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Rapid larval growth predisposes sex change and sexual size dimorphism in a protogynous hermaphrodite, *Parapercis snyderi* Jordan & Starks 1905

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The temporal relationship between growth history, sex-specific growth divergence and sex change was investigated in the haremic sandperch *Parapercis snyderi* using otolith microstructure and gonad histology. *Parapercis synderi* was found to display rapid near-linear growth with a maximum longevity of 303 days. All individuals matured first as female and later changed sex to become male (monandric protogynous hermaphroditism). Individual age-based growth histories obtained from otolith increment widths illustrated that males were larger than females at any given age. Males were found to diverge from the female growth trajectory during two ontogenetic periods; during the larval period and during the period that sex change took place. In addition, male otoliths contained a discontinuity, or 'check mark', associated with the rapid increase in otolith growth during the sex-change period. This microstructural feature was absent from all female otoliths. Accelerated growth in male otoliths lasted up to 25 days, following check-mark formation, after which time otolith growth returned to the pre-check-mark rate. Given the isometric relationship between otolith and somatic growth in *P. synderi*, and the temporal relationship between the time of check-mark formation and gonad condition, these results strongly suggest that individuals accelerate somatic growth during sex change to become the largest members of the population. Moreover, evidence suggests that the factors that determine the initial growth of larvae influence which individuals will later become males and achieve the highest reproductive success.

Key words: growth acceleration; otolith; otolith check; *Parapercis snyderi*; protogyny; sex change.

INTRODUCTION

Protogynous hermaphroditism (the sequential change in sexual function from female to male) is a reproductive strategy found in at least 12 families of tropical reef fishes, including the numerically dominant Labridae, Scaridae,

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Serranidae, Pomacanthidae and Acanthuridae. It is now widely accepted that protogynous hermaphroditism is an evolutionary consequence of the polygynous mating system, in which relatively large dominant males monopolize mating opportunities and shunt subordinate males from the breeding population (Warner, 1984, 1988; Munday *et al.*, 2006). It addition to protogyny, the polygynous mating system promotes the evolution of sexual size dimorphism (SSD), where males are larger than females at a sexual maturity (Wade & Shuster, 2004). This may be explained by the advantage of large body size in competing for extra-pair mating. Determining when SSD takes place in protogynous reef fishes is of significant importance, given that some (diandry) or all (monandry) individuals begin life as female, and that the size and age of sex change can show dramatic variability at both the population and species level (Garratt *et al.*, 1993; Adams & Williams, 2001; Munday *et al.*, 2004, 2006; Walker & McCormick, 2004). In addition, understanding the temporal relationship between sex change and SSD provides information on the proximate factors driving SSD, and thus the evolutionary context in which SSD is manifest. Studies to date suggest that the relationship between sex change and growth divergence may depend strongly on social dynamics, such that a universal pattern among species is unlikely (Adams & Williams, 2001; Munday *et al.*, 2004; Walker & McCormick, 2004).

Changes in social behaviour and growth dynamics are difficult to quantify without observing a group of fishes throughout the duration of its life. Fortunately, otoliths can provide a temporal record of life-history events through the formation of checks or discontinuities in the otolith microstructure (Wilson & McCormick, 1999; Walker & McCormick, 2004). Once the relationship between otolith and somatic growth is established, the identification of life-history events that produce checks facilitates the examination of the link between previous and subsequent growth and life-history milestones, such as metamorphosis, maturation and sex change.

The present study explores the temporal relationship between previous growth history, sex-specific growth divergence and sex change in the tropical sand perch *Parapercis snyderi* Jordan & Starks, 1905 (Pinguipedidae). *Parapercis snyderi* is a short-lived monandric hermaphrodite that lives in permanent haremic groups among coral rubble substratum throughout the Indo-Pacific (Nakazono *et al.*, 1985). In hierarchical species in which the dominant breeding status is based on relative body size, it is predicted that SSD should result from accelerated growth during sex change, due to the relaxation of growth-inhibition following motility of the dominant individual (Buston, 2003; Walker & McCormick, 2004). The primary focus of the present study was to test this hypothesis. By reconstructing individual daily growth histories from otoliths and relating this information to gonad ontogeny, it was possible to discern whether an otolith check mark occurs during sex change. This enabled an assessment of whether greater somatic growth is the cause or affect of sex change and whether faster growth is characteristic of the male tactic in general. Furthermore, the description of individual daily growth allowed the exploration of whether previous growth history influences which individuals undergo sex change to become the dominant males.

