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Fish ears are sensitive to sex change

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

Keywords: reef fish; sex change; otolith; hermaphroditism; life-history transition

1. INTRODUCTION

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

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

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

2. MATERIAL AND METHODS

(a) *Study species*

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

(b) *Experimental regime*

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

To isolate the effect of female density on otolith accretion during sex change, removal experiments were conducted in the laboratory at Lizard Island Research Station. Individuals were collected from the lagoon of Lizard Island, Great Barrier Reef (14°40.9′ S, 145° 26.8′ E). Social groups were created in individual 100–150 l microcosms containing equal quantities of rubble and algae habitat per individual: 10 polygynous social groups containing one male and four females (at 85, 80, 75 and 70±0.2 mm total length (TL)); and 10 bigamous social groups (monogamous social groups after male removal) containing one male and two females (at 85 and 80±0.2 mm TL). The male was always the largest individual. After 5 days acclimation, the male was removed from five of the 10 bigamous- and polygynous-social groups to induce sex change. The other five social groups in each treatment served as controls for male removal. At 09.00 h and 15.00 h each day, individuals were fed to satiation with brine shrimp and commercial fish food. Thirty days following the male removal, all individuals were euthanized and measured (mm TL). The sagittal otoliths were removed and stored dry, and the gonads were removed and fixed in a formalin-acetic acid-calcium chloride solution.

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

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

(c) *Otolith diagnosis and analysis*

Sagittal otoliths were processed to produce a transverse section perpendicular to the distal–rostral plane, such that daily increments could be observed from the nucleus to the outer margin of the otolith (following the methods of Wilson & McCormick 1997). Calibrated digital images of the otolith sections were then taken

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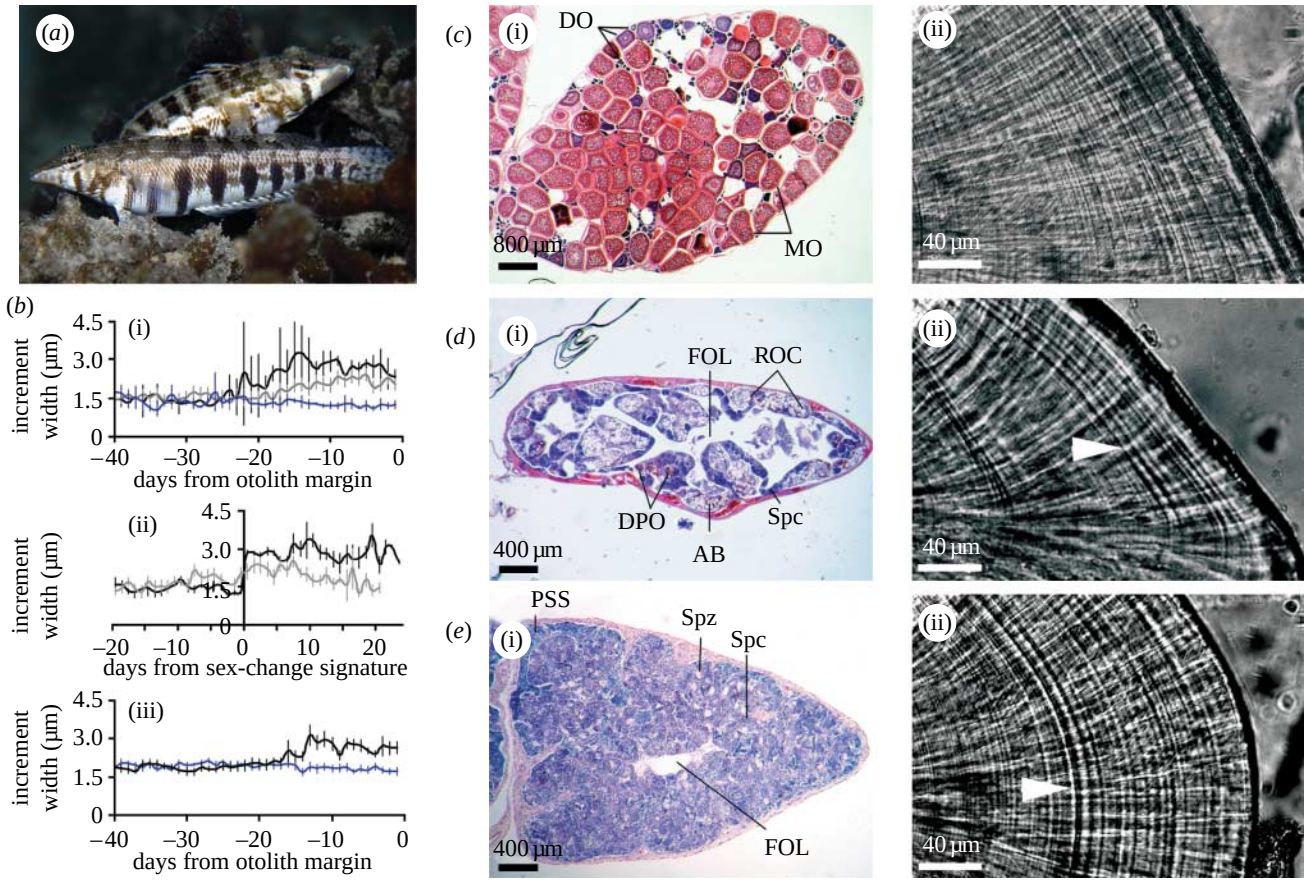

