THE SWIMBLADDER OF THE LUSITANIAN TOADFISH: SEXUAL DIMORPHISM AND HORMONAL CONTROL

T. Modesto, A.V.M. Canário

Centro de Ciências do Mar. Universidade do Algarve. Portugal.

SUMMARY. The members of the family Batrachoididae produce sounds associated with courtship and agonistic interactions. While long mating calls are only produced by nesting males, agonistic short duration grunts have been recorded from toadfishes and midshipmen in both males (nesting or not) and females (1). Sound production results from the contraction of paired sonic muscles attached to the walls of the swimbladder, which cause a rapid variation in swimbladder volume and internal pressure. Both sonic muscle growth and sonic fibre morphology have been shown to be androgen sensitive in batrachoidids (2).

INTRODUCTION

The Lusitanian toadfish, *Halobatrachus didactylus*, is an eastern Atlantic marine teleost member of the family Batrachoididae. It is a sedentary benthic species that inhabits shallow waters and lives partly buried or concealed in rock crevices, which are used as shelters and nesting sites. Seasonal profile of gonadosomatic index and plasma steroid levels (3) show that this species has a short spawning period at the end of spring and early summer (May-July) with a quiescent period during autumn. Previous studies in the Lusitanian toadfish have shown that sonic mass of type I (nesting ?) males increase significantly during the breeding season in a way that closely followed the androgen seasonal profile, suggesting a possible role of androgens in the activation and/or maintenance of seasonal sonic muscle hypertrophy. Among reproductive males, 11KT but not T is elevated in type I males compared to type II (parasitic ?) males (3). Considering the potency of 11KT at inducing secondary sexual characters in other teleosts, this steroid is a strong candidate for generation and/or maintenance of sonic muscle sexual dimorphism. In this study, the hormonal basis of the sexual dimorphism of swimbladder weights and morphologic characteristics of sonic muscle fibres has been investigated through castration and androgen/estrogen replacement.

MATERIALS AND METHODS

The adult *H. didactylus* used for the seasonal analysis of swimbladder histomorphology were collected monthly over the annual reproductive cycle in Ria Formosa (south Portugal, 37°00'N; 7°65W'). Fish collected during the reproductive season (May to July, females n=12, type I males n=12, type II

males n=7) had high relative swimbladder weights and fish collected during the non-reproductive season (December to February, females n=12, type I males n=12, type II males n=5) had low relative swimbladder weights.

Long-term effects of castration on swimbladder weight and fibre morphometry

Adult *H. didactylus* were caught in December and surgically castrated. The experimental groups were: type I males intact, i.e., not operated and not castrated (n=10), type I males castrated (n=12), type II males intact (n=9), type II males castrated (n=9) and a group of intact females (n=12). Fish were maintained in the experimental tanks for six months after castration. In June, the time of natural spawning in the field, all groups were sacrificed and sampled for swimbladder weight and sonic fibre histomorphology.

Short-term effects of castration and steroid replacement on swimbladder weight and fibre morphometry

Steroids were administrated intraperitoneally as a liquid suspension (10%) in warm coconut oil. As coconut oil becomes solid below 25°C implants formed a single long mass inside the body cavity, and acted as a slow release pellet. Fish received coconut oil implants containing 100 µg g-1 of T, 11KT or estradiol-17β (E_2) for a period of six weeks. A control group received steroid free implants. Hence, the experimental groups were: intact (n=10), sham-operated (n=10), castrated (n=8), castrated plus control implants (n=11), castrated plus T implants (n=10), castrated plus 11KT implants (n=7) and castrated plus E_2 implants (n=7).

Histology and histomorphometry

Swimbladders were weighed (total swimbladder weight, W_{TS}) and fixed in Bouin's fluid for 48 hr and then transferred into 70% ethanol. The muscle slices were embedded in paraffin, sectioned (5-7mm), and stained with haematoxylin and eosin (H&E). Differences in muscle structure among seasonal samples and experimental groups were analysed using the OPTIMAS 5.2 computerised image analysis system (BioScan, Inc. U.S.A.). Mean areas of fibre components were derived from measurements in each swimbladder of 50 randomly chosen cells. Video images of the cells were digitised to generate cross sectional areas of the muscle fibres (total fibre area), of the myofibril-containing zone (myofibril area) and of the peripheral sarcoplasm (sarcoplasm area).

