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Nutrition, quality and performance in marine fish larvae

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Resumo

O cultivo de larvas de peixes marinhos é caracterizado por altas mortalidades e por problemas de qualidade, nomeadamente malformações esqueléticas. A solução de parte destes problemas passa por uma melhor compreensão dos requisitos nutricionais. Este trabalho pretende fazer uma revisão de trabalhos recentes que demonstram como os aminoácidos, os ácidos gordos e a vitamina K, mas também o regime alimentar, podem influenciar a qualidade e a performance das larvas de peixes marinhos.

Apesar de progressos recentes, o conhecimento sobre os requisitos nutricionais em proteínas e aminoácidos (AA) das larvas de peixes é ainda limitado. As principais diferenças em comparação com peixes maiores parecem ser uma menor capacidade de digerir e / ou absorver proteínas complexas. De facto, as larvas da maioria das espécies de peixes marinhos, e sobretudo nos primeiros dias de vida, possuem um trato digestivo simples e uma capacidade limitada para digerir proteínas. Assim, as dietas de larvas de peixes necessitam de ser ricas em proteína solúvel e proteínas complexas com baixa digestibilidade devem ser evitadas. Resultados recentes revelaram ainda que o peso molecular da fração proteica da dieta tem um forte efeito sobre a expressão do proteoma das larvas, com importantes implicações na qualidade e potencial de crescimento destas. Foi ainda demonstrado que desequilíbrios no perfil de AA indispensáveis das dietas de larvas de peixes levam a um maior catabolismo proteico, o que pode causar menores sobrevivências e uma maior incidência de malformações esqueléticas.

As larvas de peixes marinhos têm altos requisitos em ácidos gordos essenciais (EFA), tais como DHA, EPA e ARA. Dietas deficientes nestes EFA afetam a sobrevivência, crescimento, pigmentação e resistência ao stress das larvas, devido a alterações na função das membranas celulares e/ou do metabolismo de eicosanóides e lípidos. Por exemplo, os níveis de ARA na dieta afetam a resposta ao stress e o metabolismo lipídico, como demonstrado em estudos de expressão génica e de marcadores radioativos. No entanto, os requisitos em EFA de larvas de peixes marinhos parecem variar com a espécie.

A vitamina K é uma vitamina lipossolúvel envolvida na coagulação do sangue e que desempenha um papel importante no metabolismo ósseo. Foi recentemente demonstrado que a suplementação da dieta de larvas de linguado em vitamina K leva a uma redução no número de peixes deformados bem como no número de deformidades por peixe, e ainda a alterações significativas da expressão do proteoma.

Em resumo, está demonstrado que tanto a qualidade como a performance de larvas de peixes marinhos podem ser melhoradas através da otimização da dieta, quer através das dosagens de nutrientes específicos, quer da melhoria do regime alimentar.

Abstract

Marine fish larval rearing is still characterised by high mortalities and larval quality problems, with a high incidence of skeletal deformities. A deeper knowledge of the larval nutritional requirements may solve part of these constrains. This work will review recent studies on how larval performance and quality may be affected by amino acids (AA), fatty acids, vitamins and feeding regime.

Despite some recent progresses, the knowledge about the proteins and amino acids requirements for marine fish larvae in is still scarce. When compared with older fish, fish larvae seem to have a lower capacity to digest and/or absorb complex proteins. In fact, the digestive tract is a simple tube during the early life of marine fish larvae of most species, conferring them a limited capacity to digest proteins. Therefore, fish larval diets should be rich in soluble protein and complex proteins should be avoided. Recently, it has been shown that the molecular weight of the dietary proteins has a strong effect on the larval proteome expression, affecting larvae quality and growth potential. Furthermore, diets with an imbalanced amino acid profile lead to higher protein catabolism, resulting in lower survival rates and a higher incidence of skeletal deformities.

Marine fish larvae have high essential fatty acid (EFA) requirements, such as DHA, EPA and ARA. Dietary deficiencies in these EFA affect larval growth, pigmentation, survival and stress resistance, due to effects on cellular membranes function and/or on the metabolism of eicosanoids and lipids. For instance, dietary ARA levels affect stress resistance and lipid metabolism in larvae, as

shown from genetic expression and radioactive tracer studies. However, EFA requirements in marine fish larvae seem to differ among species.

