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## Effect of nutrition on marine fish development and quality

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### Introduction

Fish larval stage represents a transitional period in which both ontogeny and growth cause substantial changes in structure, physiology, size and morphology. These last twenty years, most of the studies on marine fish larvae development have dealt with the ontogeny and functioning of the digestive tract in order to overcome bottlenecks in larvae culture and weaning processes (switch from live preys to compound diet feeding sequence). These studies clearly demonstrated that fish larvae are not faced to physiological or digestive deficiencies (Zambonino Infante and Cahu, 2001) although they hatch with very immature organs compared to juveniles.

More recently, the possible impact of nutritional events occurred during early larvae development stages were considered by different several research groups (Zambonino Infante and Cahu, 2007). Teleosts have similar basic mechanisms of organ development but differences can be found in the relative timing of the ontogeny. For example, the same main morphological and cellular changes occur during the ontogeny of the gastrointestinal tract in Teleost but the timing of the events will be more or less advanced depending on the natural environment temperature (tropical, temperate or cold) of the considered species (Zambonino Infante and Cahu, 2001). Several abiotic and biotic factors, such as water temperature, food availability and composition during early life stages influence the time in organ development and its associated physiological functions. The potential impact of early nutrition on larvae morphogenesis has been probably the most studied these last 5 years, mainly because morphogenesis is the most visible consequence, and therefore the most economically detrimental to aquaculture industry. However, other potential changes, less visible, that could also occurred are more and more considered since they will determine the nutritional and physiological performances of a fish, and therefore its ability to deal with challenges during its subsequent life (Fuiman, 1997).

This chapter aims to give an overview of the main developmental events in fish larvae, and describe the impact of early nutrition on the developmental processes that condition the future juvenile potentials.

## 1. The development of digestive function in fish larvae and the effect of the nutrients

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Nutritional requirements of juveniles and adult fish are now well known (Guillaume et al., 2001), and efficient compound diets are now available for several farmed fish species. But, considering that the digestive tract anatomy and function in fish larvae is different of that of juveniles, fish larvae would have nutritional requirements different from those of juvenile and adult fish as several studies showed (Watanabe and Kiron, 1994; Cahu and Zambonino Infante, 2001). Moreover, as morphogenesis and organanogenesis are developing during the early stages, they will be affected by the composition of the exogenous feeding.

### 1.1. Onset of digestive function and specificities in fish larval digestion

Fish larvae undergo major morphological, anatomical and functional changes during development. In particular, digestive tract is very immature at hatching. Particularly, the stomach is not differentiated during the first days. The development of this organ, and the development of gastric glands, appear at very different ages, depending of the species. The stomach is well differentiated as early as 7 days post-hatching in red drum *Scianops ocellata* (Holt et al. 1981), around day 9 in shi drum *Umbrina cirrosa* (Zaiss et al., 2006) but occurs only around day 25 post-hatching in sea bass *Dicentrarchus labrax* (Vu, 1983) or in sole *Solea solea* (Boulhic and Gabaudan, 1992) and later in winter flounder (Douglas et al., 1999). In fact, the digestive enzyme secreted by stomach, pepsin, seems to be not indispensable for feed digestion in fish (Zambonino-Infante and Cahu, 1994), as several species remain stomachless during all their life (Smith, 1989). Other proteolytic enzymes, secreted by pancreas, can assume the protein digestion.

The following step in digestion is achieved by enzymes secreted by pancreas. The differentiation of pancreas exocrine cells and the appearance of the excretory conduct occur around day 3 post-hatching in sea bass (Beccaria et al, 1991) thought main digestive pancreatic enzymes, trypsin, amylase, lipase, phospholipase A2 can be detected before mouth opening (Ribeiro et al., 1999, Hoehne-Reitan et al., 2001). So pancreatic enzymes are synthesized very early during development, but a maturation of pancreas occurs progressively during development, with the onset of secretion functions. The pancreatic digestive enzymes are produced in large quantity relatively to the larval weight and are not limiting for digestion as it has been shown in sea bass (Cahu and Zambonino-Infante, 1997) and in Japanese sardine *Sardinops melanoticus* (Kurokawa et al., 1998).

