Comparative Biochemistry and Physiology, Part A 181 (2015) 45–53

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Brain aromatase (*cyp19a1b*) and gonadotropin releasing hormone (*gnrh2* and *gnrh3*) expression during reproductive development and sex change in black sea bass (*Centropristis striata*)

Timothy S. Breton [a], Matthew A. DiMaggio [a,1], Stacia A. Sower [b,c], David L. Berlinsky [a,*]

[a] Department of Biological Sciences, University of New Hampshire, 38 College Road, Durham, NH 03824, United States
[b] Center for Molecular and Comparative Endocrinology, University of New Hampshire, 46 College Road, Durham, NH 03824, United States
[c] Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, 46 College Road, Durham, NH 03824, United States

ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form 21 November 2014
Accepted 25 November 2014
Available online 2 December 2014

Keywords:
Sex change
Hermaphroditism
Gonadotropin releasing hormone
Brain aromatase
Black sea bass

ABSTRACT

Teleost fish exhibit diverse reproductive strategies, and some species are capable of changing sex. The influence of many endocrine factors, such as gonadal steroids and neuropeptides, has been studied in relation to sex change, but comparatively less research has focused on gene expression changes within the brain in temperate grouper species with non-haremic social structures. The purpose of the present study was to investigate gonadotropin releasing hormone (GnRH) and brain aromatase (cyp19a1b) gene expression patterns during reproductive development and sex change in protogynous (female to male) black sea bass (*Centropristis striata*). Partial cDNA fragments for *cyp19a1b* and *eef1a* (a reference gene) were identified, and included with known *gnrh2* and *gnrh3* sequences in real time quantitative PCR. Elevated *cyp19a1b* expression was evident in the olfactory bulbs, telencephalon, optic tectum, and hypothalamus/midbrain region during vitellogenic growth, which may indicate changes in the brain related to neurogenesis or sexual behavior. In contrast, *gnrh2* and *gnrh3* expression levels were largely similar among gonadal states, and all three genes exhibited stable expression during sex change. Although sex change in black sea bass is not associated with dramatic changes in GnRH or cyp19a1b gene expression among brain regions, these genes may mediate processes at other levels, such as within individual hypothalamic nuclei, or through changes in neuron size, that warrant further research.

1. Introduction

Teleost fishes exhibit a wide range of reproductive strategies, including simultaneous, protogynous (female to male change) and protandrous (male to female) hermaphroditism. The endocrine mechanisms controlling sex change in protogynous teleosts have primarily been studied in haremic, coral reef species such as gobies and wrasses. In one of the best studied protogynous species, the bluehead wrasse (*Thalassoma bifasciatum*), removal of the harem's male results in an orchestrated series of endocrine events in the largest female, whereby she develops into a functional male within 1–2 weeks (Warner and Swearer, 1991). Considerably less is known about the mechanisms of sex change in many species with non-haremic social structures, including commercially-important snappers and groupers.

The reorganization of gonadal tissue during sex change is largely mediated by sex steroids. Dramatic increases in estradiol synthesis occur during natural sex change in many protandrous species (Frisch, 2004 and Wu et al., 2010). In contrast, protogynous species exhibit elevated 11-ketotestosterone levels during sex change, as well as a reduction in ovarian aromatase (cyp19a1a), the primary enzyme required for estrogen production (Nakamura et al., 2003; 2005 and Frisch, 2004). The activation or inhibition of the aromatase pathway is also implicated in species with bidirectional sex change (Kroon et al., 2005). Overall, an androgen or estrogen-dominated environment is required to maintain male or female gonads, respectively, in many fishes, and a shift in steroid production is sufficient to result in sex change (Cardwell and Liley, 1991, Godwin and Thomas, 1993, Perry and Grober, 2003, Bhandari et al., 2005 and Guiguen et al. 2010).

The neuroendocrine cascades that regulate gonadal steroidogenic shifts remain poorly understood. Gonadotropin releasing hormones (GnRHs), for example, are major regulators of reproduction in fishes and are associated with sexual plasticity or behavioral changes, but relatively little research has focused on gene expression changes in the brain during sex change (Foran and Bass, 1999, Volkoff and Peter, 1999, Zhang et al., 2008, Kline, 2010, Munakata and Kobayashi, 2010 and Zohar et al., 2010). Teleost brains also exhibit a high degree of endogenous estrogen synthesis, catalyzed by a brain-specific aromatase enzyme (cyp19a1b), but little is known about the role of brain estrogens

* Corresponding author. Tel.: +1 603 862 0007.
E-mail address: david.berlinsky@unh.edu (D.L. Berlinsky).
[1] Present address: Tropical Aquaculture Laboratory, Program in Fisheries and Aquatic Sciences, School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, FL 33570, United States.

http://dx.doi.org/10.1016/j.cbpa.2014.11.020
1095-6433/

in mediating sex change (Strobl-Mazzulla et al., 2008, Wu et al., 2010, Diotel et al., 2010 and Page et al., 2010). More information is needed on neuroendocrine gene regulation during sex change, to better understand these processes in a variety of teleost species.

