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Essential fatty acid requirements in Mediterranean fish species

M. Izquierdo
Grupo de Investigación en Acuicultura, ULPGC – ICCM,
P.O. Box 56, 35200 Telde, Las Palmas de Gran Canaria, Canary Islands, Spain

SUMMARY – Development of fish culture in the Mediterranean area has been partly based on the knowledge of the nutritional requirements of these species. However, formulation of specific diets for different marine fish species, restrictions in the availability of traditional ingredients and their substitution by alternative products, changes in the feeding practices and introduction of new fast growing strains require continuous updating of commercial cultured fish nutrition, including the determination of their essential fatty acid (EFA) requirements. Inadequate EFA contents in the diet give rise to several physiological, behavioural and morphological alterations, markedly reducing culture performance. EFA requirements change over the fish life cycle and, while juveniles may be able to survive for months on a diet very low in EFA, larvae may die in a few days and egg quality may be affected after only two weeks. Since environmental factors alter lipid composition of the fish tissue, EFA requirements could also be affected.

Keywords: Arachidonic acid, broodstock nutrition, docosahexaenoic acid, eicosapentaenoic acid, fish nutrition, larval nutrition, essential fatty acids.

***RESUME –** "Besoins en acides aminés indispensables chez les espèces méditerranéennes de poissons". Le développement de l'aquaculture dans la région méditerranéenne est partiellement fondé sur la connaissance des besoins nutritionnels de ces espèces. Cependant, la formulation d'aliments spécifiques pour les différentes espèces de poissons marins, le manque de disponibilité des ingrédients traditionnels et leur substitution par des produits alternatifs, les changements des pratiques alimentaires et l'introduction de nouvelles souches à croissance rapide, nécessitent une actualisation continue de la nutrition des poissons élevés commercialement, y compris la détermination de leurs besoins en acides gras essentiels (AGE). Une teneur inadéquate en AGE dans l'aliment peut donner lieu à plusieurs troubles physiologiques, comportementaux et morphologiques, réduisant de façon notable les performances de cette culture. Les besoins en AGE changent au cours du cycle de vie des poissons ; tandis que les juvéniles sont capables de survivre pendant des mois avec un aliment à très faible teneur en AGE, les larves mourraient en quelques jours et la qualité des œufs pourrait être affectée après seulement deux semaines. Puisque les facteurs environnementaux altèrent la composition en lipides du tissu des poissons, les besoins en AGE pourraient également être affectés.*

***Mots-clés :** Acide arachidonique, nutrition des géniteurs, acide docosahéxanoïque, acide eicosapentanoïque, nutrition des poissons, nutrition larvaire, acides gras essentiels.*

Introduction

Seabass (*Dicentrarchus labrax*), seabream (*Sparus aurata*) and more recently red porgy (*Pagrus pagrus*) and yellowtail (*Seriola dumerilii*) are among the most important species cultured in the Mediterranean Sea (Fig. 1). Production of two of them, seabream and seabass, has steadily increased over the last decades, even doubling over the past five years (Fig. 2). Moreover, these productions are expected to further increase in about 30% during the next five years to cope with the increased market size previsions for these species. Recently, improvements in commercial diet formulation and ingredient quality and adapted feeding strategies for both species have markedly improved growth and reduced feed conversion ratios. However, despite being two different species belonging to two different families, having in the wild different feeding habits and possibly different nutritional requirements, they still share the same commercial diet. Increased knowledge of the physiology and nutritional requirements is needed to allow the development of specific diets for each of these species once their feed markets reach an appropriate size, since at present they only account for about 300,000 MT for both of them.

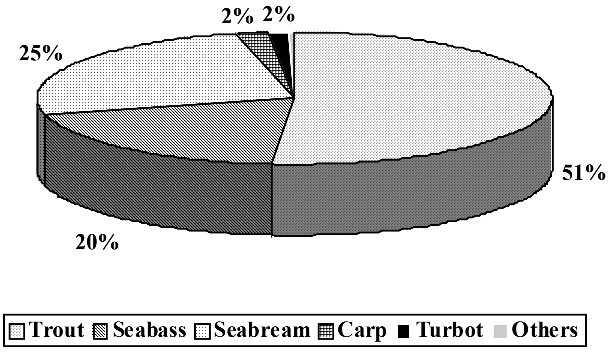

Fig. 1. Main fish species produced in Mediterranean countries in 2002.