MATERIALS AND METHODS

STUDY SPECIES AND COLLECTION

Parapercis snyderi is a 'common site-attached' reef fish inhabiting the edges of patch reefs and rubble substratum at 5–20 m throughout the Indo-Pacific (Randall *et al.*, 1997). Females form contiguous territories and males defend a territory incorporating several females, forming harems (Nakazono *et al.*, 1985). All individuals begin life as female and latter change sex to male with sex change being socially mediated (Nakazono *et al.*, 1985).

Specimens of the entire size range available were collected from the lagoon of Lizard Island, Great Barrier Reef, Australia (14°40·9′ S; 145°26·8′ E), using hand-nets and a solution of clove oil anaesthetic. Individuals were killed by cold shock, their total length recorded (±0·01 mm, L_T), and the sagittal otoliths removed, cleaned of endolymph tissue and stored dry. Gonads were removed and preserved in a formalin-acetic acid-calcium chloride solution for at least 1 week prior to processing for histological examination (Winsor, 1994).

SEX AND GROWTH

Sex was determined through examination of the gonad microstructure. Gonads were embedded in paraffin wax blocks, transversely sectioned at 5 μm and stained with Mayer's haematoxylin and Young's eosin-erythrosin (Winsor, 1994). Under a high-powered microscope, individuals were classified as female, transitional or male based on the presence of characteristic sex cells (Sadovy & Shapiro, 1987; Patiño & Takashima, 1995).

Age-based growth trajectories were constructed following the methods of Wilson & McCormick (1997). First, sagittal otoliths were sectioned such that increments could be observed from the nucleus to the otolith margin. Then, calibrated digital images of the otoliths were taken at a ×400 magnification from a high-powered microscope using polarized transmitted light. Individual increment width profiles were measured along the maximum otolith radius (R_O) from the digital images using the image analysis programme Optimus. It is assumed that increments are laid on a daily basis. This seems a reasonable assumption given the lack of studies documenting non-daily formation of increments and that similar age estimates to the present species have been found for a congeneric whose otolith increments have been validated as daily (Walker & McCormick, 2004). Average daily increment width profiles were plotted against age (increment number) for females and males to illustrate sex-specific age-based growth and to determine periods in which female and male growth diverges. Periods of growth divergence were analysed for statistical significance using repeated-measures ANOVA ($\alpha = 0{\cdot}05$). Age-based occurrence-frequency distributions for males and females were also generated to establish the age range of sex change.

To determine whether the process of sex change affects otolith accretion, otoliths were examined for abrupt discontinuities, or check marks, within the age range that sex change takes place. In otoliths where check marks were observed (all male otoliths), increment widths were compared before and after the check mark using the 'transition-centred' method described by Wilson & McCormick (1997). Average increment widths before and after the sex change-associated check mark were compared using a repeated-measures ANOVA. Average increment widths of females after the minimum age of sex change (85 days) were also compared to males after sex change using one-way ANOVA ($\alpha = 0{\cdot}05$). This was carried out to determine whether the observed shift in otolith growth is sex- or age-based.

The robustness of somatic-otolith scaling to variation in growth rate was tested following the procedure of Hare & Cowen (1995). Linear regression models were used to describe the relationships of R_O and age (A) with L_T. Heteroscedasticity was reduced by ln transformation of independent and dependant variables. Residuals from the

relationship between ln R_O on ln L_T (somatic-otolith scaling) and ln A on ln L_T (growth rate) were then compared using regression analysis ($\alpha = 0{\cdot}05$). If the relationship between somatic and otolith growth is unaffected by growth rate, then the standardized residuals from the two models should display no correlation. Prior to all statistical analysis, the assumptions of normality and homogeneity of variance were examined using residual analysis.

