news
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# FineFish

Improving sustainability of European fish aquaculture by control of malformations

**FineFish has now been running for 2 years and this newsletter contains several recommendations so as to reduce malformations in the following species: Seabass, Seabream, Rainbow Trout and Cod. More recommendations are expected from the project team for the next newsletters as well as information about training and workshops that will be provided by FineFish in 2008-9.**

## In this issue



# Effects of water current on the developments of lordosis in Cod

**Findings in commercial production of cod juveniles at Profunda were confirmed under experimental conditions by the research institute 'Nofima': high swimming speed induced by strong water current is a significant risk factor for the development of haemal lordosis in Atlantic cod juveniles.**

During a field visit to the cod hatchery of Profunda in September 2006, Dr. Koumoundouros from the University of Patras (Greece), who works mainly with seabass and seabream, noticed the high speed of current in the production tanks. Based on previous experiences with seabream, he suggested that high swimming speed might be one of the major causes for skeletal deformities seen, primarily haemal lordosis, in Atlantic cod (see figure).

This deformity is a key problem in the production of cod. In cooperation with Linde Gas, Profunda developed a new design for water inlets, providing a lower water speed in the production tanks. By the end of February 2007, fish that had been raised in tanks using these new inlets were radiographed and the resulting images confirmed a strong reduction in the incidence of skeletal deformities.

A replicated experiment was set up at the research station of Nofima (Sunndalsøra, Norway) to test the findings seen at Profunda under more controlled conditions.

### *Conclusions/Recommendations*

Swimming speed during the early feeding period of Atlantic cod must be controlled by the use of a low water current flow in the rearing tanks. Until more detailed knowledge about the swimming capacity of young cod is available, the water speed should be reduced as much as possible. For commercial purposes, the water speed must be high enough to maintain the self-cleaning properties of the rearing tanks.

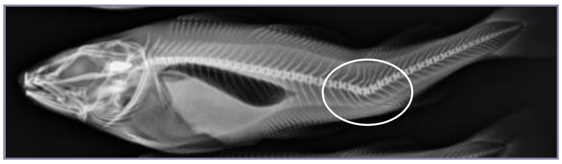

**Figure:** Haemal lordosis in a Atlantic cod juvenile reared at high water speed.

### *Experiment and results*

Atlantic cod (*Gadus morhua*) larvae were exposed to two different regimes during first feeding:

- minimal water speed
- increased water speed (adjusted twice a week)

For the latter condition, the water speed was adjusted according to fish behaviour. The speed was first increased until the cod larvae were no longer able to swim against the current; thereafter, the water speed was reduced until the larvae could just swim against the current. The experiment was terminated when the fish had a mean weight of 13 grams.

### *Summary and conclusion*

- The incidence of haemal lordosis was significantly lower in fish reared at minimal water speed (10 %) compared to fish reared at high water speed (24 %).
- There was no difference in fish weight at the end of the experiment (13,0 ± 0,2 gram).
- The results of this study confirmed that a high swimming speed induced by high water current is a significant risk factor for development of haemal lordosis in Atlantic cod juveniles.

The results do not provide specific guidelines for establishing safe limits. The fact that a significant percentage of fish with haemal lordosis was recorded in the control group demonstrates that high water speed is not the only causal factor for this deformity. However, the results provide a good starting point for further studies on the causes for deformities in Atlantic cod.

*This material is currently under preparation for scientific publication.*

## Colophon

The FineFish project aims to generate new practical knowledge on how to reduce the incidence of malformations in the major fish species used in European aquaculture production and to apply this to the professional sector. FEAP (Federation of European Aquaculture Producers), ten major European hatcheries and eight leading European scientific institutions participate in this Collective Research Project.

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fine fish news
ISSUE Nº 03
May 2008

‘’The dietary vitamin A level should be changed during larval development”

# Optimal level of vitamin A to sea bass

**Is the level of vitamin A (40000 IU retinol acetate per kg of diet) supplied by the vitamin mix appropriate? The objective of this study is to determine the optimal vitamin A level.**

***Experimental set-up***

Seven replicated groups of sea bass larvae were reared at 20°C and fed, from day 6 until day 42 post-hatching, microparticulated diets incorporating 0, 5, 10, 15, 25, 35 and 70 mg retinol acetate (RET)/kg of the diet and corresponding to 0, 16600, 33200, 50000, 83300, 116600, 233300 IU/ kg diet. The experiment was done by UMR NuAGe, IFREMER, Brest.

***Results***

The best growth and survival were observed in the larvae groups fed RET 10 and RET 15 diets, the other groups exhibiting a significant lowering in growth and survival. A partial analysis of malformations indicates that the best retinol acetate (vitamin A) levels are seen to be between 5 and 15 mg/ kg diet, which is lower than the recommended level of vitamin A.

