Nutrients interact with molecular pathways that dictate the normal development of fish larvae. Understanding these interactions is important in developing diets that lower the incidence of morphological deformities in hatchery-produced larvae. Summarising recent findings from their laboratory that link nutrition to skeletal deformities in European seabass larvae, the authors provide vital information to improve larval feed formulation.

Nutrition, Development and Morphogenesis in Fish Larvae: Some Recent Developments

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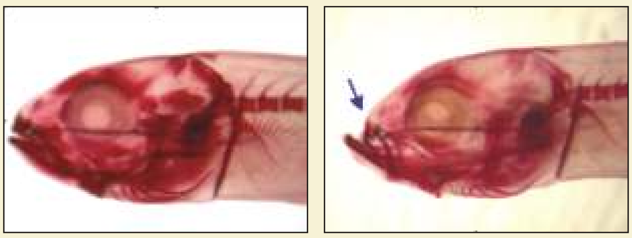
Skeletal deformities in fish larvae are due to nutritional and non-nutritional factors

Marine fish are poorly developed at hatching. They undergo important functional and morphological changes during the larval period. Several parameters could influence this development and negatively affect the larvae quality. In the wild, malformed animals rarely survive because of the forces of natural selection. But, larvae with many morphological anomalies survive in hatcheries.

The morphological deformities in larvae result from disruptions in the skeletal developmental process. They cause lowered growth, high mortalities, reduced market value of the product, and consequently a significant loss for the farmer. The most common skeletal deformities observed in hatcheries include spinal malformations (lordosis, scoliosis, coiled vertebral column), deformed operculum and head (jaw and neurocranium) malformations (Figure 1 & 2).



Normal larva | Larva with deformed neurocranium and jaw

Fig. 1: Comparison of normal larva and larva with head deformities

Larval deformities are induced in hatcheries by several environmental parameters, for example, inappropriate hydrodynamics in the tank (particularly high speed currents). High or low temperature, light intensity or salinity could also induce malformations (Johnson and Katavic, 1984). Diseases, particularly of bacterial origin, can lead to deformities as well (Madsen et Dalsgaard, 1999). High skeletal malformation rates have been observed triploid salmons (Sadler et al. 2001).

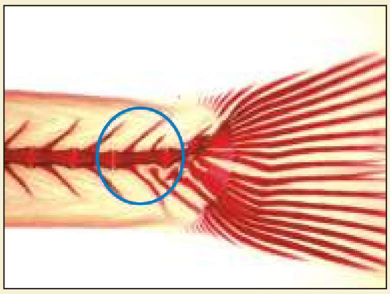


Fig. 2: Coiled vertebral column

Farmers often suspect diets to induce malformations. The link between nutrition and malformations has been difficult to demonstrate because marine larvae are fed on live prey and the nutritional composition of the live organisms cannot be easily manipulated. The recent development of

Table 1: Composition of four experimental diets containing an increasing protein hydrolysate level

	Diets			
Ingredients (%)	H0	H19	H38	H58
Fishmeal	77	58	38.5	19
Fish protein hydrolysate	0	19	38.5	58
Soy lecithin	5	5	5	5
Fish oil	5	4	3	2
Cellulose	0	1	2	3
Vitamins	8	8	8	8
Minerals	5	5	5	5
Total proteins	63	60	59	59
Total lipids	19.4	20.6	21.1	21.3
Energy(J/kg)	17830	17790	17810	17880

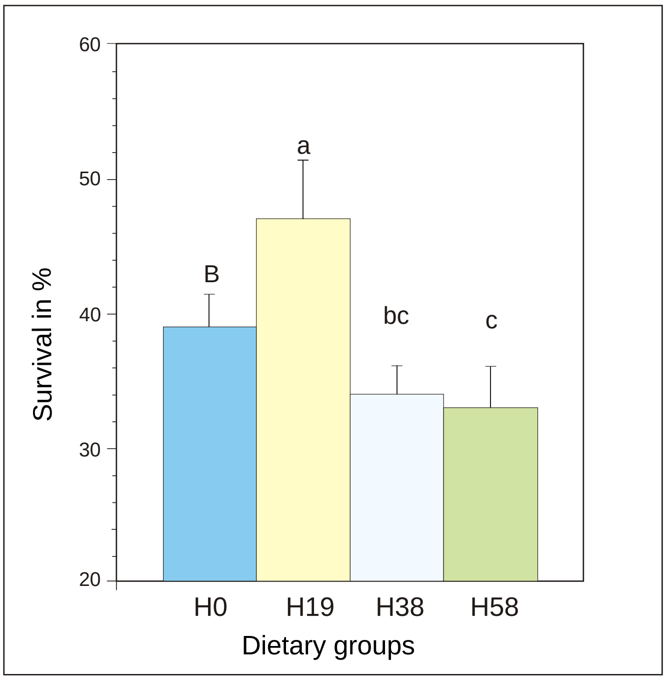


Fig. 3a: Survival rates of larvae fed diets with increasing protein hydrolysate level.(See Table 1 for formula)