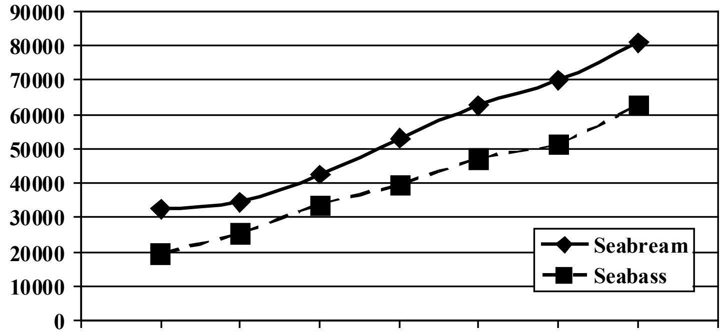

Fig. 2. Evolution of seabream and seabass production in the Mediterranean region.

The nutritional requirements of species with a longer culture tradition in Mediterranean countries [carp (*Cyprinus carpio*), trout (*Onchorhynchus mykiss*) and turbot (*Scophthalmus maxima*)] (Fig. 1) have been more extensively studied, however, changes in feed ingredients that lead to different interactions between nutrients, in feeding practices and culture conditions or the use of new fast growing fish strains will mean that these nutritional requirements will have to be redefined. For instance, dietary inclusion of products from terrestrial plants or microorganisms, although supplying important nutrients, may also contain certain fatty acids uncommon in fish composition which would potentially compete with dietary essential fatty acids (EFA) or block their tissue incorporation or function. Finally, development of culture techniques for other fish, such as tuna (*Thunnus thymus*), sole (*Solea senegalensis*) and several sparids, will also require some nutritional knowledge, including EFA requirements of these species, in order to determine optimum feed formulas and feeding practices.

Annual feed production for fish species in the Mediterranean area has reached about 700,000 metric tons, requiring about 130,000 and 100,000 metric tons of fishmeal and oil, respectively. The increased demand for these products for animal production and other uses, together with the general reduction in production over the past 12 years, has resulted in the use of ingredients of terrestrial vegetable origin. These ingredients lack certain fatty acids essential for marine fish, and this restricts their use in diets for these species; and if requirements for such fatty acids are not precisely determined it will be difficult to predict optimum levels of inclusion. Moreover, inclusion of certain levels of EFA may be high enough to promote optimum fish growth but not to prevent stress or disease resistance.

Studies on essential fatty acid requirements

Marine fish lipids are rich in a great variety of saturated and monounsaturated fatty acids, which are "de novo" synthesized, whereas polyunsaturated fatty acids must be provided in the diet. Three very long chain polyunsaturated fatty acids (PUFA[1]), namely docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) have a variety of very important functions in fish species, as in most vertebrates. Freshwater fish seem to have sufficient Δ5 and Δ6 desaturase and elongase activities to produce ARA, EPA and DHA if their precursors, linoleic (18:2n-6) and linolenic (18:3n-3) acids, are present in the diet (Fig. 3), those five fatty acids being considered essential for freshwater species (Yu and Sinhuber, 1975). However, such enzymatic activity is very restricted in marine fish and, as a consequence, DHA, EPA and ARA have to be included in the diet and are considered essential.