Figure 1. (*a*) *Parapercis cylindrica*; female front, male back. (*b*(i)–(iii)) Mean otolith increment-width profiles ±s.e. for experimental fishes. (*b*(i)) Laboratory fish. (*b*(ii)) Laboratory fish with 'day' centred to zero on the sex-change signature. (*b*(iii)) Fish from the wild. Blue, Non-sex changing control females; grey, monogamous sex changers; black, polygynous sex changers. All increment profiles are significantly different ($p<0.05$) according to the repeated measures ANOVA. (*c*–*e*); photomicrographs of the (g), gonads; and (o), otoliths from experimental fishes in the laboratory. All initial sizes = 85 mm TL. (*c*(i)) Control non-sex changing female displaying active oogenesis and (ii) regular otolith increments. (*d*(i)) Monogamous sex changer displaying partial gonad-transition and (ii) otolith-increment discontinuity (arrow). (*e*(i)) Polygynous sex-changer displaying complete gonad-transition and (ii) otolith-increment discontinuity (arrow). DO, developing oocytes; MO, mature oocytes; DPO, degenerating previtelogenic oocytes; ROC, remnant oocyte constituents; AB, atretic body; FOL, former ovarian lumen; Spc, spermatocytes; Spz, spermatozoa and PSS, peripheral sperm sinus.

at 400× magnification using a high power microscope. Increment width profiles from the otolith margin to 10 days before male removal were measured along the maximum otolith radius for all individuals from the field and laboratory experiments using the spatial analysis program Optimus. In otoliths where a discontinuity was observed within the experimental period (less than 30 days), the day of formation was recorded by back calculation from the otolith margin.

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

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

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

3. RESULTS AND DISCUSSION

All non-sex changed individuals displayed full female function and deposited regular, continuous otolith increments during the 30-day experiment (figure 1*b*,*c*). By contrast, all sex-changed individuals deposited an optically dense discontinuous zone between 6 and 20 days following male removal, followed by a rapid increase in increment width and a change in the primary growth axis (figure 1*b*,*d*,*e*). Increment width profiles were found to statistically differ between sex changers and non-sex changers in both the wild (RM ANOVA; $F_{1,17}=122.73$, $p<0.01$; figure 1*b*) and laboratory (figure 1*b*). Interestingly, female density further influenced otolith accretion among initially equal-sized sex-changed fish in the laboratory: polygynous sex-changers deposited statistically wider daily increments following the discontinuous zone compared to monogamous sex

