

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ ΥΠΟΥΡΓΕΙΟ ΑΝΑΠΤΥΞΗΣ ΚΑΙ ΕΠΕΝΔΥΣΕΩΝ ΕΙΔΙΚΗ ΓΡΑΜΜΑΤΩΝ ΕΤΠΑ & ΤΕ ΕΙΔΙΚΗ ΥΠΗΡΕΙΙΑΔΙΧΕΡΙΣΙΩ ΕΠΑΙΣΚ





Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης



- 1. Introduction
- 2. Broodstock management and sampling
- 3. Spawning induction
- 4. Spermiation enhancement
- 5. Conclusions





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greater amberjack, Seriola, broodstock, reproduction, gametogénesis, sperm, eggs, GnRHa



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#### Advances in Greater Amberjack Broodstock Management

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#### 1.- Introduction



Figure 1. Greater amberjack breeders

#### References

- Corriero A. et al (2021). Reviews in Aquaculture 13, pp. 1781-1815.
- Fakriadis I. et al. (2020) Aquaculture 519, 734880

The greater amberjack (*Seriola dumerili*)(Fig.1) is a cosmopolitan species found throughout the temperate zone, has a rapid growth rate and late reproductive maturation and large body size, characteristics that make it one of the most prominent species for Mediterranean aquaculture diversification (Corriero et al., 2021; Fakriadis et al., 2020). However, until now the greater amberjack production is still low due to inconsistent and unreliable reproduction and production of juveniles for grow out.

Although considerable progress has been made in studying the reproductive biology of greater amberjack and alleviating its reproductive dysfunctions in captivity, the control of its reproduction is still a challenge, as it exhibits relatively lower egg fertilization success compared to other species and lower sperm production compared to breeders in the wild. Additionally, greater amberjack broodstock appear to be very sensitive to repeated handling during gametogenesis, since both males and females abort reproductive development and maturation when handled before the spawning period.

In the framework of the BestBrood project that was funded by the ERA-NET Cofund BlueBio initiative, emphasis was placed on improving male spermiation by evaluating different hormonal treatments. The gonadotropin releasing hormone agonists (GnRHa), either in liquid form or in sustained release implants, and human chorionic gonadotropin (hCG) were tested. Sperm quality was evaluated and the best hormonal treatment was applied at an industrial case scenario.



#### 2. Broodstock management and sampling

Broodstock should be reared in sea cages (Fig. 2) to ensure optimal environmental conditions and welfare. The fish should be left unhandled, at least for the period of early gametogenesis - spawning (spring in the Mediterranean).

#### Anaesthesia and handling procedures

Before any fish handling, animals should be sedated and anesthetized as follows:



Trasnfer fish to a sack for clove oil sedation (10 ppm clove oil, diluted 1:10 in ethanol)



Use a stretcher to transfer and weigh the fish



Put the fish in the anaesthesia tank (30 ppm clove oil, diluted 1:10 in ethanol) in oxygenated seawater (130-150% DO)



Figure 2. Greater amberjack breeders in a sea cage



Check the PIT tag of the fish and write it down in a waterproof paper

#### Sampling procedure



Take an ovarian biopsy from the females or check for spermiation and collect sperm from the males



Check the oocyte development under a compound microscope



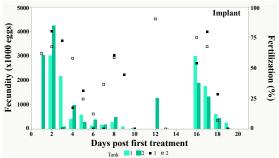
Apply a hormonal treatment to the females at late vitellogenesis and spermiating males



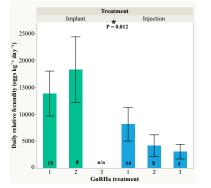
Transfer the fish to a land-based tank for spawning



#### 3. Spawning induction



**Figure 3.** Fecundity (x1000 eggs) and fertilization success (%) of greater amberjack broodstock induced to spawn with GnRHa implants on day 0 and day 14.



**Figure 4.** Mean (±SEM) daily relative fecundity in the periods between GnRHa implantations (green) or injections (blue). The "\*" indicates significant differences between the two GnRHa treatments.

#### References

Fakriadis I. et al. (2019) General and Comparative Endocrinology 279, pp. 78-87

#### 3.1 Method

Greater amberjack broodstock can produce multiple spawns after a spawning induction. Three-four females (15-20 kg each) after spawning induction with GnRHa implants can produce more than 4 million eggs in a single spawn with high fertilization success (Fig. 3). The GnRHa implants seem to be a more effective method of inducing spawning (Fakriadis et al., 2019) compared to GnRHa injections, since higher daily relative fecundity (Fig.4) can be observed with the same quality characteristics (Fig.5).

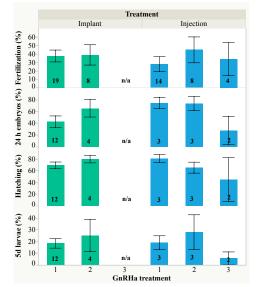
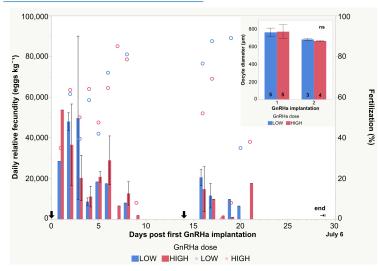


Figure 5. Mean ( $\pm$ SEM) fertilization success, 24h embryo survival, hatching and 5d larval survival in the periods between GnRHa implantations (green) or injections (blue). No significant differences between the two GnRHa treatments were observed.



**3.2 Dose** 

### 3. Spawning induction



**Figure 6.** Mean ( $\pm$ SEM) daily relative fecundity (eggs/kg) and fertilization success (%) of greater amberjack broodstock induced to spawn with GnRHa implants of low (blue) or high dose on day 0 and day 14. Insert: Oocyte diameter before the GnRHa administration.