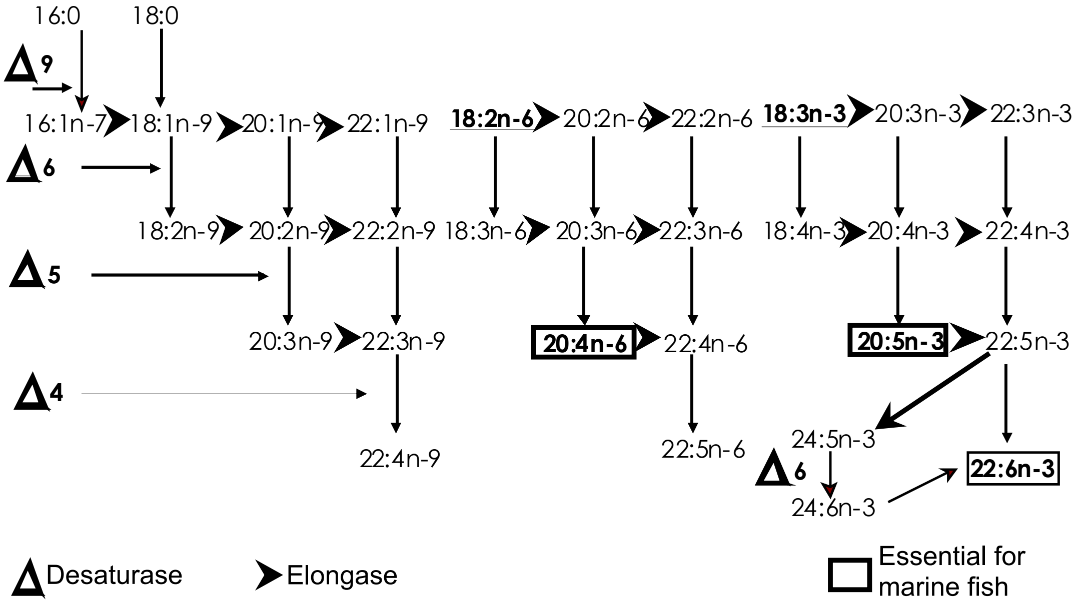

Fig. 3. Essential fatty acids synthesis pathways present in some fish species.

Inadequate contents of those EFA in the diet give rise to several behavioural and morphological alterations such as poor feeding and swimming activities, poor growth and dropping mortality (particularly in the young stages), fatty livers, hydrops, deficient swim bladder inflaction, abnormal pigmentation, disaggregation of gill epithelia, immune-deficiency and raised cortisol levels (Izquierdo, 1996). Besides, inappropriate dietary contents of such fatty acids in diets for broodstock reduce fecundity and fertilization rates, originate embryo deformities and damage larval quality (Izquierdo *et al.*, 2001a).

Despite the great effort of many research groups to determine the EFA requirements of several fish species (Wilson, 1991; Watanabe and Kiron, 1994; Izquierdo,1996; Sargent *et al.*, 1999; Izquierdo *et al.*, 2000), there is still insufficient knowledge available due to the complexity of these determinations. EFA requirements change throughout the fish life cycle and thus, whereas a gilthead seabream juvenile is able to survive for months on a diet almost completely deprived of EFA (author's own data), larvae would die in 10-15 days (Izquierdo *et al.*, 1989) and egg quality would be significantly reduced after only two weeks of feeding such an EFA-lacking diet to the broodstock (Harel *et al.*, 1994; Izquierdo *et al.*, 2001a). Besides, unlike what happens with most other nutrients, not only do EFA requirements in fish differ quantitatively between the different species, but they also differ qualitatively (Watanabe, 1982). In the wild, types and contents of EFA differ between the different steps of the trophic chain (Takeuchi, 1997), and EFA requirements would then rely on the trophic behaviour of each fish species. Thus, planktivorous fish have a potentially greater intake of EFA than icthyvorous ones, which in turn have higher intakes than those fish mainly feeding on crustaceans and molluscs.

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[1] PUFA: Polyunsaturated fatty acids with 18 or more carbon atoms and 2 or more double bonds.

Since environmental factors such as temperature (Farkas *et al.*, 1980; Olsen *et al.*, 1999), salinity (Borlongan and Benitez, 1992) and light (Ota and Yamada, 1971) affect lipid composition of fish tissue, EFA requirements could also be affected by environmental conditions. Indeed, the higher the unsaturation degree of the fatty acid the higher its fluidity, and this is particularly important at low culture temperatures (Bowden *et al.*, 1996). For instance, reduction in water temperature has been associated with an increase in the polyunsaturated fatty acid (PUFA) content in carp tissues (Kayama *et al.*, 1986), or with an increase in DHA content in salmon (Olsen and Skjervold, 1995). It has been shown that reductions in water temperature affect both lipolytic and lipogenic enzymes, increasing the oxidative capacity in rainbow trout (Guederley *et al.*, 1997) or the specific activity of delta 9-desaturase in carp (Kayama *et al.*, 1986). Higher contents of PUFA, and particularly of docosahexaenoic (DHA) and arachidonic (ARA) acids have also been found in tissue of fish cultured with increased salinity, such as guppy (*Poecilia reticulata*) (Daikoku *et al.*, 1982) and milkfish (*Chanos chanos*) (Borlongan and Benitez, 1992), denoting the important role of some of these fatty acids in osmotic regulation (Sampekalo *et al.*, 1992). Thus, although the lack of n-3 fatty acids in the diet may have caused high mortalities in seawater reared rainbow trout, it did not cause mortality in fish reared in fresh water (Lall and Bishop, 1976).

