NUTRITIONAL REQUIREMENTS FOR FISH LARVAE: PROTEIN, AMINOACIDS, PHOSPHOLIPIDS, AND VITAMINS

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Abstract

The requirement for a particular nutrient could be defined both from a physiological or a practical point of view. The determination of requirements for different nutrients should consider some general recommendations: Optimal culture conditions as close to commercial scale as possible should be used, testing a hypothesis with 5 to 6 different levels for a given nutrient by triplicates and considering several factors related to the species, the environment, type of culture, feeding strategy and feed. A standard diet should be used as a control and complete feed ingredient descriptions should be provided. In larval nutrition, fish should be fed different levels of the nutrient to be tested through enriched life prey, whose nutrient composition is difficult to control, or microdiets, which still present stability and acceptance problems. Study of the biochemical composition of the eggs, the evolution of a nutrient along the embryo development and the conservation or loss of a nutrient along larval development and during starvation and feeding of the fish can also provide very interesting information about the larvae requirements. As for other types of nutrients, protein requirements in fish larvae are higher than for juveniles or adults. Besides the 10 essential amino acids, Tyr, Cys and taurine must be frequently be included in larval diets. Phospholipids are required for growth and utilization of other dietary lipids such as essential fatty acids and fat-soluble vitamins, their inclusion in microdiets markedly improving lipoprotein synthesis and lipid transport. Most described water-soluble vitamin requirements are much higher for larvae than for juveniles of the same species, not only due to the higher metabolic demand in the former, but also for the high ratio surface/volume in larval diets making the diets more prone to oxidation and leaching. Certain fat-soluble vitamins such as Vit E, markedly improved survival, but their elevation in absence of other anti-oxidants or in inadequate molecular forms as it happens with Vit A, not only negatively affect larval growth and survival but also induces alterations in pigmentation and skeleton deformities in fish larvae.

Determination of nutritional requirements for larvae

The requirement for a particular nutrient could be defined both from a physiological or a practical point of view. Under the later we can consider the requirement for body maintenance as the minimum rate of nutrient expenditure needed to keep the animal alive. For instance, we can consider the energy for maintenance as the energy needed to maintain the basal metabolism, plus the energy used for thermoregulation, plus the energy for involuntary activity such as body movement and muscular activity. But we can also define the requirement for maximal growth, where the relation fish-diet-feeding has an important effect in the determination of the quantitative needs. Besides, we can also talk about requirement for least cost production. Different studies show that the requirements for maximal growth are always higher than the requirements for least cost production. Thus, de Silva et al. (1989) applying a second order polynomial relation to growth data compiled from different works on Tilapia, observed that the most economical dietary protein content was 28%, considerably less than the 34% protein level which supported maximum growth. Finally, it could be defined the requirement for fish health. The nutrient requirements determined for certain nutrients under optimal culture conditions increase when fish are exposed to unfavorable environmental conditions (poor water quality, stress, pathogens ...). Thus for example, the definition of the requirement for vitamin C to improve immunological defenses is related to the production conditions applied.

Defining the methodology applied to the determination of requirements for different nutrient should consider some general recommendations:

a. A practical point of view to establish nutritional requirements is preferred.
b. If optimal culture conditions for the tested species have been established, the requirements should be assayed in such conditions. If not, it should be reviewed what it is known from this species in its natural conditions.
c. As far as possible, experimental conditions similar to those used at commercial scale should be used (feed preparation technique, water quality, photoperiod or fish stocking density, among others).
d. Only one hypothesis tested per experiment is preferred.
e. At least triplicate tanks of fish should be used per dietary treatment, as one tank of fish represents a single block observation.
f. To determine quantitative requirements it is important to considerer different factors related to the species (such as age and size), related to the medium (such as temperature, salinity, culture density, to the type of culture: extensive/intensive), related to the feeding strategy and feeding regime and related to the feed (dietary energy content, nutrient availability in the ingredient source and interactions with other dietary nutrients or ingredients).
g. Complete feed ingredient descriptions should be provided, including International Feed Number (IFN), chemical composition and particle size, when reporting dietary formulations and the results of nutritional feeding trials. If a commercially prepared diet is used, the trade name and manufacturer should be indicated.
h. A standard diet should be used as a control in addition to any local diet also designed as a control. In most cases the use of different control diets makes among different authors complicates comparison of results among them.
i. A minimum of six dietary nutrient levels or treatments is recommended for nutrient requirement studies.
j. Carcass analysis should be carried out at the beginning and at the end of the experiment.
k. An appropriate statistic analysis is always necessary.
l. Fish should be fed until "apparent satiation" instead of restricted feeding rates.