Better results in ossification were also observed for vitamin A levels between 5 and 15 mg/ kg diet; these dietary vitamin A levels also represented a better compromise for reducing the incidence of

***Conclusions/Recommendations***

Data from gene expression studies strongly suggest that vitamin A may regulate the ossification/mineralisation processes and probably influence the patterning of different cell types: a down regulation of mineralisation process was observed between extreme groups as RT 35 and RT 5.

Moreover, these results also suggested that vitamin A requirements change through the development stage of seabass larvae, which would imply a change in the dietary vitamin A level during larval development so as to minimise the appearance of deformities.

**Figure:** Larvae were stained for determining cartilage (blue) and bone (red) tissues

# Test of today’s recommended/best vitamin mix in dry feed to sea bass

‘’The standard vitamin mix level induced a significant percentage of head and column deformities”

**The vitamin mix used in larvae diets is 8 times more concentrated than the vitamin mix 762 (NRC 93 recommendations) established for juvenile seabass (Dicentrarchus labrax). Is this appropriate?**

The objective of the study was to determine the optimal level of vitamin mix incorporation in diets. The experiment was done by UMR NuAGe, IFREMER, Brest.

***Experimental set-up***

Six replicated groups (three replicates per groups) of sea bass larvae were reared at 20°C and fed, from day 6 until day 38 post-hatching, microparticulated diets incorporating 0.5%, 1.5%, 2.5%, 4 % (the standard level), 5% and 8% of the 2 times concentrated vitamin mix 762.

***Results***

The NRC standard vitamin mix incorporated into dry larvae feeds at a level of 8 times the recommendation for juveniles gave the best results in terms of growth, survival and also morphogenesis.

***Conclusions/Recommendations***

Our results showed that low levels of dietary vitamins disrupted a temporal sequence of co-ordinated growth factor expression, involving different genes controlling the differentiation of osteoblasts; part of the osteoblast is then converted into adipocytes and this led to the appearance of deformities.

The standard vitamin mix level induced a significant percentage of head and 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).

The recommendation is to avoid the incorporation of high HUFA levels in larvae diets (i.e. > 2g EPA+DHA/100g DW) during early stages to prevent the conversion of osteoblasts into adipocytes.

fine fish news
ISSUE Nº 03
May 2008

# Safe temperature limits for early development in sea bream

"'Water temperature had a significant effect on the development of skeletal malformations"

**The objective of this study is to determine the optimal temperature limits for the embryonic and larval phase of sea bream (*Sparus aurata*).**

***Experimental set-up***

Six different temperature schemes were applied in duplicate during the autotrophic and exotrophic phase. Temperature conditions (16, 19 and 22° C) were applied either constantly throughout both ontogenetic phases, or in combinations at the autotrophic / exotrophic stage and until the fish reached the mean weight of ca 1g. The experiments were done in the facilities of Hellenic Centre for Marine Research (HCMR) in Crete.

***Results***

Water temperature had a significant effect on the development of skeletal malformations, as well as on growth and survival rates. Temperature was positively correlated with growth rate, while best survival rates (29.6±4.4%) were observed in the scheme of 16°C during the autotrophic and 19°C during the exotrophic phase (HCMR).

The first analysis of skeletal malformations clearly demonstrated that a water temperature of 16°C during either the autotrophic or especially the exotrophic phase significantly raised the rate of deformed fish. The analytical work was done at the University of Patras (Greece).

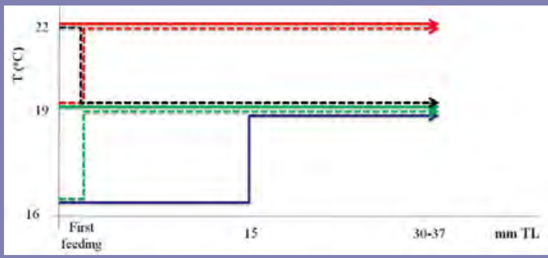


**Figure:** Experimental conditions (six different temperature schemes) which were applied in duplicate by HCMR.

***Conclusions/Recommendations***

Water temperature is a key factor for both the biological performance and the development of skeletal malformations in sea bream larvae. Up to this phase, our results suggest that the avoidance of 16°C during the hatchery phase could significantly increase the performance and quality of sea bream juveniles.

# Effect of feeding vitamin A to rainbow trout broodstock on morphogenesis of the offspring

"'Increased level of vitamin A is recommended"

**The experiment was set up to assess the influence of vitamin A level in broodstock diets on rainbow trout fry development. Vitamin A or retinol is essential for reproduction but high levels appear to be detrimental for larval development of many fish species.**

***Experimental set-up***

Three groups of rainbow trout broodstock were fed diets containing different levels of vitamin A: 20,000, 40,000 and 200,000 IU*/kg diet for 6 months before spawning. The first two levels correspond to the common levels found in commercial feeds for broodstock. The experiment was done by UMR NuAGe, INRA, St-Pée.

***Results***

The best fecundity, early growth and potential of muscle development were noticed in the group fed the highest level of vitamin A. Eggs from this group contained higher levels of retinyl palmitate, a storage form of vitamin A. However, no difference of retinoic acid level, the active metabolite of vitamin A involved in morphogenesis and early development, was recorded between eggs obtained from the 3 different groups. Likewise, no external malformation of fry originating from the 3 groups (from swim-up stage to the end of the 3-month feeding trial) was detected.

