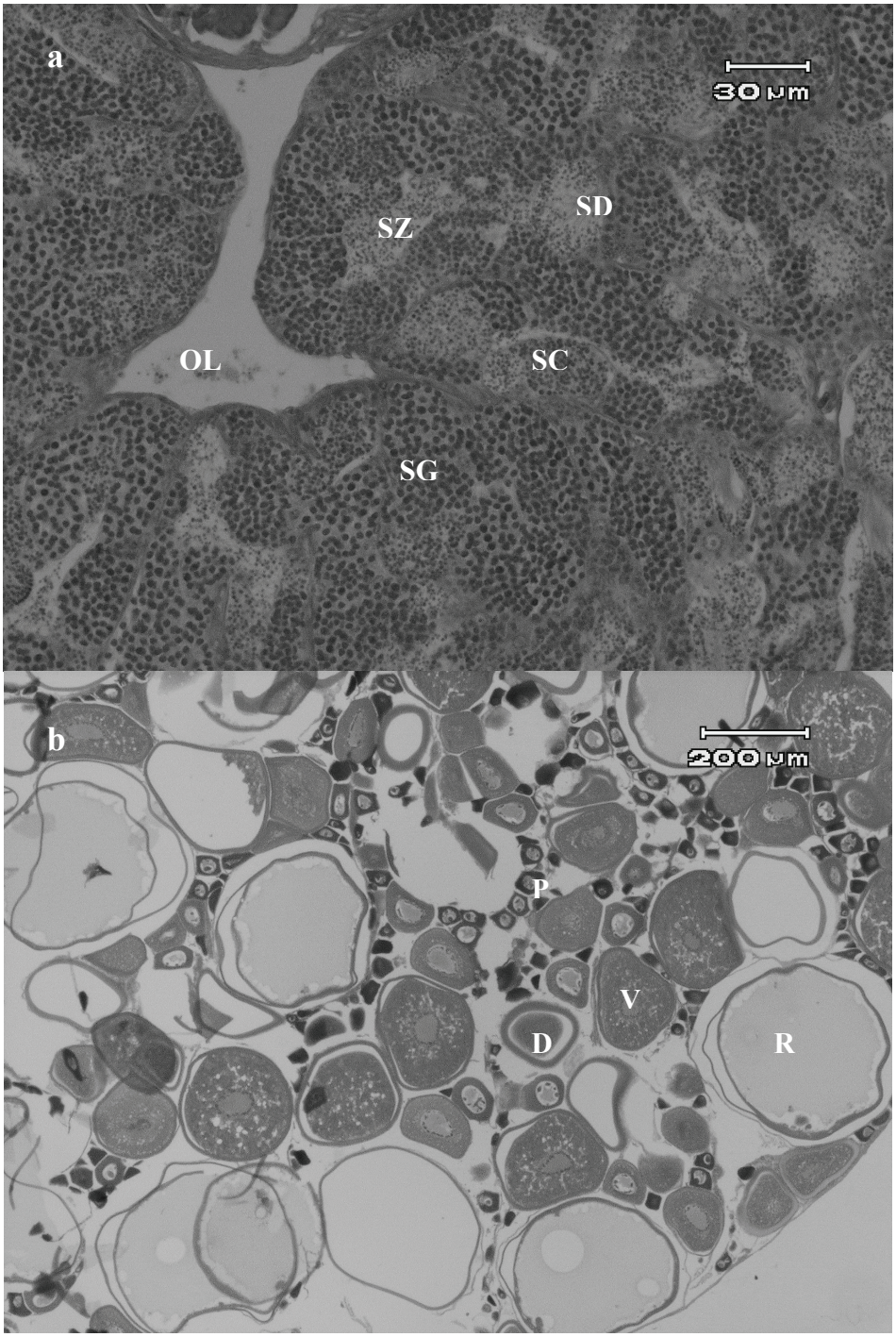

Figure 1: Gonad sections of a female that changed to male (a) and female that remained as female (b) showing spermatogonia (SG), spermatocytes (SC), spermatids (SD), spermatozoa (SZ), and remnant ovarian lumen (OL) in the male and pre-vitellogenic

oocytes (P), developing oocytes (D), vitellogenic oocytes (V), and ripe oocytes (R) in the female.

There was a significant increase in growth of females that changed sex to male compared to females that did not change sex ($F_{(1, 13)} = 6.389$, $P = 0.025$, Fig. 2). Females that changed sex grew an average of 8.51 +/- 1.40 mm SL (0.194 mm/day +/- 0.032) during the experimental period, whereas females that did not change sex grew an average of 3.32 +/- 1.37 mm SL (0.076 mm/day +/- 0.031). In all but one of the groups the largest female remained as the largest individual in the group after sex change. In group 5, however, the second largest female grew faster than the initially largest female and became the largest member of the group after sex change (Table 1).

A check mark in the form of an optical discontinuity was apparent in the otoliths of all the females that changed sex (n = 9) in the male removal experiment (Fig. 3). A similar check was not present in any of the individuals that remained female. The check mark was between 26 and 44 days (mean 40 d) of the otolith margin indicating that it was formed after the removal of the dominant male. The average increment widths in the otoliths 20 days after the identified check were significantly larger than increment widths 20 days prior to the check in those individuals that changed sex ($F_{(1,38)} = 688.62$, $P < 0.001$, Fig 4). There was no difference in otolith increment widths at corresponding days in females that did not change sex (Fig 4). Increased otolith increment width in sex-changed fish was consistent with the observed increase in growth rate of females that changed sex compared to those that did not. There was a sharp increase in average

increment width at the time of the check (repeated measures ANOVA: $F_{(2,37)} = 462.36$, $P < 0.001$, Fig 5) indicating acceleration in growth at the commencement of sex reversal.

Table 1: The size (mm SL) and age of males and females in natural social group before male removal and sizes of females that changed sex and those that remained as female 44 days after the removal of the resident male. Fish were tagged for individual identification. ← represents females that changed sex after male removal, --- missing female

	Before male removal				After male removal	
	♂		♀		♂	♀
Group	size	age	size	age	size	size
1	67.5	285	53.4	225	62.7 ←	
			52.4	182		55.9
			50.2	198		52.7
			46.3	179		51.1
			46.3	169		---
			43.8	152		47.1
2	75.5	276	57.1	250	64.6 ←	
			56.7	241	61.2 ←	
			51.8	224		54.2
3	77.2	298	56.8	230	60.3 ←	
			49.4	156		53.8
			48.7	158		50.2
			44.6	148		47.9
4	87.2	294	57.2	231	68.6 ←	
			56.2	226	67.7 ←	
			50.3	181		55.0
5	79.4	273	61.7	235	65.1 ←	
			51.9	251	67.5 ←	
			51.4	248	61.3 ←	
			49.3	192		52.1

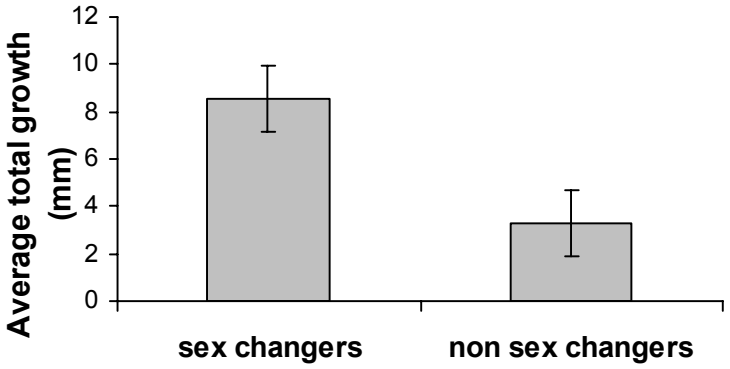

Figure 2: Average increase in length (+/- SE) after 44 days of female *H. miniatus* that changed sex to male compared to females that did not change sex.

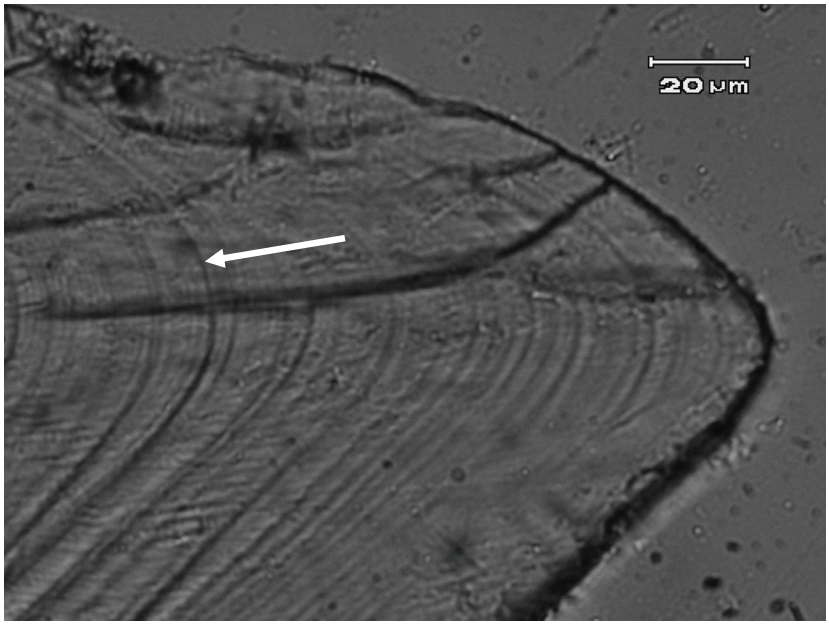

Figure 3: Otolith section from a female after sex change showing a check mark 21 days from the margin.

