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Seasonal fluctuations in androgen levels in females of the hermaphroditic gag, *Mycteroperca microlepis*, with an emphasis on juvenile animals

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Abstract

Androgens are known to play many roles in the reproductive physiology of teleosts, but less information exists on the role that they play in the development of larval and juvenile fish. This study examines an observed seasonal cycle of 11-ketotestosterone (11KT) in females of the hermaphroditic gag grouper (*Mycteroperca microlepis*). Otoliths, gonads, and plasma samples from gag were collected quarterly (spring, summer, fall, and winter), with complete data (age, sex, and androgen levels) obtained from a total of 225 individuals. Ages ranged from zero to 11 years, and all individuals were female. Testosterone (T) peaked in the spring, coincident with spawning, and was low throughout the remainder of the year. The androgen 11KT peaked in summer and declined through the following spring. 11KT levels were negatively correlated with fish size in both the summer and winter, while T was negatively correlated in the summer and positively correlated in the winter. T levels showed little seasonal variation in juveniles (0–1 and 2–3 age groups), but showed a seasonal increase from fall through spring in older fish (4–5 and 6+ age groups). Age 0–1 fish had significantly higher levels of 11KT than the age 4–5 group during the summer and both the 4–5 and 6+ age groups in the winter. The gag is a protogynous hermaphrodite that goes through several ontogenetic shifts during its life, and this seasonal fluctuation in plasma levels of 11KT may play a role in the growth and development, behavior, or control of sex change of gag.

Keywords: Gag; Grouper; 11-ketotestosterone; Androgen; *Mycteroperca*; Development; Sex change; Female

1. Introduction

Androgens have been demonstrated to play many roles in the development and reproductive physiology of teleosts (Fostier et al., 1983; Borg, 1994; Sullivan et al., 1997). In fish undergoing sexual maturation androgenic steroids, primarily testosterone (T) and 11-ketotestosterone (11KT), have been shown to be involved in the growth of gametes in both ovarian and testicular tissues (Fostier et al., 1983; Jackson and Sullivan, 1995; Sullivan et al., 1997; Heppell and Sullivan, 1999). Androgens, particularly 11KT, are also involved in the generation of male typical reproductive behaviors in teleosts, including aggressiveness and territoriality, and the development of secondary sex characteristics such as the breeding coloration and kidney and skin hypertrophy (Kindler et al., 1989; Mayer et al., 1990; Cardwell and Liley, 1991; Hourigan et al., 1991; Borg et al., 1993; Borg, 1994). Demonstration of high levels of 11KT in female teleosts is less common, although 11KT has been correlated with a rise in vitellogenin in females of some species (Idler et al., 1981).

Less information exists on the role that androgens play in the development of larval and juvenile fish. It has been suggested that in early developmental stages and up through the first few months of life, androgens produced by the developing gonad are involved in the differentiation of these tissues into testes and ovaries (Fostier et al., 1983; Chang and Chen, 1990; Feist et al., 1990). The role of 11-oxygenated androgens in female fish in particular is not well studied, although Lokman and co-workers recently reviewed the potential role of 11KT and other oxygenated androgens in migratory behaviors of teleosts (Lokman et al., 2002). Elevated levels of 11KT have been demonstrated in

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female shortfin eels (*Anguilla australis*; Lokman et al., 1998).

In the course of a study on the reproductive physiology of gag grouper (*Mycteroperca microlepis*), a seasonal summer peak of 11KT in juvenile and adult female gag was observed. This peak is offset from the spring spawning season by approximately three months. Summer is marked by elevated water temperatures, lengthened photoperiod, and maximum population density as juveniles recruit to the inshore seagrass beds (Koenig and Coleman, 1998; Ross and Moser, 1995) and which most likely also corresponds with a time of elevated growth rates and increased intraspecific interactions. Androgens may coordinate some of these events through their identified role in the control of growth, development, and territorial interactions. Seasonal fluctuations of 11KT in gag is particularly interesting because (1) this species is a protogynous hermaphrodite and 11KT has been indicated as a hormone involved in the control of sex change (Cardwell and Liley, 1991; Borg, 1994), and (2) there is no testicular source of 11KT in juvenile gag because all fish are born as females. This paper presents an analysis of size and seasonal changes in 11KT levels in juvenile gag grouper, and evaluates some potential reasons for the patterns observed.