However, the expressions of the different pancreatic digestive enzymes follow different pattern during development. Trypsin specific activity (i.e., relative to larvae protein content) is high during the early development then decrease, the other proteolytic enzymes appearing. Trypsin is roughly regulated by the amount of ingested diet (Pedersen et al., 1990) and by dietary protein content (Peres et al., 1997), but not very accurately, since trypsin activity does not follow the pattern of mRNA coding for this protein (Peres et al., 1998). Trypsin activity is highly regulated by the nature of protein (Peres et al., 1998). Moreover trypsin and chymotrypsin are affected by the size of peptide chains, trypsin being enhanced by native protein when chymotrypsin is induced by small size peptides (Zambonino Infante et al., 1997). This will have consequences on larval protein nutrition. On the opposite, amylase expression is regulated by the dietary glucide level, starch or glycogen as early as the youngest stages (Peres et al., 1998). Amylase has been detected from hatching in red porgy *Pagrus pagrus* (Darias et al. 2006). Data collected in literature on different species (Buddington et al., 1997, Ribeiro et al., 1999; Lazo et al., 2000) suggest that the amylase expression decreases during development down to a low level in carnivorous fish as lactase does in mammals (Henning, 1994). This decrease is less pronounced in omnivorous fish such as mullet *Chelon labrosus* (Zouiten et al., in press), and can even be avoided in carnivorous fish fed diet contained glucides during the larval development (Ma et al. 2005). This characteristic will have consequences on the possibilities of programming fish to digest glucides after early stages, and then to be fed with diet containing glucides in the aim to spare protein resources.

The two main lipolytic pancreatic enzymes, lipase and phospholipase A2, can be detected very early during the development, 6 days after hatching in turbot *Scophthalmus maximus* (Hoehne-Reitan et al. 2003). PLA2 synthesis is modulated by diet composition and regulated at transcriptional and post-transcriptional level, by an hormonal mechanism involving cholecystokinin (Cahu et al., 2004). On the opposite, the response of pancreatic lipase to the amount of dietary triacylglycerides is not linear. Interestingly, the source of triacylglycerides, i.e. the chain length and saturation degree of fatty acids constituting the triacylglycerides, seems to affect lipase activity (Morais et al., 2004). Again, the regulation mode of these enzymes will have consequences on larval nutritional requirements.

The third step of digestion is achieved by the enterocytes constituting the brush border membrane of intestine. Hydrolysis of small peptides or di-saccharides is assumed by the enzymes located on the brush border membrane of enterocytes in juveniles fish as in other vertebrates. Microvilli of brush border membrane are very thin at hatching, and the expression of the enzymes of the brush border membrane is very poor. On the opposite, the expression of enzymes located in cytosol is high. Cytosolic enzymes are mainly peptidases, and the high capacity of young stages to digest peptides affects diet acceptance and utilization. A maturation occurs in intestine, characterized by a sharp increase in brush border enzyme activity. Especially, the developmental pattern of alkaline phosphatase, located for 90% on brush border membranes, and aminopeptidase N is considered as an indicator of this maturation which allows the transition of a larval to an adult mode of digestion. This maturation process, well known in mammals (Henning 1994), has been described in several fish species, and occurs at different times post hatching: around day 25 in sea bass (Cahu and Zambonino Infante, 1994) and in sole *Solea senegalensis* (Ribeiro et al., 1999), some day earlier in yellow croaker *Pseudosciaena crocea* (Ma et al., 2005), up to day 50 in cod and halibut (Kvåle et al., 2007). This time is related to the duration of larval development in fish species, associated with rearing temperature.

## **1.2. The effect of the nutrient on fish larval development**

Exogenous feeding progressively begins from mouth opening (day 5 post-hatching in sea bass, day X in halibut), concurrently with endogenous feeding. When yolk and oil globule are totally depleted (day 15 in sea bass, day XX in halibut), exogenous feeding becomes the only source for providing nutrients. The effect of some of them on larval development has been extensively studied during the last two decades, since the formulation of compound diets allowed nutrition experimental studies.

### **1.2.1. Effect of protein fraction supply**

Growth is particularly high during larval stages, and growth is mainly related to muscle protein deposition. However, total requirement in protein has been poorly studied. It was generally supposed to be higher than in juveniles (Person et al. 1991) and Peres et al., (1996) showed that diet containing 50 to 60% protein induced better growth than a similar isocaloric diet containing only 30 and 40% protein. These proteins were supplied as fish meal, which is considered as the protein source providing the best amino acid profile for fish requirement. However, it appeared that both mRNA coding for trypsin and trypsin activity were not regulated by dietary fish meal concentration at day 18. The modulation of trypsin activity by dietary fish meal occurred only from day 35. The regulation of this enzyme, as other digestive enzymes in fish larvae, is age dependant. In the same way, it was showed that native protein are better absorbed in 31 day old halibut rather than in 25 day old larvae (Tonheim et al., 2005).