Besides these general considerations, other ones specifically related with the larval production must be taken into account. The most direct method to evaluate the several nutrients requirements in fish larvae is to feed the larvae with different levels of these nutrients. Although these may seem simple, in fact it is quite complicated. For this purpose we must feed the larvae with enriched life prey or directly on microdiets containing different levels of the nutrient to be tested. Although it is possible to control the live preys content on some nutrients such as fatty acids, the precise amount of certain nutrients such as proteins, amino acids, vitamins and minerals is difficult to manipulate in live preys, whose own metabolism modifies the nutrients provided through the enrichments. In this sense, microdiets are a preferred method to determine the larval requirements, but stability and acceptance are yet problems to be solve before using them in mass production of fish larvae. Once several levels of the nutrient are provided through the diet, their effects in several parameters are studied: a) Growth rate (much affected by some nutrients but not by others) b) survival rate and resistance to stress (again some species are very sensible but no others and molecular markers of stress would be needed to precise the effects of certain nutrients), c) biochemical composition of the fish larvae.

Other additional methods which can provide interesting information about the requirements are:

- Study of the biochemical composition of the eggs. Marine fish eggs should contain all the nutrients which are essential for the embryo and the larvae development up to the stage of yolk-sac absortion. So their biochemical composition should give us some information about which nutrients are essential for this development.
- Study of the evolution of a nutrient along the embryo development. The evolution in the changes in the free amino acid composition during the embryo development should say us which of these fatty acids are preferentially incorporated into proteins and which ones are used a source of energy.
- Study of the conservation or loss of a nutrient along larval development and during starvation and the feeding of the fish.

But when comparing the determined quantitative nutrient requirements for a given species, results sometimes differ among the different studies (Izquierdo, 1996). These differences are particularly found during larval stages and can be related not only to differences in the culture conditions (i.e. the presence or not of different types of microalgae in different concentrations in the rearing tank) or larval nutrient original levels (coming from either broodstock feeding or previous larval feeding) but also to the several dietary aspects which have been discussed above.

Protein and amino acid requirements

Fast growing fish larvae have a high demand for protein and require more elevated dietary contents than juveniles and adults, microdiets designed for larval rearing containing between 50 and 70% protein. From the 20 most common aminoacids 10 have been found to be essential or indispensable for all studied fish and are required for optimum growth despite fish are not able to synthesize them: Leu, Ile, Val, Thr, Phe, Met, Trp, Arg, His, and Lys. Another two amino acids, Tyr and Cys are only non essential if Phe and Met are present in the diet. At least all those aminoacids should be also required by fish larvae. Moreover, the importance of other minor amino acids such as taurine, recently pointed out as essential for best growth and survival of several species of sparids should not be neglected.

Methods to determine quantitative requirements of each of those aa in fish larvae include feeding microdiets with gradded levels of one amino acid at a time in a test diet containing either all cristaline amino acids, a mixture of casein, gelatin and cristalline amino acids, or a semipurified diet using an imbalanced protein (zein, corn gluten) formulated so that the amino acid profile is identical to the test protein except for the amino acid being tested. As studied by Kanazawa and co-workers for fish larvae of several species, diets are designed to contain protein levels at or slightly below the optimum protein requirement for that species to assure a maximum utilization of the limiting amino acid. Hence, quantitative requirements of several aa have been determined for red sea bream and japanese flounder larvae (López-Alvarado, 1995). Relations among aa, such as competition or common synthesis pathways, need also be considered. Moreover, aa leaching in the relatively long water staying microdiets, cause difficulties to accurately determine physiological requirements. Hence other methods previously utilized in juveniles have been applied to fish larvae. For instance, from the early 80´s it has been shown that there is not difference between the relative proportions of individual essential aa required in diet and the relative proportions of the same 10 aa present in fish carcass. Since the essential aa profile of fish muscle protein does not differ greatly between individual fish species the pattern of requirement for individual species will also be similar. Thus, analysis of the larval aa composition have been frequently used to predict its essential aa requirements (Watanabe and Kiron, 1994).