2. Materials and methods

Gag were collected on a quarterly basis (spring, summer, fall, and winter) between December 1994 and June 1996 by hook and line, fish trap, speargun, and otter trawl in the Atlantic Ocean from Beaufort, North Carolina to Cape Canaveral, Florida, and in the Florida Gulf of Mexico from Sarasota to Panama City (Heppell and Sullivan, 2000). The actual sampling dates for each period were as follows (date inclusive, pooled for both years): summer, 11 April to 27 June; fall, 13 September to 27 September; winter, 11 November to 29 December; spring, 24 February to 22 March. All animals were bled by caudal puncture into heparinized syringes <10 min after capture. Blood, which was also being used to study vitellogenin dynamics, was mixed with 0.87 trypsin inhibitor units (T.I.U) per mL of the protease inhibitor aprotinin (Sigma Chemical Co.), held on ice until the plasma was collected by centrifugation, and the plasma was frozen on dry ice and then transferred to a −80 °C freezer until laboratory analyses were performed.

Gonads were collected and immediately placed in 10% neutral buffered formalin for histological analysis to identify sex. All gonads were processed as previously described (Heppell and Sullivan, 2000). Sagittal otoliths were removed, dried, then mounted with thermoplastic on glass slides and sectioned for use in age determination (Collins et al., 1987).

Existing steroid radioimmunoassays (RIAs) for T and 11KT were previously validated for use with gag plasma (Heppell and Sullivan, 2000). The high specificity of the antisera used in the RIAs was reported previously (Korenman et al., 1974; Hourigan et al., 1991). Extraction efficiencies were 92±7 and 91±4%, for T and 11KT, respectively (triplicate samples of tritiated steroids) — therefore steroid results were not corrected for extraction efficiency. Both T and 11KT measured in samples of gag plasma diluted parallel to the standard curves over the working ranges used in this study (ANCOVA, $p>0.05$ for all three steroids). Conservative upper and lower limits of detection for the RIAs ($B/B_0=20\%$ and 80%) were 0.1 and 3.2 ng/mL and 0.2 and 4.6 ng/mL for T and 11KT, respectively. Duplicate 20 µl aliquots of plasma were triple ether extracted and dried at 37 °C under nitrogen gas, resuspended in phosphate buffered saline containing 1% gelatin (PG), and T and 11KT levels were measured by RIA (Woods and Sullivan, 1993; Heppell and Sullivan, 2000).

Regression analyses for steroid levels versus fish length were run using the Microsoft Excel Statistical Analysis Toolpak (Microsoft Corp.). Statistical significance for androgen levels between age groups and quarters was assessed using Tukey's honest significant difference test, contained within the Statistica software package (StatSoft, Inc.). All differences were deemed significant at the $p<0.05$ level.

3. Results

Two hundred sixty nine female gag were collected, from which age was determined for 225 individuals. Ages ranged from zero to 11, as determined by counting presumed annuli on cross-sections of the sagittal otoliths (Harris and Collins, 2000; Manooch and Haimovici, 1978). All age zero and age one fish were collected from inshore, estuarine habitat, age two and three fish came from a mixture of inshore and nearshore habitats, while all fish older than age four came from offshore reef habitat. No age six or older fish were collected during the summer quarter. Sample sizes for each age group for each quarter are shown in Table 1.

When all age groups were pooled, there were distinct patterns in the seasonal fluctuations of both T and 11KT. Testosterone levels peaked in the spring (Fig. 1B), coincident with the natural spawning season (Collins et

Table 1
Sample sizes for each age group for each quarter (spring, summer, fall, and winter)

Quarter	Age group			
	Ages 0–1	Ages 2–3	Ages 4–5	Ages 6+
Spring	34	7	17	11
Summer	9	30	5	0
Fall	14	12	16	10
Winter	11	32	13	4

Total $n=225$.