Considering that trypsin regulation is poor in fish larvae, and that fish larvae express high levels of peptidases, it was assumed that larvae should use peptides better than native protein. Indeed, an incorporation of a moderate concentration of peptides does improve growth, survival and general development in fish larvae. Casein is a protein of poor nutritional value for fish larvae but a partial replacement of native casein by casein hydrolysate improved growth and survival in carp (Carvalho et al., 1997). A replacement of 10 to 20% of native fish meal by hydrolysate manufactured with the same fish meal induced a better development in European sea bass. This was observed both with peptides chains of 10 to 20 amino acids (Cahu et al. 1999) and di- and tripeptides (Zambonino et al., 1997). In this last study, it was shown that replacement of 40% fish meal by fish protein hydrolysate led to a large reduction in skeletal malformation, lordosis, scoliosis and lower jaw deformities. Peptidase activity was induced by dietary medium chain hydrolysates and di- and tri-peptides. The other indicators of digestive tract development, such as the increase in brush border membrane activity showed a better development of the animals. Nevertheless, an excess of dietary peptides led to negative effects (Zhang et al., 2006), a burst of peptides and amino acids in the intestine inducing a saturation of transporter mechanisms. Indeed, in addition to amino acid transporters, it has been shown that PTPT1-type peptide transporters are expressed in fish larvae before first feeding (Verri et al. 2003). Besides, other data suggest that the capacity of utilizing peptides is related to larval characteristics of digestive tract in fish: peptides seems to be inefficient to improve growth in wolffish, *Anarhichas minor* (Savoie et al. 2006), which species hatching with very few larval characters and especially with a well developed digestive tract (Falk-Petersen and Hansen, 2001).

Fish larval requirements in indispensable amino acids, their changes during ontogenesis and the differences between species, have been extensively studied during the last years (Aragão et al. 2004). Recent studies have demonstrated that the conventional live prey sequence, rotifer and *Artemia*, would supply some indispensable amino acids in deficient or limiting amount for growth. Indeed, histidine appears to be the limiting amino acid at two day post-hatching in the white seabream (*Diplodus sargus*), threonine at day 12 post-hatching, then cysteine and methionine were considered as limiting later in the development (Saavedra et al., 2006). The enrichment of live prey with amino acids is hazardous, and it will be more rational to formulate compound diets with balanced amino acid profile. As demonstrated above, fish meal though having a convenient amino profile, is not the best supply form when provided alone. But peptides, eventually synthetic as recommended by Dabrowski et al. (2005), could be added in diet to provide a convenient concentration in the different amino acids. Results concerning the absorption and metabolism of free amino acid are until now controversial: it seems that a mixture of free amino acid added to diet is inefficient to improve growth, though enhancing secretion of pancreatic digestive enzymes (Cahu and Zambonino Infante, 1995). But the use of tube-feeding technique, recently developed for fish larvae with radiolabelled compounds, showed that free fatty acids would be absorbed much faster as protein and peptides and would have a higher assimilation efficiency (Rojas-Garcia and Rønnestad, 2003). This technique allows also to confirm that indispensable amino acids such as lysine are mainly anabolised, until 70% of absorbed lysine in the herring *Clupea harengus*, when the dispensable amino acids such as glutamate would be catabolised as energy substrate at more than 75% (Conceição et al., 2002).

### 1.2.2. Effect of lipid fraction supply

Lipid constitutes with free amino acids the most important energy reserve in fish embryos (Vetter et al., 1983; Rønnestad et al. 1999). Studies conducted on different species agree to show that larval development requires high dietary lipid level: 18% lipid related to diet dry matter in sea bream (Salhi et al., 1999), 25% for *Paralichthys olivaceus* (Furuita et al., 1998), 25 to 30% in sea bass (Zambonino Infante and Cahu, 1999). It is assumed that young larvae require high energy, around 20 KJ/kg of diet. But the effect depends on the nature of the lipids. Diets containing 26% lipid can lead to very poor growth and low survival if lipids are mainly neutral lipid, when a similar diet containing 14% neutral lipid and 12% phospholipid induce high growth and survival in sea bass larvae (Cahu and Zambonino Infante, 2003). This growth depressing effect of high dietary neutral lipid levels has been reported in different marine fish species (Morais et al., 2007). Indeed, high neutral lipid levels result in an accumulation of large lipid droplet in the enterocytes, mainly in anterior intestine. This accumulation has not been described as pathological, but reflects a limited capacity for assembling triglycerides with apolipoprotein, necessary for the transfer of triglycerides into the general circulation (Zambonino Infante and Cahu, 2007). In the same way, we saw above that lipase is not finely regulated by the dietary neutral lipids, maximal activity level in level being reached with 20% dietary lipid. So the high lipid level requirement in larvae does not correspond only to a high energy requirement, but also to a specific requirement in some fatty acids and phospholipids.

