

Investigation on Early Development, the Feeding Ability and Larval Survival under Starvation in Common Meagre, *Argyrosomus regius* (Asso 1801)

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Abstract The development of *Argyrosomus regius* early development stages (embryonic, yolk sac and newly larval) was studied, with emphasis on yolk and lipid absorption. The effect of a progressively delayed initial feeding on the feeding ability and survival of this species was also examined. The number of days from hatching after which the feeding rate dropped to half the initial highest feeding rate or the point of no return (PNR) was determined. In May of 2010, the experimental eggs were obtained by natural spawning from captive broodstock (F₁ generation cultured specimens). The embryo, yolk sac and larval stages of meagre were reared in laboratory conditions on temperature of 19 °C and 35ppt salinity. Eggs present a diameter of 1.056±0.010mm, a volume of 0.616mm³ and a wet weight of 0.718±0.033mg, while they contain one lipid globule of 0.265±0.005mm diameter and 0.010mm³ volume. The total length of meagre larvae after hatching and at the onset of exogenous feeding was measured 2.621±0.037mm and 3.492±0.051mm, respectively. The yolk sac reserves were consumed 60h after hatching, while the reserves of oil globule exhausted 156h after hatching. The percentage of larvae with visible gut contents was maximal 108h after hatching and decreased to half (PNR) just 12h after this point or 120h after hatching. The findings indicate a high rate of food demand for this species.

Keywords: Sciaenidae, *Argyrosomus regius*, larva starvation, PNR, yolk and lipid absorption, survival

1. Introduction

Common meagre *Argyrosomus regius* (Teleostei) is a sciaenid species, inhabiting depths ranging of 15-200m and distributed along the eastern coast of Atlantic Ocean from Norway to Gibraltar and Congo, including the Mediterranean (although not very common around Italy and Greece) and the Black Sea, while migrated to the Red Sea via the Suez Canal [4,8]. This species can weigh up to 103Kg and reach 230cm in length [28]. It is gonochoristic, attains puberty at 2 (males) and 3 (females) years of age, with a spawning period from May to July at a temperature range of 17-22 °C [31], in which period the fish produce characteristic sounds [16]. *A. regius* has been proposed as one of the most promising candidate for marine finfish diversification on commercial Mediterranean and Eastern Atlantic coasts aquaculture [17,32] mainly because of the ease of cultivation [6,30], its high growth rate and food conversion efficiency [12,27] and the fresh and fillet quality traits [9,26].

The *A. regius* has pelagic eggs, spherical in shape, transparent, telolecithal, with homogenous and unsegmented vitellus. After yolk resorption, the remaining lipid globule reserves support the fish larvae for a limited period, [25] depending on species, spawn quality, egg and larval dimensions and temperature [14,20]. The onset of

exogenous feeding is a crucial moment in developing fish larvae [34], since after the exhaustion of yolk reserves, the delay or deprivation of food has been associated with massive mortality in marine fish culture [7,33]. Blaxter & Hempel [1] identified the point-of-no-return or PNR (this point has also been called "irreversible starvation"), which is denoted as the number of days from hatching after which the feeding rate drops to half the initial highest feeding rate or the 50% of maximal feeding incidence during progressive starvation. A lot of attempts have been made about feeding ability and survival in relation to the PNR, as also to examine the starvation efficiencies on fish larvae [24,33,35].

In the present study, the main object was to give information on embryonic and early larval development of *A. regius*, with emphasis on yolk and lipid absorption, and also to study the effect of the progressively delayed initial feeding on the feeding ability and survival of this species, determining the PNR, for giving solutions and improve the quality on its rearing.

2. Materials and Methods

The experiment was performed on May of 2010, when the natural temperature of the seawater was 17.5 °C, and was conducted in the Technological Educational Institution of Messolonghi, in the Department of

Aquaculture and Fisheries Management. Eggs were obtained by natural spawning (maturation and spawning were performed spontaneously under natural photoperiod and temperature conditions) from captive broodstocks, descended from culture specimens (F_1 generation), from commercial Greek hatchery. On stage of pre-early gastrula (onset of gastrula), the eggs were transferred to two tanks of 35L water volume each, with a density of 150 eggs L^{-1} , and with a water exchange rate of 50% of the tank volume per hour, via recycled system. Temperature was kept at 19 ± 0.2 °C and salinity at 35 ppt. Constant slight aeration was provided, while the experiment was conducted under natural light condition.

