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1. Introduction

Malformations in seabass and gilthead seabream hatcheries were extensively studied in the FineFish project, with a major focus to the abiotic factors of the tank environment and nutritional aspects during early life stages. Abiotic factors included the effect of temperature and salinity and a combination of these. The focus on these topics was based on previous knowledge for both species. Rearing temperature during early stages is one of the most acknowledged factors implicated in the development of malformations and, during the FineFish project, we were able to do “fine tuned” experiments in order to recommend safe limits. Nutritional studies included the role of vitamins (A, C & D) and lipids with regard to malformations..

These vitamins were chosen because they can have physiological effects:

- Vitamin A is involved in night vision, it is an antioxidant, it is associated with cell differentiation, and it controls the expressions of many genes involved in morphogenesis (can be teratogenic when overdosed).
- Vitamin D is a hormone that maintains the Ca homeostasis and directly acts on bone cells..
- Vitamin C is an antioxidant, is essential for collagen synthesis, and it participates in several metabolic processes..

Lipids were chosen because:

- Phospholipids (PL) are essential components of the cell membranes and are involved in cellular Ca metabolism.
- Phosphatidylcholine (PC) and phosphatidylinositol (PI) levels in diet were reported to be associated with reduced deformities, and improvement of many zootechnical parameters.
- Lipids are an important component of the larval diets.

General recommendations on hatchery management for both sea bass and sea bream can be found in the *'Manual on Hatchery Production of Seabass and Gilthead Seabream: Volume 1' (FAO 1996)*.

In this chapter we provide new species specific recommendations for reducing malformations in gilthead seabream and seabass production based on results of the FineFish project.

2. Seabass

Abiotic factors — Temperature

Growth and survival were directly affected by changes in temperature. Low temperature at the early stages reduced growth rates, but resulted in higher survival and lower malformation incidences. Haemal lordosis was evident at any deviation from early water temperature of 15°C. However, at a given optimal temperature for minimal haemal lordosis, there was an increased incidence of pugheadness which underlines the need for further research on the combined effect of temperature and other factors (e.g. nutrition) for normal bone development.

Based on the results of FineFish experiments, we recommend that the best rearing performance of seabass is achieved within the known thermal range (18-21°C), while a low temperature (15°C) from epiboly up to metamorphosis increases the resistance to swimming induced haemal lordosis.

Nutritional factors

Vitamins

The standard vitamin mix level used by most of the hatcheries induced a significant percentage of head and vertebral column deformities showing the need to further refine the proportions of certain vitamins (particularly those known to be involved in bone and collagen synthesis, i.e. vitamin A, D and C).

High dietary vitamin A levels strongly affect early larval development (before day 20); the effect is less marked after this stage. The dietary vitamin A level should then be changed during larval development in order to minimise the appearance of deformities: The optimal dose for the early developmental stage should be ~ **15 µg/g DW** until day 20 post-hatching. A higher dose ~ **45 µg/g DW** could be used afterwards. If there is a high incidence of cranial deformities we recommend that the vitamin A levels are checked, especially during the rotifer feeding phase

Low vitamin D levels in feeds could represent a risk for larval development; however this risk is unlikely considering the amount of vitamin D present in the live prey enrichments or in larval feed incorporating marine products. Low levels of vitamin D influences bone mineralisation.



Figure 1: European seabass (*Dicentrarchus labrax*)

Photo: NCM

Low levels of vitamin C induce abnormalities while high levels of vitamin C affect bone formation and lipid metabolism. The level of vitamin C in the feeds has to be accurately defined concurrently to the dietary lipids, in order to prevent a poor mineralisation linked to a disruption of bone formation induced by the metabolism of lipids. Therefore, the vitamin C level should be assessed concurrently with lipids.

Lipids

The larval nutritional requirement in HUFA depends on temperature. Inadequate HUFA supply influence the process of morphogenesis. Molecular pathways influenced by HUFA were not totally similar to those usually studied with vitamins.

3. Feeding regime for seabass

Live feed phase

Commonly, seabass larvae are start fed with enriched *Artemia* (80 one-day old *Artemia*/larva) 4 days after mouth opening and before the total resorption of the yolk-sac. For the following days, the amount of *Artemia* is adjusted according to the appetite of the larva and could reach 200 one-day old *Artemia* at day 20 post-hatch.

Weaning phase

From this date, co-feed *Artemia* and formulated feed for 5 days, during which the amount of live prey is gradually reduced and the amount of formulated feed is increased. Do look at the larvae regularly to check that they are eating the formulated feed and particularly control whether the size of the microparticulated diet is convenient.

Alternative and new feeding protocol

The availability of new microparticulated diets that can be used from mouth opening offer an alternative feeding sequence, based on a co-feeding *Artemia* and formulated diet from day 9 post-hatching until day 15, and then followed by a progressive reduction of *Artemia* until day 20. The amount of *Artemia* and formulated diet must be adjusted by the hatcheries themselves.

Growth expectancy

Seabass larvae usually reach at least 50mg at the end of the larval period, whatever the feeding protocol (classical or alternative). The percentage of deformities is however slightly different, and it can be expected less than 4% deformities with the classical feeding protocol versus less than 10% with the alternative one.

4. Gilthead seabream

Abiotic factors — Temperature

Growth and survival are also affected by temperature for seabream. The FineFish experiments have confirmed that the best rearing performance of gilthead seabream is achieved between 19-22°C (One study show that the incidence of skeletal deformities was independent of the rearing temperature within the range of 19-25°C). These temperatures also result in lower deformity rates for the operculum and the caudal fin. The development of haemal lordosis seems to be independent of temperature.

Abiotic factors — Salinity

High salinities (40‰) result in poorer swim bladder inflation and consequently in lower survival and reduced juvenile quality. Optimum salinity for gilthead seabream according to the FineFish experiments is 25‰. At this salinity better survival rates and juvenile quality (in terms of malformation) were achieved.

Recommendations

For the best rearing results, in terms of malformation and growth, rear at 22°C and 25 ppt for the entire larval period of 35 DPH (770 degree days).

Nutritional factors—Vitamins

Similar effects of the dietary levels of vitamin A were observed in seabass and seabream larvae. High dietary vitamin A levels strongly affect early larval development (before day 20); the effect is less marked after this stage. The dietary vitamin A level should be then changed during larval development in order to minimize the appearance of deformities: The optimal dose for the early developmental stage should be 30 ng/mg DW of rotifers (3.9 µg/g WW) and 45 ng/mg DW in Artemia nauplii (3.4 µg/g ww Artemia nauplii) until day 20 post-hatching.

Nutritional factors—Lipids

For best larval growth performance, when microdiets are used, the recommendation is to never exceed the ratio of 5 between PC/PI in the phospholipid fraction (5% total phospholipid).

The best PC/PI ratio is 1.28 and a PI dietary level of 3.04g 100g-1 diet (DW)



Figure 2: Gilthead seabream (*Sparus aurata*)

Photo: NCM