Studies concerning phospholipid requirements in fish larvae have been initiated by Kanazawa et al. since 1981. Phospholipids constitute the cell membranes, and so are essential for development of fish larvae which weight is more than 20 fold multiplied during the first month. Fish have the ability to synthesize phospholipids from precursors, but at a rate insufficient to sustain larval development (Coutteau et al. 1997). Moreover, dietary phospholipids affect lipid absorption and transport, as they have a specific role in the synthesis and secretion of chylomicrons and VLDL (Very Low Density Lipoproteins) from the intestinal mucosa into the circulatory system. In fish larvae fed live preys, superior performance of copepod nauplii compared to rotifer and *artemia* was attributed for a large part to their phospholipid content, 50% of total lipid (Mc Evoy et al., 1998). Moreover, sea bass fed from mouth opening with similar isocaloric and isolipidic diet, growth and survival in sea bass was directly related to dietary phospholipid level, graded from 3 to 12%. Interestingly, phospholipid appeared to prevent skeletal malformations, lordosis, scoliosis and lower jaw deformities, prevalence of skeletal malformation in larvae being 35% with the lower phospholipid level and only 2% with the higher (Cahu et al., 2003). Besides, digestive enzyme activities revealed a proper maturation of digestive tract in larvae fed high phospholipid levels. Geurden et al (1997) suggested that phosphatidylcholine has a growth promoting effect in carp, when phosphatidylinositol prevents deformities.

In Cahu et al. (2003) experiment, phospholipid was supplied as soybean lecithin, so the global beneficial effect of phospholipid cannot be attributed to additional highly unsaturated fatty acids (HUFA) supply. Nevertheless, it is now well known that HUFA have a major role in fish larval development. Sargent et al. (1999), considering the HUFA content in fish eggs, recommended an optimal level of total HUFA, mainly eicosapentaenoic acid EPA and docohexaenoic acid DHA, around 3% of dry weight of larvae diet. This recommendation was supported by experimental data, showing an optimal value of 2.5% EPA and DHA for sea bass larvae. But this amount is inefficient to promote growth in sea bass larvae if brought by triglycerides, and can even lead to total mortality (Villeneuve et al., 2005a). At the opposite, the same quantity of EPA+DHA brought as phospholipids induces good growth and survival. Growth and survival are depressed when EPA+DHA level move away of this optimal value : diets containing 1% and 5% EPA+DHA induce poor development in sea bass larvae. High prevalence of skeletal deformities was observed in sea bass fed 5% EPA+DHA, almost 35% of the larvae exhibiting skeletal deformities and 10% other deformities (neurocranium, maxilla, operculum). These malformation were associated with a down regulation of the retinoid X receptor  $\alpha$ , retinoic acid receptor  $\alpha$ , retinoid acid receptor  $\gamma$  and bone morphogenetic protein-4 genes in early stages, showing that retinoid pathways can be influenced by dietary lipid, leading to skeletal malformation during fish development. HUFA are known to modulate the transcription of genes involved in their metabolism, through their nuclear receptors, the PPARs (Peroxisome Proliferator Activated Receptors) (Kliwer et al., 1997). These receptors form heterodimers with retinoid X receptors to regulate the expression of more than 500 genes, involved in lipid metabolism, energy balance, morphogenesis, bone synthesis (Kliwer et al., 1997, Balmer and Blomhoff, 2002). This explains why a unbalance dietary fatty acid composition can affect fish larval development in such large extend. It is interesting to observe that an adverse result was obtained when an EPA+DHA level only twice of the optimal ration. Thus, the teratogenic effect of HUFA appears to be very potent and should incline us to be more cautious when incorporating high levels of lipids in fish and larvae diets.

The relative concentration of the different HUFA, supplied as pre-formed phospholipids, also affects the onset of the pigmentation of flat fish. A negative effect of ARA (arachidonic acid) and a positive effect of EPA on flat fish pigmentation has been reported. In summary, improvement of dorsal pigmentation in turbot and halibut can be achieved by providing ratio of DHA/EPA of  $> 2/1$ , and EPA/ARA ratio  $> 5/1$  during the "pigmentation windows" (Bell et al. 2003). A reduced rod/cone ratio in eye of halibut fed *Artemia* compared to those fed copepod has been attributed to the low phospholipid DHA/EPA ratio in *Artemia*, and would be responsible of a poor pigmentation (Shield et al., 1999).

### 1.2.3. Effect of vitamins

Accurate data concerning vitamin requirements in fish larvae are very recent. Indeed, up to the last decade, these requirements were studied using live prey enrichment, which gave some interesting indications, but non comprehensive data. Effects of vitamin C (ascorbic acid) have been described firstly: ascorbic acid would reduce the incidence of opercular abnormalities in milkfish *Chanos chanos* larvae (Gaspasin et al., 1998). These abnormalities, associated with distortion of gill filament cartilage are characteristic of scorbutic fish (Soliman et al. 1986) and is the result of a decalcification (Dabrowski et al., 1990). Nevertheless, vitamin studies conducted with live prey can lead only to limited conclusion, the ascorbic concentration in non- enriched *Artemia* can reach up to 500 mg equivalent ascorbic acid per gram dry matter and is sufficient to meet the nutritional requirements of developing larvae (Merchie et al. 1996). Thus, a deficiency effect was quite impossible to describe with experiment using live prey feeding sequence.