As fish larvae are visual feeders, larval trophic behaviour is closely related to the development of visual capacity. In sparids, such as gilthead seabream and red porgy (Roo *et al.*, 1999), the most important changes in the eye structure occur during lecitotrophic stage as a preparation for prey capture, the rod photoreceptors necessary for accurate vision at low light intensity appearing in gilthead seabream about 18 days after hatch. N-3 PUFAs, and particularly DHA, play a critical role in neural and retinal tissue functions. Bell and Dick (1993) found that both rod and cone photoreceptors in herring eye, accumulate and selectively retain DHA, and thus, feeding herrings a DHA-poor *Artemia* during the period of rod development resulted in impaired vision at low light intensities. Moreover, higher dietary DHA and eicosapentaenoic acid (EPA) increased eye diameter in gilthead seabream (Izquierdo *et al.*, 2000; Roo *et al.*, submitted), and this fact, together with a high density of cone photoreceptors in these larvae, implied greater number of cones and a potentially improved visual accuracy (Roo *et al.*, submitted). Thus, restriction in light intensity applied in some commercial hatcheries, particularly during the first two weeks of larval development when only cone type receptors of maximum light capture effectiveness at high light intensity are sufficiently developed, may impose higher DHA requirements in broodstock and larvae than in fish cultured at higher light intensities. Besides, inadequate lighting regimes may constitute a stress factor in larval culture conditions, which in turn increase the EFA demand in this fish.

Determined EFA for Mediterranean fish species

Essential fatty acid requirements, and particularly those for highly unsaturated fatty acids with 20 or more carbon atoms and 3 or more double bonds (HUFA) including mainly DHA and EPA (n-3 HUFA), have been extensively studied in some species such as gilthead seabream or turbot throughout the whole life cycle. Studies about the requirements of other species, such as the red porgy, *Dentex dentex, Diplodus sargus,* or *Pagellus bogavareo,* started only few years ago. Amazingly, and despite its importance in Mediterranean aquaculture, seabass is a species very sensitive to handling and disease, and this could be related to nutritional unbalance in this species, since studies on lipid and essential fatty acid requirements of seabream are much more common in seabream than in seabass (Oliva-Teles, 2000).

n-3 HUFA requirements throughout the life cycle

By analysing the above-mentioned studies in detail it was found that in gilthead seabream, for instance, several authors (Fernández-Palacios *et al.*, 1995; Tandler *et al.*, 1995) already described broodstock requirements of about 1.5-1.6% n-3 HUFA on a dry basis to obtain the highest production of high quality eggs, higher inclusions of these essential fatty acids leading to a reduction in egg and larval quality. However, a further increase of dietary n-3 HUFAs up to 2.2% enhanced egg quality only when dietary α-tocopherol was also increased from 125 to 190 mg/kg (Izquierdo *et al.*, 2001b), showing the importance of considering interactions with other nutrients to precisely determine EFA requirements. In rainbow trout, although n-3 fatty acids are also essential for good spawning quality, no differences in spawn were found when broodstock diets contained either corn or cod liver oil

(Corraze *et al.*, 1993) in a fish meal based diet (minimum dietary n-3 HUFA of 0.64% on a dry basis). In another study with these same diets, the substitution of dietary fish oil for different vegetable lipid sources did modify rainbow trout semen composition, but it did not affect motility, even after thawing (Labbe *et al.*, 1993; Gasco *et al.*, 1999). Subsequent studies with lower basal n-3 HUFA levels showed that, on the contrary, fish oil in rainbow trout broodstock diets protected better from cryopreservation damages than vegetable oils such as safflower (McNiven *et al.*, 2000; Pustowka *et al.*, 2000), suggesting that the basal 0.64% n-3 HUFA in the previous studies was enough to cover the EFA requirements. Published studies to define EFA requirements in other Mediterranean species broodstock are very scarce. In seabass, it has been reported that the presence of PUFA, and particularly ARA, improves maturation and spawning quality (Sorbera *et al.*, 2001), whereas in carp broodstock requirements for essential fatty acids did not seem to particularly differ from those of the juveniles (Nandi *et al.*, 1997).