Egg diameter (samples of 150 eggs), lipid globule diameter (samples of 150 eggs) and wet weight (7 samples of 75 to 412 mg) were measured. From these traits the volume of egg and lipid globule were estimated, using the equation of the sphere $(4/3) \pi (LD/2)^3$. The early development of *A. regius* was studied *in vivo* using a stereoscopic microscope (Leica ICCA), while morphometric development with photographs via digital camera (Leica DM100) adapted to microscope, using the ImajJ program. For the embryonic period, 20 eggs were sampled every 30min, while for the yolk sac larvae, 15 specimens were sampled and photographed every 4 hours. The morphometric characters which were measured throughout the entire larval development were TL, prAnl, pstAnl, YsL, YsD, BD, ED, and LD to the nearest 0.001 mm, while YsV and LV, were estimated (Table 1). All lengths were measured parallel to the longitudinal axis of the body, and all depths, perpendicular to this axis (Figure 1).

Table 1. Description of the morphometric characters of *A. regius* measured in the present study

| Abbreviation | Character | Description |
|--------------|------------------------|---|
| TL | Total Length | From tip of snout to the posterior margin of body |
| prAnl | Pre-anal length | From tip of snout to anus |
| pstAnl | Post-anal length | From anus to posterior margin of body |
| YsD | Yolk-sac depth | Maximum |
| YsL | Yolk-sac length | Maximum |
| BD | Body depth | Body height just posterior to anus |
| ED | Eye diameter | (Maximum + minimum) / 2 |
| LD | Lipid globule diameter | Maximum |
| YsV | Yolk-sac volume | $(\pi/6) YsL \times YsD^2$ (Blaxter and Hempel, 1963) |
| LV | Lipid globule volume | $(4/3) \times \pi \times (LD/2)^3$ |

For the determination of the point-of-no-return (PNR), 50 starving larvae were sampled every 12 hours and were placed to a beaker 2L volume, supplied with sea water, algae and rotifers *Brachionus plicatilis* at a density of 10 individual's ml^{-1} approximately. The percentage of feeding larvae was determined 2 h after supplying food. The PNR was determined as the number of days from hatching after which the feeding rate dropped to half the initial highest feeding rate (or defined as 50% of maximal feeding incidence) [1]. The study of starvation continued up to total mortality of the experimental population.

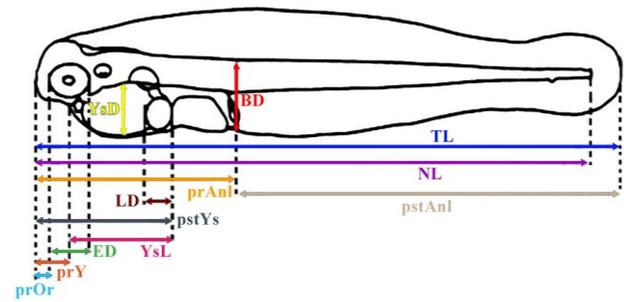


Figure 1. The measured morphometric characters on the larvae of *A. regius*

3. Results

The fertilized eggs present a diameter of 1.056 ± 0.010 mm (1.040 to 1.081 mm), a volume of 0.616 ± 0.017 mm³ (0.589 to 0.661 mm³), a wet weight of 0.718 ± 0.033 mg (0.678 to 0.767 mg) and contain a single, un-pigmented lipid globule of 0.265 ± 0.005 mm (0.255 to 0.271 mm) diameter and 0.010 ± 0.001 mm³ (0.009 to 0.011 mm³) volume (Table 2).