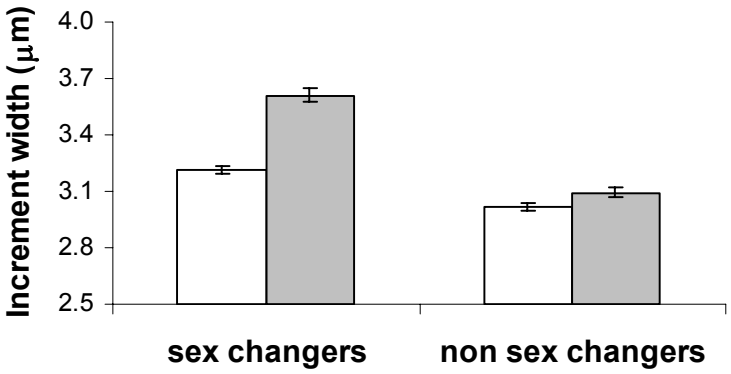

Figure 4: Average otolith increment width (+/- SE) in sex changed and non sex changed *H. miniatus* for the period 20 days before and 20 days after the identified otolith check mark. Before sex change is shown in white and gray is after sex change.

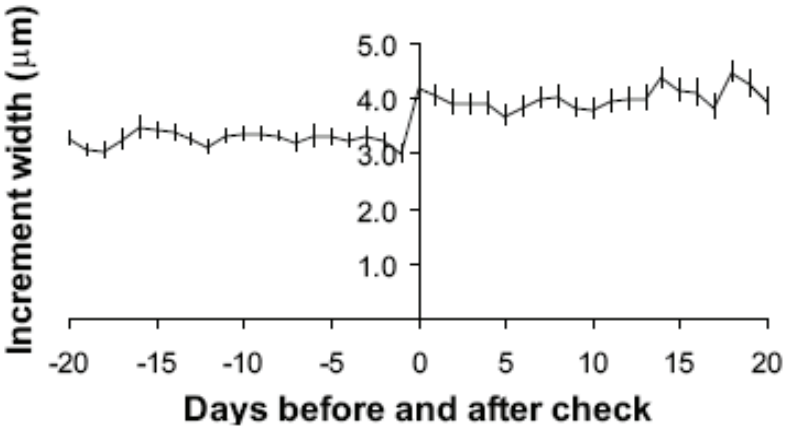

Figure 5: Average daily otolith increment width (+/- SE) of *H. miniatus* individuals from 20 days before to 20 days after sex change in females that changed to male.

3.4.2 Behavioural changes

The focal observation group displayed a haremic structure prior to the removal of males. Less formal observations of the other four experimental groups indicated a similar social structure in each. Males aggressively defended non-overlapping territories from other males and each male territory encompassed multiple, smaller, female territories.

Males maintained their dominance through displays and chases toward the females within their territory. Females remained site attached and rarely moved outside their territory. Larger females displayed to and chased other females that entered their territory but largely ignored the small females already within their territory. Near spawning time males visited female territories more frequently until spawning took place.

The study area encompassed the territory of the largest male in the area. The average territory size over three days for the dominant male (79.4 mm SL) was 120.48 m^2. Three neighbouring males in the area had territories of 88.4 m^2, 87.52 m^2 and 19.64 m^2 (male size 74.2 mm, 66.4 mm, and 63.1 mm, SL respectively). The male interacted regularly with all of the females within his territory. Before male removal, the largest females in the group had an average territory size of 13.25 m^2. After male removal, the territory sizes of the three largest females changed (Fig. 6). Female territories initially became indistinguishable for three days during which time all the females roamed throughout the study area, often in one group. Territories were re-established after approximately three days at which time the second largest (and initially the most isolated) female began to assume the dominant male role and maintain the largest territory (Fig. 5, 6a). By the end of the experimental period, this female and two other large females had changed sex to male. At the end of the 44 days the dominant female had grown larger than the other two and controlled the largest territory while the other two new males maintained territories similar in size to their original territories on the periphery of the dominant's territory (Fig. 6, 7a).

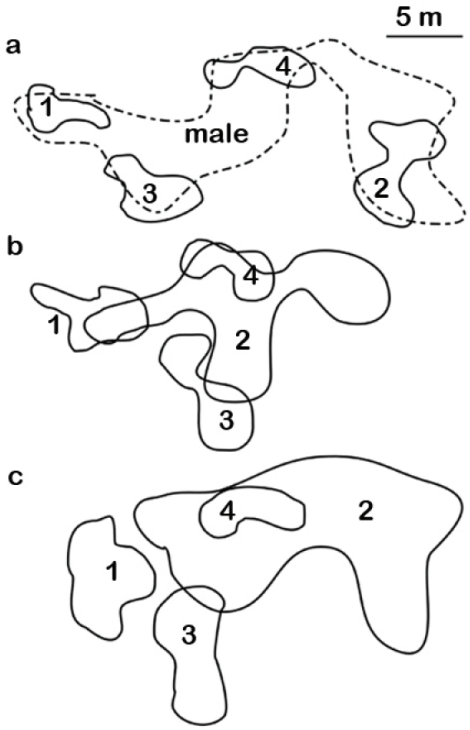

Figure 6: The shape and relative size of the male and largest female territories in the focal study group, (a) before male removal, (b) 22 days after male removal, and (c) 44 days after male removal. Territory numbers match females in Figure 7. Territory 1 belongs to the largest female and 4 to the smallest female and the dashed lines represent male territory.

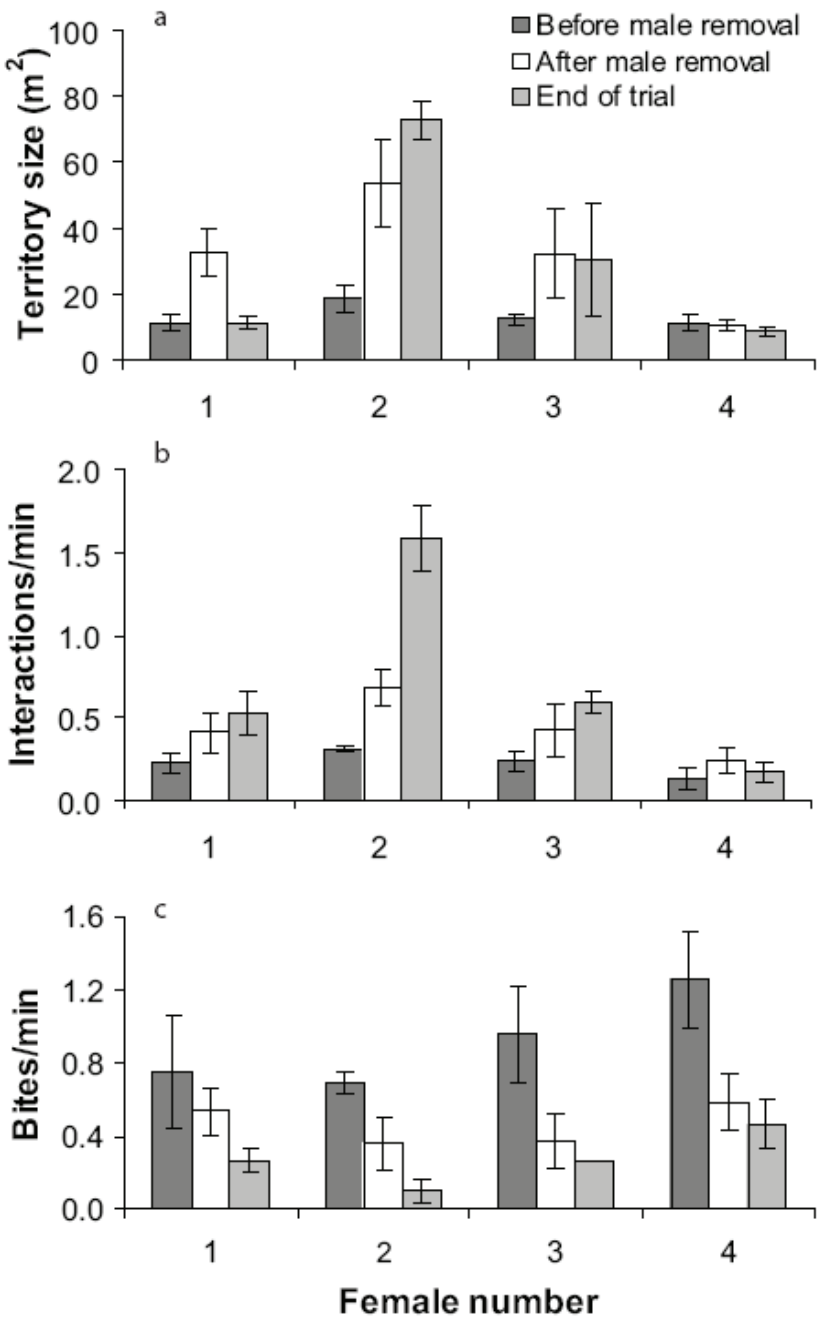

Figure 7: Mean (+- SE) territory size (a), frequency of female-female interactions (b) and feeding rates (c) before and after male removal for the four largest *H. miniatus* females in the focal observation group. Females are in order from largest to smallest. The first three females changed sex to male and the fourth remained as female.