Comparison of live prey and fish larvae aa profile would also allow us to predict if such feed would cover the larval aa requirements. For instance, when turbot larvae and live food eaa profiles are compared, the profile of the latter seems to be deficient in some eaa such as leucine, arginine, threonine or methionine (Conceiçao *et al.,* 1997), depending on the larval age and type of prey, whereas rotifers seem to be deficient in threonine and leucine for larval seabream.

Other methods utilized in juveniles consider that when an essential amino acid is deficient in a diet the major proportion will be used for protein synthesis and only a little fraction will be oxidized to carbon dioxide to obtain energy, whereas if that amino acid is supplied in the diet in excess plasma levels will increase and it will be more available for oxidation. A force feeding method including labelled eaa has been recently developed for fish larvae (Conceiçao *et al.,* 2003), denoting a high retention of labelled doses of eaa in the body (>60%), and low catabolism as measured by liberated 14CO2 (< 25%). In contrast, non essential aa were faster catabolized (>40%).

Phospholipids

Feeding larvae low dietary contents of PL reduces growth and lipid transport from larval enterocytes to hepatocytes (Kanazawa 1993; Izquierdo *et al.,* 2000). For instance, feeding larval gilthead seabream diets without lecithin supplementation produces accumulation of lipidic vacuoles in the basal zone of the enterocyte and esteatosis in the hepatic tissue, both of them being markedly reduced by a 2% addition of soybean lecithin (Figure 1), denoting an enhancement in the lipid transport activity in gut and liver (Izquierdo *et al.,* 2000). This reduction in lipid transport could be related with a limited capacity for "de novo" synthesis of phospholipids in the larvae. Reacilation of phospholipids in the enterocyte is known to occur through the glycerol-3-phos-

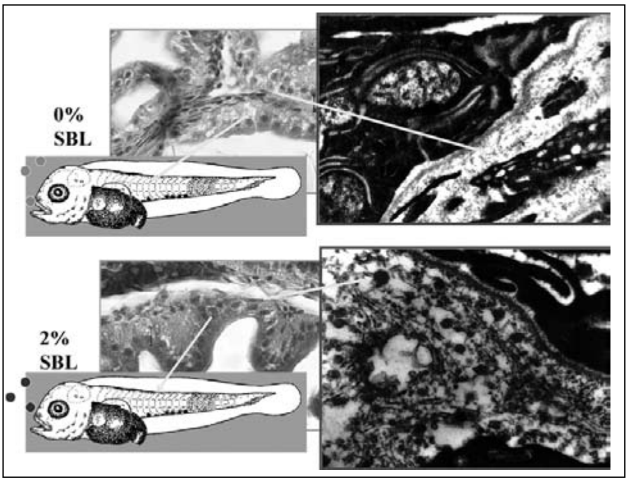

Figure 1. *Enhancement of lipoprotein production by inclusion of 2% soybean lecithin in microdiets for marine fish larvae.*

phate pathway in both the rough and the smooth endoplasmic reticulum (Izquierdo *et al.,* 2000). But since marine fish larvae fed microdiets show enterocytes with a poor development of endoplasmic reticulum and Golgi system, reacilation capacity may be limited in these larvae. Moreover, inappropriate dietary lipids have been found to markedly affect re-esterification pathways in seabream gut (Caballero *et al.,* 2005), modifying the type of lipoprotein formed. For instance, addition of soybean oil promotes PC synthesis by both gycerol-3-phosphate acyltransferase and monoacylglycerol pathways, thus providing material for VLDL formation, whereas addition of rapeseed oil inhibits lipid re-esterification, particularly into TG (Caballero *et al.,* 2005).