Compound diets were initially formulated with high vitamin levels, in order to avoid any possible vitamin deficiency. As exemple, Escaffre et al (1997) and Cahu et al. (1998) incorporated 240 mg equivalent vitamin C in larval diet, i.e. 8 times more than for juveniles. This high vitamin requirement was supported by an experiment conducted with purified compound diet incorporating a vitamin C gradient which indicated that the dietary requirements of carp larvae is between 45 mg ascorbic acid equivalent per kg of diet (value to obtain optimal growth and survival) and 350 (value to obtain vitamin C maximum concentration in tissues) (Gouillou-Coustans et al. 1998). Moreover, Dabrowski et al. (1996) showed that larvae are more sensitive to vitamin C deficiency than juveniles.

More recently, in relation to skeletal abnormalities affecting fry production in hatcheries, attention has been paid on vitamin A and its active metabolite, retinoic acid. Experiments conducted by immersing Japanese flounder, *Paralichthys olivaceus*, embryos in retinoid acid solution showed a repressed expression of sonic hedgehog (shh) and hoxd-4 genes, inducing severe pharyngeal

cartilage malformation (Suzuki et al., 1999). It was assumed that *shh*, involved in the development of various skeletal system and in organogenesis would affect not only embryogenesis in but also metamorphosis in flat fish, as it does in *Xenopus* (Stolow and Shi, 1995). An experiment conducted on sea bass fed from first feeding up to day 42 post-hatching with a compound diet incorporating graded levels of vitamin A allowed to precise the effects of vitamin A during larval development. Taking account growth, survival and development of digestive function in intestine, the optimal level was determined at 30 mg all-*trans* retinol/kg diet dry matter. Higher or lower levels of vitamin A induced poorer growth and survival. Interestingly, an linear relationship between dietary vitamin A and skeletal malformation incidence in fish was demonstrated. In the group fed the maximum level, 196 mg all-*trans* retinol/kg diet dry matter, 80% of larvae exhibited a skeletal deformity, either in neurocranium, maxilla, operculum or vertebral column (Villeneuve et al., 2005b). It has to be pointed out that head skeletal was mostly affected, when deformities induced by HUFA excess concern mainly vertebral column. Malformation incidence was associated to an upregulation of retinoic acid receptor  $\gamma$ , expressed 82 times more in larvae fed highest vitamin A level than in those fed optimal level at day 10 post-hatching

Skeletal malformations can be also induced by an inadequate dietary vitamin D3 level. However, the recent papers focused on the effect of vitamin D3 on pigmentation, default in pigmentation being a major problem for flat fish hatcheries. Hasegawa et al. (1998) showed that an excess in vitamin D3 (20 000 IU /100g diet) induced hypermelanosis in the Japanese flounder, especially on the blind size of the fish. Haga et al. (2004) confirmed that the form 1,25-dihydroxyvitamin (1,25(OH)<sub>2</sub>D<sub>3</sub>) is the active metabolite of this vitamin, acting affecting fish pigmentation. Beside its role in calcium homeostasis and bone formation, this vitamin acts on cell proliferation and differentiation (Walter 1992). Moreover, the skin is a target organ of this vitamin, which is has been showed to be involved in stimulation of melanogenesis in human melanocytes (Sakai et al. 2001).

Thus, it appears that specificities in digestion in fish larvae induce specificities in nutritional requirements. Fish are especially sensitive to dietary nutrient supply, compared to juveniles. A slight imbalance or maladjusted supply form in some nutrients such as amino acids, fatty acids or vitamins will impair development (Figure 1). The nutrients are used to build body mass, but also act as modulators of genes involved in organogenesis and skeletal development, which need to be better investigated in fish. From this point of view, fish larvae could also constitute an interesting model for studies on the impact on nutrition on higher vertebrate development, since very precocious stages are accessible for nutritional experiments.

