EFFECT OF CULTURE SYSTEM AND DIET IN EARLY LARVAL STAGES OVER SKELETAL DEFORMITIES DEVELOPMENT IN GILTHEAD SEA BREAM.

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ABSTRACT

There are some evidences that main deformities considered as quality descriptors in commercial marine fish fry production as lordosis, opercular deformities and upper/lower jaws shortening are related with larval culture conditions in early larval stages. The aim of this work was to obtain information about the contribution of the diet and rearing system to the apparition of these abnormalities in gilthead sea bream (*Sparus aurata*) larvae in semi-industrial scale facilities. Two different treatments were designed, in the former all the culture conditions were equal and two commercial rotifers enrichment emulsions were tested; posterior artemia and weaning phase were perform with the same feeding regimes and products. For the later, feeding regimes and products were the same for the whole larval period, although tank design and larval density were different. Biochemical composition of larvae, preys and commercial products were assessed. At 50 days post hatching six hundred fish per treatment were individually check under stereoscope and abnormalities frequency recorded, 95 DPH fry were soft X ray monitored as well. Survival and malformation frequency were significantly different between treatments, the effect of diet and system are discussed.

MATERIAL AND METHODS

Experiment I: System treatment.

Two different cylindroconical fibber glass larval tanks designs were use, two 40 cubic meter tanks for the larval phase with a semi-intensive method (7 eggs/l-1) and 2 cubic meter tanks with the intensive method (125 eggs/ l-1). Larval rearing were conducted under continuous photoperiod, combining both natural and artificial light. Natural salt (37‰), almost constant temperature (20 + 1ºC) water, previously pass through a sand filter and a UV sterilizer was used.

From 2 days post hatching (dph) 5-7 enriched rotifers per ml were added twice a day, when larvae reached 5,5 mm 0.25-0.5 artemia nauplii per ml were added and enriched nauplii thereafter. From 20dph a starter commercial diet was combined with live food. During rotifers phase, phytoplankton (Nannochloropsis oculata) was added daily keeping a concentration of 250.000 cells/ml in the tanks.

Experiment II: Diet treatment.

Four 2 cubic meters cylindroconical fibber glass larval tanks were use with intensive larval rearing method (125 eggs/l-1). Culture conditions were the same than the previous treatment although two commercial emulsions for rotifers enrichment were tested (R1 ,R2).

Abiotic parameters as dissolved oxygen and temperature were recorded daily and light intensity and ammonia levels weekly. Every 5-7 days the total length of a 25 larvae sample were measured by a profile projector (Nikon V-12A, Nikon, Tokyo, Japan), these larvae were used after to determine fresh and dry weight. A 1000 larvae sample per tank were collected at 12, 20 dph and two hundred larvae at 33 and 46 dph from each tank for biochemical analysis. Samples of rotifers, artemia, pellets and enrichment products were taken twice during the larval phase for biochemical analysis as well.

Biochemical analysis.

Total lipid extraction was carried out using a chloroform/methanol (2:1, v/v) mixture as described by Folch *et al.*,1956. Fatty acid methyl esters (Fame's) were prepared by transesterification. Individual Fame's were identified by reference to a standard and quantified by a Shimadzu C-R4A integrator.

Survival:

Survival at the weaning phase were determined after 112 dph. Survival in larval phase (50 dph) were determined by addition to the final number of fry (112 dph), the daily died counted fish for the whole weaning and pre-growing period.

Deformities characterisation:

For skeletal characterisation, six hundred larvae per treatment were killed with an anaesthetic overdose, the internal and external morphology were individually monitored. Observed abnormalities were recorded, for statistical analysis purposes, we established an acuity scale that just takes in consideration the most critical deformation. In order to determine accuracy the effect after 95 dph, fry samples from each treatment were soft X ray monitored again to check the abnormalities presence.

RESULTS AND DISCUSSION: LARVAL GROWTH AND SURVIVAL

One of the major problems in aquaculture is the production of high quality fry. Skeleton anomalies are a considerable problem for hatchery industry, with an important negative economic impact.

Both rearing systems used in present trials were appropriated for seabream larval rearing, although semi-intensive conditions provided a more natural environment where none factor was critical for the larval development, which growth better from the very beginning of life stages, these rearing conditions improved as well larval survival. Semi-intensive system improved survival obtained in the intensive one at very high levels (78%) with significant differences from hatching rates to the end of the experimental period (Figure 1). Clear significant differences in total length were found between systems from 5dph to the end of the larval phase (Figure 2). High stocking densities have been shown to produce a wide variety of effects on culture fish populations such as alterations in behaviour and poor feed utilisation, resulting in mortality and poor growth (Montero *et al.*,1999)

In diet treatment, significant differences ($P<0.05$) were found too, thus R1 improved survival 60% more than R2 (Figure 3). At the early larval stages no significant differences were found on growth between diets. However from 20 dph to the end of the larval period, larvae from R2 showed significant better growth than larvae from R1 (Figure 4).