Several authors showed that throughout larval development EFA requirements of gilthead seabream are very close to 1.5% n-3 HUFA in dry matter when larvae were fed either live preys (Rodríguez *et al.*, 1998) or microdiets (Salhi *et al.*, 1999), regardless of the dietary lipid level (Salhi *et al.*, 1994). Much higher requirements are estimated in the literature when EPA contents are 2 or 3 times higher than those of DHA (Rodríguez *et al.*, 1994, 1997), due to the very high incorporation of EPA into the larval polar lipids and the displacement of DHA from certain polar lipids (Izquierdo *et al.*, 2000). However, as it happens in the other life stages and provided other nutrients such as antioxidants are also balanced, an increase of dietary n-3 HUFA up to 8% –keeping a DHA/EPA ratio of 1.7– further improves larval growth and survival (Liu *et al.*, 2002). High n-3 HUFA requirements have also been estimated for red porgy (3.39% at 1.35 DHA/EPA, Hernández-Cruz *et al.*, 1999) and *Dentex dentex* (Mourente *et al.*, 1999), despite the fact that in the latter the high EPA content in Artemia may have caused an overestimation of the requirements as we have seen in gilthead seabream (Rodríguez *et al.*, 1997). On the contrary, carp larvae seemed to require as low as 0.05% n-3 fatty acids from cod liver oil (Radunz-Neto *et al.*, 1993) to cover the essential fatty acid requirements throughout this period of life.

In juvenile gilthead seabream, Ibeas *et al.* (1994) and Montero *et al.* (1998) have recommended minimum inclusion levels of 1.5 and 1.8% n-3 HUFA in diets with 12-15% lipids in order to promote maximum growth and stress resistance, respectively. In these studies the whole body weight of gilthead seabream increased about 2.5 times over more than 2 months of experimental trials, being considered very appropriated for a short term experiment. Under a practical point of view, recent studies in our laboratory (Izquierdo *et al.*, submitted) have shown that feeding semi-commercial diets with 22% of lipids and 1.8% n-3 HUFA during more than 6 months of feeding trial is not sufficient to maintain the best growth (Fig. 4), feed efficiency and stress resistance, several immune parameters being also negatively affected by such low levels (Montero *et al.*, 2003). Thus, considering the whole on-growing period and the high lipid contents of commercial diets at present, dietary n-3 HUFA contents over 2.5% on a dry basis are highly recommended (Table 1) for gilthead seabream juveniles. This requirement is higher than that determined for rainbow trout (Takeuchi and Watanabe, 1976) and turbot (Gatesoupe *et al.*, 1977), and lower than those found by Lanari *et al.*, (1999) for European seabass (Table 1), whereas for carp, linoleic and linolenic acids seemed to be able to sufficiently cover the EFA requirements (Watanabe *et al.*, 1975). Available data for juvenile Mediterranean yellowtail (author's own data) and red porgy (Schuchardt, Robaina and Vergara, unpublished data), have to be confirmed with further studies, although they are presented in Table 1 as guidance.

Docosahexaenoic acid requirements

The particular structure of DHA provides this fatty acid with many important functions in fish metabolism. Its incorporation into cell membranes regulate membrane integrity and function, this fatty acid being an important component of phosphoglycerides, particularly phosphatidyl ethanolamine and phosphatidyl choline, in gonads, larvae and juveniles. It is specifically retained in starved or low-EFA fed fish, possibly due to the lower cell oxidation rates than other fatty acids (Madsen *et al.*, 1999). It is necessary for reproduction, growth, survival, flat fish metamorphosis and disease prevention. It may be a substrate for some lypoxigenases (Asturiano, 1999) and several studies have shown that it has a greater potential as an essential fatty acid for marine fish larvae than EPA (Watanabe *et al.*, 1989; Watanabe, 1993), its requirement being more limiting for growth and survival than those for n-3 HUFA (Izquierdo, 1996).