## **2. Consequences of larval nutrition on further development**

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Events at critical period of early life could influence long-term outcome in two different ways: 1/ induction, deletion or impaired development of a somatic structure resulting from a stimulus or injury and 2/ physiological “setting” by an early stimulus or injury. These physiological responses reflect a biological mechanism which is turned irreversibly “on” or “off” only once during an individual’s lifetime in response to condition prevailing at some critical stage. The term of programming is often used to describe this biological mechanism (Lucas 1991). A key challenge is to identify stressors that are capable of permanently altering organ development and function, and also stages of development of greatest vulnerability. As we showed in the first part of this chapter, the effect of early nutrition on finfish larvae and juvenile development has been mainly studied with the aim to reduce fish morphological abnormalities that adversely affect the image and profits of fish aquaculture. However, the possibility to orientate specific metabolic pathways or functions in juvenile fish, for example to facilitate the use of substitutes to fish meal and oil and to promote sustainability in fish aquaculture, has just begun to be considered in fish larvae.

### **2.1. Morphogenesis process**

Morphogenesis is a process that implies a precise spatial and temporal expression of some specific genes, like Hox genes that are major actors in body patterning (limbs, vertebrae, craniofacial structures) (Krumlauf, 1994). These genes may be then particularly sensitive to dietary modulations of some transcription factors during these temporal windows of development. Therefore, a same diet could have a different impact on morphogenesis if given at different periods to larvae.

Dedi et al. (1997) have fed larvae Japanese Flounder with *Artemia* containing high concentrations of vitamin A at different developmental stages. They reported that vertebral deformities occurred in larvae when exposed to these high vitamin A doses during day 25-27 post-hatching, period that corresponds to the notochord segmentation. After this period, the exposition of larvae to high dietary vitamin A levels did not induce any malformations. This study clearly showed how the timing of vitamin A intake influenced the risk of appearance of deformities in vertebrae.

More recently, Villeneuve *et al.* (2006) have shown that inadequate vitamin A or high HUFA levels in diets highly affected sea bass larvae morphogenesis when given earlier than 18 dph (day post hatching). Hypervitaminosis A delayed development, reducing the number of vertebrae (25 is most frequently observed in European sea bass) and negatively affecting bone formation in the cephalic region. These malformations were correlated to an upregulation of retinoic acid receptor  $\gamma$ , retinoid X receptor (RXR- $\alpha$ ) and bone morphogenetic protein (BMP-4). An excess of HUFA accelerated the osteoblast differentiation process through the upregulation of RXR- $\alpha$  and BMP-4, leading to a supernumerary vertebra. When larvae are older than 18 dph, hypervitaminosis A or high HUFA levels in diets did not significantly influence sea bass morphogenesis. This work indicated that the first 18 days post-hatching constitute a vulnerable period for European sea bass larval development. Rearing temperature during early stages has been described as another parameter affecting vertebra number in fish (Lewis et al., 2004).

## 2.2. Metabolic processes

### 2.1.1. Glucose metabolism

Carnivorous teleosts have an inefficiency to use high levels of dietary carbohydrates. In rainbow trout, diets incorporating more than 20-30% of digestible carbohydrate induce prolonged postprandial glycemia and adversely affect growth (Moon, 2001). The incorporation of carbohydrates in fish feeds has as main objective to spare proteins that are naturally firstly used for energy purposes in carnivorous fish. Although the general mechanisms for the digestion of carbohydrates, absorption and metabolism of glucose in carnivorous fish are not different from those in herbivorous or omnivorous fish (Krogdahl et al., 2005), the enzymes involved in carbohydrate utilization seem to be less expressed and roughly regulated in the former species (Buddington et al., 1997; Hemre et al., 2002). Stimulating effects of dietary starch on intestinal disaccharidases have been described in on-growing salmonids (Krogdahl et al., 2004) indicating a certain adaptive ability of the digestive enzymes; moreover, high levels of amylase and maltase are expressed during larval stages of omnivorous fish and are modulated by dietary glucides (Péres et al., 1996; Ma et al., 2005). However, this sort of adaptation do not allow juvenile carnivorous fish to deal with high carbohydrates loads since high dietary glucose do not induce a retro inhibition of gluconeogenic enzyme expression as usually observed in omnivorous fish and non diabetic animals (Panserat et al., 2002). An acute but short (few days) nutritional stimulus, i.e. hyperglucidic diet, applied in early post-hatch life of trout induced a persistent positive effect on carbohydrate utilization at the digestive level, revealed at the juvenile stages by a higher persistent expression of amylase and maltase in early-stimulated fish compared to controls (Geurden et al., 2007). Nevertheless, this study failed to demonstrate any persistent effect on glucose transport or metabolism. These data show that the concept of early nutritional programming also exists in fish, but needs further investigations in order to better define 1/ the developmental windows where the nutritional stimulus could have the maximal effect on trout larvae 2/ the duration of the glucidic stimulations and 3/ the possible mechanisms of this process.