Following the series of embryonic development of *A. regius* after the pre-early gastrula, the blastoderm expanded gradually over the surface of yolk mass, and after epiboly, the neural groove formation started with a mass of cells clearly visible in the anterior side of blastopore. The first somites observed soon after closure of the blastopore, while the number of somites finally reached 25-28. The duration of the above stages (gastrula, neurula and organogenesis of embryo) was 12, 3 and 27 hours, respectively. The pigmentation throughout the embryonic organogenetic stage was characteristically strong along almost the entire body. Within an hour the process of hatching was completed.

Table 2. Eggs traits of *A. regius* measured in the present study

| Traits | Aver. | S.D. | Max. | Min. | n |
|---|-------|-------|-------|-------|------|
| Egg's diameter (mm) | 1.056 | 0.010 | 1.081 | 1.040 | 150 |
| Egg's volume (mm ³) | 0.616 | 0.017 | 0.661 | 0.589 | 150 |
| Egg's weight (mg) | 0.718 | 0.033 | 0.767 | 0.678 | 2125 |
| Lipid globule's diameter (mm) | 0.265 | 0.005 | 0.271 | 0.255 | 150 |
| Lipid globule's volume (mm ³) | 0.010 | 0.001 | 0.011 | 0.009 | 150 |

The newly hatched larvae of *A. regius* floated at the water, while characterized by a large yolk sac extending from the tip of the snout to the middle of the body, with the lipid globule located at the posterior ventral part. The head and anterior part of the body were curved over the yolk sac, and the transparent primordial marginal finfold surrounded the body from the dorsal area of the head to the posterior margin of the yolk. The pattern of pigmentation included big mass of pigment cells in the dorsal area of the body especially on head, with three characteristic spots at 1-3 somites, at 9-10 somites and at 18-20 somites. During the yolk sac larval stage (in the present study this duration was 60 hours), the digestive system was completed following the Sparidae species pattern, but the oil globule did not adhere on the body of the larvae.

The TL immediately after hatching was measured 2.621 ± 0.037 mm (1.211 ± 0.055 mm and 1.409 ± 0.059 mm, prAnl and pstAnl, respectively) (Table 3). This character increased rapidly at first 24 hours (approximately 88% of total increase of stage), followed by a slow growth the

next 24 hours, and then kept almost constant up to the end of stage, where it measured 3.492 ± 0.051 mm. The increase of TL determine at the area after anus (prAnl length), while the prAnl length kept a constant rate.

Table 3. Morphometric characters (average and standard deviation) of *A. regius* yolk sac larvae (character abbreviations are explained in Table 1). The time is given as hours after hatching (A.H.)

| Hours A.H. | TL (mm) | | prAnl (mm) | | pstAnl (mm) | | BD (mm) | | ED (mm) | |
|---------------|---------|-------|------------|-------|-------------|-------|---------|-------|---------|-------|
| | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. |
| 0 | 2.621 | 0.037 | 1.211 | 0.055 | 1.409 | 0.059 | 0.493 | 0.045 | 0.219 | 0.015 |
| 12 | 3.152 | 0.055 | 1.254 | 0.030 | 1.898 | 0.064 | 0.538 | 0.031 | 0.242 | 0.009 |
| 24 | 3.383 | 0.039 | 1.263 | 0.026 | 2.120 | 0.045 | 0.563 | 0.015 | 0.263 | 0.012 |
| 36 | 3.427 | 0.054 | 1.265 | 0.035 | 2.162 | 0.049 | 0.565 | 0.012 | 0.273 | 0.007 |
| 48 | 3.471 | 0.050 | 1.262 | 0.030 | 2.209 | 0.048 | 0.564 | 0.018 | 0.282 | 0.012 |
| 60 | 3.492 | 0.051 | 1.262 | 0.040 | 2.230 | 0.060 | 0.590 | 0.020 | 0.281 | 0.008 |

The yolk sac at newly hatched larvae of *A. regius* measured 1.127 ± 0.057 mm YsL and 0.852 ± 0.040 mm YsD (representing a volume of 0.430 ± 0.054 mm³), while the diameter of lipid globule was 0.264 ± 0.010 mm (0.010 ± 0.001 mm³ volume) (Table 4). These measures show that the yolk consumption during the embryonic period was 29%, while the lipid reserves were not utilized during this time. The consumption of the yolk sac reserves followed similar pattern with the TL increase, as at the first 24 hours the YsV was rapidly decreased (approximately 75% of total decrease of stage), followed by a slower rate of decrease the next hours, to be consumed totally at the end of stage.