On the contrary, when gilthead seabream larvae are fed TG of marine origin, rich in n-3 HUFA it was observed an accumulation of lipid vacuoles in the basal zone of the enterocyte and hepatic steatosis, denoting the good absorption of dietary TG but also a reduced lipid transport to peripheral tissues, whereas feeding with marine PL markedly reduced lipid accumulation in both type of tissues. A higher lipid content due to accumulation of TG and cholesterol esters was found in larvae fed marine TG, whereas in larvae fed marine PL relative proportions of PC and phosphatidyl-ethanolamine (PE) were higher and richer in n-3 HUFA (Salhi *et al.,* 1999). These results agree well with the higher incorporation into larval polar lipids of fatty acids from dietary polar lipids than from dietary triglycerides. In studies with labelled fatty acids dietary n-3 HUFA PL, significantly improved the incorporation of free eicosapentaenoic acid, but not of free oleic acid, into larval polar lipids in comparison to n-3 HUFA rich TG. This especific incorporation of eicosapentaenoic acid when dietary polar lipids are rich in n-3 HUFA could be related to the enhancement of lipid transport, mobilization and deposition in the peripheral tissues by n-3 HUFA rich dietary phospholipids. As a consecuence, growth of larval gilthead seabream was improved when they were fed microdiets containing marine PL instead of marine TG despite the slightly lower dietary n-3 HUFA levels of the former (1.5% versus 1.8%, respectively) (Salhi *et al.,* 1999).

But incorporation of dietary free fatty acids seems to be even lower than that of triglycerides. Thus, labelled oleic acid was better incorporated into both polar or neutral lipids of seabream larvae when it was provided in the diet esterified in a triglyceride than as a free fatty acid, suggesting again a limited capacity of reacilation or transport for dietary long chain free fatty acids or its preferential utilization as energy source in the enterocyte.

Enzymatic, histological and biochemical evidences suggest that marine fish larvae are able to digest and absorb n-3 HUFA rich TG more efficiently than free fatty acids, but feeding with PL, particularly if they are rich in n-3 HUFA, will enhance PL digestion and specially lipid transport alowing a better n-3 HUFA incorporation into larval membrane lipids and promoting fish growth. This confirms former studies which suggest that in addition to the dietary level of essential fatty acids, the molecular form in which they are present in the diet is also important for good growth and survival of marine fish larvae (Izquierdo, 1988; 1996; Izquierdo *et al.,* 1989).

Accumulation of lipidic vacuoles in the basal zone of the enterocyte caused by feeding diets without lecithin supplementation in gilthead seabream disappeared when 0.1% PC was added regardless of its (squid or soybean) origin (Izquierdo *et al.,* 2000). However, squid PC was more efficient in reducing hepatic steatosis than soybean PC, suggesting a combined effect of dietary PC and n-3 HUFA to further enhance hepatic lipid utilization. Indeed both types of molecules have been found to promote lipoprotein synthesis.

Vitamins

The improvement in production of microdiets for larval feeding has greatly facilitated the determination of the vitamin requirements in fish larvae, allowing to experimentally isolating vitamin deficiencies and describing several types of abnormalities. Most described water-soluble vitamin requirements are much higher for larvae than for juveniles of the same species, not only due to the higher metabolic demand in the former, but also for the high ratio surface/volume in larval diets making the diets more prone to oxidation and leaching. Thus, whereas in juveniles vitamin premix accounts for about 2-3% of the diet, in larval microdiets they may reach up to 6-8% of the diet.

Most water soluble vitamin contents of hatchery microalgae and live prey seem to be able to match the requirements of fish larvae, except for the low levels of pyridoxine described in certain studies (González, 1997). However, fat soluble vitamin contents of microalgae and live prey greatly varied among sample batches and with culture conditions, frequently originating hypo and hypervitaminosis.

Vitamin E and vitamin A decreased in seabream from fertilization to the onset of exogenous feeding and a continuous uptake of both nutrients from live preys is observed from day 10th after hatching (Figure 2). However a decrease in the larvae vitamin A content is found when rotifers are substituted by *Artemia* nauplii. Enrichment of *Artemia* nauplii with fat-soluble vitamins improves amber-jack growth (*Seriola dumerilii*) and seabream microdiet supplementation with 1756 IU of a retinol and beta-carothene mixture significantly improves larval growth. However, bioavailability of beta-carotene seems to be very poor in gilthead seabream in comparison with retinol and astaxanthin which seems to have a provitamin A function in larvae of this species. Regarding vitamin E requirements, progressive elevation of dietary alpha-tocopherol acetate levels from up to 1500 mg/kg in larval seabream diets containing free ascorbic acid significantly reduced larval survival, whereas the same increase in alpha-tocopherol when vitamin C was supplemented as ascorbic acid polyphosphate caused a significant improvement in larval growth without affecting survival, suggesting a pro-oxidative effect of alpha-tocopherol over vitamin C in the former (Figure 3).

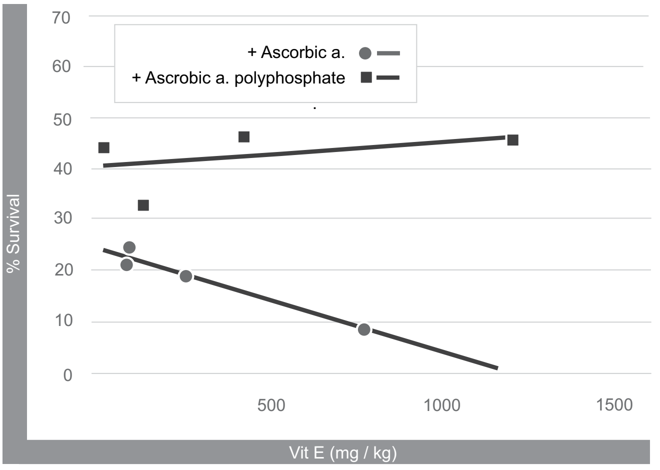

Figure 2. *Effect of dietary Vit E in seabream performance in diets containing ascorbic acid in a free or polyphosphate form.*

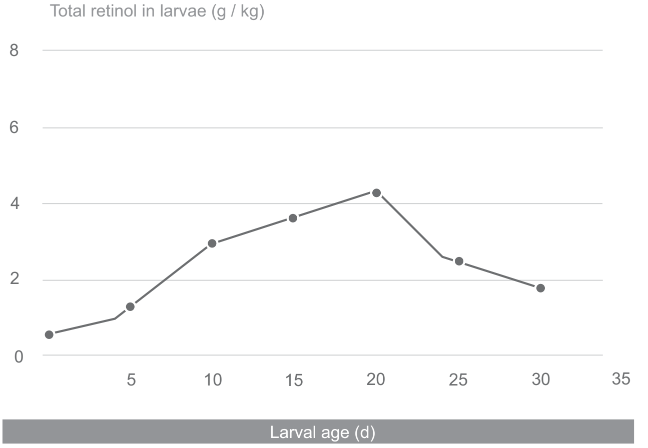

Figure 3. *Evolution of total retinol content in seabream larvae along development.*

BIBLIOGRAPHY

Bell, M. V., Dick, J.R., 1993. The appearance of rods in the eyes of herring and increased di-docosahexaenoyl molecular species of phospholipids. J. Mar.Biol.Ass.U.K. 73, 679 – 688.

Benítez, T., Izquierdo, M., Masuda, R., Hernández-Cruz, C., Valencia, A. and Fernández-Palacios, H. Modification of larval gilthead seabream swimming behaviour by dietary essential fatty acids. J. Fish Biol., submitted.

Bessonart, M., Izquierdo, M.S., Salhi, M., Hernandez-Cruz, C.M., Gonzalez, M.M. Fernandez-Palacios, H., 1999. Effect of dietary arachidonic acid levels on growth and survival of gilthead sea bream (*Sparus aurata L.*) larvae. Aquaculture, 179, 265 – 275.