These differences could be related with the high mortality incidence in R2 at day 11 and 25 which reduced significantly survival for this diet (10%) and consequently larval density, increasing food availability and vital space for the remaining larvae which grew better. Early larval mortality could be associated with dietary requirements, especially lipids and essential fatty acids of the diets used. Dietary requirements become more critical from 10dph to 17dph when both lipoproteic-glycogenic activity of the liver and enterocytary absorption of lipids are important (Dhert *et al.*, 1998).

One of the main differences found between the enriched rotifers used were the higher values of n-3 HUFA especially Docosahexaenoic acid (DHA) and Docosapentaenoic acid (DPA) levels in R2 (Table I). The latter was especially rich in some products that are being used as fish oil alternatives as Essential Fatty Acid (EFA) providers. The level of this fatty acid (FA) is extremely higher in R2 than in R1, and it was clearly reflected on larval composition in previous experiences, and probably related with the mortality observed for this diet treatment (Roo *et al.*, 2004, in press). In the same way Koven *et al.*, (2001) obtained the worst survival results after rotifers phase for larvae reared with rotifers enriched with products with a high levels of DPA. However these authors related these findings with the Arachidonic acid (ARA) values in diets. Blair *et al.*, (2003) rearing haddock larvae, found that artemia enriched with some commercial products showed high bio-concentration levels of DHA, DPA y ARA suggesting a specific role as EFA for the DPA, as well as the role as ARA precursor, suggested by Barcley and Seller (1996) and Sargent *et al.*, (1997). On the other hand Robin *et al.*, (2003) did not find this precursory capacity using microdiets in seabream larvae, showing independence of ARA incorporation from DPA content in diets. In present study ARA levels were 80% less in R1 than R2, so probably the effect of ARA deficiency in mortality was limited.

Table I. Total lipids fatty acid composition (% of total fatty acids) of the rotifers used R1,R2.

Fatty acids	R1	R2
16:0	15,180	18,387
16:1 n-7	14,028	7,433
18:0	3,308	2,033
18:1 n-9	17,309	7,611
18:2 n-6	8,960	1,808
18:3 n-3	3,044	0,300
20:4 n-6	0,691	3,306
20:5 n-3	5,177	3,577
22:5 n-6	0,456	9,827
22:6 n-3	10,014	28,573
Saturated	22,334	26,927
Monounsaturated	40,313	19,664
n-3	22,029	34,768
n-6	10,952	16,118
n-9	23,200	10,122
n-3HUFA	18,270	34,062
AA/EPA	0,133	0,924
EPA/DHA	0,517	0,125
DHA/DPA	21,938	2,907
oleic/DHA	1,728	0,2664
oleic/n-3HUFA	0,947	0,223
n-3/n-6	2,011	2,157

LARVAL GROWTH AND SURVIVAL

Figures 1,2: Survival after 50 and 112 days post hatching, and total length evolution at system treatment.

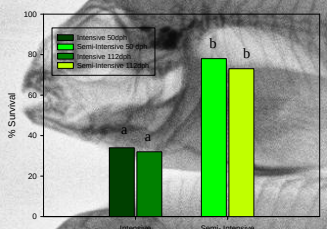

Figure 1

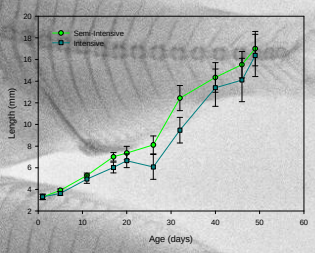

Figure 2

Figures 3,4: Survival after 50 and 112 days post hatching, and total length evolution in diet treatment.

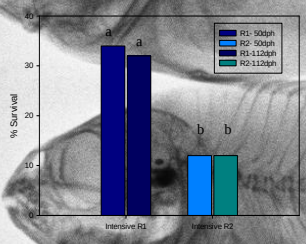

Figure 3

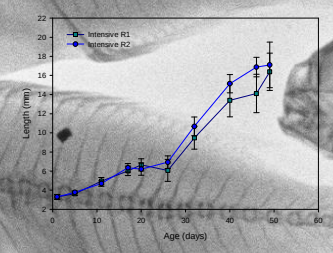

Figure 4

LARVAL DEFORMITIES

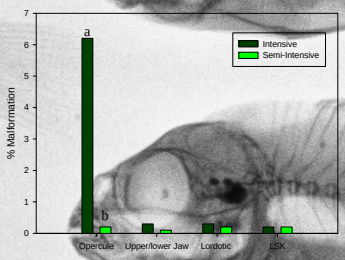

Figure 5: Acute deformities at 50 dph.

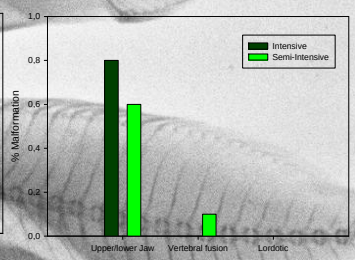

Figure 6: Light deformities at 50 dph.