RESULTS

SIZE AND LONGEVITY

Eighty-one *P. snyderi* were collected and processed, comprising 62 females, 17 males and two transitional individuals. Male size ranged from 3·2 to 6·7 g and from 42·1 to 90·1 mm L_T. Females ranged from 0·1 to 4·7 g and from 22·1 to 78·4 mm L_T. Ages ranged from 71 to 260 days for females and 142 to 303 days for males. No males were found in age classes <121 days and females were most abundant in the age classes from 61 to 150 days [Fig. 1(a)]. No males were found in size classes <40 mm L_T and females were most common in size classes from 45 to 65 mm L_T with only males in the largest size class [Fig. 1(b)]. The gonads of two individuals were found to be in the process of sex change and contained an ovarian lumen, degenerating oocytes and

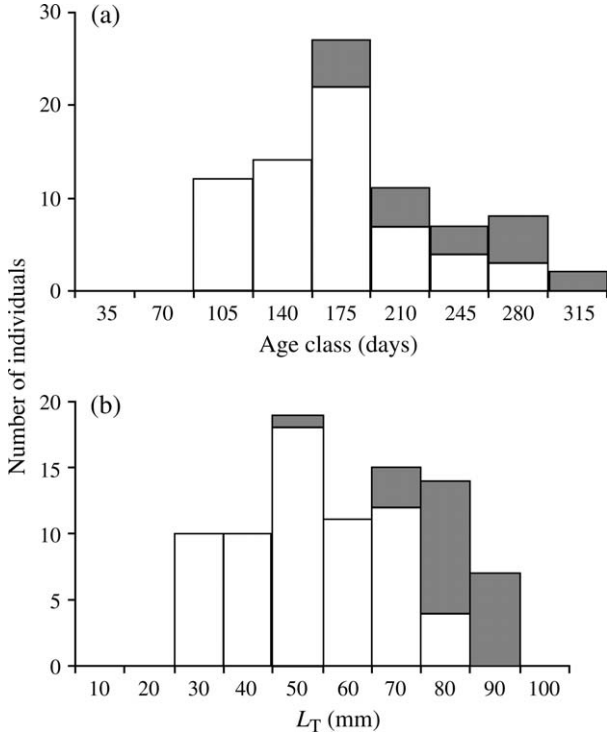

FIG. 1. Age (a) and (b) total length (L_T) frequency distributions of male (■; $n = 17$) and female (□; $n = 62$) *Parapercis snyderi* from Lizard Island, Great Barrier Reef.

proliferating testicular tissue. Furthermore, male gonads contained vestiges of ovarian tissue indicating that they were derived from females (Sadovy & Shapiro, 1987).

OTOLITH–SOMATIC SCALING

The relationship between otolith growth and somatic growth was near-consistent for varying rates of somatic growth. A very weak correlation was found between the standardized residuals of the otolith–somatic relationship ($\ln R_O$ and $\ln L_T$; the amount of otolith growth per unit of somatic growth) and the standardized residuals of somatic growth rate ($\ln A$ and $\ln L_T$) (linear regression, $n = 81$, $r^2 = 0{\cdot}076$, $P < 0{\cdot}05$). This illustrates that for varying somatic growth rates, the relationship between otolith and somatic growth remains isometric and validates the use of otolith increment widths as an age-based proxy for somatic growth in *P. snyderi*.

SEX CHANGE-ASSOCIATED CHECK MARKS

In male otoliths, a check mark was identified within the age range that the female and male age-frequency distributions overlap (Fig. 2). This check mark was not found in female otoliths. Furthermore, high congruency was observed between the age-frequency distribution for males and the check-mark frequency distribution among males (Fig. 3). These results suggest that check marks are formed during the course of sex change. The time lag between the check mark and male age distributions suggests that the check mark may be formed at the initiation of sex change. This is supported by the examination of the two fish that possessed transitional gonads. In both individuals, there was a check mark <20 days from the margin of the otolith.

The average otolith increment width significantly increased following check-mark formation (repeated-measures ANOVA, d.f. = 1, 40, $P < 0{\cdot}05$), illustrating a marked and rapid increase in otolith growth during sex change. Since individuals change sex at varying ages, this shift in otolith growth is best

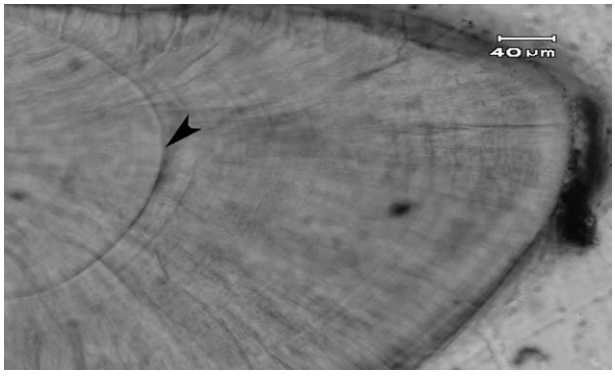

FIG. 2. Otolith section showing a check mark at the suggested time of sex change in a male *Parapercis snyderi* (×400 magnification).