Before male removal the largest females in the group generally encountered other females infrequently. Interaction rates increased after male removal for all three females that changed sex to male, with the second largest female ending up with the highest number of interactions (Fig. 7b). Feeding rates decreased at the same time (Fig. 7c) suggesting a shift in time budget from feeding to social interactions and territory exploration. Before male removal all of the largest females in the focal group were observed to spawn with the male territory holder. Spawning behaviour within the group involving several different pairs of resident females was observed the same evening as the male was removed. After several days the second largest female began to monopolize all spawning activity. Colour phase in the dominant female (second largest) began to change within a week of male removal. After three weeks the two other largest females also began to change colour. At the end of the 44 days these three large females exhibited male colouration and histological analysis of the gonads confirmed that all three had become functional males.

3.4.3 Laboratory validation of sex change

Only the largest female changed to male in all three female groups without a male present. No females changed sex in groups where a male was present. The colour phase of the sex-changing female started to change within several days. At the end of the experiment (35 days) the gonads of sex changed females had changed from ovaries to testes. There was no significant increase in size in any of the fish in the aquaria, which was most likely a result of the small aquarium size constraining growth. Daily otolith

increment widths during the experimental period were highly compressed and could not be resolved using light microscopy for these fish.

3.4.4 Growth and sex change in the natural population

A total of 33 females and 16 males were collected from the natural population. All initial phase individuals were female and no males were found in the smaller size classes, therefore, all males appear to be derived through sex change, as previously reported. The initial size and age distributions were representative of a protogynous species, with males only found in the larger size classes (Fig 8a). There was more variation in the age distribution of males (Fig 8b) compared to the size frequency distribution, indicating that females change sex to male at a wide range of ages. The youngest male was 197 days old, only marginally older than the youngest female in the sample (192 days old).

The histological examination of the gonads confirmed that all terminal phase (male colouration) individuals were functional males. Testes contained only male sex cells, although most also exhibited a remnant ovarian lumen and some also contained degraded oocyte follicles. One individual had transitional colouration with only the beginnings of male facial markings. The gonad of this individual was also transitional in nature, being composed almost entirely of degenerating oocytes. A check mark was identified five days from the margin of the otolith for this individual.

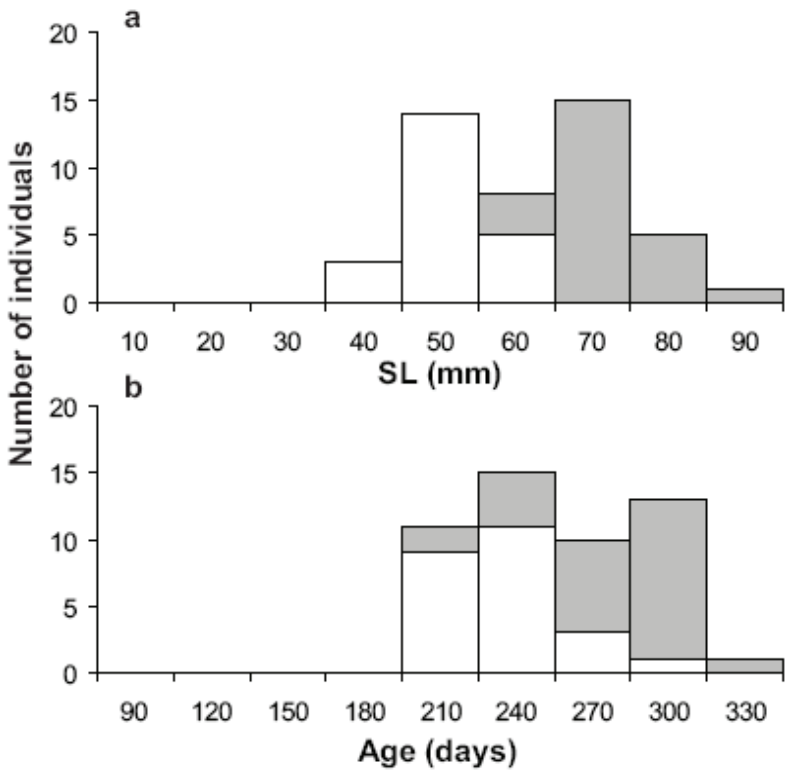

Figure 8: Size (a) and age (b) frequency distributions of *H. miniatus* at Lizard Island, GBR. White represents females and gray is males.

A discernable optical discontinuity in the otolith, consistent with the sex-change check mark observed in experimental fish was identified in 14 of 16 (87.5%) males from the wild population. A clear check was not evident in the otoliths of two males, possibly due to otolith sectioning flaws; however a change in the otolith accretion rates was still distinguished in these fish. The age at sex change based on the check mark was between 141 and 269 days (mean 209 d). Daily otolith increment width was significantly greater 20 days after the check compared to 20 days before the check, (Fig 9; repeated measures ANOVA: $F_{(2, 37)}$=296.11, P<0.001), indicating a growth spurt associated with sex change as observed in the experimental manipulation. Otolith increment widths of males after the

sex-change check mark were significantly larger than otolith increment widths of females of the same age that had not changed sex (one-way ANOVA: $F_{(1,38)}$=181.92, P<0.001).

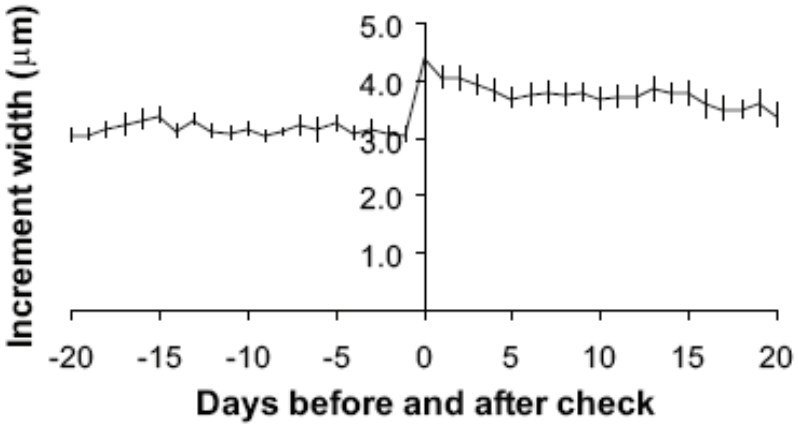

Figure 9: Average daily otolith increment width (+/- SE) of *H. miniatus* individuals from 20 days before to 20 days after the identified check in the otoliths of wild caught individuals.

3.5 Discussion

In protogynous species, females are expected to compete for the opportunity to change sex following the loss of a dominant male and may exhibit traits that help them maintain their dominant status after sex change. I experimentally demonstrated that the largest female(s) in social groups of the haremic wrasse *H. miniatus* change sex following the loss of the dominant male. All sex changers exhibited a transitional growth spurt. This rapid increase in size likely improves the competitive ability of new males. I also experimentally identified the presence of an optical discontinuity (check mark) in the otolith of sex changed fish and an increase in otolith accretion that corresponded with the growth spurt. A similar check mark was found in males, but not females, for the natural

population, demonstrating that a growth spurt associated with sex change is a common feature of *H. miniatus* growth dynamics.

A transitional growth spurt has been reported in several other haremic sex-changing fish (Walker and McCormick 2004, Rodgers et al. 2005, Walker et al. 2007, Walker and McCormick unpublished data). Increased size during and after sex change most likely benefits new males by increasing their ability to defend the harem against other males. Bachelor males or other territorial males frequently attempt to take over a harem after loss of the dominant male (Sakai 1997, Taborsky 1998) and accelerated growth would increase the new males' ability to prevent such takeovers. Large size may also help the new male maintain a dominance hierarchy within the harem and prevent other females reaching a size where they could challenge for the dominant position (Robertson 1972, Hattori 1991, Rodgers et al. 2005, Wong et al. 2007). Harem males usually have much higher reproductive success than females, because their reproductive output is the combined fecundity of all the females in the harem, therefore I expect that new males will use their size advantage and other behavioural tactics to defend their dominant position.

Behavioural changes in the largest females began almost immediately after the removal of the male. This has been observed in other species shortly after male removal and is likely to be important in maintaining or establishing dominance relationships among prospective sex changers (Kuwamura et al 2002, Sakai et al 2003, Rodgers et al 2005). Interaction rates and movement of females within the harem increased substantially, whereas feeding decreased slightly. A similar pattern of increased home range size and social interaction rates as well as a decrease in foraging behaviour was

found to be associated with sex change in protogynous hogfish (*Bodianus* spp.) demonstrating a time budget shift in social, reproductive and feeding demands (Hoffman 1983). Importantly, I found that sex-changing females exhibited an increase in growth at the same time that feeding decreased, suggesting that growth acceleration may be fuelled by stored energy or changes in metabolic efficiency rather than increased energy intake.