Vitamin K is a liposoluble vitamin involved in blood coagulation and with an important role in bone metabolism. Recently, it has been shown that diets supplemented with vitamin K reduced the number of sole larvae with deformities, reduced the number of deformities per larva and significantly affected the proteome expression.

In summary, there is evidence that both larval quality and performance in marine fish may be enhanced through diet optimisation, either by adding doses of specific nutrients or by improving the feeding regime.

1. Introduction

Marine fish larval rearing is still characterised by high mortalities, with values 10-15% survival at 60 days of age being considered acceptable for many species farmed at the commercial scale. In addition, larval quality problems arise for many species, with an emphasis on high incidence of skeletal deformities, which may reach 60% or more of the reared population. Skeletal deformities can affect growth, morphology, fish survival and decreased market value leading to a higher production cost of many commercial marine fish species. Several studies have focused on the identification of causes of deformities and associated them to nutrition, but also to rearing environment and genetics (Boglione et al, 2003; Cahu et al, 2003). Another common quality problem is malpigmentation, which affects mostly flatfish species such as turbot, Japanese flounder and Atlantic halibut (Shields et al., 1999). In addition to mortality and quality problems, sub-optimal growth and stress/disease resistance are also major issues in marine fish larvae rearing. A deeper knowledge of the larval nutritional requirements may solve part of these constrains. This work will review recent studies on how larval performance and quality may be affected by protein/amino acids, fatty acids, vitamins and feeding regime.

2. Dietary protein and larval quality

2.1 Quality of dietary protein

When compared with older fish, fish larvae seem to have a lower capacity to digest and/or absorb complex proteins (Rønnestad and Conceição, 2005). In fact, the digestive tract is a simple tube during the early life of marine fish larvae of most species, conferring them a limited capacity to digest proteins. Therefore, fish larval diets should be rich in soluble protein and complex proteins should be avoided (Conceição et al., 2011). Recently, it has been shown that the molecular weight of the dietary proteins has a strong effect on the larval proteome expression, affecting larvae quality and growth potential (Richard et al., 2011).

2.2 Effects on AA metabolism and larval performance

Larval quality is influenced by the quality and composition of the dietary protein. Dietary AA imbalances compromise a maximised protein deposition, since this requires that all indispensable AA occur at an optimal ratio with respect to each other (Carter and Houlihan, 2001). Since growth is essentially muscle protein deposition, dietary AA imbalances affect larval growth and performance. Recent studies showed that dietary AA imbalances increase protein catabolism (Aragão et al., 2004) and consequently increase nitrogen excretion (Saavedra et al., 2009), leading to lower survival rates during rearing experiments using microdiets (Aragão et al., 2007; Saavedra et al., 2009).

Although the major fate of AA is towards protein synthesis, many AA regulate key metabolic pathways that are crucial to maintenance, growth, and immune responses (Li et al., 2009). Therefore, dietary supplementation with individual AA has also shown to affect larval quality. For instance, it has been shown that feeding gilthead seabream (*Sparus aurata*) larvae with rotifers enriched with lysine or methionine tends to stimulate intestinal maturation and triggers native endocrine factors to complete the digestive process in the developing digestive tract (Naz and Türkmen, 2009a,b). Furthermore, even when diets are well balanced in terms of AA, dietary supplementation with some individual AA beyond the expected requirement has proven to increase larval quality. For instance, when white seabream (*Diplodus sargus*) larvae were fed rotifers with an AA balanced profile but enriched with a surplus of phenylalanine, a decrease in the percentage of vertebral compressions compared with larvae fed rotifers with an AA balanced profile was observed (Saavedra et al., 2010). Phenylalanine is the precursor of tyrosine and the latter play important physiological roles in fish, being involved in the synthesis of several compounds with important roles in stress response, such as the thyroid hormones and cathecolamines. A significantly higher survival to a temperature stress test was found for white seabream larvae fed diets supplemented with phenylalanine and tyrosine (Saavedra et al., 2010). Additionally, short-term experiments revealed that Senegalese sole (*Solea senegalensis*) postlarvae are able to maintain a normal tyrosine metabolism under stressful conditions, if a dietary tyrosine supplement is given (Aragão et al., 2010). Furthermore, since metamorphosis in flatfish species are highly dependent on thyroid hormones, some studies suggest that aromatic AA supplies may be required during flatfish larval metamorphosis in order to achieve a successful transition to the juvenile stage (Aragão et al., 2010, Pinto et al., 2010a).

Another AA that has been the subject of interest in the latest years is taurine, a β-sulphur AA that is one of the most abundant organic constituent with low molecular weight in fish. Taurine participates in several physiological functions, such as membrane stabilization, modulation of neurotransmitters, antioxidation and detoxification, and is known to affect the early development of brain, eye and heart (Huxtable, 1992). Positive correlations between dietary taurine levels and larval growth have been demonstrated for turbot, *Scophthalmus maximus* (Conceição et al., 1997), red seabream, *Pagrus major* (Chen et al., 2004), Japanese flounder, *Paralichthys olivaceus* (Chen et al., 2005), Pacific cod, *Gadus macrocephalus* (Matsunari et al., 2005) and cobia, *Rachycentron canadum* (Salze et al., 2011). However, the underlying mechanisms leading to growth enhancement have not been clarified, since taurine, lacking a carboxyl group, cannot be incorporated into tissue proteins. Nevertheless, the few studies available with fish larvae show that dietary taurine supplementation affect larval quality, through an enhancement on the ontogenetical development of the sensory systems, as observed in cobia (Salze et al., 2011), or through an enhancement in metamorphosis completion in flatfish species, such as Japanese flounder (Chen et al., 2005) and Senegalese sole (Pinto et al., 2010b). Furthermore, the increased larval quality is also reflected by the increased resistance to starvation in red seabream larvae (Chen et al., 2004) and by the increased survival in cobia larvae (Salze et al., 2011) fed taurine supplements.

2.3 Effects on Skeletal deformities

The amino acid profile of the diet of fish larvae has repercussions on larval development, notably on skeletogenesis. Recent studies conducted on white seabream larvae showed that a microencapsulated diet balanced in amino acid offered at as an early stage as 8 DAH and co-fed with live feed, induced a decrease in the frequency of larvae affected by skeleton deformities at 25 DAH when compared to an unbalanced amino acid profile (containing a higher proportion in dispensable amino acids) or a live feed diet (Saavedra et al. 2009a). Interestingly, a higher incidence of deformed larvae was registered in that experiment in larval groups fed on live feed compared to those fed on the microencapsulated diet presenting an unbalanced amino acid profile, and this was reflected in a higher number of vertebral fusions (Saavedra et al., 2009a).

Besides the effect of the global dietary amino acid composition, some specific amino acids and in particular the indispensable ones, can have an effect in themselves on the appearance of vertebral deformities in fish larvae. For example, a dietary supplementation in phenylalanine has been shown to influence skeleton development, lowering the frequency of vertebral compression in the trunk region of the vertebral column in white seabream larvae (Saavedra et al., 2010). Tryptophan content of the diet of fish larvae was reported to be important for a proper skeleton development in salmonids, scoliosis events being more frequent in individuals fed a deficient diet (Akiyama et al., 1986). However, a balanced diet supplemented in tryptophan did not produce any beneficial effects in the prevention of skeletal deformities appearance in white seabream larvae (Saavedra et al., 2009b). Also, the form in which amino acids are delivered to fish larvae has been reported to affect skeleton development. Thus, purveying a part of dietary amino acids in the form of protein hydrolysates rather than intact protein has been shown to induce a decrease in skeleton deformities appearance in seabass larvae (Zambonino et al., 1997; Cahu et al., 1999).