Caballero, M.J., Izquierdo, M.S., Gallardo, G., Montero, D., Robaina, L., Fernández, A. Is the intestinal biosynthesis of triacylglycerols and phospholipids affected by the inclusion of vegetable oils in diets for sea bream (*Sparus aurata*)? J.Fish Dis., submitted.

Castell, J.D., Bell, J.G., Tocher, D.R., Sargent, J.R., 1994. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*) Aquaculturre 155, 149 – 164.

Conceiçao, L.E.C., Grasdalen, H., Rønnestad, I., 2003. Amino acid requirements of fish larvae and post-larvae: new tools and recent findings. Aquaculture 227, 221 – 232.

Dantagnan, P., Izquierdo, M., Bórquez, A., Valdebenito, I. Lipid and fatty acid composition along embryo and larval development of puye (*Galaxias maculatus* Jenyns, 1842), obtained from estuarine, fresh water and cultured populations. Journal of fish Biology (accepted).

Estévez, A., Izhikawa, M., Kanazawa, A., 1997. Effects of arachidonic acid on pigmentation and fatty acid composition of japanese flounder, *Paralychthys olivaceous* (Temmink and Schlegel). Aquaculture Research 28, 279 – 289.

González, M. Dietary vitamin E, vitamin A and carotenoids for gilthead seabream (*Sparus aurata*) larvae. European Ph.D. Dissertation, Las Palmas Univ. 234 pp.

Hagve, T.A., Woldseth, B., Brox, J., Narce, M., Poisson, J.P., 1998. Membrane fluidity and fatty acid metabolism in kidney cells from rats fed purified eicosapentaenoic acid or purified docosahexaenoic acid. Scandinavian J. Clin.Lab. Inves. 58, 187 – 194.

Hashimoto, M., Hossain, M.S., Yamasaki, H., Yazawa, K., Masumura, S., 1999. Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. Lipids 34, 1297 – 1304.

Hernandez-Cruz, C.M., Salhi, M., Bessonart, M., Izquierdo, M.S., Gonzalez, M.M., Fernandez-Palacios, H., 1999. Rearing techniques for red porgy (*Pagrus pagrus*) during larval development. Aquaculture 179, 489 – 497.

Izquierdo, M.S. 1996., Essential fatty acid requirements of cultured marine fish larvae. Aquaculture Nutrition 2, 183 – 191.

Izquierdo, M.S., 2004. Essential fatty acid requirements of Mediterranean fish. Cah. Opt. Med. 67, 34 – 52.

Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J. Rosenlund, G., Ginés, R. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture. Submitted.

Izquierdo, M.S., Tandler, A., Salhi, M., Kolkovski, S., 2001. Influence of dietary polar lipids' quantity and quality on ingestion and assimilation of labelled fatty acids by larval gilthead seabream. Aquaculture Nutrition 6, 153 – 160.

Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, T., Kitajima, C., 1989 Requirement of larval red seabream *Pagrus majo*r for essential fatty acids (Nippon Suisan Gakkaishi) Bull. Japan. Soc. Scien. Fish 55, 859 – 867.

Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernandez-Cruz, C.M., 2000. Recent advances in lipid nutrition in fish larvae. Fish Physiol. Biochem, 22, 97 – 107.

Koven, W., Barr, Y., Lutzky, S., Ben-Atia, I., Weiss, R., Harel, M., Behrens, P., Tandler, A., 2001. The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. Aquaculture, 193, 107 – 122.

Liu, J., Caballero, M.J., Izquierdo, M.S., El-Sayed Ali, T., Hernández-Cruz, C.M., Valencia, A., Fernández-Palacios, H., 2002. Necessity of dietary lecithin and eicosapentaenoic acid fro growth, survival, stress resistance and lipoprotein formation in gilthead seabrema Sparus aurata. Fisheries Science 68, 1165 – 1172.

López, J., 1995. Amino acid nutrition of two marine fish larvae: the red seabream (*Pagrus major*) and the Japanese flounder (*Paralycthis olivaceous*). Ph.D. Dissertation, Kagoshima Univ. 158 pp.

Madsen, L. Rustan-Arild, C., Vaagenes, H., Berge, K., Dyroy, E., Berge, R., 1999. Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference. Lipids 34, 951 – 963.

Mourente, G., Tocher, D.R., Diaz-Salvago, E., Grau, A., Pastor, E. 1999. Study of the high n-3 higly unsaturated fatty acids requirement and antioxidant status of *Dentex dentex* larvae at the Artemia feeding state. Aquaculture 179, 291-307.

Radunz-Neto, J., Corraze, G., Charlon, N., Bergot, P. 1993. Essential N-3 fatty acid requirements of carp (*Cyprinus carpio*) larvae. FROM-DISCOVERY-TO-COMMERCIALIZATION. Carrillo, M., Dahle, L., Morales, J., Sorgeloos, P., Svennevig, N., Wyban, J. European-Aquaculture-Soc 19.

Rodríguez, C., Pérez, J.A., Diaz, M., Izquierdo, M.S., Fernandez-Palacios, H., Lorenzo, A., 1997. Influence of the EPA/DHA ratio in rotifers on gilhead seabream (*Sparus aurata*) larval development. Aquaculture 150, 77 – 89.

Rodríguez, C., Pérez, J.A., Izquierdo, M.S., Lorenzo, A., Ferández Palacios, H., 1994 "The effect of n-3 HUFA proportions in diets for gilthead sea bream (*Sparus aurata*) larval culture." Aquaculture 124, 284 p.

Rodríguez, C., Pérez, J.A., Badia, P., Izquierdo, M.S., Fernandez-Palacios, H., Hernandez, A.L., 1998. The n-3 highly unsaturated fatty acids requirements of gilthead seabream (*Sparus aurata L.*) larvae when using an appropriate DHA/EPA ratio in the diet. Aquaculture 169, 9 – 23.

Roo, F., Socorro, J., Izquierdo, M.S., Caballero, M.J., Hernandez-Cruz, C.M., Fernandez, A., Fernandez-Palacios, H., 1999. Development of red porgy *Pagrus pagrus* visual system in relation with changes in the digestive tract and larval feeding habits. Aquaculture 179, 499 – 512.

Roo, F.J., Izquierdo, M. S., Socorro, J., Hernández-Cruz, C.M., Valencia, A. Rearing gilthead sea bream *Sparus aurata* larvae under different lighting regimes and dietary n-3 HUFA levels. Importance of dietary n-3 HUFA for eye development and cone formation along gilthead seabream Sparus aurata larval development. Aquaculture. Submitted.

Salhi, M., Hernandez-Cruz, C.M., Bessonart, M., Izquierdo, M. S., Fernandez-Palacios, H., 1999. "Effect of different dietary polar lipid levels and different n-3 HUFA content in polar lipids on gut and liver histological structure of gilthead seabream (*Sparus aurata*) larvae". Aquaculture 179, 253 – 263.

Salhi, M., Izquierdo, M.S., Hernández-Cruz, C.M., González, M., Fernández-Palacios, H., 1994. Effect of lipid and n-3 HUFA levels in microdiets on growth, survival and fatty acid composition of larval gilthead sea bream (*Sparus aurata*). Aquaculture 124, 275 – 282.

Seiliez, I., Panserat, S., Corraze, G., Kaushik, S., Bergot, P., 2003. Cloning and nutritional regulation of a D6-desaturase-like enzyme in the marine teleost gilthead seabream (*Sparus aurata*). Comp. Biochem. Physiol. 135, 449 – 460.

Watanabe, T., 1993. Importance of docosahexaenoic acid in marine larval fish. J. World Aqua. Soc. 24, 152 – 161.

Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture 124, 223 – 251.

Watanabe, T., Izquierdo, M.S., Takeuchi, T., Satoh, S., Kitajima, C., 1989. Comparison between eicosapentaenoic and docosahexaenoic acids in terms of essential fatty acid efficacy in larval Red Seabream (*Nippon Suisan Gakkaishi*) Bull. Japan. Soc. Scien. Fish. 55, 1635 – 1640.