The examination of the mechanisms involved in the transcriptional regulation of lactase-phlorizin hydrolase gene in mammals could likely help for understanding the mechanisms involved in the nutritional programming of (amylase and maltase) gene in fish larvae. Lactase-phlorizin hydrolase (LPH) is a  $\beta$ -glycosidase located in the enterocyte brush border membranes, as maltase. After weaning, LPH is normally down-regulated in most mammals, but continuous lactose challenge enhances lactase protein expression (Peuhkuri et al., 1997) and in the case of human lactase, persistent high lactase expression can be observed (Flatz G., 1989). In pigs, this enzyme gene expression is primarily regulated at the transcriptional level (Motohashi et al., 1997), involving several transcription factors such as NF-LPH 1, HNF 1 $\alpha$  (hepatic nuclear factor) and Cdx2 (caudal related homeodomain protein); the decreased expression in NF-LPH coincided with the post-weaning decline of LPH (Troelsen et al., 1992), while the presence of both Cdx and HNF1a leads to a much higher level of transcription than the sum of the activation by either factor alone (Mitchelmore et al., 2000).



Cdx2 and HNF1 $\alpha$  are both expressed in intestinal cells from an early stage of development, with a crucial role during crypt-villus differentiation process since HNF1 $\alpha$  has been implicated in chromatin remodelling of target genes (Pontoglio et al., 1997). This particular property of HNF1 $\alpha$  may lead to different methylation patterns of crypt stem cells in case of lactose challenge, recording nutritional events in somatic cell and consequently producing, all along animal life, differentiated enterocytes pre-programmed for a specific nutrient (Kim et al., 2005). Interestingly, Cdx2 and HNF1 $\alpha$  also interact with several intestine-specific genes including maltase (Mitchelmore et al., 2000). The possible involvement of Cdx2 and HNF1 $\alpha$  in nutritional programming of fish larvae intestinal genes appears then very likely but has never been studied in fish, and would constitute an interesting field for future research.

### **2.1.2. Highly polyunsaturated fatty acid synthesis.**

Very long chain fatty acids containing more than 18 carbons are often called HUFA, highly unsaturated fatty acids, and play a number of essential roles in animal metabolism. n-3 HUFA determine the biophysical properties of membranes (particularly in retina and central nervous system), and they are also precursors of several biologically active eicosanoids including prostaglandins, thromboxanes and leukotrienes (Gill and Valivety, 1997). These metabolites bind to specific G-protein-coupled receptors and signal cellular responses that mediate fever, inflammation, vasodilation, blood pressure and pain (Funk, 2001). There is notable variation between farmed fish species in their ability to synthesize HUFA. For example, salmonids are able to synthesize EPA and DHA by fatty acid elongation and desaturation from short feed n-3 fatty acids, when this pathway is not totally functional in marine fish. The incorporation of such n-3 HUFA in marine fish feeds is then absolutely necessary for marine fish survival and also for maintaining a certain level of n-3 HUFA in fish flesh (for both marine and fresh water species). Current dietary sources of n-3 HUFA are in decline since quotas for fisheries captures are severe and at the same time there is an increasing need for fish oils in feeds for fish aquaculture. Consequently, there is a considerable interest in producing fish species or fish strains able to synthesize n-3 HUFA, in order to lower the dependency of fish aquaculture on fisheries.

Although many fatty acid desaturase genes may be found in some marine fish species, the apparent deficiency of these fish in the fatty acid desaturation pathway could be the result of relaxation of constraints on a prototypic pathway in an environment providing a diet naturally rich in HUFA (Hasting et al., 2001). Recent work has shown that an enhanced expression of the delta-6-desaturase gene, which controls the first step of the n-3 HUFA synthesis pathway, could be obtained when feeding gilthead sea bream a HUFA-deprived diet (Seiliez et al., 2003). Interestingly, Vagner et al. (2007) have shown a persistent elevated expression of delta-6 desaturase gene in European sea bass that have been fed during larval period with a diet incorporating a low level of n-3 HUFA, compared to a control group without any early nutritional conditioning. These authors hypothesized that post-transcriptional events could have improved the functioning of the delta-6 gene itself or some of its modulators, in particular SREBP-1a and SREBP-1c, and concluded that it is possible to influence juvenile fish metabolism by a nutritional conditioning during the larval stage. However, as already evoked for glucose metabolism, the early-programming process is very promising in terms of practical applications, but needs further studies in fish larvae.