*1 international unit = 0.3 µg retinol equivalent.

**Figure:**
Trout fingerlings
@ Aquatour

***Conclusion/Recommendations***

For rainbow trout, increased level of dietary vitamin A (around 200,000 IU/kg diet) is recommended for broodstock as the level of retinoic acid in eggs is well controlled and does not increase with increasing levels of vitamin A in broodstock diets.

fine fish news
ISSUE Nº 03
May 2008

# Testing disinfection products on Rainbow trout eggs

**Preliminary results from the FineFish project have indicated that trout stocks appear to have higher malformation rates than salmon and many of the other fish species being investigated within the scope of the project. In view of the maturity of the trout industry, this finding has been a surprise to many within FineFish and is a concern to all trout producers and of particular relevance to the trout sector participants.**

"Use of chemicals may have an impact on farm malformations"

Disinfection protocols form an integral part of on-farm biosecurity and farm management health plans. The wide-scale use of chemicals for this purpose may have an impact on farm malformation rates and has been targeted for investigation by the Finefish project. Earlier work by Viviers de France (VdF) looked into eyed egg disinfection using peracetic acid, hydrogen peroxide, glutaraldehyde and Incimaxx DES*. Recent work done by Brow Well Fisheries (BWF) looked into the use of formalin and Pyceze*.

### *Field experiment*

Trout and salmon egg production requires adequate fungal control as well as disinfection capability. Using two identical production batches of green eggs, BWF treated each batch daily, 48 hours after fertilization, with either Pyceze (132 ppm for 30 minutes) or formalin (203 ppm for one hour) using a water flow through scheme. After egg picking (to remove dead eggs) at the "eyed" stage, the batches were again disinfected, before being transferred to the hatchery, by using 250ppm hydrogen peroxide for 10 minutes. Survival rates and all types of malformations were monitored until the first feeding of the fry. The results showed very similar survival and malformation rates to first feeding when using typical treatment regimes for Pyceze or formalin.

### *On farm monitoring*

On-farm monitoring of water and environmental conditions by the SMEs form an integral part of the Finefish project. Industrial monitoring by BWF has provided some interesting results. Trout eggs that are imported into mainland Britain must be disinfected with an iodophore. However, one of the greatest trout health concerns throughout the EU is the prevention of infection of the hatchery facilities by Flavobacteria psychrophlum. Disinfection by iodophores has shown to be ineffective against this bacterium so further treatments may be necessary to reduce this risk. Successive treatments, for example using hydrogen peroxide after an iodophore treatment, may result in a "premature hatch" (see figure 1).

**Figure 1.** Premature hatch

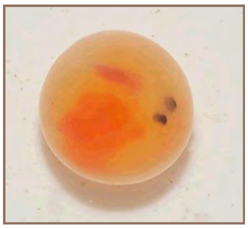

**Figure 2.** "Pin eyes"

If premature hatch is an issue on a particular site, it is recommended that antifungal treatments and disinfection procedures should be reviewed.

Monitoring of commercially available rainbow trout egg stocks at BWF also brings to light some surprisingly high background losses due to malformations that are regularly experienced in hatchery situations. "Pin" or "piggy" eyes can occasionally account for 1-2 % of batches (see figure 2) and "blue sac" regularly represents 2-5% of batches (see figure 3).

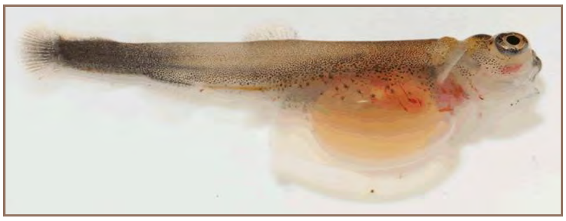

**Figure 3.** "blue sac"

It has also been found that the strain "blue trout" has higher levels of blue sac incidence and it could be inferred from this information that there is a genetic element to this condition. A reduction of oxygen levels during egg incubation from 95 to 79% saturation has also been associated with a doubling in the rates of "ball" and "curly" types of alevin malformations. (see figure 4 and 5).

**Figure 4.** "ball" type

**Figure 5.** "curly" type

### *Recommendation*

In addition to the genetic diversity of the broodstock, it is also recommended that high oxygen levels are maintained and regularly monitored during the egg incubation stage.

**Incimaxx DES is a proprietary disinfectant made by ECOLAB; Pyceze is a wide-spectrum fungicide and bactericide made by Novartis Animal Vaccines.*

## Brow Well Fisheries Ltd.

The BWF group consists of five trout farms and is long established in the UK as a leading producer of fry, fingerlings and fish for the restocking market.

Their operations also include a broodstock breeding programme for the production of rainbow and brown trout ova for sale and on-growing within the group.

Web page: www.brow-well.co.uk