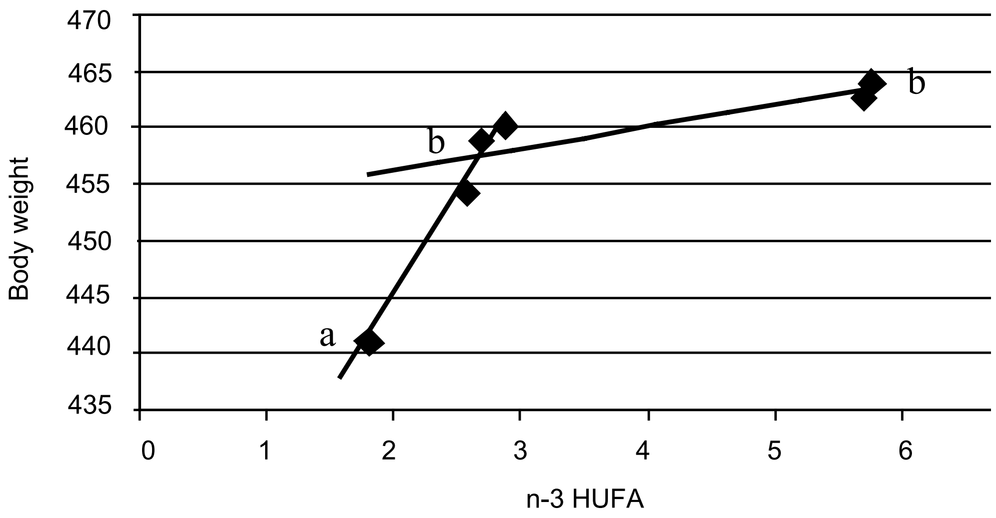

Fig. 4. Effect of dietary n-3 HUFA in gilthead seabream growth over a 6 month feeding period (data from Izquierdo *et al.*, submitted).

Table 1. n-3 HUFA requirements of Mediterranean fish species throughout the life cycle

	Broodstock	Larvae	Juveniles
S. aurata	2.2	>3	2.5
S. maximus	–	3.2[2]	0.8[3]
D. labrax	–	–	>3.5[4]
S. dumerilii	–	4[1]	2.5[1]
P. pagrus	–	<3.4[5]	<5[1]
C. carpio	–	0.05[6]	–
S. raidneri	0.64[7]	R	1[8]
D. dentex	–	4[9]	–

[1]Author's own data; [2]Le Milinaire, 1984; [3]Gatesoupe *et al.*, 1977; [4]Lanari *et al.*, 1993, 1999; [5]Hernández-Cruz *et al.*, 1999; [6]Radunz-Neto *et al.*, 1993; [7]Corraze *et al.*, 1933; [8]Takeuchi and Watanabe, 1976; [9]Mourente *et al.*, 1999; R: required.

Minimum dietary levels in diets for gilthead seabream seem to be 0.6% (Fernández-Palacios *et al.*, 1995), 0.8% (Rodríguez *et al.*, 1998; Salhi *et al.*, 1999) and 0.6% (Montero *et al.*, 1996), respectively, for broodstock, larvae and juveniles. In larvae, high levels (5% in dry basis) of dietary DHA in the microdiets for gilthead seabream did not cause any problem, but further promoted growth and larval survival (Liu *et al.*, 2002).

Regarding other sparids, requirements during larval development seem to be about 1.5% for red porgy larvae when DHA/EPA ratios were about 1.4 (Hernández-Cruz *et al.*, 1999) and close to 2.3% for *Dentex dentex* fed a very low DHA/EPA ratio (0.32) (Mourente *et al.*, 1999). For seabass juveniles, increase in dietary lipid levels from 15 to 19%, implying an increase in DHA of 1 to 1.5%, significantly improved growth, regardless of the non fat energy dietary levels, suggesting again higher EFA requirements than in gilthead seabream juveniles (Lanari *et al.*, 1999).

DHA is very effective as an EFA for rainbow trout, 1% on a dry basis leading to such good weight gain and feed conversion when feeding 1% n-3 HUFA, and being more effective as an EFA than 18:3n-3 (Takeuchi and Watanabe, 1976). Besides, 22:6 n-3 seems to be particularly required in diets for first feeding rainbow trout, since their absence resulted in reduced larval growth (Wirth *et al.*, 1997).

Eicosapentaenoic acid requirements

Eicosapentaenoic acid is also particularly important for larval growth (Watanabe *et al.*, 1989) and broodstock fertility (Fernández-Palacios *et al.*, 1995), playing general and particular roles in fish metabolism. Its presence in rotifers enhances non-specific lipase activity in larval seabream (Izquierdo *et al.*, 2000), neutral lipids esterified with EPA being a preferred substrate for this enzyme. In marine fish it is a main component of polar lipids and it regulates membrane integrity and function, indeed its incorporation into phosphoacylglycerides enhances fluidity of the cell membrane (Sipka *et al.*, 1996) much more than ARA (Hagve *et al.*, 1998), but less than DHA (Hashimoto *et al.*, 1999). Moderate dietary levels of this fatty acid also enhance DHA incorporation into larval PL (Izquierdo *et al.*, 2000, 2001b), causing a sparing effect on such an important fatty acid. It is a good substrate for some cycloxygenases, being precursor of some prostanoids in marine fish and also a main substrate for some lypoxygenases, being the main precursor for leukotriene synthesis in some species. Its competition with ARA for these two types of enzymes enables it to be an important regulator of eicosanoid synthesis.