Daily relative fecundity, fertilization success and 24h embryo survival was not significantly different between the two doses, while less eggs were produced in terms of total relative fecundity after the 2<sup>nd</sup> GnRHa administration regardless of dose (Fig.7).

#### References

Fakriadis I. et al. (2020) Aquaculture 521, 735011

When it comes to the GnRHa dose to be used (Fakriadis et al., 2020), 50  $\mu$ g/kg seems to be effective for the females, since a higher or lower dose had similar results in terms of daily relative fecundity and fertilization success (Fig.6).

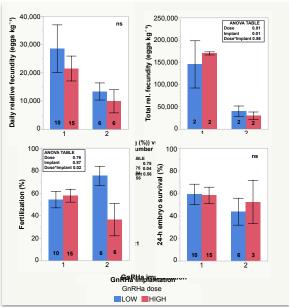


Figure 7. Mean  $(\pm$ SEM) daily relative fecundity, total relative fecundity, fertilization success and 24h embryo survival in the periods between GnRHa implantations.



### 3. Spawning induction

#### 28 ns 800 26 Oocyte diameter (µm) Temperature ("C) 600 24 400 22 200 20 0 18 Ô. 5 10 20 30 15 25 35 40 45 50 100 000 80 Fertilization (%) 00 0 60 0 0 20 end 0 5 10 15 20 25 30 35 40 45 50 May 30 Days of the experiment (2017) July 19 2nd 3rd 4th • 1st • 2nd 3rd · 4th

**Figure 8.** Mean (±SEM) oocyte diameter and temperature, daily relative fecundity (eggs/kg) and fertilization success (%) of greater amberjack broodstock induced to spawn with GnRHa implants at different times in spawning period.

### 3.3 Timing

Regarding the timing of GnRHa administration, between the end of May and mid July in the Mediterranean, when temperature was between 19-24°C, spawning induction was effective. Less spawns were produced after each implantation when the temperature increased (Fig.8) and this had an impact on total relative fecundity (Fig. 9), but no significant differences were observed in daily relative fecundity and fertilization success among the different time of GnRHa application.

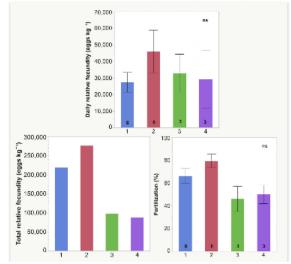


Figure 9. Mean  $(\pm SEM)$  daily relative fecundity (eggs/kg), total relative fecundity and fertilization success (%) of greater amberjack broodstock induced to spawn with GnRHa implants at different times in spawning period.



#### 4. Spermiation enhancement

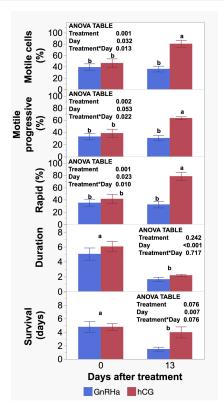


Figure 10. Mean ( $\pm$ SEM) motile, motile progressive and rapid spermatozoa (%), duration of motility (min) and survival under cold storage (days) of sperm collected from greater amberjack males before (day 0) and after (day 13) GnRHa (blue) or hCG (red) administration. Letter superscripts indicate statistical significant differences. Sperm production is a limiting factor in greater amberjack, as captive fish produce less sperm than wild ones. In order to enhance spermiation, the use of human chorionic gonadotropin is recommended since it increases both the number of individuals producing sperm and the quality of sperm in some parameters. The use of 1000 IU/kg hCG compared to 100 µg/kg GnRHa increased the percentage of motility parameters (Fig. 10) 13 days after the administration. while velocitv parameters, well as as straightness and sperm density remained the same 11). (Fig. Motility duration and survival under cold storage were reduced for both hormonal treatments for the same time period.

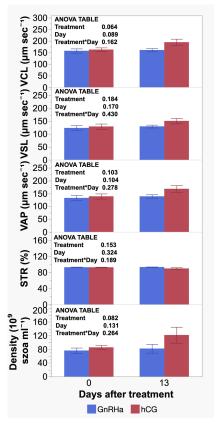


Figure 11. Mean ( $\pm$ SEM) curviliear velocity (VCL), straight line velocity (VSL), average path velocity (VAP)(µm/sec), straightness (STR)(%) and density (10<sup>9</sup> szoa/ml) of sperm collected from greater amberjack males collected before (day 0) and after (day 13) GnRHa (blue) or hCG (red) administration. 8/10



### 5. Conclusions

- Maintain broodstock in sea cages for optimal environmental conditions
- Leave the fish unhandled during the period of gametogenesis
- At the expected spawning period, select eligible fish based on gonadal development evaluation
- Induce females with GnRHa implants using a 50 µg/kg dose and transfer them to land-based tanks for spawning, when temperature is between 19-24°C
- Enhance spermiation of males with hCG using a 1000 IU/kg dose













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ΥΠΟΥΡΓΕΙΟ ΑΝΑΠΤΥΞΗΣ ΚΑΙ ΕΠΕΝΔΥΣΕΩΝ ΕΙΔΙΚΗ ΓΡΑΜΜΑΤΕΙΑ ΔΙΑΧΕΙΡΙΣΗΣ ΠΡΟΓΡΑΜΜΑΤΩΝ ΕΤΠΑ & ΤΣ ΕΙΔΙΚΗ ΥΠΗΡΕΣΙΑ ΔΙΑΧΕΙΡΙΣΗΣ ΕΠΑΥΕΚ





Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης



