Recent Developments in the Essential Fatty Acid Nutrition of Fish

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Abstract. Because of competitive interactions in the metabolism of polyunsaturated fatty acids, tissue and bodily requirements for each of the three dietary essential fatty acids in marine fish, 22:6n-3, 20:5n-3 and 20:4n-6, cannot be meaningfully considered in isolation. Rather, it is necessary to consider requirements in relative as well as absolute amounts, i.e. in terms of the ratio of 22:6n-3 : 20:5n-3 : 20:4n-6. This is illustrated by recent research in our laboratories which has suggested that the optimal dietary ratio of 22:6n-3 : 20:5n-3 in sea bass larvae is circa 2:1 with the optimal dietary ratio of 20:5n-3 : 20:4n-6 being circa 1:1. The optimal dietary ratio of 22:6n-3 : 20:5n-3 in turbot and halibut larvae is similarly circa 2:1 but the optimal dietary ratio of 20:5n-3 : 20:4n-6 in these species is 10:1 or greater. In addition, studies with salmon parr point to dietary 18:3n-3 and 18:2n-6 being important in determining the optimal tissue ratio of 20:5n-3 : 20:4n-6 for successful parr - smolt transition. We deduce that differences in essential fatty acid requirements for different species of fish reflect different dietary and metabolic adaptations to different habitats, and consider how such knowledge can be exploited to develop improved diets for fish, especially in their early stages of development.

I. Introduction.

Fish like all other vertebrates studied so far require three long chain polyunsaturated fatty acids (PUFA) for their normal growth and development including reproduction: docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (AA, 20:4n-6) (Sargent et al. 1993a, 1995, 1997). The biochemical, cellular and physiological functions of these three PUFA are broadly the same in fish as in other vertebrates and fall into two categories: (a) an apparently generalised role in maintaining the structural and functional integrity of cell membranes; (b) a more specific role as precursors of the group of highly biologically active paracrine hormones known collectively as eicosanoids.

In fish, as in terrestrial mammals, DHA, EPA and AA are all involved in maintaining cell membrane structure and function. However, in fish DHA and EPA and not AA are the major PUFAs of cell membranes, the converse being true in terrestrial mammals. An exception to this statement in terrestrial mammals is neural tissues including the eye where DHA can be very abundant in some cell membranes, especially rod cell outer segment membranes and the membranes of synaptic junctions. These cell membranes in fish are particularly rich in DHA. Consequently, fish tissues in general have much higher concentrations of DHA and EPA than AA and fish have correspondingly high dietary requirements for n-3 PUFA, a situation reflected historically in the marked emphasis on DHA and EPA, the so-called "n-3 HUFA", in fish nutrition. This should not, however, obscure the fact that AA, though generally a minor component of fish cell membranes, can and does fulfil a membrane structural role in fish which, although largely unassessed, is not necessarily trivial.

The eicosanoids are a range of highly active C20 compounds formed in small or even trace amounts by virtually every tissue in the body and involved in a great variety of physiological functions. In broad terms they are produced in response to stressful situations, both at a cellular and whole body level. The major precursor of eicosanoids in fish, as in mammals, is AA with eicosanoids formed from EPA being less biologically active than those formed from AA. Moreover, EPA competitively inhibits the formation of eicosanoids from AA. Likewise eicosanoids formed from EPA competitively interfere with the actions of eicosanoids formed from AA. Therefore, eicosanoid actions in the body are determined *inter alia* by the ratio of AA : EPA such that high tissue ratios of AA : EPA result in enhanced eicosanoid actions, e.g. in cardiovascular functions including blood clotting and in the inflammatory response, whereas high tissue ratios of EPA : AA tend to damp down eicosanoid actions. Consequently EPA has an important physiological function in modulating eicosanoid action. The optimal ratio of AA : EPA remains uncertain in higher terrestrial vertebrates, though a dietary ratio of n-6 / n-3 PUFA of circa 5:1 probably generates close to the optimal tissue ratio of AA : EPA. The situation in fish is even less certain, though fish physiology clearly operates with a much lower tissue ratio of AA : EPA than does terrestrial mammalian physiology.