Best growth, survival, resistance to stress and spawning quality have been obtained in gilthead seabream with EPA dietary levels of 1.2 (Fernández-Palacios *et al.*, 1995), 0.7-0.8 (Rodríguez *et al.*, 1998; Salhi *et al.*, 1999) and 0.7 (Montero *et al.*, 1996) on a dry basis for broodstock, larvae and juveniles, respectively. In larvae, increase of EPA up to 2.9% on a dry basis when DHA/EPA levels where high (1.72) and ARA contents were only 0.05 significantly improved growth, survival and resistance to a shock temperature stress of gilthead seabream (Liu *et al.*, 2002), denoting its high value as an EFA. However, increase of dietary EPA up to 1.8 reduced growth when ARA levels were as high as 1.8% (Fig. 5) and DHA/EPA about 1.3, denoting how the EFA value of EPA is dependant on the dietary levels of DHA and ARA.

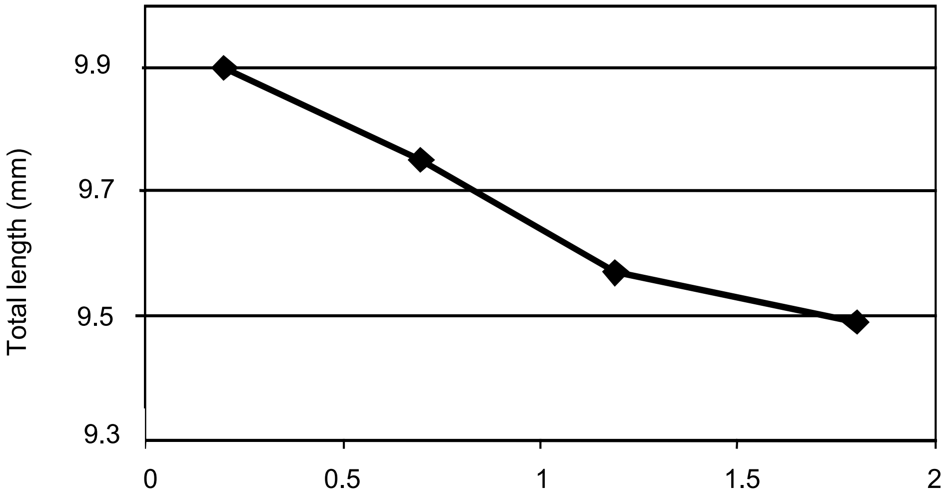

Fig. 5. Effect of dietary EPA in larval seabream growth when dietary ARA is high (1.8%)

Arachidonic acid requirements

Arachidonic acid is a main component of a minor but very important polar lipid class, phosphatidyl inositol (PI). *In vitro*, ARA is a preferred substrate for most cycloxigenases, being the main precursor for prostaglandin synthesis, whereas in some marine fish *in vivo*, EPA is the main substrate due to its high presence in the diet. ARA also constitutes a good substrate for several lypoxigenases, its derivative hydroxi-fatty acid having important physiological functions in marine fish. Its content in the PI of cell membranes possibly regulates eicosanoid synthesis.

In gilthead seabream larvae, an increase of ARA up to 1% enhances survival and growth when DHA and EPA dietary contents are 1.3 and 0.7, respectively (Izquierdo, 1996; Bessonart *et al.*, 1999). An increase in ARA contents in the rotifers also prevents post-stress mortality (Koven *et al.*, 2001). ARA seems to play also important roles in turbot juveniles (Castell *et al.*, 1994) and in flatfish pigmentation (Estévez *et al.*, 1997).