2.4 Effects on proteome expression

Analysing the proteome of a given organism or tissue is a useful explorative step in finding molecular indicators of nutritional status, and provides also untargeted information that could help elucidate the mechanistic details of metabolic pathways. The purpose of one of our recent studies was to investigate the impact of the dietary protein hydrolysate content on proteome expression of the entire body of white seabream larvae after 15 days of feeding (Richard et al., 2011). The results of this experiment revealed that the molecular weight of the dietary protein hydrolysate fraction had a strong effect on larval proteome expression, modulating the abundance of 339 protein spots over 709 protein spots detected and analysed within the pH range 4 to 7. Among proteins whose abundance was significantly affected by the dietary molecular weight fractions of protein hydrolysates, some were analysed by liquid chromatography-tandem mass spectrometry and subsequently identified as being involved in various processes, as diverse as the cellular contractile system, energy metabolism, collagen maturation, carbohydrate metabolism, protein folding, protein catabolism, mRNA processing, immunity, lipid transport, calcium metabolism, aldehyde metabolism and nucleotide metabolism. Consistent with our study, Sveinsdóttir and Gudmunsdóttir (2010) also reported that administering fish protein hydrolysates to Atlantic cod larvae (*Gadus morhua*) modified the abundance of several proteins in larval body.

3. Dietary lipid and larval quality

3.1 Effects on larval performance and fatty acid profile/metabolism

Lipids have multiple key roles in fish nutrition. They provide metabolic energy, are essential to the maintenance of the structural and functional integrity of cell membranes, and function as vehicles for lipid-soluble micronutrients (Tocher et al., 2003). Lipids are also precursors of metabolites, like hormones and eicosanoids, which play an important part in cellular signaling, including the regulation of gene transcription (Tocher et al., 2003).

In general, marine fish, especially during the larval stages, appear to require a dietary supply of long-chain polyunsaturated fatty acids (LC-PUFA), particularly DHA, EPA and ARA, and of phospholipids for normal growth and development since they are unable to synthesise them from C18 precursors (linoleic and linolenic acids), at least at a sufficiently high rate to satisfy their metabolic needs (Sargent et al., 1999; Cahu et al., 2009). Due to higher abundance in cell membranes relative to other fatty acids , DHA and EPA requirements have been the main subject of studies on the EFA requirements in fish larvae nutrition. Nonetheless, in recent years, the importance of ARA supply in fish nutrition has been recognized especially on growth, survival, pigmentation success, stress resistance, and health.

Dietary EFA deficiencies in larval stages are often associated with poor growth and increased mortality, but also with higher susceptibility to stress and abnormal behaviour (Watanabe et al., 1993; Izquierdo, 1996). It has become generally accepted that the EFA requirements are species-specific and that besides a minimum dietary supply, the relative ratios between EFA must be considered (Izquierdo et al., 2000). In gilthead seabream for example, a sparid with high commercial interest in southern Europe, larval requirements for n-3 HUFA have been reported to vary between 1.5 and 5.5% of dry diet depending on the DHA:EPA ratio, as reviewed by Tocher (2010).

Fish tissues commonly reflect dietary fatty acid profiles and diet-induced changes in phosphoacylglyceride fatty acids are likely responsible for major dietary outcomes on fish physiology (Sargent et al., 1999). Between EFA, competition between ARA and EPA for inclusion into cell membrane phospholipids and subsequent eicosanoid formation and action is often reported. In a recent study, we have shown the displacement of EPA from seabream larvae tissues caused by increasing dietary ARA supply, associated to higher production of ARA-derived eicosanoids, prostaglandin E_2 and leukotriene B_4 (Alves Martins et al., in press). Considering the high digestibility of LC-PUFA in seabream larvae (Morais and Conceição, 2009), reduced EPA deposition in the tissues relative to constant dietary supply level also indicated that it was increasingly directed for metabolic processes such as β-oxidation or conversion into other metabolites. Fatty acids are either selectively retained or metabolized, and preferential metabolism often occurs when a particular fatty acid is supplied at high concentrations in the diet (Karalazos et al., 2007). A study where marine lecithin was replaced by soybean lecithin in seabream microdiets revealed up-regulated expression of the hormone-sensitive lipase gene with the later diet (Alves Martins et al., 2010). Reduced EFA (EPA and ARA) but especially linoleic acid abundance in the larvae influenced the expression of a key gene in lipolytic activity, which was associated to a reduction in larval growth potential.