Because the marked chemical similarities of the three long chain PUFA lead to competitive interactions in the plethora of biochemical and physiological

reactions they and their precursors and products undergo, the optimal tissue requirement for any individual long chain PUFA cannot be considered meaningfully in isolation. Neither, therefore, can the optimal dietary requirements for the different PUFA. Rather, the relative amounts, i.e. the ratios of all three long chain PUFA, DHA : EPA : AA, must be considered and defined, whether at a specific tissue or whole body level. This is difficult to achieve in practice and the problem is exacerbated by the fact that many vertebrate species, including many or most freshwater fish, are capable of producing DHA and EPA from linolenic acid (LNA, 18:3n-3) and AA from linoleic acid (LA, 18:2n-6). In such species LNA and LA are dietary essential fatty acids and the dietary ratios of LNA : LA are major determinants of the final tissue ratios of DHA : EPA : AA. However, competitive interactions exist between LNA and LA (and also other PUFA including C20 and C22 PUFA, and monounsaturated fatty acids) in the conversions of these C18 PUFA to their end product C20 and C22 PUFA. Therefore, the final tissue outcome from a given dietary ratio of LNA : LA for a given species is difficult to predict with confidence, especially as nearly all natural or commercial diets themselves contain complex blends of PUFA and other fatty acids that are difficult to manipulate experimentally in a controlled manner. In other species, including all marine fish so far studied, the rates of conversion of LNA to EPA and DHA and of LA to AA are insufficiently fast, negligible or zero, such that in these species DHA, EPA and AA themselves are dietary essential fatty acids. This simplifies the situation somewhat but still leaves the possibility that other dietary fatty acids, especially other dietary PUFA such as LA and LNA, themselves may interfere with the cellular functions of AA, EPA and DHA.

Against this background, we review recent research in our laboratories on four species of farmed fish studied as part of an ongoing programme aimed at understanding factors determining optimal tissue and bodily ratios of DHA : EPA : AA in vertebrates. Studies on the essential fatty acid nutrition of fish, especially marine fish, were dominated in the early years by “n-3 HUFA” whereas more recent emphasis has been on optimising dietary levels of DHA. However, the theoretical considerations above dictate that AA is an essential dietary fatty acid for marine fish and this has been experimentally confirmed for the turbot (Castell et al. 1994; Bell, J.G. et al. 1995). AA may well be a quantitatively minor constituent of marine fish oils, the standard source of essential fatty acids for

marine fish and increasingly freshwater fish culture. It is nonetheless an important constituent that deserves as much consideration as DHA and EPA in determining the optimal essential fatty acid requirements of fish and any other vertebrate species.

II. Sea Bass.

Thrush et al. (1993) reported that sea bass broodstock reared on locally available "trash fish", mainly *Boops boops*, yielded higher quality eggs, as assessed on the basis of successful hatching, than broodstock reared on a defined diet containing fish meal and a blend of vegetable oil and northern hemisphere fish oil, i.e. a fish oil derived from species such as herring, sandeels or capelin and being relatively rich in EPA and DHA and also in 20:1n-9 and 22:1n-11, as is used routinely in salmon farming (see Sargent and Henderson, 1995). Fatty acid analyses of total lipid from the sea bass broodstock diets established that the diet based on *Boops boops* contained the relatively high level of 4.6 % AA, in contrast to the 0.6 % AA in the vegetable oil - fish oil diet, and this difference was reflected in corresponding differences in the PUFA compositions of total lipid extracted from the eggs of the two broodstock groups (Thrush et al. 1993). Subsequent detailed analyses of these eggs (Bell et al. 1997a) established that the phospholipids of eggs of broodstock fed *Boops boops* were also elevated in AA, the elevation being particularly marked in phosphatidylinositol, which in fish is atypically enriched in AA and which is a likely source of the AA precursor of eicosanoids in fish (see Sargent et al. 1993a). Thus, the ratio of EPA : AA in phosphatidylinositol changed 7 fold from 0.7 in the oil - fed broodstock to 0.1 in the *Boops boops* - fed broodstock (Bell et al. 1997a). The importance of AA in sea bass broodstock nutrition was supported by the findings of Bell et al. (1996) that spermatozoa from both wild sea bass and sea bass fed *Boops boops* had higher levels of AA in their phospholipids than spermatozoa from sea bass fed diets containing a vegetable oil - fish oil diet.