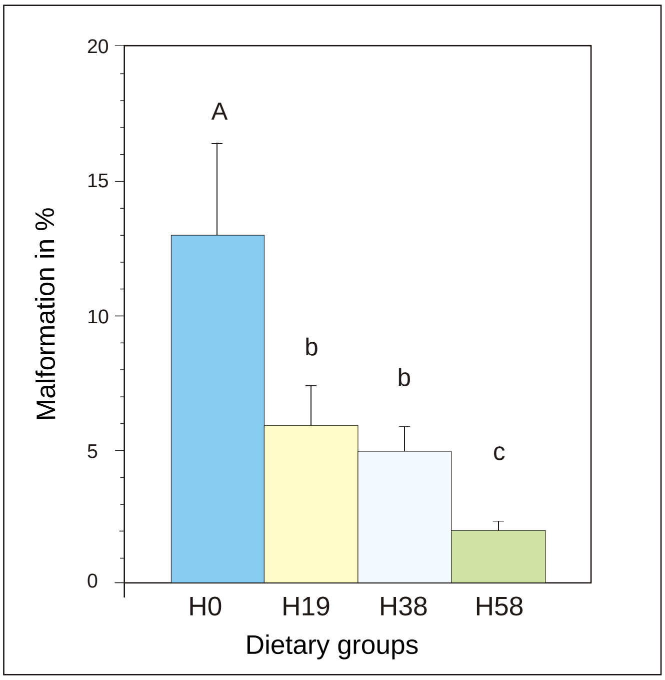


Fig. 3b: Percentage of malformed larvae in the four experimental dietary groups.(See Table 1 for formula)

appropriate microparticulated compound diets (Cahu et al. 2003) allows investigation of the influence of nutrients on fish larval morphogenesis. It is now becoming clear that dietary proteins, lipids and vitamins act on developmental processes that affect the morphogenesis of fish larvae.

1. Protein hydrolysate enhances larval morphogenesis

Cahu et al. (1999) designed four iso-protein experimental diets that differed only in the level of fish protein hydrolysate substituting native proteins (Table 1). When the diets were fed to European sea bass larvae, the fish fed 19% protein hydrolysate had the highest survival (Figure 3a). The results further demonstrated that the higher the protein hydrolysate level, the lower the malformation rate (Figure 3b).

The molecular form of the dietary protein supply, native proteins or hydrolyzed into oligopeptides (around 20 amino acids), has probably an indirect effect on morphogenesis. It is now well known that protein hydrolysates are better digested than native proteins by some specific peptidases that are active during the larval stages.

2. Fish morphogenesis is affected by dietary lipid composition

Dietary lipids play an essential role in larvae growth and survival. Our team has shown that a high dietary lipid level, around 20% of the feed dry matter, improved the growth and survival of European sea bass larvae (Zambonino & Cahu 1999). Recent experiments have demonstrated that dietary phospholipids are extremely crucial for larval development and morphogenesis. Phospholipids are structural lipids and are constituents of the cell membranes, in contrast to neutral lipids that are considered as reserves. Successful first feeding with diets incorporating high levels (12%) of vegetable phospholipids (supplied as soybean lecithin) has been reported in European sea bass larvae (Cahu et al., 2003). Growth and normal morphogenesis increased as the dietary inclusion of phospholipids increased (Figure 4 a&b). It is possible that different classes of phospholipids have specific physiological roles. Geurden et al (1998) have shown in carp that phosphatidylcholine improves growth while phosphatidyl-inositol acts positively on morphogenesis.

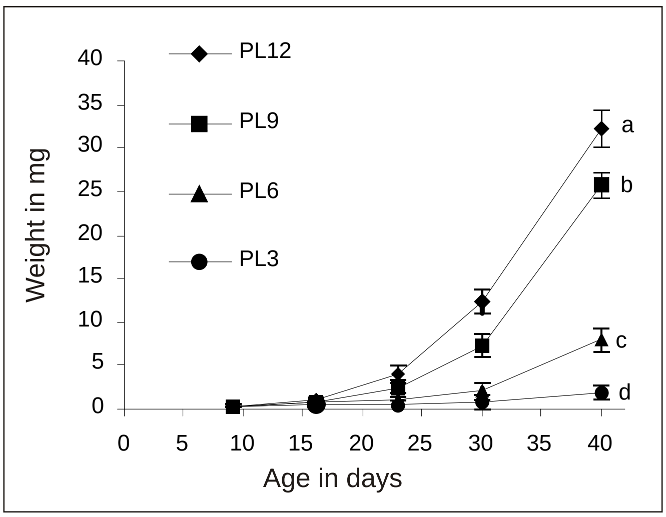


Fig. 4a: Growth of European sea bass larvae fed diets incorporating phospholipid levels ranging from 3% to 12%.

