Available online at www.sciencedirect.com
ScienceDirect
General and Comparative Endocrinology 156 (2008) 628–632
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Sex change of adult initial-phase male wrasse, *Halichoeres trimaculatus* by estradiol-17β treatment

Y. Kojima [a], R.K. Bhandari [b], Y. Kobayashi [a], M. Nakamura [a,c,*]

[a] *Tropical Biosphere Research Center, Sesoko Station, University of the Ryukyus, Sesoko 3422, Motobu, Okinawa 907-0227, Japan*
[b] *Center for Reproductive Biology, Washington State University, Pullman, WA 99164-4231, USA*
[c] *CREST, and SORST, JST (Japan Science and Technology) Corporation, Saitama, Japan*

Received 1 June 2007; revised 15 August 2007; accepted 4 February 2008
Available online 14 February 2008

Abstract

Sex steroids are considered major regulators of sex change processes in fish. Estrogen depletion is shown to be crucial for female–male sex change initiation; however, its role in male–female sex change is largely unknown. In the present study, we examined the effects of estradiol-17β (E2) treatments on testes of initial-phase (IP) males of the three-spot wrasse (*Halichoeres trimaculatus*), which naturally do not undergo male–female sex change. Sexually mature IP males were fed a diet containing E2 (low, 20 μg/g feed; high, 200 μg/g feed) for 6 or 12 weeks, and changes in gonadal structures were examined. Percentage of sex change varied with the dosage of E2 and the duration of treatment. All individuals treated with high-dose E2 for 6 weeks had ovaries with many immature oocytes; whereas 75% of individuals treated with low-dose of E2 for 6 weeks and sampled on the 12th week had ovaries with yolky oocytes and an ovarian cavity indicating a typical mature ovary. No testicular tissue was observed in sex-reversed gonads in both treatment groups. Contrary to the previous assumptions, present results suggest that IP male wrasses have the potential to undergo male–female sex change in response to exogenous estrogen. How the presence or absence of estrogen creates sexual plasticity in gonadal germ and somatic cells remains to be clarified.

Keywords: Initial-phase wrasse; Sex change; Estrogen

1. Introduction

All vertebrates express some degree of plasticity throughout the period of sexual differentiation. In particular, fishes encompass a broad spectrum of sexual plasticity ranging from gonochorists (mature as separate sexes with a single gamete type) to functional hermaphrodites (sex changers after maturation) (Devlin and Nagahama, 2002). Many marine hermaphrodite fish change sex in response to social cues, while a few change when growth thresholds are met (Ross, 1990; Shapiro, 1990). Both factors likely alter endogenous pools of sex hormones, which consequently regulate the proper functioning of germ and supporting somatic cells, leading to anatomical changes in the gonads (Nakamura et al., 2003). It is widely believed that, during the course of sex change, populations of new germ cells appear, or that already present spermatogonial stem cells differentiate into spermatogonia or oogonia, depending on the nature of the sex change process. In contrast, very little is known about development of sex-specific somatic cells, such as Sertoli or Leydig cells in the sex changed testis and theca or granulosa cells in the sex changed ovary.

It is well-known that most wrasses change their sex from female to male during their life spans. Following sex differentiation, initial-phase (IP) wrasse males maintain the same sex throughout their life spans (gonochoristic), whereas females undergo sex change sometime in their life cycle. The most important factor in initiating sex change is the

* Corresponding author. Address: Tropical Biosphere Research Center, Sesoko Station, University of the Ryukyus, Sesoko 3422, Motobu, Okinawa 907-0227, Japan. Fax: +81 980 47 6072.
E-mail address: masaru@lab.u-ryukyu.ac.jp (M. Nakamura).

0016-6480/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2008.02.003

social cue; i.e. in the absence of a male, the dominant female in the group changes sex into the male. Some drastic endocrine changes have been reported for some species of wrasses undergoing sex change naturally. In Saddleback wrasse, *Thalassoma duperrey*, serum E2 levels dropped significantly during female to male sex change (Nakamura et al., 1989). Artificial inhibition of estrogen production via aromatase inhibitor (AI) treatments resulted in complete sex change in three-spot wrasse (*Halichoeres trimaculatus*), and co-treatment with AI and E2 prevented sex change (Higa et al., 2003). Similar results have been reported for the Protogynous (female to male sex changer) groupers, *Epinephelus sp.* (Bhandari et al., 2003, 2004, 2005; Li et al., 2005), goby (Kroon et al., 2005) and gilthead seabream (Wong et al., 2006). Conversely, estrogen treatment is shown to induce male to female sex change in protandrous hermaphrodites (male to female sex changers) (Chang et al., 1994; Wu et al., 2005). Together, it is likely that endogenous estrogen plays a critical role in female to male sex change.