Such findings pointed strongly to the desirability of increasing the AA content of sea bass broodstock diets without compromising their naturally and desirably high content of DHA, i.e. altering the DHA : EPA : AA ratio of the diets to achieve a better performance. In analysing the problem we noted that the DHA : EPA ratio of the *Boops boops* broodstock is 3.3 and the EPA : AA ratio is 1.5. The DHA : EPA ratio of a broodstock diet based solely on northern hemisphere

fish oils is 0.8 and the EPA : AA ratio is 20:0, reflecting the typically high and low concentrations of EPA and AA respectively in northern hemisphere fish oils. Clearly, the ratios of DHA : EPA : AA generated by the *Boops boops* diet cannot be achieved using northern hemisphere fish oil. However, the speciality oil derived from the eye socket of the tuna, which is commercially available, has a DHA : EPA ratio of 5.6 and an EPA : AA ratio of 3.1, i.e. more similar to the values generated by *Boops boops* than the northern hemisphere fish oil. Recent analyses in our laboratory (Bruce et al. 1998) have established that the phosphatidylethanolamine of eggs of broodstock sea bass fed tuna orbital oil has a DHA : EPA ratio of 8.6 and an EPA : AA ratio of 2.2. The corresponding values for eggs from broodstock sea bass fed *Boops boops* are 7.0 and 1.5 respectively, and those for eggs from sea bass broodstock fed northern hemisphere fish oils are 6.6 and 6.1 respectively. Therefore, tuna orbital oil is recommended as a more suitable dietary oil for sea bass broodstock than northern hemisphere fish oil. Recent results from our laboratory confirm that broodstock fed this oil generate higher quality eggs compared to fish fed northern hemisphere oil, at least on the basis of their polyunsaturated fatty acid profile (Bruce et al. 1998). It had already been reported that *Artemia* nauplii enriched with tuna orbital oil generate very satisfactory ratios of DHA : EPA : AA in phospholipids of the eyes of sea bass larvae and that the larvae perform well on this diet (Navarro et al. 1995).

The full implications of these findings for sea bass production await evaluation of the longer term survival and growth performance outcomes of fish reared on the apparently improved diet. No less important is to understand the underlying biochemical basis of these largely empirically - derived findings. The notion that eicosanoid production and structural membrane considerations are both involved is currently under study in our laboratory, in developing eggs as well as in rapidly growing and developing larvae and juveniles.

III. Halibut and Turbot.

A major consideration in recent efforts to improve the essential fatty acid nutrition of halibut larvae was the earlier finding, with juvenile herring, that *Artemia* nauplii supplemented with oils that generated a low percentage of DHA in their lipid resulted in fish with a lower ability to capture prey than fish fed *Artemia* nauplii supplemented with oils that generated a high percentage of DHA (Bell, M.V. et al. 1995). Such a reduced “predation efficiency” occurred

particularly at low but natural light intensities where rod vision was essential. It was accompanied by a markedly decreased percentage of DHA, and specifically of di-DHA molecular species of phospholipids, in the eyes of the juvenile herring (Bell, M.V. et al. 1995). This result was predictable on the basis of the well known high contents of DHA in rod outer segment membranes and also in synaptosomal membranes in vertebrates in general (see Sargent et al. 1993b).