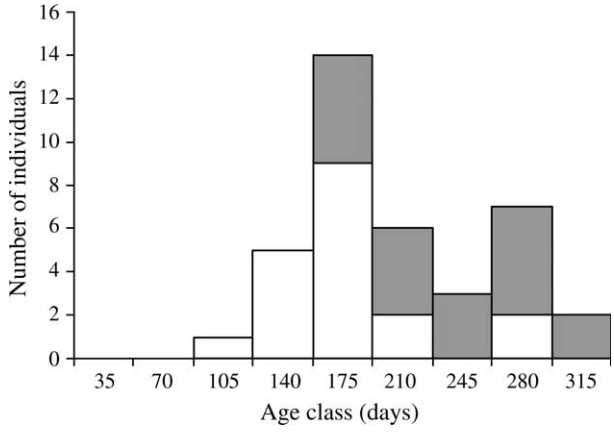

FIG. 3. Frequency distributions of age (■; $n = 17$) and sex change-associated check marks (□; $n = 17$) in male *Parapercis snyderi*.

illustrated when the increment width profiles are centred on the check mark (Fig. 4). Enhanced otolith growth following check-mark formation is maintained for *c.* 25 days, after which time otolith growth declines to nearly the pre-check-mark rate.

SEX-RELATED GROWTH

The sex-specific size-at-age plot in Fig. 5(a) illustrates that males are larger than females for any given age. Only by comparing the otolith increment widths, however, is it possible to discern when this growth divergence takes place, since all males are derived from females. The increment-width profiles illustrate that there are two periods in which male growth exceeded that of females; the first is an initial period during the larval phase (*c.* 20 days), and the second is the period within the age range that sex change takes place. This suggests that larvae of individuals that later became males may have been

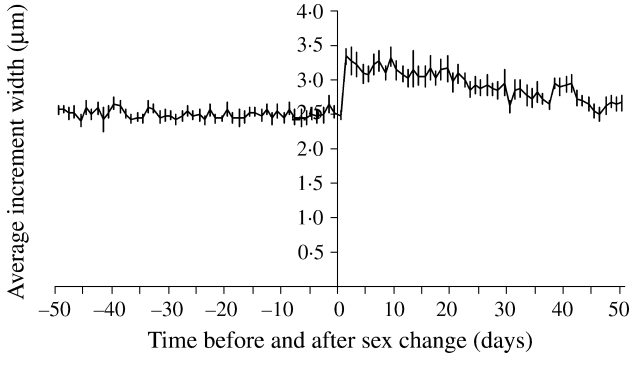

FIG. 4. Mean ± S.E. daily otolith increment width in male *Parapercis snyderi* ($n = 17$) before and after check-mark formation.

