

Hormonal induction of Atlantic dusky grouper (*Epinephelus marginatus*) broodstock

by

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ABSTRACT. - Immature fish and young females were sex reversed using hormonal induction in two consecutive years. In the first year one permanent male was obtained. Collected sperm of both years ranges from 30 μ l to 300 μ l and cell concentration varies from 3 to 6.5 $\times 10^9$ spermatozoa/ml. Atlantic dusky grouper can successfully be induced to functional males with 17 α -methyltestosterone implants.

Key words. - Dusky grouper - Hormonal induction - Broodstock management.

Introduction

Dusky grouper is a protogynous monandric hermaphrodite and only large individuals (more than 9 kg) are present as males in the wild (Chauvet, 1988). Due to this fact, it is difficult to maintain a sex-balanced broodstock in captivity without considering sex ratio control. The use of hormonal induction as a reproductive strategy for balanced broodstock management of this species has been successfully applied in individuals from the Mediterranean (Spedicato *et al.*, 1995; Marino *et al.*, 2003; Sarter *et al.*, 2006). The aim of this study was to verify if such a technique would be successful in Atlantic dusky grouper.

Methods

Broodstock (11 fish) was constituted during the period of 2001-2003 from fish captured in the Atlantic Ocean, most of them in the Azores islands. First reproductive attempts in Portugal started in 2005 and 2006. Gonad biopsy was done monthly to determine sex, gonad maturation and oocyte size. Immature fish and young females were sex reversed using 17 α -methyltestosterone implants (α -MT) two months prior to gamete extraction using a dosage of 2.5mg α -MT/kg fish. Females were induced with gonadotropin-releasing hormone agonist (GnRH α) EVAc implants (Mylonas and Zohar, 2001) when the diameter of oocytes was $> 325 \mu$ m. The implants were used to target a dose of 45 μ g GnRH α /kg fish. Gametes were collected daily and by abdominal massage. The frequency of ovulation and oocyte/sperm quality were recorded during the season. Part of the sperm samples was used in cryopreservation trials. Fertility rates were determined 2 h after, and only fertilized eggs were incubated until hatching (48 h later).

Results and Discussion

In 2005 one natural male, 8 females and two immature fish were studied. Of the three reversed individuals (young females), two (66%) were functional males (Tab. I). After a

Table I - Sex-reversed fish with hormonal induction of dusky grouper broodstock from two consecutive years (2005 and 2006).

Fish	2005		2006	
	Before α -MT	After α -MT	Before α -MT	After α -MT
A	♀	♂ functional	♀	♂ functional
B	♀	♂ functional	♂	♂ functional
C	♀	♂ or ♀	♀	-
D	immature	immature	immature	♂ functional
E	immature	immature	immature	♂ functional

9-month period, one permanent male was obtained. In the second year, 6 females, three males and two immature fish were studied. All sex-reversed fish (one female and two immature) were successfully reversed into functional males (100%). One female was naturally reversed into a male, changing the sex-ratio to 6 males: 5 females. Sperm collected from both years ranged from 30 μ l to 300 μ l and cell concentration varied between 3 to 6.5 $\times 10^9$ spermatozoa/ml. Sperm motility was 35 min and decreased in cryopreserved sperm. Males produced sperm within 24 h after Lucrin Depot injection. A total of 4 and 8 batches were obtained in 2005 and 2006, respectively. Relative fecundity was 66.8 and 114.7 for each year. In the second year the frequency of ovulations was 1.6 per female, with a maximum of 3 batches, these values were lower than the values reported by Marino *et al.* (2003) and probably related to the lower female weight in the present work. In the 2005, 79.5% of eggs were fertilized, with a 8-100% hatching rate. In 2006, only 22.4% of eggs were fertilized, and no hatching occurred.

Conclusions

Young female and immature Atlantic dusky grouper can successfully be induced to functional males with α -methyltestosterone implants. Hormonal induction of females is also possible, as in Mediterranean dusky grouper. Sex ratio control is possible in dusky grouper with close control of the broodstock at the beginning of Spring.

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