In social systems where sex change and growth are regulated by interactions between individuals I might expect to find a one-to-one replacement of males by the largest dominant females (Robertson 1972, Nakazono et al. 1985, Hattori 1991). In this experiment the largest females always changed sex to male and exhibited a growth spurt, as has been observed in other haremic species in association with sex reversal (Rodgers et al. 2005). Surprisingly, one to two of the next largest females also changed sex to male in three of the five groups. This suggests that the presence of the male, and not necessarily a dominance relationship among females, is responsible for the timing of sex change in *H. miniatus*. This perspective is reinforced by the low level of interactions between females in the focal harem before the male was removed. Even more surprisingly, in one of those groups the second largest female changed sex first and became the dominant male. Territory sizes initially increased for all three of the females in the focal group that changed sex, however only the second largest females' territory remained permanently increased in size after sex change. The new territory of the dominant sex changer encompassed the territory of the remaining large female and the other small females present in the area. The other females that changed sex did not permanently expand their territories; instead they remained in small territories which only incorporated one or two small females in the immediate area. In this particular harem, the second largest female

was initially the most isolated of the large females in the harem. This may have resulted in fewer interactions with the dominant male, imposing less suppression of sex change candidature and giving her the opportunity to change sex first and usurp the dominant position.

Given the potential high pay-off associated with becoming a harem master, it might be to the advantage of lower ranked females of sufficient size to change sex and challenge the dominant female for the male position. If the reproductive success of a harem master is much greater than the average reproductive success of females, challenger females need only succeed in a relatively small number of instances for this to be an effective strategy (Rodgers 2003). Natural social groups of *H. miniatus* appeared to be haremic at this location, with only one male resident in each harem. Therefore, subordinate males that do not succeed in a challenge to the new harem master may become bachelor males and wait for an opportunity to takeover another harem (Lutnesky 1994, Sakai 1997, Taborksy 1998).

An alternative explanation for why more than one female changed sex in some groups is that new males were smaller than the resident male and, therefore, unable to defend a harem of the size of the original males'. In the present system, as a female changed to male she attempted to integrate the previous male's territory into her own. However, the territory size of the new dominant male did not reach the size of the initial male territory. This is most likely related to the larger size of the initial male. The changing female, being smaller than the original male, might not be able to maintain a territory of the same size as the original male. Reduced territory size of the new male and lower levels of interactions between peripheral females and the new male might stimulate

peripheral females to change sex, resulting in the initial fragmentation of the original male's territory (Ross et al. 1983, Hoffman et al. 1985, Sakai 1997). A similar occurrence has been observed in the angelfish, *Centropyge ferrugatus*; the removal of a dominant male initially resulted in the takeover of the harem by either a sex changing female or a neighbouring male and then shortly after, the fragmentation of the large harem by sex changing females (Sakai 1997, Sakai and Kohda 1997). Presumably, however, the new dominant male would expand his territory as he increases in size and ultimately exclude peripheral males. The result would be a dynamic of territory fragmentation and expansion following the loss of dominant males.

The presence of other females in addition to the removal of a dominant male is known to induce sex change in species of damselfish (Fishelson 1970), wrasses (Robertson 1972), anthias (Shapiro 1981), and gobies (Reavis and Grober 1999). With the loss of the dominant male the female interaction rates increased most likely as they had more time to allocate to intrasexual interactions. The larger females in different areas of the original territory may have changed in response to not only the absence of the dominant male but also the more noticeable presence of the other females in the area. (Did female-female interactions after male removal increase over the male-female interactions before removal?)

In wrasses, sexual dichromatism is usually associated with mating systems where females have the potential for mate selection (Robertson and Choat 1974, Robertson and Hoffman 1977). In contrast, males and females usually have similar colouration in strictly haremic systems because spawning is monopolized by a dominant male and females have limited potential for mate choice. In the present system, males had distinctly brighter

colouration than females even though a male-dominated haremic social system was observed. This suggests that other populations of *H. miniatus* might display a social structure based on female selectivity, so that bright male colouration is retained in this species even though it does not appear to be important for mate choice under the present conditions. Multiple social structures have been observed in other species (Fricke 1980, Warner 1982, Sakai and Kohda 1997) and this may result in the retention of a characteristic favourable in one of the systems. An alternative possibility is that the system observed here is not as strictly haremic as it appears and that female mate choice may be an important component of social organisation. For example the choice of territory locations by females might be influenced by the perceived quality of the male in the area.

The validation of a sex-change check mark in the otoliths of *H. miniatus* provides a unique and effective way to identify the age of sex change and examine growth patterns associated with sex change without time-consuming and logistically difficult individual tracking. The present study validated a mark incorporated into the otolith at the start of the sex reversal period and growth acceleration associated with sex change was recorded in otolith accretion. Comparisons to natural populations revealed a similar check in the otoliths as well as a similar increase in daily increment width. As many reef fish are known to change sex, other species that share similar life history characteristics may also acquire a mark in the otoliths indicating the time of sex change. Implications for the use of otoliths as a tool for estimating growth history have been described before (Shafer 2000, Hare and Cowen 1995). These results provide stronger evidence for the use of otoliths as a way to estimate growth history and mark life history events. The largest

females in the manipulated groups changed sex providing the possibility for the use of this species and similar species in further experimentation on the influences of social systems on the individuals within the group.

Chapter 4: A comparative analysis of mechanisms responsible for sexual size-dimorphism in two populations of a sex-changing fish

4.1 Abstract

Variability in the density of populations within a patchy environment leads to differences in interaction rates, growth dynamics and social organization. In protogynous hermaphrodites there is a hypothesized trade-off between sex-specific growth, reproductive output and mortality. When differences in density lead to changes to social organization the link between growth and the timing of sex-change is predicted to change. The present study explores this prediction by comparing the social organisation and sex-specific growth of two populations of a protogynous tropical wrasse, *Halichoeres miniatus,* which differ in density. At a low density population a strict harem structure, where males maintained a tight monopoly of access and spawning rights to females, was found. In contrast, at a high density site a loosely organized system was found, where females could move throughout multiple male territories. There was considerable overlap in male territories at high density site (35.7%) while there was no overlap of male territories at the low density site. Intraspecific interaction rates also differed between the two sites. Otolith microstructure revealed the species to be annual and deposit an otolith check associated with sex-change. Growth trajectories suggested that sex-changing females in both populations underwent a growth spurt at sex-change; with this spurt being less well marked in the high density population. Moreover, in the high density, less socially controlled population, sex-changing females were those individuals that had the

largest otolith size at hatching and consistently deposited larger increments throughout early larval, juvenile and female life. This study demonstrates that previous growth history and growth spurts can be responsible for the sexual dimorphism, typically found in sex-changing species, and that the relative importance of these may be socially constrained.

4.2 Introduction

Sex-allocation theory suggests that the timing of the transition between genders in a sequential hermaphrodite is dependent upon a trade-off between sex-specific reproductive output, growth and mortality (Charnov 1982, Iwasa 1991, Munday et al. 2006a). When individuals are brought together by a common requirement for limited resources, dominance hierarchies lead to the monopolisation of some resources, differential growth of individuals and the social control of sex-ratios (Buston 2003, Wong et al. 2007). In the marine environment, resource availability can be unpredictable due to environmental patchiness and variability in population density. The complex life-history of most marine organisms also means that juveniles enter social environments that may be very different from their natal state. This unpredictability has lead to plasticity in the way individual fitness is maximised; different populations may change sex at different sizes and ages due to the different ways that they can trade-off sex specific fertility, growth and mortality (Munday et al 2006a).

In fishes there is a strong link between growth, the sex of an individual and the mating system it operates within. In protogynous mating systems where males

monopolize matings with many females, male reproductive success is strongly linked to size (Warner 1988). Males tend to be larger than similar aged females within the social group. This size difference can either be due to a history of higher growth in sex changing individuals (Walker et al 2007), or a product of a growth spurt that occurs coincident with sexual transition (Walker and McCormick 2004, Walker and McCormick unpublished data). While it is commonplace for males to be larger than females, the mechanisms by which this sexual dimorphism is manifested have seldom been explored (see for exceptions Adams and Williams 2001, Munday et al. 2004, Walker and McCormick 2004, Walker and McCormick unpublished data, Walker et al 2007).

Recently, the microstructural increments within otoliths have been used to clarify the link between sex-change and growth history. Once the deposition of increments has been appropriately validated (Campana 2001), the width of increments can be used as a proxy for somatic growth. Abrupt changes in increment structure, or checks, associated with key life history transitions, such as settlement (Wilson and McCormick 1999) and sex-change (Walker and McCormick 2004, Walker et al 2007), allow a growth history to be interpreted with respect to key life events. This powerful tool gives researchers the opportunity to explore the link between growth history, sex-change and their mating system in a detail not previously possible.