Marine larvae may in fact tolerate a relatively wide range of dietary EFA supply such that growth and survival may not be affected within usual experimental periods of 2-3 weeks. This could be due partly to a successful retention of EFA in the tissues. However, adaptation to dietary lipid composition may involve changes on eicosanoid production, cellular signaling and expression of genes which are only starting to be explored. Therefore, dietary deficiencies in EFA may present long-term effects on health and growth performance parameters of marine fish.

3.2 Effects on larval stress resistance

Intensive fish farming can negatively impact animal welfare. Coping with stress requires energy which may be driven away from other biological processes, such as growth. Resistance to stress has been increasingly recognised as a valuable parameter for the evaluation of physiological condition in fish larvae, along with growth performance and survival. The importance of the dietary supply of phospholipids and both n-3 and n-6 series LC-PUFA on stress resistance in fish larvae has been reported in several species (Dhert *et al.*, 1990; Kanazawa, 1997; Bessonart *et al.*, 1999; Liu *et al.*, 2002; Koven *et al.*, 2003), although the physiological mechanisms potentially associated are unclear at present. Dietary fatty acids can affect cellular signalling through the modulation of various functional aspects of cell membranes. These include the activity of enzymes, receptors or ion channels in membranes, metabolite permeability, eicosanoid synthesis and action, and gene expression, either directly or through interactions with transcription factors. Due to the fact that LC-PUFA are not usually preferentially oxidised for energy production, it is likely that their effects on stress resistance are caused by their influence on cellular signalling or on functional aspects of cell membranes.

Recent studies have explored the role of dietary fatty acids on the cortisol response and/or the expression of glucocorticoid receptors in fish (Montero *et al.*, 2003; Van Anholt *et al.*, 2004; Ganga *et al.*, 2006; Alves Martins *et al.*, 2010, 2011; Lund and Steenfeldt, 2011). This area of research has gained particular relevance within the current context of fish oil replacement by alternative vegetable oils in commercial diets in order to assure that the health and welfare of farmed fish are not compromised. Nonetheless, few studies involving the testing of vegetable lipids have focused on fish response to a stressor (Montero *et al.*, 2003; Oxley *et al.*, 2010; Ganga *et al.*, 2011). It is known that increased C18 fatty acid deposition and lower LC-PUFA content in fish tissues, typically observed with diets containing vegetable ingredients, may lead to significant mortality in both juvenile (Bell *et al.*, 1991) and larval stages (Alves Martins *et al.*, 2010; Lund and Steenfeldt, 2011).

It is generally hypothesized that the effect of dietary lipids on fish stress response is mediated by eicosanoids and that ARA-derived eicosanoids in particular present important bioactivity. Therefore, a number reports show effects of ARA supply on stress resistance and cortisol production in fish larvae (Bessonart et al., 1999; Koven et al., 2003; Alves Martins et al., 2011). In our lab, two recent experiments have explored the potential of ARA on the regulation of genes involved in stress response in gilthead seabream and Senegalese sole larvae. These included: steroidogenic acute regulatory protein (StAR), glucocorticoid receptors (GR), phosphoenolpyruvate carboxykinase (PEPCK), and enzymes involved in eicosanoid synthesis. In seabream, an increase in dietary ARA from 0.4 to 3.0% dry diet decreased the expression of all genes analysed which could be due to elevated ARA signaling and/or lesser EPA deposition in the tissues and lower EPA-derived eicosanoids. Besides, the role of C18 polyunsaturates in this process should not be disregarded. Similar whole-body basal cortisol levels, growth performance, and survival after acute stress between groups suggested seabream larvae could tolerate a wide range of dietary ARA supply. This indicates that adaptation to a wide range of dietary ARA levels may involve the modulation of genes related with eicosanoid synthesis, lipid metabolism, and stress response. A second study with Senegalese sole post-larvae revealed that cortisol levels at 3h post-stress increased with dietary ARA supply (up to 1.7% dry diet) and that at a higher dietary level (2.3%) basal cortisol could be elevated and the stress response pattern affected. Nonetheless, differences in gene expression seemed much less pronounced than in seabream. Senegal sole is known to have relatively low EFA requirements compared to most marine fish studied. Tolerance to a wide range of dietary ARA/EPA ratios was previously shown in Senegalese sole post-larvae as well as the importance of ARA in steroidogenesis regulation and the modulatory role of EPA in this process (Alves Martins et al., 2011). Specifically, the acute stress coping response may be more efficient in post-larvae fed low ARA/EPA ratios and, under these particular conditions, a faster recovery of cortisol to basal values could be indicative of rearing conditions (undisturbed vs. repeatedly stressed).