### **2.1.3. Muscle development**

Nutrition status has profound effects on the growth and development of somatic tissues, particularly the skeletal muscle. Muscle development during larval period is characterized by hypertrophy (increase of muscle diameters) and by hyperplasia (recruitment of new muscle fibers) from undifferentiated myoblasts. The proliferation and differentiation of these cells lead to the formation of new fibers, a process regulated by several myogenic regulatory factors that can be also influenced by environmental and nutritional parameters (Johnston, 2006). Rodgers et al. (2003) showed that myostatin mRNA levels in tilapia larvae were sometimes elevated after a short-term fast (3 days) and significantly reduced with prolonged fasting (6 days). Myostatin is known to regulate muscle growth and development, by inhibiting specifically myoblast proliferation via cell-cycle arrest (Thomas et al., 2000). However, the effect of nutrition has been mostly studied comparing extreme nutritional conditions, i.e. fasting/feeding, which do not allow to understand how to orientate (and improve) further skeletal muscle development. Some dietary nutrients, like ascorbic acid, could be worth studies; indeed, this nutrient is a precursor of collagen and then could have a crucial role for muscle development in fish, particularly in the white myotomal muscle which exhibited a certain plasticity during larval period (Alami-Durante et al., 2006).

#### **2.1.4. Reproduction**

Reproduction of animals in adult life can be affected by several influences acting at different stages of development, which are mediated by changes in the hypothalamic-pituitary-gonadal (HPG) axis (Davis and Norman, 2002). Gonadotropins constitute an important group of pituitary regulatory hormones which comprise luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The secretion of Gonadotropin hormones is controlled by the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator that is an integrator of hormonal, metabolic and neural signals. Experimental data in mammals (and observations in humans) revealed that early life exposures influence the development and functioning of the HPG from a programming perspective. Effects of nutrition on the reproductive health of fish have not been studied until now. However, studies conducted in other vertebrates could be very useful for further focus. They have shown that, at earlier stages of foetal development, the normal ontogeny of gonadal development and function can be disrupted by undernutrition or the influence of endocrine-disrupting compounds. Clearly, any effect of undernutrition on the process of tissue differentiation, gonad formation and the establishment of associated enzyme systems is likely to have a fundamental effect on the subsequent function of these organs (Rhind et al., 2001). Specifically, in female fetuses, the onset of meiosis is delayed, whereas, in male fetuses, testosterone synthesis is increased as a result of enhanced testicular steroidogenic enzyme activity. As evoked above, most of the nutritional studies undertaken in this area have compared fasting to feeding conditions. It is then necessary to investigate the effects of different dietary components as proteins and other specific nutrients, like HUFA and vitamins. It is then well established in mammals that the reproductive performance can be influenced by factors acting during the earliest developmental periods; however, much further work is required to identify these critical developmental periods and the relationships between developmental abnormalities and adult reproductive performance.

### **3. Conclusion**

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These last 20 years, most of the studies on fish larvae nutrition aimed to develop a compound diet able to replace efficiently live preys in the larvae feeding sequence.

It was hypothesized that fish larvae lacked a functional digestive system. Consequently, the functioning of the digestive tract during the larval period was mainly examined and, unexpectedly, revealed that larvae have a primary but efficient mode of digestion that progressively matures during development. The knowledge of these larvae digestive features allowed to develop a compound diet that can be used from the earliest larval stages. Then, it is now possible to refine the nutritional requirements of the fish larvae, and identify the potential physiological effects of some particular nutrients. Indeed, it has been shown in upper vertebrates that some nutrients, even at low dietary concentrations, may influence morphogenesis particularly by acting on bone differentiation.

These nutrients, such as vitamins and HUFA, recognize nuclear receptors which are transcription factors for several genes involved in tissue differentiation and organogenesis. These nuclear receptors can link together and form heterodimers, particularly with RXR. In consequence, HUFA and the oxidative balance of lipids, itself regulated by vitamin C and E, may act on retinoid pathways and, in the same way, molecular pathways of vitamin A and D can interact together (Figure 1). The formation of nuclear receptor heterodimers leads to cross-talks between metabolic processes and then, may affect the quality of the future juvenile fish.

It is then possible to orientate the metabolic pathways of the juvenile fish by an appropriate nutritional conditioning during the larval period. This conditioning offers the possibility to have fish with an enhanced immunological system, fish able to use efficiently some vegetable diets...

For this, nutritional studies on fish larvae need to take into account the global effect of nutrients on developmental processes. Important information on genome and gene expression is now available for several fish species, and this information allows to undertake genomic studies on fish development. Fish could then constitute an interesting model for studying the effect of nutrition on development processes in upper vertebrates.

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Figure 1. Possible impact of dietary nutrients on some metabolic pathways controlling development and morphogenesis of marine fish larvae. RAR: Retinoic Acid Receptor, RXR: Retinoid X Receptor, PPAR: Peroxisome Proliferator Activated Receptor, VDR: Vitamin D Receptor, SVCT: Vitamin C transporter system, BMP: gene coding for Bone Morphogenetic Protein, IGF: gene coding for Insulin-like Growth Factor; SHH: sonic hedgehog gene, hox: hox genes, cdx: caudal-related homeobox genes. (from Zambonino Infante and Cahu, 2007)