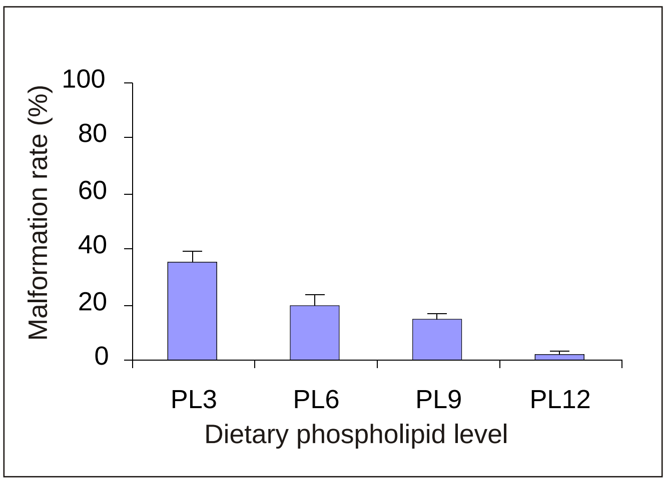


Fig. 4b: Malformation rate observed in European sea bass larvae fed diets incorporating phospholipid levels ranging from 3% to 12%.

The nutritional requirements in polyunsaturated fatty acids, in particular EPA (20:5n-3) and DHA (22:6n-3), for sustaining growth have been extensively studied (Sargent et al., 1999). Recent studies (unpublished) have shown that dietary intake of polyunsaturated fatty acids also affects the morphogenesis of European sea bass larvae, particularly the vertebra number. The mean vertebra number in this species is 25. A diet containing 5% EPA+DHA, instead of 2%, induced the appearance of an additional vertebra (Figure 5). These highly unsaturated fatty acids probably act through a nuclear receptor, Peroxisome Proliferating Activated Receptor (PPAR) and other nuclear receptors of the retinoid pathway that are involved in the bone differentiation process.

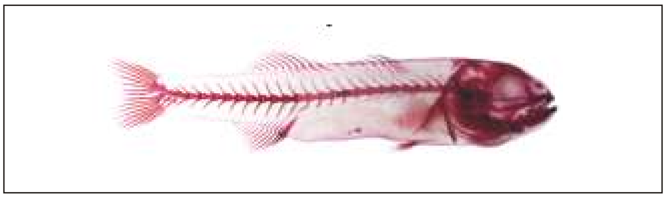


Fig. 5: Skeleton of European sea bass larvae.

3. Vitamins, particularly vitamin A, act on fish morphogenesis

Vitamin requirements of juvenile fish are better understood than those of larval fish. Consequently vitamins are incorporated in excess in larval diets to ensure that the requirements will be met. This excess could lead to hypervitaminosis for some vitamins such as vitamin A (active derivate is retinoic acid).

In a recent experiment, malformation rates in European sea bass were positively correlated to vitamin A levels in the larvae (Villeneuve et al., 2005). Vitamin A acts directly on the expression of genes coding for the nuclear receptors of the retinoid pathway, RAR and RXR. These receptors are involved in osteoblast differentiation. RAR gene expression increases during the normal development of larvae. A high dietary vitamin A level disrupts the normal expression sequence and leads to abnormal bone development. The involvement of RAR genes in bone differentiation has been proven by the in-situ hybridization detection of RNA messengers coding for RAR in developing jaw as early as day 5 (Villeneuve et al. 2004). Using Real-Time RT-PCR technique, the authors demonstrated that high dietary vitamin A levels disrupted RARgene expression, little before the appearance of jaw deformities.

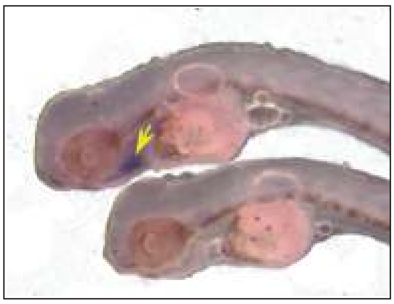


Fig. 6: In situ hybridization of RAR in developing jaw (arrow) of 4 day-old sea bass larva (larva below constitutes a negative control for hybridisation).

Conclusions

Understanding how nutrients act on the molecular pathways controlling fish larvae morphogenesis is helpful to development of dietary formulations that lower the incidence of malformed larvae in hatcheries. Ongoing investigations are considering nuclear receptors such as those involved in the retinoid pathway and Peroxisome Proliferating Activated Receptors because they could provide useful tools for understanding molecular mechanisms of nutrient-morphogenesis interactions. These investigations have to be conducted in addition to studies focusing on other potential sources of abnormalities in hatcheries, in particular those concerning the larval rearing techniques.

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Dr José Zambonino graduated with a MS degree in Biochemistry and a Ph.D. in Protein Nutrition and Digestion in mammals at the University of Montpellier (France). He was recruited in 1992 by IFREMER, French Institute for Research and Exploitation of Sea Resources. He conducted research on the effect of feeds on the digestive tract development in marine fish larvae for more than 8 years. These studies led to a patent for a compound diet that can efficiently and totally replace live preys in the feeding sequence of marine fish larvae. He currently studies the influence of some nutrients on the molecular pathways controlling morphogenesis in marine fish larvae for optimal fish juvenile production.