Halibut larvae fed marine copepods such as *Eurytemora* commonly have a higher survival rate through metamorphosis and beyond and a better pigmentation pattern than halibut larvae fed *Artemia* nauplii supplemented with various oils (Shields, R., Gillespie, M., personal communications and unpublished data). Recent experiments (McEvoy. L. and Shields, R., unpublished) have established the following values for phosphatidylethanolamine from the eyes of halibut larvae fed a mixture of marine copepods, largely *Centropages hamatus*:

% DHA 30.9 % EPA 7.0 %AA 2.7
DHA : EPA, 4.4 EPA : AA, 2.6.

In phosphatidylethanolamine from the eyes of halibut larvae fed *Artemia* nauplii supplemented with a SuperSelco^{TM} preparation, the values were:

% DHA 21.3 % EPA 12.7 % AA 1.9
DHA : EPA 1.7 EPA : AA 6.7

Thus, the natural marine copepod diet generated higher percentages of both DHA and AA and a lower percentage of EPA than the supplemented *Artemia* nauplii diet. In separate but related experiments, larvae fed *Artemia* nauplii supplemented with SuperSelco^{TM} had significantly decreased numbers of rod cells relative to cone cells in their eyes (rod/cone ratio of 1.3 ±0.6, n=6) as compared to larvae cultured on *Eurytemora* (rod/cone ratio of 2.5±0.7, n=6) (Luizi, F. and Shields, R., personal communication, unpublished data), suggesting a diminished performance of the *Artemia* - fed larvae at low light intensities (which has yet to be tested experimentally). The decreased percentage of DHA in the eyes of the latter larvae can, at least partially if not wholly, account for the decreased numbers of rod cells. However, the decreased percentage of AA

and the increased percentage of EPA in the larvae's eyes cannot be excluded as causative factors, on the grounds that eicosanoid production in retinal cells is a normal part of vertebrate vision (as it is in neural functions in general, including signal transduction processes in synaptosomal functions). The PUFA composition of the phosphatidylethanolamine of halibut larvae reared on *Artemia* nauplii supplemented with tuna orbital oil is:

% DHA 22.9 % EPA 8.0 % AA 2.6
DHA : EPA 2.9 EPA : AA 3.1

On the basis of EPA : AA ratios, tuna oil - supplemented *Artemia* generate a very similar value to *Eurytemora*. However, on a percent DHA basis, both tuna oil - supplemented *Artemia* and SuperSelcoTM - supplemented *Artemia* are inferior to *Eurytemora*. The effects of the tuna oil - supplemented *Artemia* on rod cell development in halibut have yet to be tested. However, it is predicted that, should eicosanoid production be a factor in rod development, then the tuna orbital oil is likely to be beneficial. In contrast, should DHA limitation be the main PUFA factor in rod development, then the tuna oil will not be effective and a dietary oil with a higher concentration of DHA will be required for normal rod development.

These considerations emphasise the importance and complexity of PUFA functions in neural tissues which include, as well as rod cell membranes, the rapidly and very extensively developing DHA - rich synaptic junctions that generate the critical neural networks in developing animals. It is inevitable, therefore, that perturbing the long chain PUFA and especially the DHA content of the diet will seriously perturb neural development in marine fish larvae and, in principle, virtually any aspect of the developing larvae's physiology and behaviour may be affected.

A problem in cultured halibut, turbot and flatfish in general is malpigmentation. The problem in halibut larvae is commonly reported to be more prominent in larvae fed *Artemia* nauplii supplemented with oils than in larvae fed natural copepods such as *Eurytemora* (Shields, R. and Gillespie, M., personal communications and unpublished data). This problem may or may not involve suboptimal essential fatty acid nutrition. It may or may not have a neural origin since innervation and neuroendocrines are both involved in pigmentation.