To date, an array of the literatures explains the mechanisms of female to male sex change in wrasses and other sex changing fishes; however, there is no report on sex change of IP males of protogynous hermaphrodites. This is partly owing to the long-held belief that IP males are gonochoristic in nature. In contrast, we tested the hypothesis that adult IP male wrasses have the potential to change sex into typical females, and that estrogen induces their sex change.

2. Materials and methods

2.1. Experimental animals and rearing

The three-spot wrasse, *H. trimaculatus*, was used as an experimental model. This species is among the most abundant fishes at Sesoko beach in Okinawa, Japan. It exhibits diandric protogyny, with populations consisting of small initial-phase (IP) males, initial-phase females, and large

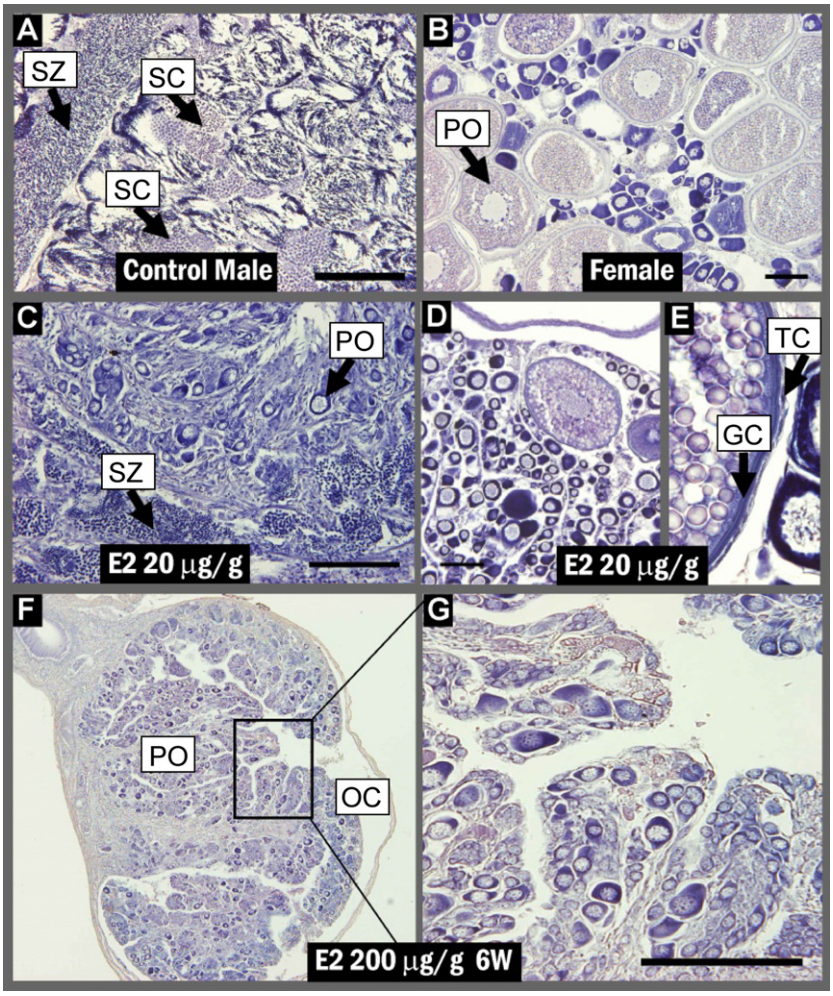

Fig. 1. Estradiol-17β treatments reverse the sex of IP male wrasses to that of typical functional females. (A and B) Show representative sections of IP male testis and female ovary, respectively, before start of experiment. Treatment with a low-dose of E2 (20 μg/g) for 6 weeks caused partial sex reversal (C), whereas the same treatment induced complete sex reversal in 12 weeks when stopped at the 6th week and followed with normal feeding for an additional 6 weeks. Sex changed ovaries contained oocytes typical of untreated initial females (D), where outer theca and inner granulosa cells can be clearly seen (E). A higher dose of E2 (200 μg/g) caused sex change in 6 weeks but lacked properly growing oocytes (F and G). Abbreviations: GC, granulosa cells; OC, ovarian cavity; PO, primary oocytes; SC, spermatocyte; SZ, spermatozoa; TC, theca cells; YO, yolky oocytes. Scale bars (100 μm).