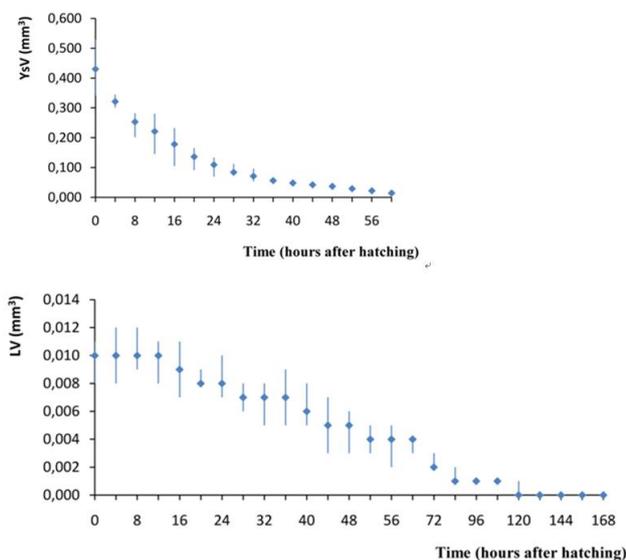


Figure 2. Evolution of the yolk sac volume (YsV) and lipid globule volume (LV) (mean values with SE) of *A. regius* in relation to time (hours after hatching)

In contrast, the consumption of lipid reserves started about 16 hours after hatching (the increase in the rate of oil globule utilization 16 hours after hatching corresponded to extensive cranial differentiation), and followed stable rate during the remainder yolk sac larval stage, while the LD was decreased at the end of yolk sac larval stage up to 60%, as the LV from 0.010 mm³ at the begging of yolk sac stage reduced to 0.004 mm³ at the end of stage (Table 4) (Figure 2). This pattern of lipid reserves consumption changed on the larval stage. Specifically, in

first 12 hours of larvae stage it was consumed the 50% of larval oil globule reserve (the LV from 0.004 mm³ reduced to 0.002 mm³), and after 24 hours the 75%, while the total consumption occurred 7 days after hatching. Another character that determines the quality of fish is the swim bladder inflation. At the present study, the swim bladder inflation of *A. regius* started 24 hours after the onset of larval stage (10% of observed individuals had inflated swim bladder) and into 36 hours completed the inflation with 100% success.

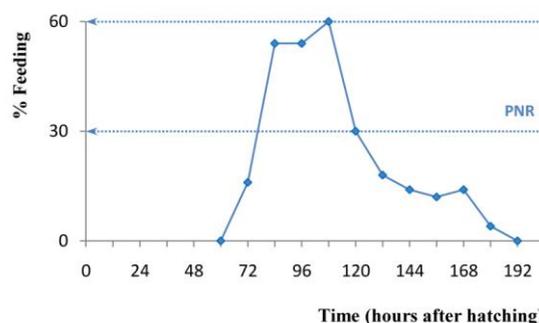


Figure 3. The percentage of *A. regius* fed larvae in relation to time (hours after hatching). PNR is the point-of-no-return (defined as 50% of maximal feeding incidence)

The food ingestion ability was affected by the amount of time that larvae of *A. regius* were kept without diet (Figure 3). The larvae started to eat (the percentage of fed larvae was 16%) 72 hours after hatching (12 hours or ½ day of larval age) and not from the onset of larval stage, although the mouth was already open. The highest percentage of fed larvae (60%) was obtained when diet was offered 108 hours after hatching (48 hours or 2 days of larval age). 12 hours after this point, the percentage of larvae able to start feeding was 30% (50% of the above mentioned maximum). Thus, 120 hours or 5 days after hatching (60 hours or 2 ½ days of larval age), the larvae were in condition of irreversible starvation, characterized as point-of-no-return PNR. All the unfed larvae had died by 192 hours or 8 days after hatching (144 hours or 6 days of larval age), while the first mortality observed at the time of the highest percentage fed larvae.