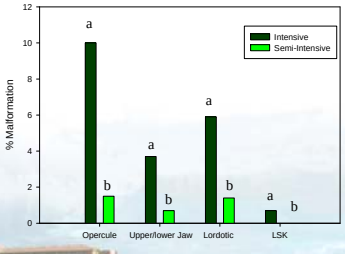

Figure 7: Acute deformities at 95 dph.

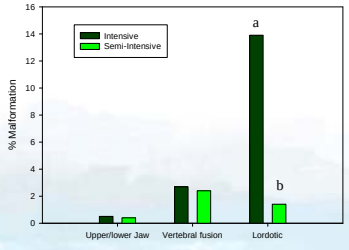

Figure 8: Acute deformities at 95 dph.

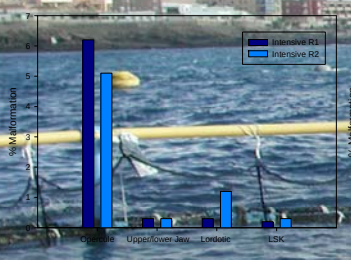

Figure 9: Acute deformities at 50 dph.

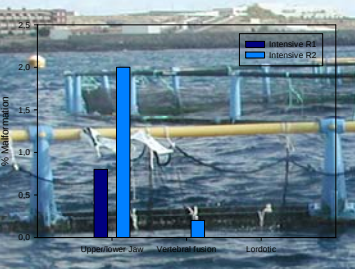

Figure 10: Light deformities at 50 dph.

RESULTS AND DISCUSSION: LARVAL DEFORMITIES

After 50 days of culture, the first deformity evaluation was conducted. At this age loglineal statistical analysis, shows dependence of the abnormalities recorded from the rearing system ($\chi 2$: 78.64; $P>0.05$). Relation between deformities and rearing system is mainly due to significantly different rates in opercular abnormalities. Intensive system reached values up to 6 % at this age, and no more than 0.2% of this deformity was recorded in the semi-intensive one. No significant differences were shown in other abnormalities considered (Figures 5,6).

Opercular deformities are common in different fish species, and are frequent in reared seabreams (Koumoundouros *et al.*,1997) affecting negatively biological functions, as respiration because of a reduced efficiency of the bucal pump, gills exposition to physical damage and infection by fungi, bacteria and parasites (Andrades *et al.*, 1996; Beraldo *et al.*, 2003). All these factors could affect final survival percent obtained in system and diet treatments. Several opercular deformities occur at the beginning of chondrogenesis and ostheogenesis. According with Beraldo *et al.*, 2003, the first signs of the abnormality were observed between 20-40dph, showing a nearly folding of the loose edge of the operculum, in the gill chamber. However, different structures that constitutes the operculum starts to ossified at earlier stages, from 3.7 mm to 5.4mm total length (Almeida, 2001). Divanach, P (pers. comm) suggests that fish reared on semi-intensive systems can be move to intensive one just after 20-25 dph without an increase in frequency of the ostheological deformations. So, according with these references the differences obtained between rearing systems suggested that factors involves in the development of this deformation are mainly affecting in the 3 first weeks of larvae life. Efforts to identify the causes of this deformity have to be focus on these early stages.

Ninety five days old fry checked with soft X ray shown that, log lineal statistical analysis of the recorded ostheological malformation frequency between the two rearing systems used, were not independent ($\chi 2$: 239; $P>0.05$). Opercular, acute upper/lower jaws shortening as well as acute lordosis were significantly higher in the intensive system, increasing the recorded frequency from 50dph. The most important frequency of lordosis apparition (90%) was positioned between the 14th and 16th vertebra. The association Lordosis-Scoliosis-Kyphosis fish were superior in the intensive one affecting 0.7 % of the population (Figure 7). Soft upper/lower jaws shortening and vertebral fusion were not affected by the culture system. Soft lordosis (only detected on the Rx, not visually) were recorded in up to 13% of the juvenile reared in the intensive system, no more than 3.5% were recorded in the Semi-intensive one (Figure7,8).

Larvae reared in the intensive system and feed with the different diets at the rotifers stage, after data statistical loglineal analysis, showed the independence of the abnormalities recorded from the diet used at this stage. Nevertheless the effect of the diets were reflected at opercular, lordotic and LSK abnormalities although with no significant differences. Thus, R1 slightly increased the opercular deformity level (6.2% diet 1 vs 5.1%, R2) on the other hand more relevant differences were found with the lordotic larvae, R2 showed four times more lordotic individuals (4.2%) than R1(0.2%) as well as LSK larvae, which were 3 times higher in this diet (0.2% diet 1 vs. 0.7% diet 2) (Figure 9,10).

Semi-intensive system used reduce 35% the use of life food per fry produced, besides high survival an quality fry in terms of ostheological deformities were produced, allowing farmers sold fry without previous individual sorting with an important cost reduction.

On the other hand the intensive technology used at these trial and previous experiences shows a high reproducibility in terms of survival an fry quality, both are sometimes limiting factors at commercial scale.