Optimum ratios between essential fatty acids

Evidence of competition between two or more of these essential fatty acids has been suggested for digestive enzymes (Iijima *et al.*, 1998, Izquierdo *et al.*, 2000) fatty acid binding proteins, phosphoacylglicerides synthetases (Bell *et al.*, 1995; Izquierdo *et al.*, 2002), lypoxigenases and cyclooxigenases (Hwang, 1989), and probably in beta-oxidation, as it happens in rats (Totland *et al.*, 2000). Not only absolute dietary values for each of these essential fatty acids but also optimum dietary ratios between them must be defined since both factors will affect at least their incorporation into tissue lipids and hence membrane fluidity and function, the energy values obtained from their beta-oxidation and the production of metabolically active compounds. Thus, optimum DHA/EPA ratios have been defined for turbot larvae around 2 (Reitan *et al.*, 1994) and for seabream around 1.2 at least (Rodríguez *et al.,* 1997). Considering both the sum of the three EFAs and the ratios between them, if the dietary value of the ratio (DHA+EPA+ARA)*DHA/EPA/ARA is plotted against growth, a significant correlation can be found in some of our recent studies (Fig. 6). If the same equation is applied to dietary fatty acids in other gilthead seabream studies (Rodríguez *et al.*, 1994, Salhi *et al.*, 1994, Liu *et al.*, 2002, Koven *et al.*, 2001, Fernández-Palacios *et al.*, 1995 and others), we found that for ARA values higher than 0.5% the closer the value of the equation (DHA+EPA+ARA)*DHA/EPA/ARA to 50 the better the growth performance.

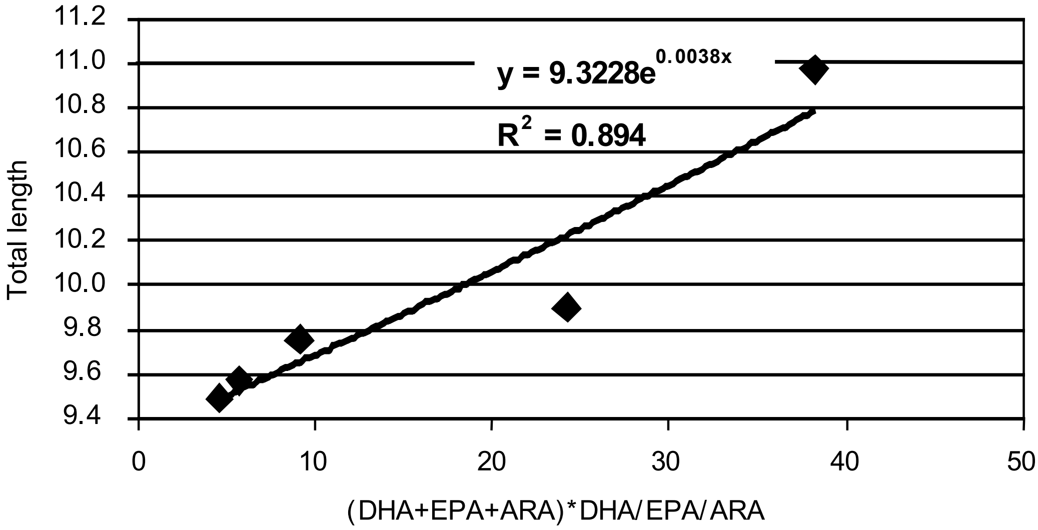

Fig. 6. Effect of dietary (DHA+EPA+ARA)*DHA/EPA/ARA on growth of gilthead seabream larvae.

Conclusions

Essential fatty acid requirements have been extensively studied in some Mediterranean species such as seabream, turbot, carp or rainbow trout, whereas in others such as red porgy, sole, *Dentex* sp., yellowtail, tuna and groupers, published data are very scarce. Determined n-3 HUFA requirements in Mediterranean fish species range between 0.64 and 2.2 in broodstock diets, 0.05 and 4 in live preys and starter diets and 0.8 and 3.5 in diets for juveniles. Optimum EFA levels are found around 0.6-2.3 for DHA, 0.7-2.3 EPA and up to 1% ARA. However, due to several examples of competition between two or more of these essential fatty acids, the ratios between them have to be carefully adjusted. For instance, optimum DHA/EPA ratios of 2 and 1.2 have been defined for turbot and gilthead seabream larvae. Considering both the sum of the three EFAs and the ratios between them (DHA+EPA+ARA)*DHA/EPA/ARA a significant correlation with growth and broodstock performance is found for seabream and when ARA values are higher than 0.5% the closer the value of the ratio to 50 the better the performance. Further studies are needed, particularly in larval nutrition, to carefully determine requirements for each EFA and the optimum dietary ratios among them. Finally, restrictions in the availability of traditional ingredients, such as fish oil, and their partial or complete substitution by alternative products, may lead to light or severe EFA deficiencies reducing fish culture performance and disease and stress resistance.

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