RESULTS

Seasonal changes in morphology of sonic fibres

Morphometric measurements of sonic fibres showed that during the reproductive season type I males had smaller myofibril contracting zones surrounded by larger areas of peripheral sarcoplasm compared to females, while type II males showed intermediate (but not significantly different) values of sarcoplasm area/myofibril area ratio between type I males and females.

There were no differences in the ratio sarcoplasm area/myofibril area between sexes during non-reproductive season.

Effects of castration on the swimbladder

Six months after surgery castrated fish had lighter W_{TS} than non-castrated fish (p<0.01). Castration increased total fibre area (marginally significant, p=0.048), and myofibril area (p<0.001) in both morphs but had no effect on sarcoplasm area (p=0.836). This is reflected by the ratio sarcoplasm area/myofibril area, which showed a significant decrease in castrated groups (p<0.001)

Effects of steroid replacement on the swimbladder

Neither the incisions nor the castration had significant effects on any of the structural features of sonic fibres (p>0.100) or in W_{TS}. The hormone treatment had no significant effect on total fibre area (p=0.471), myofibril area (p=0.627) or sarcoplasm (p=0.059), but had a significant effect on sarcoplasm area/myofibril area ratio (p<0.01). Only E_2-treated animals showed sarcoplasm area/myofibril area ratio significantly lower compared to control (p<0.05).

DISCUSSION

During the breeding season the general hypertrophy of sonic muscle mass in *H. didactylus* males was accompanied by structural modifications in muscle fibres: males had thinner myofibrils and more sarcoplasm, and therefore, a bigger sarcoplasm area/myofibril area ratio than females. This was interpreted as an adaptation to the increased speed and fatigue resistance necessary for production of the long mating call, since small size and concomitant large surface to volume ratio would facilitate rapid fluxes of glucose, O_2, lactic acid and CO_2. Additionally, enlargement of the sarcoplasm area permits larger mitochondria content around the myofibril zone essential for the energetic demands of long mating calls. In *H. didactylus* only long-term (six months), and not short-term (six weeks) castration, induced reduced swimbladder weights and a decrease in sarcoplasm area/myofibril area ratio. Furthermore, long-term castrated males showed morphological characteristics of fibres similar to those of intact females. As castration of *H. didactylus* was carried out in December, before the period of natural increase of sonic mass, results obtained after 6 months may be indicative that testicular factors are required to initiate seasonal sonic muscle hypertrophy. However, these factors do not seem to be necessary for sonic hypertrophy, at least for short periods, since short-term castration did not induce any alteration in sonic mass or fibre morphology. The effects of androgens either on sonic mass or muscle fibre structure of *H. didactylus* were not as pronounced as reported in other teleosts (2). The failure of the sonic muscle in *H. didactylus* to respond to androgen is unexpected since androgen sensitivity is a common feature of sonic muscle in related sound producing teleost species and of skeletal muscle in most, if not all, vertebrates. However, it may be a consequence of several factors

such as: an inadequate dose of androgen and/or duration of the experiment, the muscle was already at its maximum stimulation when implants were given, or in this species other factors and not androgens are responsible for sonic muscle hypertrophy and hyperplasia. In this study the specific involvement of androgens has not been completely clarified. Further studies are needed to establish whether a different dose of androgen implants and/or prolonged exposure period or even other hormones are effective in promoting the expression of swimbladder type I male phenotype.

Acknowledgments

REFERENCES

1. Brantley, R. K., Bass, A. H. 1994 Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae). *Ethology* 96: 213-232.
2. Brantley, R. K., Marchaterre, M. A., Bass, A. H. 1993 Androgen effects on vocal muscle structure in a teleost fish with inter- and intra-sexual dimorphism. *J Morphol* 216: 305-318.
3. Modesto, T., Canario, A. V. M. 2003 Morphometric changes and sex steroid levels during the annual reproductive cycle of the Lusitanian toadfish, *Halobatrachus didactylus*. *Gen Comp Endocrinol* 131: 220-231.