Table 4. The consumption of yolk sac and lipid globule reserves during the early life stages (yolk sac larvae and larvae) of *A. regius*. The time is given as days or hours after hatching (A.H.) (character abbreviations are explained in Figure 1 and Table 1)

| Developmental stage | Days | Hours | YsL (mm) | | YsD (mm) | | YsV (mm ³) | | LD (mm) | | LV (mm ³) | |
|---------------------|--------|-------|----------|-------|----------|-------|------------------------|-------|---------|-------|-----------------------|-------|
| | | | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. |
| Yolk sac larvae | 0 | 0 | 1.127 | 0.057 | 0.852 | 0.040 | 0.430 | 0.054 | 0.264 | 0.010 | 0.010 | 0.001 |
| | | 4 | 0.955 | 0.022 | 0.801 | 0.014 | 0.321 | 0.018 | 0.264 | 0.016 | 0.010 | 0.002 |
| | | 8 | 0.913 | 0.031 | 0.726 | 0.037 | 0.253 | 0.032 | 0.266 | 0.009 | 0.010 | 0.001 |
| | | 12 | 0.865 | 0.051 | 0.696 | 0.046 | 0.221 | 0.038 | 0.264 | 0.010 | 0.010 | 0.001 |
| | | 16 | 0.808 | 0.030 | 0.644 | 0.064 | 0.178 | 0.038 | 0.254 | 0.012 | 0.009 | 0.001 |
| | 1 | 20 | 0.751 | 0.029 | 0.586 | 0.036 | 0.136 | 0.019 | 0.253 | 0.005 | 0.008 | 0.001 |
| | | 24 | 0.675 | 0.040 | 0.553 | 0.033 | 0.109 | 0.017 | 0.253 | 0.011 | 0.008 | 0.001 |
| | | 28 | 0.601 | 0.019 | 0.516 | 0.035 | 0.084 | 0.012 | 0.241 | 0.007 | 0.007 | 0.001 |
| | | 32 | 0.574 | 0.042 | 0.484 | 0.033 | 0.071 | 0.011 | 0.236 | 0.010 | 0.007 | 0.001 |
| | | 36 | 0.510 | 0.026 | 0.458 | 0.025 | 0.056 | 0.007 | 0.233 | 0.012 | 0.007 | 0.001 |
| | 2 | 40 | 0.462 | 0.017 | 0.445 | 0.021 | 0.048 | 0.006 | 0.223 | 0.010 | 0.006 | 0.001 |
| | | 44 | 0.445 | 0.017 | 0.425 | 0.030 | 0.042 | 0.006 | 0.213 | 0.015 | 0.005 | 0.001 |
| | | 48 | 0.429 | 0.029 | 0.404 | 0.021 | 0.037 | 0.005 | 0.208 | 0.013 | 0.005 | 0.001 |
| | | 52 | 0.392 | 0.014 | 0.378 | 0.027 | 0.029 | 0.005 | 0.197 | 0.014 | 0.004 | 0.001 |
| | | 56 | 0.374 | 0.024 | 0.334 | 0.024 | 0.022 | 0.003 | 0.189 | 0.014 | 0.004 | 0.001 |
| | Larvae | 60 | 60 | 0.352 | 0.042 | 0.280 | 0.015 | 0.014 | 0.002 | 0.189 | 0.008 | 0.004 |
| 72 | | | | | | | | | 0.162 | 0.008 | 0.002 | 0.000 |
| 84 | | | | | | | | | 0.141 | 0.011 | 0.001 | 0.000 |
| | | | | | | | | | 0.121 | 0.008 | 0.001 | 0.000 |
| 96 | | | | | | | | | 0.111 | 0.006 | 0.001 | 0.000 |
| | | | | | | | | | 0.087 | 0.015 | 0.000 | 0.000 |
| 108 | | | | | | | | | 0.054 | 0.012 | 0.000 | 0.000 |
| | | | | | | | | | 0.053 | 0.018 | 0.000 | 0.000 |
| 120 | | | | | | | | | 0.045 | 0.016 | 0.000 | 0.000 |
| | | | | | | | | | 0.039 | 0.006 | 0.000 | 0.000 |
| 132 | | | | | | | | | | | | |
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| 168 | | | | | | | | | | | | |
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4. Discussion

