

## REPRODUCTIVE DEVELOPMENT IN WILD AND CAPTIVE-REARED GREATER AMBERJACK *Seriola dumerili* (RISSO, 1810)

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### Introduction

The greater amberjack *Seriola dumerili* (Risso, 1810) is a large teleost fish with rapid growth and excellent flesh quality, whose domestication represents an ambitious challenge for aquaculture. A proper commercial aquaculture production of this species has not developed so far, mainly due to its inconsistent and unpredictable reproduction in captivity (Kožul et al., 2001; Mylonas et al., 2004). A research effort is currently ongoing ([www.diversifyfish.eu](http://www.diversifyfish.eu)) with the final goal to develop the necessary broodstock management, larval rearing and on growing husbandry methods to convert the present experimental greater amberjack rearing into a true aquaculture industry. The aim of the present research was to study the reproductive development of wild greater amberjack and investigate the effects of rearing in captivity on gametogenesis.

### Materials and methods

Adult wild (n = 33) and captive-reared (n = 24) greater amberjack were sampled during three different phases of the reproductive cycle: early gametogenesis (late April-early May), advanced gametogenesis (late May-early June) and spawning (late June-July). Wild fish were caught by a commercial purse seine fishing vessel around the Pelagie Islands (Sicily, Italy); captive-reared fish belonged to a broodstock reared in a sea cage of Argosaronikos Fishfarming S.A. (Salamina Island, Greece). Fish reproductive state was evaluated through the calculation of the gonado-somatic index ( $GSI = 100 \frac{GM}{BM}^{-1}$ ), routine histological analysis of the gonads and determination of plasma sex steroids (testosterone, 11-Ketotestosterone,  $17\beta$ -estradiol,  $17,20\beta$ -dihydroxypren-4-en-3-one) by ELISA assays. In females, liver vitellogenin (VtgA, VtgB and VtgC) gene expression was assessed by qRT-PCR. In males, proliferating and apoptotic germ cells were identified throughout the immunodetection of Proliferating Cell Nuclear Antigen (PCNA) and the TUNEL method, respectively.

### Results

Gonado-somatic index of wild and captive-reared greater amberjack increased from the early to the advanced gametogenesis period and decreased thereafter. Both GSI and plasma steroid levels were lower in captive-reared compared to wild fish, particularly during the advanced and the spawning phases. During the early gametogenesis phase, ovaries showed primary growth and early vitellogenic oocytes, and testes contained germ cells in all spermatogenic stages. During the advanced gametogenesis phase, the ovaries of wild greater amberjack showed oocytes at the late vitellogenic stage and post-ovulatory follicles (POFs; a sign of recent spawning), whereas major  $\alpha$  atresia (> 50% of vitellogenic oocytes in  $\alpha$  atresia) was found in the ovaries of most captive-reared fish. In the advanced gametogenesis phase, wild males had all stages of spermatogenesis in the germinal epithelium, as well as large amount of luminal spermatozoa. On the other hand, half of the captive-reared males had already ceased their spermatogenic activity, having

only residual sperm cysts in the germinal epithelium and abundant spermatozoa in the lumen of seminiferous lobules. In the spawning phase, all the wild females were in spawning condition, showing late vitellogenic oocytes together with POFs or hydrated oocytes, whereas all the captive-reared females were in a regressed condition, showing primary growth oocytes and extensive atresia of vitellogenic oocytes. Spermatogenic activity was still active in wild greater amberjack sampled in this period, whereas all the captive-reared males had ceased their spermatogenic activity, still showing a moderate amount of spermatozoa in the lumen of seminiferous lobules. The liver expression levels of the three vitellogenins did not differ significantly between captive-reared and wild females in any of the three phases of the reproductive cycle and no difference was found between wild and captive-reared fish in the amount of yolk globules contained in vitellogenic oocytes. The analysis of germ cell proliferation and apoptosis showed an abnormal, progressive, reduction of spermatogonial mitosis during the reproductive season in captive-reared males, and very high levels of apoptosis during the early gametogenesis phase.

## Discussion and Conclusions

Gonad development of wild greater amberjack was well described by the GSI, which increased from early May to late May and decreased thereafter. Spawning occurred from late May to early July at surface water temperatures of 19-24°C. Greater amberjack reared in captivity showed a scarce gonad development, evidenced by lower GSI values and sex steroid plasma levels compared to the wild population. Moreover, gametogenic activity of captive-reared greater amberjack showed an early cessation in late May, evidenced by an extensive atresia of vitellogenic follicles along with an increased apoptosis and a reduced proliferation of male germ cells. In females, the observed reproductive dysfunction was not associated to a damaged vitellogenesis, since both liver vitellogenin gene transcription and oocyte yolk accumulation occurred normally. Reproductive dysfunctions often occur in fish caught from the wild and reared in captivity. The most common type of reproductive dysfunction is the absence of oocyte maturation at the completion of vitellogenesis in females and a reduced sperm volume and quality in males (Mylonas et al., 2010). A very high incidence of atretic vitellogenic follicles was recorded in Atlantic bluefin tuna *Thunnus thynnus* females that underwent severe panic frenzy caused by improper manipulation (Corriero et al., 2011). The reproductive dysfunctions described in the present study were probably associated to the repeated handling operations necessary for the sampling of the captive-reared fish and underlay the extreme susceptibility of this species to manipulation, thus suggesting the need for a careful handling of the breeders during the reproductive season.

## Acknowledgments



Co-funded by the Seventh  
Framework Programme  
of the European Union



Financial grant provided by the European Union's Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY).

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