The mating system adopted in a given circumstance can be dependent upon the density of individuals that are potential members of a reproductive group. Monopolisation of resources by a small number of males may be difficult at high densities since interactions may be too frequent to allow a stable social group to form (Robertson and Hoffman 1977, Fricke 1980, Petersen 1990). In contrast, at low densities,

males may be able to visit females sufficiently often to reinforce a social hierarchy, suppress growth and monopolise environmental resources and females (Robertson 1972, Hattori 1991, Lorenzi et al 2006). It is predicted that growth of subordinate females should be associated with the strength of social control. Furthermore, at low densities the previous growth history of an individual should be less important in determining which females change sex, and its timing, because transition will be triggered by the relaxation of social control through the loss of a dominant male.

The present study compares the social organisation and sex-specific growth of two populations of a protogynous tropical wrasse, *Halichoeres miniatus,* which differ in density. The social organisation of the populations is first described by examining the space use and interaction regime of individuals within the groups. The presence of otolith checks associated with sex-change (Chapter 2) enables the examination of sex-specific growth in a detail not previously possible. It is predicted that differences in space use and interaction rates between populations will result in different relationships between growth and sex-change. Higher densities may lead to a breakdown in dominance suppression and may allow the individuality in growth to be expressed, leading to an association between growth history prior to sex-change and which females change sex. Prior growth history is predicted to be less important in influencing sex-change candidature in low density populations, where males can exercise strict dominance control of subordinate females.

4.3 Materials and methods

4.3.1 Study species and habitats

The small coral-reef wrasse *H. miniatus* is a common component of the Indo-Pacific fish fauna that inhabits the macroalgal zone and shallow reef flats. Males of this short-lived protogynous hermaphrodite are larger than females and display brightly coloured markings. A previous study of the species has shown that many females have a change in their pattern of increment deposition as they change sex into males (Chapter 2). This check is characterised by a change in optical density and increment width and has been experimentally validated as being associated with sex change (Chapter 3). The production of a check mark gives a powerful tool with which to examine the link between social conditions, growth and sex-change.

Study sites were located on the mid-shelf reef at Lizard Island (14°40'S 145°28'E) and the inner-shelf reef at Orpheus Island (18°37'S 146°29'E), both on the Great Barrier Reef (GBR), Australia. At Lizard Island the study population inhabited isolated patches of rubble and algae in shallow water separated by open sand flats. *H. miniatus* was common but not densely populated on the rubble patches with an average density of 0.12 individuals/m^2. In contrast, at Orpheus Island, the study site was part of a continuous macroalgal zone on shallow reef flat and *H. miniatus* was highly abundant in the area, with an average density of 0.45 individuals/m^2. Both sites were located at the leeward side of the fringing reefs.

4.3.2 Demography and social structures

Size and age distributions were constructed for *H. miniatus* at each location in order to determine if distributions reflected patterns characteristic of protogynous hermaphrodites. Fish were collected from five sites located haphazardly around each island (49 individuals from Lizard Island and 69 from Orpheus Island). Age was determined by counting the increments in the transverse sections through the nucleus of one sagittal otolith from each fish. Increments have been validated as daily (Chapter 2) and methodology followed that of Wilson and McCormick (1997). Sex for each individual was initially determined by the colour patterns (terminal or initial phase) and then by macroscopic examination of the gonads under a dissection microscope. Testes were identified by their smooth surface and cream colouration while ovaries were indicated by their yellow colouration and a rough surface texture, indicating the presence of developed eggs (Sadovy and Shapiro 1987).

To determine the social structure at each study site, behavioural observations were made on all males and the largest females in a group of fish from each location (4 females and 3 males at Lizard Island, 6 females and 7 males at Orpheus Island). To facilitate mapping the location and movement of individuals, areas were grided with nylon string at both study sites (15 x 25m at Lizard Island; 15 x 30m at Orpheus Island). Males and females within the grids were collected using hand nets and a dilute clove oil solution (Munday and Wilson 1997), transferred to a small clip-seal bag and measured with callipers (standard length (SL), mm). All the males and the largest females were tagged under the epidermis with a fluorescent elastomer (Northwest Marine

Technologies) using a 27 gauge hypodermic needle to facilitate individual identification. Upon recovery, fish were released at the point of capture.

Behavioural observations began the day after tagging and were made over three days for five hours per day. Male/female interactions (displays, chases, physical contact), feeding (strikes to the substrate), movement and spawning were recorded. Each observation period followed one individual for 15 minutes and all interactions were recorded during that time. Behaviour was compared between populations with a one-way MANOVA, with location and interaction rates used as the variables. The nature of significant differences in behaviour found by MANOVA was explored using t-tests.

Following the location of individuals in relation to the reference grid allow the production of maps of the areas used by tagged individuals. Territory sizes and the degree of overlap of territories for males and the largest females were measured and compared between the two sites using a one-way analysis of variance (ANOVA). Residual analysis was used to test whether data conformed to the assumptions of homogeneity of variance and normality.

4.3.3 Growth patterns

Microstructural increments on the sagittal otoliths were used to describe the growth history of the tagged fish. At the end of the observation period, fish were collected using a hand net and a dilute solution of clove oil again and euthanized by cold shock. Otoliths were removed, cleaned of endolymph tissue and stored dry. Cross-sections of the sagittal otoliths were prepared using the methods of Wilson and McCormick (1997). Digital images were taken at a 400x magnification and an image

analysis program (Optimus 6.5) was used to measure the distances between the daily otolith increments. The daily periodicity of increments has been validated in a previous study (Chapter 2). The short-lived, fast-growing nature of the species is a good indication that the increment widths can provide a reasonable estimate for relative daily growth (Shafer 2000). In this study otolith increment width profiles were used to describe the shapes of male and female growth trajectories and compare these between populations; no attempt was made to compare the magnitude of growth between populations since different relationships may exist between somatic growth and otolith growth between populations.

Increment width profiles were compared between sexes and locations using t-tests to look for differences between male and female increment widths at different stages. Any significant results were interpreted from increment graphs. Profiles were plotted using only ages where $n \geq 2$.

To explore whether there was an increase in increment width associated with sex change the otoliths of males were re-examined and increment widths were re-plotted so that they were centred on the check in the otolith associated with sex-change (see Chapter 2). This accentuates changes in otolith growth that occur at the time of sex-change and gets around the problem of masking through the averaging of increments widths of fish that undergo the transition at a variable age. Increment widths were compared from 20 days before and after the check using repeated measures MANOVA.

4.4 Results

4.4.1 Demography

A total of 69 fish were collected from Orpheus Island, 51 female and 18 male, and 49 fish from Lizard Island, 23 female and 26 male. The size and age and size-at-age distributions for both locations were characteristic of a protogynous species (Fig. 1), with no males in the smaller size and younger age classes. Examination of the gonads also revealed no initial phase males, suggesting that *H. miniatus* in these populations are monandric (i.e. males exclusively derived from females) protogynous hermaphrodites. Overall, there was more overlap in the age-frequency distributions of female and male age than size, suggesting that sex change is more closely linked to size than age. Males and females at Orpheus Island showed a greater overlap in size and age classes than at Lizard Island indicating greater variability in the size and age at sex change in the Orpheus Island population. The average male size at Orpheus Island was 65.6 mm SL and 71.1 mm SL at Lizard Island, the smallest males being 55.7 mm SL and 60.0 mm SL respectively. Average female sizes were 48.4 mm SL and 49.5 mm SL at Orpheus and Lizard Island, respectively. Several females were larger than the smallest males at Orpheus Island but males were always larger than females at Lizard Island. The average age of males at Orpheus Island was 200 days (youngest = 152 days) and at Lizard Island was 263 days (youngest = 196 days), while the average age of females was 178 days and 193 days at Orpheus and Lizard Island, respectively. At both locations no individuals were over 365 days indicating that, at these locations, *H. miniatus* is an annual species.

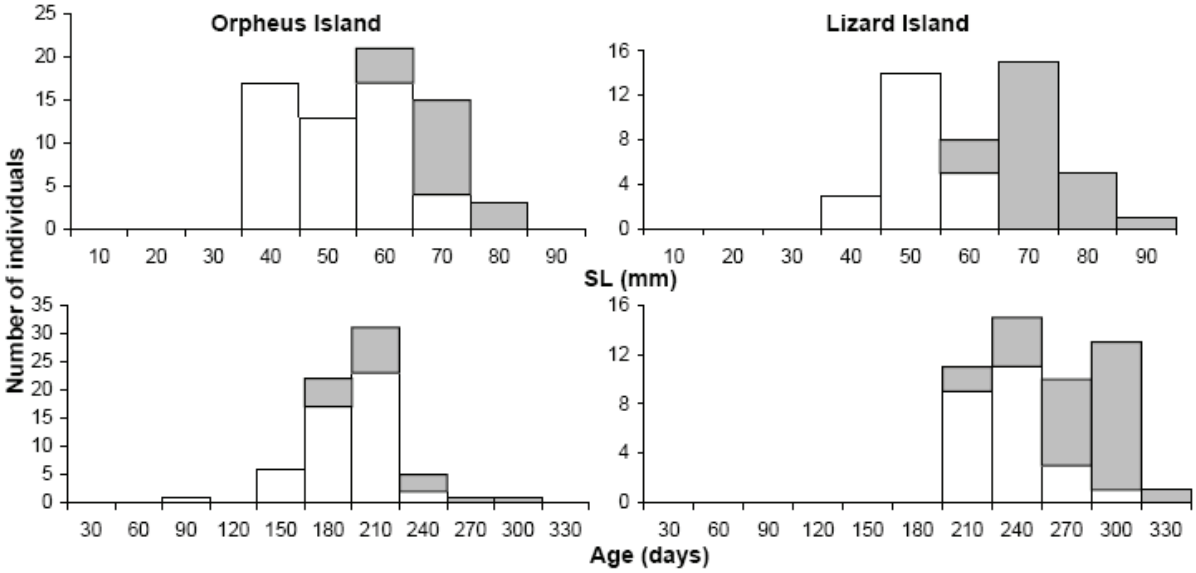

Figure 1: Size and age distributions for two populations of *H. miniatus,* at Lizard Island and Orpheus Island. Females are shown in white and males in gray.