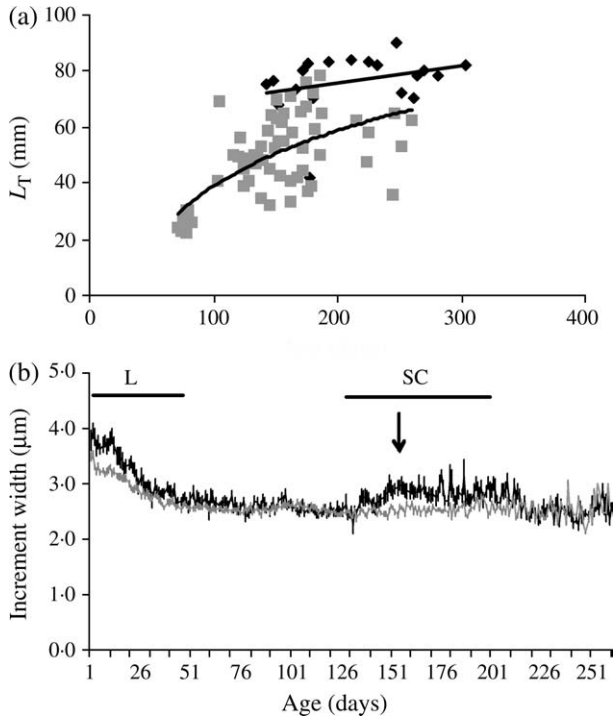

FIG. 5. (a) Size-at-age plots for male (◆; $n = 17$) and female (■; $n = 62$) *Parapercis snyderi* [male: $y = 0{\cdot}062x + 63{\cdot}263$; $r^2 = 0{\cdot}631$ and female: $y = 28{\cdot}584 \ln(x) - 92{\cdot}663$; $r^2 = 0{\cdot}416$]. (b) Mean ± S.E. daily otolith increment width of male (——; $n = 17$) and female (——; $n = 62$) *P. snyderi*. L, the larval stage; SC, the age range of sex-change associated check-mark occurrence in males (mean ± S.E. = 161·8 ± 41·3 days).

larger as late-stage larvae than those that remained females. The period of enhanced growth within the age range of sex change was that associated with check-mark formation. Within the age range that all individuals function as female, male and female increment widths were statistically indistinguishable (repeated-measures ANOVA, d.f. = 1, 66, $P > 0{\cdot}05$). Within the age range that individuals change sex, however, males displayed significantly greater otolith growth compared to females (repeated-measures ANOVA, d.f. = 1, 38, $P < 0{\cdot}05$; Fig. 6). In addition, otolith growth during the larval phase was statistically greater in males compared to females (repeated-measures ANOVA, d.f. = 1, 79, $P < 0{\cdot}05$).

DISCUSSION

The majority of polygynous reef fishes are SSD. The processes driving the timing of SSD in these animals are poorly understood due to their complex sexual ontogenies that frequently involve sex change from female to male (sequential protogynous hermaphroditism). The focus of this study was to investigate the temporal relationship between sex change and SSD in the haremic *P. snyderi*. Relatively large male body size was found to be manifest

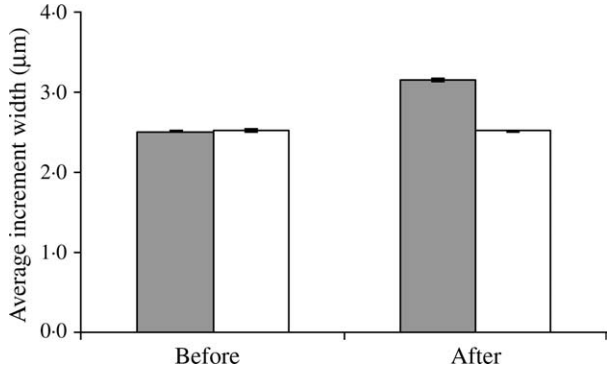

FIG. 6. Mean ± S.E. daily otolith increment width for male (■; $n = 17$) and female (□; $n = 62$) *Parapercis snyderi* 20 days before and after the estimated time of sex change.

during the course of sex change through a period of accelerated growth. The acceleration in growth appears to last throughout the duration of the sex-change process (*c.* 25 days) but declines shortly afterwards. Sex change in the closely related *Parapercis cylindrica* (Bloch, 1792) occurs within a relatively short time period (*c.* 20 days; Walker & McCormick, 2004) and this is most likely also true for the present study species. This would indicate that the increase in growth during sex change is due to the relaxation of growth inhibition in subordinate females by the dominant male. Backcalculated growth trajectories from the otoliths of females and males illustrated that growth is similar among individuals throughout the period in which all individuals function as female. It is, therefore, likely that growth during the female stage does not play a significant role in determining which individuals change sex or the age of sex change. It is likely that sex-change candidature is related to dominance status whereby the effect of body size is relative, as was demonstrated for the congeneric *P. cylindrica* (Walker & McCormick, 2004). However, it is interesting to note the unique finding that males also displayed greater otolith growth during the larval phase (0–30 days) compared to females. This suggests a follow-on affect from early growth performance; it is possible that the largest individuals at settlement secure dominance within the cohort, and maintain that dominance throughout the female ontogenetic period. This would precipitate a higher chance of becoming the dominant individual of the social group and eventually changing sex to become male.