The results of this study, although demonstrated that the *Argyrosomus regius* exhibited significantly larger eggs and lipid globule comparatively to the common species produced in Mediterranean aquaculture [13,4,19], seemed to follow the same ontogenetic pattern with the above species.

The egg size was found as an important factor affecting the survival of fish larvae through the influence on larval size, growth rate, and length of time from hatching to irreversible starvation [10,23]. Larger eggs provide more energy for growth and development, while produce larger larvae capable to avoid predators more effectively, with longer survival under unfed conditions, able to exploit larger water volume for prey search [10]. If growth rate is positively related to initial body size, larger larvae will spend less time at the most early size [20]. On the other hand, larger eggs require more time to incubate [11,37].

Larvae from bigger eggs are usually bigger while hatching, as well as while beginning exogenous feeding. As the TL is directly related to the mouth opening and the prey size that larvae are able to consume [21,30], the increased size at first feeding of *A. regius* affecting the successful rearing techniques of this species.

The larval development of *A. regius* during yolk resorption is similar to that of other species produced in Mediterranean aquaculture [13,14,15,19,36], with a yolk utilization pattern similar to the other marine larvae that contain visible oil globule [2,36]. Because of the rapid increase of *A. regius* TL after hatching, the consumption of vitelline reserves was higher in the same period, while the lipid reserves exhibited a stable consumption rate during this stage.

The slower utilization rate of the oil globule reserves to the yolk sac stage supports the hypothesis of the primary energy source at the onset of exogenous feeding [25,29].

In addition, the transition feeding period in fish larvae is defined as an interval in which feeding ability has been developed and also feeding commences, with some lipid reserves still present to content the energetic demands of prey capture [22]. The period of time a larvae has to find food is directly related to the transitional feeding stage.

Compared to the other studied cultured species [2,14,15], the oil globule never adhered to the body, without this to be a problem for the growth and survival of *A. regius*. There are a lot of causes reported that lead to a low proportion of adhered lipid globule [36], as also the importance of lipid droplet localization [2,36], but the complete lack of adherence may be a typical character of this species.

For the *A. regius* total yolk absorption occurred 2 ½ days or 60 hours after hatching and leads from endogenous to exogenous feeding driven by the survival instinct. In nature there is a period of starvation until the start of the exogenous feeding. In aquaculture, several studies showed that different species of fish differ in their ability to withstand delay in first feeding of food deprivation [5], additionally the maternal conditions affected the early life history species traits. Chambers *et al.* [3] found that an increase in oil globule volume at hatching extended the time of survival under starvation conditions at the onset of larval period, as also, the lipid stored around the organs was an important contribution to the fasting endurance because the high weight ratio was due mainly to the increasing lipid tissue content around the intestine [11].

The *A. regius* delayed 12 hours to start the exogenous feeding, while the PNR was attained 5 days after hatching and only 12 hours after the point with the highest percentage fed larvae. In consequence, larvae had only 2.5 days to begin feeding. The PNR obtained in this study (7.5 to 8 days after egg fertilization) almost agrees with the 8.8 days predicted from the regression of PNR against temperature for marine fishes [18]. On the other hand, the

small period of time between the highest percentage of fed larvae and the irreversible starvation timing, indicates the high rate of food demand on mass culture of common meagre.

The present study indicates the need to improve the biologic knowledge of this new culturing species. Understanding the endogenous energy reserves consumption pattern in fish larvae, especially in new cultured species, helps aquaculturists to construct an appropriate management technique to maximize larval rearing success in massive aquaculture systems. The study of the influence of lipid droplet absorption on larval survival and its resistance to starvation can be used as an early indication of the batch quality.

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