# HEMATOLOGICAL AND PLASMA BIOCHEMICAL PARAMETERS IN F1 GENERATION GREATER AMBERJACK (Seriola dumerili) DURING SPAWNING INDUCTION WITH GNRHa DELIVERY SYSTEMS

M.V. Martin. 1\*, I. Fakriadis<sup>2</sup>, S. Jerez<sup>1</sup>, A. Misol<sup>1</sup> and C.C. Mylonas<sup>2</sup>

#### Introduction

The greater amberjack (*Seriola dumerili*) is a species with high potential for the aquaculture. However, the industrial production is still negligible due to several bottlenecks, among which the absence of reliable reproduction is one of the most important. Agonists of gonadotropin-releasing hormone (GnRHa) have been used to overcome the reproductive dysfunctions in several species. The present study shows the effects of a hormonal spawning induction method using optimal doses of gonadotropin-releasing hormone agonist (GnRHa) on hematological and plasma biochemical parameters in F1 generation greater amberjack, in order to assess the physiological condition and stress indices of treated broodstock.

#### Materials and methods

A group of 9 greater amberjack broodstock born in captivity (average weight of 18.7±6.0kg) was maintained in an outdoor covered raceway (500m³) supplied with 6 renewals day¹¹ of seawater, under natural photoperiod at the Instituto Español de Oceanografía, Tenerife, Spain. The fish (4 males of 16.9±5.0kg and 5 females of 20.1±6.8kg) were sampled five times during the 2016 spawning season (from June to October). Ovarian biopsies were obtained and a wet mount was examined under a microscope to evaluate the stage of oogenesis and the mean size of the largest, most advanced vitellogenic oocytes (n=10). Maturation of the males was confirmed by the release of sperm upon application of gentle abdominal pressure. If this was not possible, a sperm sample was obtained by inserting a plastic catheter. The collected sperm was stored (4°C) until evaluation of sperm density, sperm motility and motility duration.

Blood was collected from the caudal vessels using heparinized syringes. Total erythrocytes and leucocytes were estimated from fresh samples of blood by counting using a Neubauer haemocytometer, and hematocrit was carried out by capillary diffusion and centrifugation. Plasma samples were separated after centrifugation and stored at -80°C until analysis for plasma levels of protein, triglycerides, cholesterol, glucose, lactate and enzymes (GPT, GOT, alkaline phosphatase, cholinesterase and amylase), measured by enzymatic colorimetric assays (Biosystems, Spain), and sodium and potassium, determined by standard spectrophotometric assays (Spinreact, Spain).

Fish were treated with an Ethylene–Vinyl acetate (EVAc) GnRHa implant (Mylonas and Zohar, 2001) in June, July, August and September. Although there were variations in the effective GnRHa dose applied to each fish (due to the implants are loaded with fixed amounts of GnRHa), the females and males were treated with a dose of  $\sim 75$  and  $50 \mu g$  GnRHa  $kg^{-1}$  body weight, respectively. At the time of GnRHa implantation, females were in advanced vitellogenesis and males had intra-testicular sperm.

#### Results

Spawning of greater amberjack started 24-48h after each hormonal treatment and a total of 61 spawning were obtained during a period of 103 days, between June and September. The oocyte diameter ranged between  $440\pm240\mu m$  in July to  $720\pm470\mu m$  in October.

<sup>&</sup>lt;sup>1</sup> Centro Oceanográfico de Canarias, Instituto Español de Oceanográfia, Vía Espaldón, Dársena Pesquera PCL 8, 38180 Santa Cruz de Tenerife, Spain. E-mail: virginia.martin@ca.ieo.es

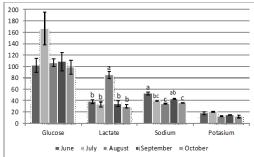
<sup>2</sup> Institute of Marina Pictora Pictora

<sup>&</sup>lt;sup>2</sup> Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, P.O. Box 2214, Iraklion, Crete 71003, Greece

Mean sperm motility was  $54\pm29\%$  during the reproductive period and no differences were observed between the samplings. Mean motility duration was  $2.3\pm0.9$ min, and in June and August were significantly lower than in September. Moreover, the sperm density decreased after the  $1^{st}$  treatment, remaining lower from July to September.

The measured hematological and biochemical parameters along the spawning season in females greater amberjack showed values considered to be within the normal range for greater amberjack and only the number of erythrocytes, leucocytes and plasma protein changed slightly during the experimental period (data not shown). Stress secondary responses include changes in plasma ions and metabolite levels (e.g., increases in glucose, lactate, and decreases in plasma sodium and potassium). In this study, an increase in plasma lactate was observed in August, at the 3<sup>th</sup> treatment-sampling moment, together with a drop in plasma sodium (Figure 1).

Bivariate correlation analysis between plasma parameters and gamete quality indicators from June to September showed that plasma triglycerides were significantly correlated with oocyte diameter (P=0.016) (Figure 2) and plasma protein with duration of sperm motility (P=0.007) in broodstock males.



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Figure 1. Plasma glucose (mg dl<sup>-1</sup>), lactate (mg dl<sup>-1</sup>), sodium (mg dl<sup>-1</sup>) and potassium (mg dl<sup>-1</sup>) in greater amberjack during experimental spawning period.

Figure 2. Linear relationship between plasma triglycerides (mg dl<sup>-1</sup>) and oocyte diameter (μm) in female greater amberjack from June to September.

## Conclusions

No significant variations in hematological and biochemical parameters were observed in F1 greater amberjack after repeated treatment with implants of GnRHa. However, several changes in secondary stress indicators as lactate and sodium were detected during the experimental spawning period, but were not directly associated with the hormonal spawning induction method. Moreover, significant correlations were observed between serum biochemical parameters and gamete quality.

#### References

Mylonas and Zohar. 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. Rev. Fish Biol. Fish. 10: 463-491.

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