Research to date suggests that social structure is a major determinant of when SSD takes place in relation to sex change. The period of accelerated growth during sex change found in *P. snyderi* is typical of the trend found for haremic sequentially hermaphroditic species studied to date. These include the protogynous wrasse *Thalassoma duperrey* (Quoy & Gaimard, 1824) (Ross, 1987), the protandric clownfish *Amphiprion percula* (Lacepède, 1802) (Buston, 2003) and the protogynous *P. cylindrica* (Walker & McCormick, 2004). In these species, dominance hierarchies are formed and growth is suppressed by the dominant through direct aggressive interactions, such as chases or physical contact. When the male is removed or displaced, the largest female is induced to change sex and growth inhibition is relaxed. In species where dominance

hierarchies are absent or temporary, variance in growth performance between individuals may occur earlier in life due to a lack of growth inhibition. SSD may therefore be realized before sex change, where the largest females are the ones to change sex. This pattern has been suggested for the serranids *Plectropomus maculatus* (Bloch, 1790) (Adams & Williams, 2001), *Plectropomus leopardus* (Lacepède, 1802) (Ferreira, 1995) and *Epinephelus rivulatus* (Valenciennes, 1830) (Mackie, 2003) and several parrotfishes (Munday *et al.*, 2004).

The presence of check marks associated with sex change greatly enhances the ability to relate growth to sex change. Sex change-associated checks were first noted in otoliths of male *P. cylindrica* (Walker & McCormick, 2004), and similar check marks were found in the otolith of the congeneric, *P. snyderi*, in the present study. Several pieces of evidence suggest that the check marks in *P. snyderi* are associated with sex change. Firstly, check marks were present in all male otoliths but absent in female otoliths within the age range that sex change takes place. Secondly, the check-mark frequency distribution coincided with the age range that males enter the population. Lastly, the two males that were found to have deposited a check mark <20 days prior to capture were still in the process of sex change.

One of the most exciting findings of the study was that males had higher otolith growth during the first 2 weeks of larval life. There are two possible explanations for this result. Firstly, since males tended to be older, and hence had on average earlier hatch dates than females, the difference in otolith increment widths may be due to a difference in the otolith and fish size relationship among spawnings. Unfortunately, the lack of larval samples of *P. snyderi* prevented a detail examination of this. When the comparison was restricted to similar aged males and females, however, the trends for males to have larger increments in the early larval period was maintained, suggesting it is not an artefact of a variable otolith and fish size relationship. The second explanation is that the difference in larval increments among sexes mirror larval growth rates and the fish that had higher larval growth were larger for a given age in keeping with the size-advantage model of larval dynamics (Bailey & Houde, 1989; Leggett & Deblois, 1994). These larger fish were better able to monopolize resources and became competitive dominants (possibly due to a covariance between growth and behavioural traits; Biro *et al.*, 2004), which as a result predisposed an opportunity to change sex to males. Although higher otolith size at hatching has been associated with a larger larval size or higher metabolism at hatching (Vigliola & Meekan, 2002; Armstrong *et al.*, 2004; Gagliano, 2007), and with higher larval and juvenile survival (Vigliola & Meekan, 2002), this is the first study to provide evidence that early larval growth may pro-rate individual fitness.

Males had larger otoliths from the very first increment, suggesting that processes operating prior to spawning may have pre-determined which fish would later become the dominants in a harem. These initial differences are likely to be generated through genetic and non-genetic parental effects. Recently, the early larval development, growth and trait covariation in the tropical damsel fish *Pomacentrus amboinensis* Bleeker, 1868, has been shown to be strongly influenced by the stress regime of the mother at the time of gametogenesis (McCormick, 1998, 1999, 2006; McCormick & Necheav, 2002; Gagliano &

McCormick, 2007). In species that form hierarchically structured social systems such as *P. snyderi*, it may well be the case that parental stress plays an important role in influencing individual fitness of the next generation, due to the effect of initial condition (such as body size) in securing intra-cohort dominance.

Sex-change associated check marks not only aid in discerning the temporal relationship between sex change and SSD but may also be used to assess variance in the age of sex change in relation to demographic, environmental, social and cross-generational determinants. Further studies are needed to explore the extent to which parental effects influence which individuals will change sex and the timing of that change. Future research should be directed towards establishing the prevalence of sex-change associated check marks among reef fish taxa, and their suitability in addressing key areas in life history and sex-allocation theory, and fisheries science.

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