4.4.2 Social structures

Females at Orpheus Island used larger areas on average than females at Lizard Island (134.4 m^2 and 13.2 m^2 respectively; t = -4.38, df = 27, $P < 0.0001$; Fig. 2). In contrast, males had similar mean territory sizes at both locations (69.8 m^2 at Orpheus Island and 92.8 m^2 at Lizard Island; t = 1.10, df = 18, $P = 0.286$; Fig. 2). Females at Orpheus Island were far less site attached than at Lizard Island and moved through multiple male territories on a regular basis. At Lizard Island, the females remained within their territories and were relatively isolated from similar-sized females. There was a large amount of overlap (average of 35.7%) between male territories at Orpheus Island and territory perimeters were not rigorously maintained. Male territories at Lizard Island had no overlap and perimeters were defended aggressively from other males, as was access to females within the territory.

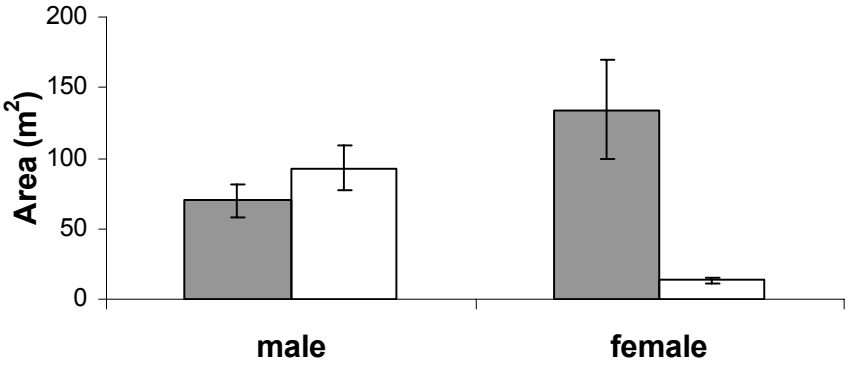

Figure 2: Comparison of average male and female territory sizes (+/- SE) between Lizard Island and Orpheus Island. Orpheus Island shown in white and Lizard Island in gray.

The behaviour of *H. miniatus* differed between the two locations (MANOVA: Pillai's trace = 0.699, df = 2, $P < 0.0001$). Male and female interaction rates differed between locations (Fig. 3). Males interacted with females 26-times more often at the Lizard Island site (0.713 interactions/min) compared to the Orpheus Island site (0.027 interactions /min; t = -1.984, df = 73, $P < 0.001$). The largest females interacted with other females six-times more often at the Lizard Island site than at the Orpheus Island site (0.219 interactions/min and 0.035 interactions/min respectively) but there was no significant difference between locations. There was higher male to male encounter rate at Orpheus Island than at Lizard Island (t = 4.04, df = 45, $P < 0.0001$; Fig. 3), and there were distinct differences in the male behaviour between locations. Male encounters were always highly aggressive at Lizard Island while at Orpheus Island encounters were characterised by displays and few chases or contact. There was no significant difference in the feeding rates of males and females between the two locations (two-way ANOVA:

F = 3.28, df = 1, 90; *P* = 0.073), with an average feeding rate of 1.81 bites/min for females and 0.979 bites/min for males.

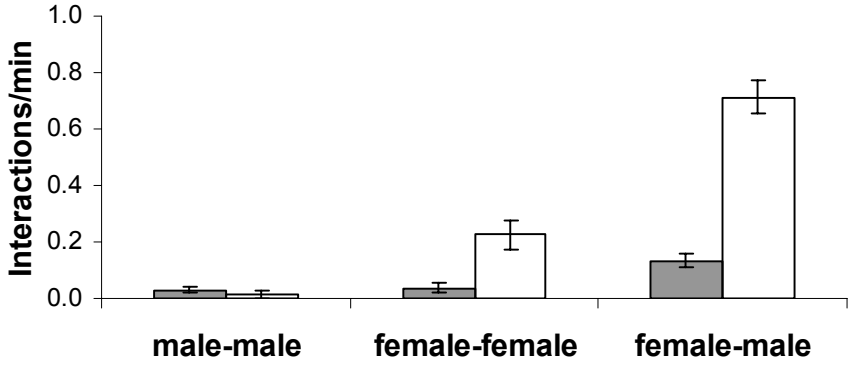

Figure 3: Comparison of the frequency of encounters per minute (+/- SE) for *H. miniatus* at Orpheus and Lizard Islands on the GBR. Orpheus Island shown in gray and Lizard Island in white.

4.4.3 Growth patterns

Comparisons of the growth estimates derived from the otolith increments revealed differences in the average daily increments between sexes, which differed between locations (Fig. 4). At the Orpheus Island site those females that had changed sex into males generally had wider increment widths than females that did not change sex (Fig. 4a). A t-test on the width of a larval stage increment (day four) showed that those females that changed into males had larger sagitta when that increment was laid down compared to females that did not change sex later in life (t = 2.48, df = 66, p = 0.016). When the same data were plotted as cumulative increment widths it is evident that the small differences accumulate into a divergence of otolith growth trajectories between males and females (Fig. 5a). A significant difference was found between male and female increment

sizes at 100 days ($t = 3.106$, $df = 65$, $P = 0.003$). In contrast, at Lizard Island males and females displayed similar increment width profiles until later in life (~220 days), when those females that changed sex showed an increase in increment widths compared to the non sex-changing females (Fig. 4b). The cumulative growth trajectories for males and females at Lizard Island were almost identical until after about 200 days when the female plot began to asymptote (Fig. 5b).

By focusing on the period when sex-change occurred and centring the increment width profiles on the check associated with sex-change, the increase in increment widths later in life was shown to be tied to sex-change (Fig. 6). In males from the Lizard Island site (Fig. 6b), increments widths rapidly increase in association with sex-change (repeated measures ANOVA: $F_{(26, 13)} = 21.867$, $p < 0.0001$). Although this increased otolith growth is still evident in the Orpheus Island population, it is not as dramatic (repeated measures ANOVA: $F_{(18, 21)} = 5.8937$, $p = 0.0001$, Fig. 6). The age of check mark occurrence also differed between the two sites (Fig. 7). The average age of check occurrence at Orpheus Island was 158 days and at Lizard Island was 209 days. The age distributions of the check marks were also skewed in the opposite direction; the Lizard Island population exhibited a negative skew, while the Orpheus Island population displayed a positive skew.

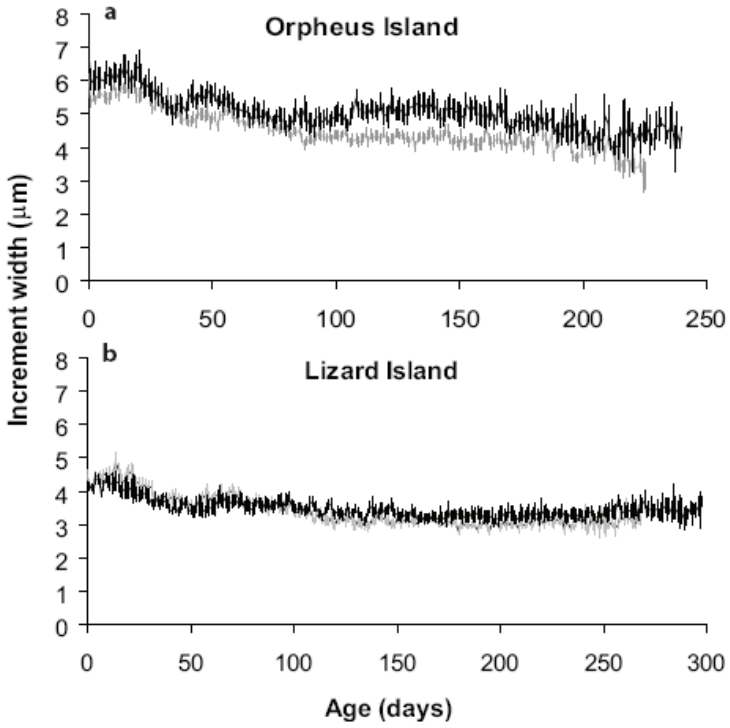

Figure 4: Mean (+/- SE) daily increment widths of *H. miniatus* males and females collected from (a) Orpheus Island (b) and Lizard Island. Females shown in gray and males in black.