Table 1. Statistical results for the experiment in the wild. (Regression analysis on the relationship between female density and somatic growth during sex change, otolith growth during sex change, the time lag between sex-change induction and signature formation.)

predictor	dependant	*n*	model	r^2	$F_{d.f.}$	*p*-value
female density (100 m^2)	otolith growth (μm)	12	$y=5.3063(x)+37.303$	0.37	$F_{1,10}=6.92$	0.03
	somatic growth (mm TL)	12	$y=1.6205(x)–4.0741$	0.56	$F_{1,10}=13.89$	0.01
	time of signature formation (days since sex-change induction)	12	$y=-0.8649(x)+22.486$	0.44	$F_{1,10}=8.56$	0.02

changers (RM ANOVA; $F_{1,8}=113.69$, $p=0.032$; figure 1*b*), and mean time-lag between sex-change induction and signature formation were statistically shorter among polygynous sex changers (10.4 ± 1.7 days) compared to monogamous sex changers (17.8 ± 2.4 days; 1-way ANOVA; $F_{1,9}=7.5$, $p=0.025$). Similar patterns were observed among sex-changed fish in the wild (table 1).

The greater otolith growth of polygynous sex changers compared to monogamous sex changers paralleled a faster gonad-transition rate and greater somatic growth during sex change (figure 1*d*,*e*; table 1). Thus, the otolith signature not only acts as a temporal marker of sex change, but also provides information on the magnitude of masculinization during sex change.

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

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

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Alonzo, S. H. & Mangel, M. 2005 Sex-change rules, stock dynamics, and the performance of spawning-per-recruit measures in protogynous stocks. *Fish. Bull.* **103**, 229–245.

Buston, P. M., Munday, P. L. & Warner, R. R. 2004 Sex change and relative body size in animals. *Nature* **428**, 783. (doi:10.1038/nature02512a)

Frisch, A. J., Walker, S. P. W., McCormick, M. I. & Solomon-Lane, T. K. 2007 Regulation of protogynous sex change by competition between corticosteroids and androgens: an experimental test using sandperch, *Parapercis cylindrica*. *Horm. Behav.* **52**, 540–545. (doi:10.1016/j.yhbeh.2007.07.008)

Munday, P. L., Buston, P. M. & Warner, R. R. 2006 Diversity and flexibility of sex change strategies in animals. *Trends Ecol. Evol.* **21**, 89–95. (doi:10.1016/j.tree.2005.10.020)

Patiño, R. & Takashima, F. 1995 Gonads. In *An atlas of fish histology: normal and pathological features* (eds F. Takashima & T. Hibiya), pp. 129–150, 2nd edn. Tokyo, Japan: Kondansha Ltd.

Randall, J. E., Allen, G. R. & Steene, R. C. 1997 *Fishes of the Great Barrier Reef and Coral Sea*. Honolulu, HI: University of Hawaii.

Thorrold, S. R. & Hare, J. A. 2003 Otolith applications in reef fish ecology. In *Coral reef fishes: dynamics and diversity in a complex ecosystem* (ed. P. F. Sale), pp. 243–264. New York, NY: Adecemic Press.

Walker, S. P. W. & McCormick, M. I. 2004 Otolith-check formation and accelerated growth associated with sex change in a haremic reef fish. *Mar. Ecol. Prog. Ser.* **266**, 201–212. (doi:10.3354/meps266201)

Wilson, D. T. & McCormick, M. I. 1997 Spatial and temporal validation of settlement-marks in the otoliths of tropical reef fishes. *Mar. Ecol. Prog. Ser.* **153**, 259–271. (doi:10.3354/meps153259)

Wright, K. J., Higgs, D. M., Belanger, A. J. & Leis, J. M. 2005 Auditory and olfactory abilities of pre settlement larvae and post settlement juveniles of a coral reef damselfish (Pisces: Pomacentridae). *Mar. Biol.* **147**, 1425–1434. (doi:10.1007/s00227-005-0028-z)

Zar, J. H. 1999 *Biostatistical analysis*. Upper Saddle River, NJ: Prentice-Hall.