Black sea bass (*Centropristis striata*) are a high-valued, marine teleost (Serranidae) that support important commercial and recreational fisheries along the Atlantic coast of the United States. Due to high demand and limited, seasonal availability, considerable research has been conducted to develop black sea bass as an aquaculture species (Berlinsky et al., 2000, Atwood et al., 2003 and Alam et al., 2008). Similar to related grouper species, black sea bass are protogynous hermaphrodites, and in the wild, change from females to males between 2 and 5 years of age (Shepherd and Idoine, 1993). During hatchery production, however, black sea bass sex change is often accelerated in wild-caught adults brought into captivity (Howell et al., 2003 and Colburn et al., 2009) and hampers maintenance of breeding stock. Although the reproductive social structure of black sea bass is unknown in the wild, the presence or absence of mature males has been shown to influence the rate of sex change in captivity, and additionally, sex change was induced with implantation of aromatase inhibitors and androgens (Benton and Berlinsky 2006).

Recently, full-length cDNA sequences for two GnRH forms (*gnrh2* and *gnrh3*) were characterized in black sea bass, which provided new genetic resources to understand sex change in this species (Morin et al., in press). The purpose of the present study was to examine expression of GnRHs and brain aromatase in male and female black sea bass, as well as fish undergoing spontaneous sex change, to further understand the neuroendocrine mechanisms of sex change in this species. To this end, partial cDNA sequences for brain aromatase and eukaryotic elongation factor 1 alpha (*eef1a*; a commonly used reference gene) were identified, and expression was assessed, along with *gnrh2* and *gnrh3*, using a real time quantitative PCR approach.

2. Material and methods

2.1. Animal sampling

Adult black sea bass ($n = 100$) were wild-caught from Rhode Island waters (2012) and transported to the University of New Hampshire's (UNH) Aquaculture Research Center. All fish were maintained in recirculating systems under UNH Institutional Animal Care and Use committee guidelines and fed a commercially prepared diet ad libitum (Skretting, Stavanger, Norway). Fish were kept at a simulated natural photoperiod and thermal regime (13–18 °C) for approximately 9 months and sampled thereafter at 1–3 month intervals. Experimental fish were euthanized via acute immersion in a 200 mg/L buffered tricaine methanesulfonate bath (Argent Chemical Laboratories, Redmond, WA, USA), and brains, pituitaries, and gonads were subsequently removed for analyses.

Since gonadal sex change in black sea bass is initiated in the posterior ovary (Cochran and Grier, 1991), four representative fragments from each individual (anterior and posterior sections/gonad) were dissected for histological evaluation of reproductive stage. Samples were preserved in 10% neutral buffered formalin for 24–48 h, processed for

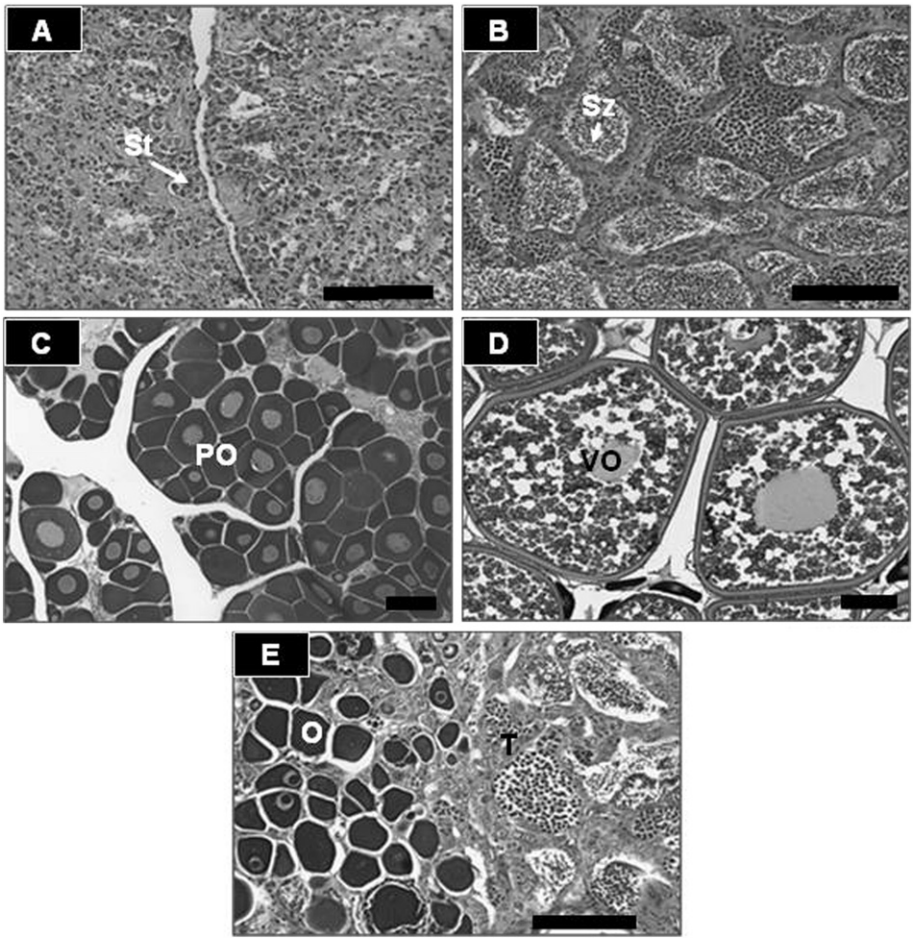

Fig. 1. Gonadal histology of male early testicular development (A), male spermiating (B), female early ovarian development (primary oocyte growth, [C]), female vitellogenic growth (D), and sex change (E). Scale bars represent 100 μm. St, spermatids; Sz, mature spermatozoa; PO, primary oocyte; VO, vitellogenic oocyte; O, ovarian tissue; T, testicular tissue.