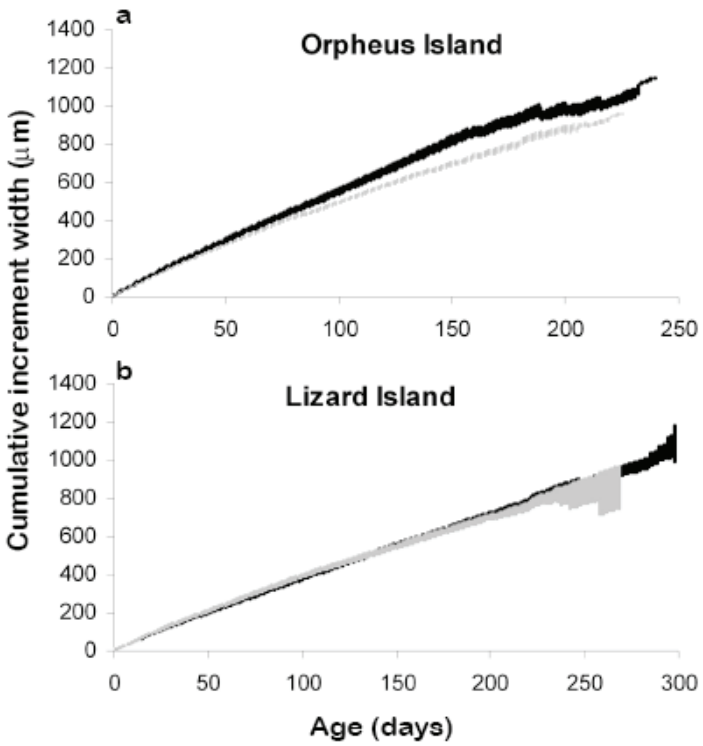

Figure 5: Mean (+/- SE) cumulative increment widths of *H. miniatus* male and females collected from (a) Orpheus Island and (b) Lizard Island. Females shown in gray and males in black.

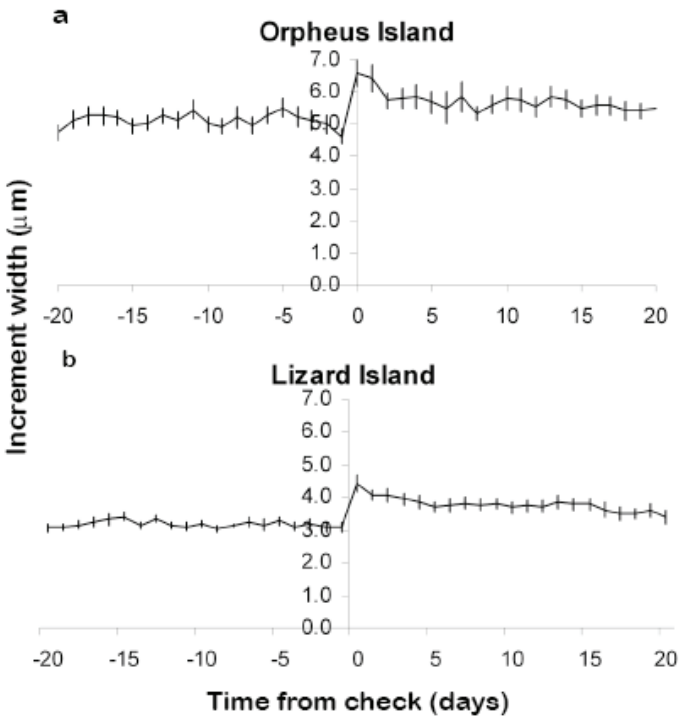

Figure 6: Mean (+/- SE) otolith increment profiles of males centred on the check-mark associated with sexual transition for fish collected from (a) Orpheus Island and (b) and Lizard Island.

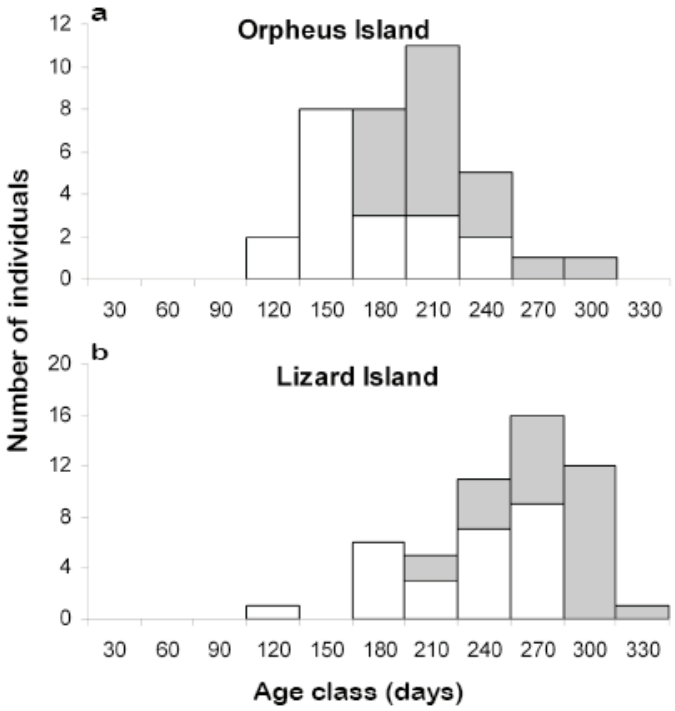

Figure 7: The age distribution of the check marks associated with sex change compared to the male age distributions from (a) Orpheus Island and (b) Lizard Island. Male age shown in gray and check age in white.

4.5 Discussion

In tropical fishes, social organisation and mating systems are influenced by the physical and behavioural environment the group experiences (Petersen 1990, Gust et al 2002). As the patchiness of resources and the composition of the interacting community changes, the intraspecific interactions, sex-ratios, size ratios of the sexes, spawning strategies and the social organisation of groups can also change, within phylogenetically determined limits (Warner and Hoffman 1980a, Warner 1984, Shapiro 1984, Lutenesky 1994, Munday et al. 2006b). The present study describes the occurrence of two very

different social organisations in the same species and their consequences for growth and sex-change. At the densely populated Orpheus Island site, female *H. miniatus* roamed throughout multiple male territories and encountered multiple males and females on a regular basis. Males maintained loosely defined territories with extensive overlap with other male territories and encounters were not overly aggressive. This social system may be closer to a loose form of resource defence polygamy than a strongly haremic social organisation. In contrast, the females at the low density Lizard Island site maintained small territories within large male territories and defended them from other females. Females did not cross between male territories and only encountered a single male on a regular basis. Males at Lizard Island actively defended large territories encompassing multiple females. There was no overlap between male territories and male encounters were always highly aggressive. These observations suggest a strictly haremic society (i.e. female defence polygyny) with strong internal control of space use. The finding of different levels of social control of females within the two study sites, leads to the prediction that the link between growth and sex-change should also differ.

Differences in social organisation with density and habitat have been found in other studies. The density of the population affects the daily intraspecific interactions of the group (Fricke 1980, Lutnesky 1994). The higher the density, the more frequently individuals encounter one another and the more difficult it is for dominant individuals to maintain control over access to spawning partners (Warner and Hoffman 1980b). Depending on the environmental conditions multiple male spawning tactics can evolve within the same species (Mank and Avise 2006). Many wrasse species within the genus *Thalassoma*, for example, have two general male spawning modes: (a) males remain in

the initial phase (similar in size and colouration to females) and participate in mass spawning aggregations and (b) larger brightly-coloured terminal phase males defend temporary spawning sites to which females are attracted and perform pair spawns (Roberston and Hoffman 1977, Warner 1982). Differences between environments, high to low density and continuous to patchy substrate, determine which mode will be more effective and control the distributions of initial and terminal phase males within the population (Warner and Hoffman 1980a, b, Warner 1982, Munday et al. 2006b).

Density can operate through changes in social organisation to influence the relationship of growth history and the occurrence, timing and speed of sex-change. A recent study comparing 4 species of wrasse that exhibit different social systems illustrates that the relationship between growth and sex change can vary with the strength of the social control (Chapter 2); the stricter the social scheme, the less influence of early growth history on sexual size dimorphism and which individuals change sex. The present study highlights that the same pattern can occur within an individual species when groups are exposed to different environmental and behavioural regimes. The population at Orpheus Island had a much more loosely organized social structure and the males showed increased early growth compared to females, while the study population on Lizard Island showed little difference between the male and female growth rates until later in life.

The present study elucidates the mechanism by which females that change sex quickly become larger than non sex-changing females of similar age to give the sexual dimorphism that is typically found in protogynous hermaphrodites. Detailed growth information from otoliths suggests that sex-changing females rapidly increase their growth at the time of sex change and maintain an elevated growth rate compared to non

sex-changing females for some months afterwards. This shows that for both high and low density populations, growth spurts that coincide with sexual transition are integral to the production and maintenance of sexual dimorphism. These findings are similar to a number of recent studies of tropical protogynous sandperch (Pinguipedidae) that show growth spurts coincident with sex-change (Walker and McCormick 2004, unpublished data, Walker et al. 2007).