4. Dietary vitamins and larval quality

4.1 Effect of Vitamin A and other retinoids

Vitamin A (VA) is an essential nutrient during the lifecycle of all chordates, playing numerous functions in vision, maintenance of epithelial surfaces, immune competence, reproduction and embryonic development (Ross et al., 2000). There are two main different dietary sources of VA compounds, vegetable (carotenoids) and animal (retinyl esters) compounds. There are approximately 600 different forms of carotenoids, but only about 50 of those have the pro-vitamin A activity (Olson, 1989). However, VA levels marginally above the recommended dietary intake are associated with embryonic malformations, reduced bone mineral density, and increased risk for bone fractures (Melhus et al., 1998). Several studies have shown that larval fish fed diets with deficient or excess VA had a compromised development, showing reduced growth and survival rates, delayed digestive system maturation and high incidence of skeletal deformities (Cahu et al, 2003). In particular the most active form of vitamin A, retinoic acid, have been proven to cause marked effects over the skeleton, with treatments in larvae causing a high teratogenicity even at very low concentrations. Gilthead seabream fed VA deficient diet showed that body shape was affected by the level of dietary VA, increasing the incidence of specimens with lordosis, kyphosis and/or scoliosis, affecting mostly cranial elements, prehaemal and caudal vertebrae and also the skeletal elements composing the caudal fin (Fernandez et al., 2009). In addition, high dietary VA advanced ossification of vertebral centrums and resulted in the development of supernumerary vertebrae and the appearance of high frequency of vertebral compressions and/or fusions. Fernandez and Gisbert (2011) suggested to use lower levels of dietary VA since, high incidence of skeletal deformities is observed in fish fed 0.66 108IU Kg-1 that is a common level in diets. These authors propose that VA content should be less than 1.0*108 IU kg-1 in gilthead sea bream fed with rotifers and less than 42,666 IU kg-1 VA in Senegalese sole fed Artemia.

4.1 Effect of Vitamin K

Vitamin K is a liposoluble vitamin classically thought as involved in blood coagulation, and until recently largely disregarded in aquaculture nutrition. Vitamin K is known to play an important role in bone metabolism by acting as a coenzyme for vitamin K-dependent gamma carboxylation of matrix proteins as Osteocalcin (Oc) and Matrix gla protein (Mgp) and by regulating Msx gene expression, through the recently identified vitamin K binding Pregnane X receptor (PXR). The known tetrapod target homeobox gene Msx2 is known to play a role in skeletogenesis and in morphogenesis. Still, the mechanisms underlying the association of vitamin K with the changes of skeletal microanatomy are still not fully understood. It is thought that vitamin K may be delivered to osteoblasts by triglyceride-rich lipoproteins, although the precise mechanisms are not described (Shea et al., 2007).

Roy and Lall (2007) showed that dietary supplementation with menadione (Vitamin K3) have positive effects over the incidence of deformities during juvenile growth in haddock (Melanogrammus aeglefinus). Conversely, dietary Vitamin K nutritional deficiency caused a decrease in bone mineralization and bone mass, related to an increasing susceptibility to bone deformity. In mummichog (Fundulos heteroclitus), fish fed vitamin K rich diets showed better skeletal quality than vitamin K deprived ones, while mature fish fed vitamin K rich diets resulted in better skeletal quality in progeny (Udagawa, 2001, 2006, 2007). The minimum feed level of vitamin K, to promote normal skeletal growth and correct mineralization of the structures was recently determined for haddock to be 40 mg/kg (Roy and Lall, 2007). These authors showed that supplementation with Vitamin K3 was related to a decreased incidence of deformities during juvenile growth of haddock.