It has been observed that pigmentation of halibut larvae fed *Artemia*

nauplii supplemented with SuperSelco^{TM} rich in PUFA is similar to that of larvae fed *Artemia* nauplii supplemented with tuna orbital oil but, in both cases, the pigmentation is inferior to that of larvae fed *Eurytemora* (Shields, R., McEvoy, L., unpublished data). On the basis of the argument developed above for rod development, this points to DHA provision and not eicosanoid production being the more likely PUFA candidate in causing malpigmentation. Thus, the two *Artemia* supplementation procedures generate the same level of DHA in the larvae's neural phospholipids and lower than that generated by *Eurytemora*. On the other hand, the ratios of EPA : AA in the larvae's phospholipids are very similar after feeding *Eurytemora* or *Artemia* supplemented with tuna oil, but differ from those after feeding *Artemia* supplemented with SuperSelco^{TM}.

To test directly the possible involvement of eicosanoids in malpigmentation, the ratio of EPA : AA in turbot larvae has been altered by feeding rotifers followed by *Artemia* larvae, both supplemented with either EPA - rich or AA - rich oil emulsions (McEvoy, L. and Estevez, A., unpublished data). The results show that increasing the EPA : AA ratio in the oils used for supplementation from 4.1 to 38.0 yielded larvae whose percentage pigmentation was not significantly different (85.9% ±5.8 n=3 and 82.1% ±10.7 n=3, respectively). However, decreasing the EPA : AA ratio in the oils from 4.1 to 0.3 induced marked malpigmentation in the larvae (85.0% ±5.8 n=3 and 0.9% ±0.9 n=3, respectively), the effect becoming particularly marked between oils with an EPA : AA ratio of 2.0 (pigmentation of 78.8% ±13.5, n=3) and 0.7 (pigmentation of 32.6% ±17.4, n=3). Thus, increasing the EPA content of the supplementing oil emulsion (from 6.6% to 68.4% of the total fatty acids present, at respective AA % of 1.6% and 1.8%) does not cause malpigmentation, whereas increasing the AA level of the supplementing oil emulsion (from 1.6% to 15.1% of total fatty acids in the oil, at respective EPA % of 6.6% and 4.1%) does. In these experiments the DHA content of the AA - rich oil was greater than the EPA - rich oil (15.5% and 9.8% respectively), so that DHA deprivation is unlikely to be a factor in the malpigmentation induced by the AA - rich diet. One interpretation of the findings is that excess AA in the supplementing oil subsequently generates excess eicosanoids in the larvae, i.e. they are experiencing experimentally (dietarily) - induced stress which leads, *inter alia*, to malpigmentation. Larval diets with the high levels of AA used here are difficult to generate and will seldom if ever be encountered in commercial larval culture. However, stress induced by means

other than diet, e.g. fluctuating environmental factors, over crowding or sub-optimal husbandry practice, is not unusual in marine flatfish larval production. Indeed, it may be inevitable given the high stocking densities required for economically viable larval production. Such stress is likely to be at least as important as suboptimal PUFA nutrition, especially DHA provision, in causing malpigmentation in flatfish larvae.

IV. Atlantic Salmon.

Salmon parr are reared in fresh water before transfer to seawater immediately after the parr - smolt transition. It has been noted earlier (Bell et al. 1994) that the natural diets of salmon parr, which include a preponderance of freshwater crustaceans and insects, generally have enhanced levels of 18:3n-3 and 18:2n-6 and decreased levels of 22:6n-3, i.e. decreased dietary ratios of n-3 : n-6 and 22:6n-3 : C18 PUFA, as compared to marine zooplankton and northern hemisphere fish oils. However, salmon parr are commonly reared in fresh water on diets containing northern hemisphere fish oils. Salmon parr, like trout, are well capable of elongating and further desaturating C18 PUFA to C20 and C22 PUFA, reflecting the abundance of C18 PUFA in their natural diet, although these conversions will be greatly reduced by feeding the end product C20 and C22 PUFA, i.e. by feeding fish oil. It was considered, therefore, that farmed salmon parr, normally fed fish oil diets in fresh water in current salmon practice, might benefit from increased dietary inputs of 18:3(n-3) and 18:2(n-6), not least from the resulting increased tissue levels of AA and derived eicosanoids.