This rapid increase in growth around sex-change was not the only mechanism that resulted in larger male size. The growth trajectory of sex-changing females at the high density Orpheus Island site was consistently higher throughout most the fish's life prior to sex-change. At this site, it appears that those individuals that started off their larval life with larger sagittal otoliths maintained a growth advantage and were those individuals that changed sex. The general link between otolith and somatic growth (e.g. Gagliano et al. 2007; made more complex through its link to metabolism, Armstrong et al. 2004) suggests that this result can be cautiously interpreted with respect to somatic growth (Schafer 2000, Walker and McCormick 2004, Walker et al. 2007); those individuals that changed sex may have been those individuals that were slightly larger at hatching (when the first otolith increment is often deposited), and went on to be larger for a given age at the time of sex-change. The history of higher growth, combined with an acceleration in growth at the time of sex reversal, provide the mechanisms by which males eventually become the largest individuals in the higher density population.

A similar relationship between otolith increment size and the likelihood of sex change was not evident in the low density population, with no difference in growth trajectories between sex-changing and non sex-changing females prior to sex change.

Only at the time of sex change did the changing females display an increase in growth rate over other members of the population. The acceleration in growth at sex change in the low density population was more dramatic than in the high density population, which might be due to the release from a stronger degree of social growth suppression in the low density population. Recently, an experiment which manipulated the harem size in a protogynous sandperch, *Parapercis cylindrica*, found a similar increase in the magnitude of the growth spurt associated with sex change as the number of interacting females increased (Walker and McCormick unpublished data). The difference in the importance of growth history to sex-change candidature in both these studies may be related to the social systems in which the fish live. In the low density, strictly haremic social groups, growth and sex change in subordinate individuals is suppressed by dominant individuals and growth in potentially fast growing individuals is stifled. In contrast, the high density, loosely organized social system provided less growth suppression and greater opportunity for females to grow closer to their intrinsic potential growth rate.

In the present study I have demonstrated that sexual dimorphism can arise through differential growth by several mechanisms: a growth spurt coincident with sex-change; accumulation of a historical growth advantage for sex-changing individuals; or a combination of both. I suggest that the social organisation of a group will determine the relative importance of previous growth history, and that growth advantages may only be realised when social control is relaxed; as shown at high population densities. The initial growth advantages evidenced here may be due to genetic or non-gentic parental (particularly maternal) effects, which have recently been shown to carry over into the juvenile phase to influence growth and survival (Green and McCormick 2005, Gagliano

et al. 2007). My findings suggest that I should be targeting the level of individual groups if I am to obtain a detailed understanding of the link between physical and behavioural environments, growth history and sex-change.

Chapter 5: General discussion

Sexual size dimorphism in animal species has been demonstrated to be a result of differences in the reproductive advantage of body size between the sexes (Ghiselin 1969, Kueller and Reeve 1994). In most species of vertebrate size differences are an intrinsic feature of their biology; the differences in male and female growth patterns are fundamental aspects of development and strongly influenced by their genotype. However, there are many species that can change sex during their life (Policansky 1982) and it is likely that the mechanisms by which sexual dimorphisms are produced in these sequential hermaphrodites are very different. In these organisms, sexual dimorphism must be the product of either an advantageous growth history for those individuals that change sex (thereby making them larger than non sex changers), a result of a growth spurt at sex change, or a combination of the two growth scenarios. Since growth has been shown to be dependent upon population density and is often influenced by social status and societal type (Buston 2003, Lorenzi et al. 2006, Wong et al. 2007), I predicted that there should be an association between the strength of social control within a population of sex-changing individuals and the growth mechanism responsible for the sexual dimorphism. Coral reef fishes were chosen as an ideal group to explore this prediction because they are one of the few groups that commonly undergo sex change and they also exhibit a large diversity of social systems (Shapiro 1987, Ross 1990, Munday et al. 2006a).

Previous studies have recognized three possible, potentially inclusive, hypotheses explaining how males become the largest individuals in populations of protogynous reef fish, an acceleration in growth at the time of sex change (i.e. a growth spurt), faster

growth rates in eventual sex changers after female maturation, and initially larger size or growth rates in eventual sex changers from hatching through the juvenile stage (Walker and McCormick 2004). The first idea has been explored previously but the other two ideas have received little attention. The findings from this study provide evidence for both of the two latter cases as well as a cumulative effect of growth differences throughout life.

Through the detailed examination of the growth history of 4 species of annual wrasse this study found different patterns of growth lead to sexual dimorphisms among species and within a single species between populations. These differences in growth patterns appeared to be related to the differences in social systems found within the study populations. The two species with the strongest social control, *Coris batuensis* and *Stethojulis strigiventer*, exhibited a marked growth spurt at sex change and there was no evidence of growth differences between sex-changing and non-sex-changing individuals in their growth histories prior to sex-change. In the species that had the weakest social organisation, *Cirrilabrus exquisitus*, prior growth history appeared to play a major role in influencing not only how sexual size dimorphism was manifest, but also appeared to determine which individuals would change sex to males. Meanwhile, in the species that had an intermediate strength of social organisation, *Halichoeres miniatus*, both a growth spurt and an advantageous growth history played a role in producing the sexual size dimorphism that was evident in the species.

Interestingly, it appears that there is plasticity in the determinants of sex-change candidature since even within a species (*H. miniatus*) there can be differences in growth patterns between populations displaying different social organisations. Once again, in

populations that displayed strong social control, sexual size dimorphism was the result of a growth spurt that coincided with sex change. In contrast, sexual size dimorphism in a high density population with less social control was a product of both previous growth history and a growth spurt.

In two of the four species, eventual sex-changers could be predicted from earlier growth. In three of the four species there was an initially larger otolith size which, using otolith size as an indication of body size, means that initial size or growth rates could predict which females could change to male. Larger initial size in sex-changing individuals has been demonstrated recently in the reef fish *P. snyderi* (Walker et al. 2007) and this initial size or growth advantage in certain individuals may be a result of differences in the condition of the mother or may be traits passed on through the parents. This size advantage appears to carry over to later life stages increasing the likelihood that those individuals will change sex sooner than other similarly aged fish.

Wrasses and other tropical reef fish display a much wider variety of social systems than those looked at in this study. Examining the effects of the a broader range of systems would give a greater understanding on how much influence the social organization of the group can have on individual growth. In one of the study species two different systems were observed under different conditions. Further investigation of the interactions between environmental conditions such as density, habitat type, and resource competition may reveal what influences the different social structures and growth patterns which result in selection for sex change and larger size in males. Wrasses and other tropical reef fish similar to the species studied here provide a good opportunity to experimentally manipulate various groups and observe variations in growth patterns

under different conditions. By changing the densities of different populations of the same species for example, we may observe different social systems arising within the groups which could in turn result in different growth patterns of individuals. Variation in individual size has also been shown to possibly be linked to population density (e.g. Gust et al 2002). In this case, variable density in manipulated populations could directly affect changes in individual growth regardless of the social structure resulting in variations in sex change candidature.

Although this study has shown the potential for social control of differences in sex-specific growth and the possible influences of early stage growth on which individuals in a group change sex, only one species was looked at in detail. Further experimental manipulations of changes in social structures within species would allow for a greater understanding of specific mechanisms behind variable male and female growth patterns. Otoliths may be able to reveal individual growth trajectories, however they merely provide estimates. Many reef fish such as gobies are remarkably short-lived (eg. Depczynski and Bellwood 2005, 2006) and some can change sex multiple times throughout their life (St Mary 1993, Nakashima et al. 1995, Munday 2002). Such short-lived species that can change sex multiple times would allow for observations on how multiple changes to social systems affect populations of sequential hermaphrodites. This also could provide an exceptional opportunity for manipulating social structures and observing patterns of growth and sex change under different conditions throughout the duration of life. By using such short-lived species one could follow growth throughout the duration of life and under variable social conditions within a reasonable time period. The finding of a check mark associated with sex change also provides a useful tool for

the analysis of sex specific growth history through the otoliths of reef fish and this may prove useful in longer lived fishes. However, it is unlikely that check marks associated with sex change will be found in fish with longevities beyond 2 years as the compression of the increments makes resolution of a check mark difficult (Walker and McCormick 2004).

Understanding the mechanisms behind sexual size dimorphism and sex change patterns is essential to understanding population dynamics, especially in sequentially hermaphroditic reef fish. Such information may aid our prediction of how protogynous tropical fishes of commercial importance may respond to sex- and size-selective fishing pressures and the impacts of marine protected areas on fish populations. The reproductive output of a population is a combined result of individual size, life history, mating patterns, social behaviour, individual fertility as well as other factors and these all need to be incorporated in the management of a species (Alonzo and Mangel 2004). In areas of high fishing pressure there can be a decrease in large individuals which leads to a smaller average size of sex change and reduces the proportion of terminal phase males in populations of protogynous hermaphrodites (Hawkins and Roberts 2003). Without an understanding of the demography of a species, management regulations are placed on the species which do not protect the breeding populations (Pears et al 2006). By recognizing how social systems influence growth and sex determination one may be able to predict how an individual will develop given the social organisation in which they are found and how changes to the population will affect individual development and overall reproductive output. While providing substantial evidence for the social mediation of variable growth patterns, this study by no means delivers a comprehensive understanding

of how males become the largest individuals in protogynous species. Further studies incorporating a broader range of species and social organizations and integrating other environmental factors are necessary to be able to formulate predictions on the proximate factors that determine which individuals change sex in protogynous fish populations.

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