Our team has recently identified beneficial effects of supplementing enriching media with phylloquinone (K1) and using life preys as vehicles for delivering this nutrient to sole larvae. Vitamin K1 supplemented to sole larvae had no influence on growth or mortality, but these specimens showed a marked reduction of skeletal abnormalities and a differential pattern of expression for the proteome (Richard et al, 2008).

5. Feeding regime and larval quality

Inert diets are nutritional balanced, ready to use, have a long shelf life and have a stable composition, when compared to live feed that may vary according to culture/enrichment conditions. Nowadays suitable larval inert diets are available for several marine fish species that can be used from mouth opening. That is the case of European seabass (Cahu and Zambonino Infante, 2001), gilthead seabream (Yúfera et al., 2000; Robin and Vincent, 2003), red seabream (Takeuchi, 2001) and red drum (Lazo et al., 2000). Still, in most marine fish species, *Artemia* replacement regime, i.e. a co-feeding regime with live prey and inert diet during a period prior to weaning, is still needed to sustain larval growth at earlier stages and is a feeding strategy widely used in marine fish hatcheries.

Artemia replacement regimes can improve survival and growth performance of several marine fish larvae even in early larval stages (Holt, 1993; Rosenlund et al., 1997; Baskerville-Bridges and Kling, 2000; Alves et al., 2006) and may enhance digestive maturation (Kolkovski et al., 1993; Kolkovski et al., 1997b; Rosenlund et al., 1997; Baskerville-Bridges and Kling, 2000). This feeding strategy is also known to stimulate feeding rates in Dover sole (Knutsen, 1992) and gilthead seabream (Kolkovski et al., 1997a), and to pre-condition larvae onto inert diet (Hart and Purser, 1996; Brown et al., 1997; Callan et al., 2003; Curnow et al., 2006a; Fletcher et al., 2007). Nevertheless, weaning of marine species might be accomplished with relative success with different strategies. In Asian seabass survival rates had a four-fold increase when start of weaning was delayed two weeks (Curnow et al., 2006b). In Atlantic cod, a co-feeding strategy produced a two-fold increase in larval weight, in comparison to an inert diet strategy (Fletcher et al., 2007). Alves et al. (2006) noticed that doubling the co-feeding period in fat snook would promote a two-fold increase in larval length. An earlier co-feeding regime in cobia larvae produced larger fish than a later co-feeding (Nhu et al., 2010).

In Atlantic halibut (*Hippoglossus hippoglossus*) a shorter or longer co-feeding period produces similar survival rates, higher than 80% (Mæland et al., 1999; Næss et al., 2001; Hamre et al., 2005). In greenback and winter flounder survival rates are always above 80%, when fish is weaned at younger ages with a longer co-feeding period (Hart and Purser, 1996; Lee and Litvak, 1996; Geurden et al., 1997). In tongue sole the addition of inert diet to live feed alone promote a two-fold increase in larval weight (Chang et al., 2006). Senegalese sole postlarvae can be weaned with two different feeding strategies, sudden weaning and *Artemia* replacement regime (Engrola et al., 2007; Engrola et al., 2009). However, an *Artemia* replacement with 20% of inert diet from mouth opening promoted better sole growth and quality at weaning (Engrola et al., 2009; Engrola et al., 2010).

6. Conclusions

There is clear evidence that both larval quality and performance in marine fish may be enhanced through dietary levels of amino acids, fatty acids and vitamins, as well as by feeding regime. Dietary effects may not be apparent in the short term, but may have major consequences in terms of postlarvae/juvenile quality, growth potential and stress/disease resistance. Such long term effects are still poorly studied. Moreover, the interaction between different nutrients during the larval stage needs further study as non-additive effects may occur, be it synergistic or antagonistic.

These nutritional effects on larval quality and performance are more relevant when one considers the tremendous growth potential of fish larvae together with their limited physiological development, e.g., immature digestive, immune and endocrine systems.

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