To investigate this possibility, salmon parr were reared in fresh water on a standard diet of northern hemisphere fish oil and an experimental diet containing a blend of rapeseed and linseed oils to generate increased dietary inputs of 18:2n-6 and 18:3n-3. The following was observed (Bell et al. 1997b): (a) hepatocytes of parr fed the vegetable oil diet had an enhanced ability to further chain elongate and desaturate 18:2n-6 and especially 18:3n-3 to their respective C20 and C22 end product PUFA, as compared to parr fed the fish oil diet; (b) only moderately elevated levels of 18:2n-6 and 18:3n-3 and moderately depressed levels of 20:5n-3 and 22:6n-3 occurred in liver and gills phospholipids of the fish fed the vegetable oil diet, i.e. these fish were well capable of generating and maintaining typically high cell membrane levels of 20:5n-3 and 22:6n-3 from the vegetable oil diet; (c) the ratio of AA : EPA was substantially elevated in liver and gills

phospholipids of the parr fed the vegetable oil diet; (d) the levels of eicosanoids produced from AA were substantially increased in gills of parr fed the vegetable oil diet; (e) when challenged in sea water 6 weeks prior to final transfer to sea water, fish fed the vegetable oil diet achieved greater control over their serum chloride ion concentrations than fish fed the fish oil diet, i.e. fish fed the vegetable oil diet were better able to osmoregulate their body fluids in sea water.

These experiments provide direct evidence that the parr - smolt transition and subsequent osmoregulation in sea water may be improved in Atlantic salmon parr by simple dietary manipulation designed to elevate tissue levels of AA and its derived eicosanoids.

V. Overview.

The work outlined here has raised two important issues for marine fish farming: first, the importance of high dietary levels of DHA for neural development; second, the importance of the dietary ratio of EPA : AA for determining eicosanoid actions. These issues hold also for freshwater fish except that here the dietary precursor 18:3n-3 and 18:2n-6 PUFA must now be considered as well as end product 20:5n-3, 22:6n-3 and 20:4n-6. The two issues are inexorably linked because all dietary oils rich in DHA (marine fish oils) contain substantial but variable amounts of EPA and also minor and variable amounts of AA. Therefore, it can be difficult practically to achieve the desired blend of dietary PUFA to a provide a pre-determined dietary balance, even if that balance is known, which it seldom is. Changing the percentage of one PUFA in the diet automatically changes the percentages of the others and alters the ratios of all three!

The issue of DHA in neural development holds for all fish species, whether marine or freshwater (and probably all vertebrates), and the case for providing adequate dietary levels of DHA for all early developing fish is now overwhelming. However, the issue of optimal EPA : AA ratios in eicosanoid production is likely to be much more species - specific. The present studies strongly suggest that the elevated levels of dietary and tissue levels of AA appropriate for sea bass, and also possibly for the salmon parr - smolt transition, are not appropriate for the halibut and turbot. Optimal AA levels and eicosanoid production rates in tissues must relate directly or indirectly to the life cycle and life style of a particular species. For example, smoltification in salmon and

subsequent migration from fresh water to sea water is undoubtedly a stressful period in the fish's normal development, during which the demand for eicosanoid production from AA is likely to be elevated. Similarly, the sea bass is a euryhaline, eurythermic species, commonly found in turbulent inshore waters, i.e. a species thriving in stressful environments. This can not be said to the same extent for the turbot and certainly the halibut which occupy more constant, less turbulent environments. The best clues as to what constitutes an optimal ratio of DHA : EPA : AA in a particular species have come thus far from analysing the natural prey of a given species in its natural environment, unsurprisingly, since both predator species and prey species are, *a priori,* well adapted to the same environment in terms of achieving optimal behaviour by optimal neural development, and by responding optimally to environmental stress by optimal eicosanoid production. Understanding the dietary and tissue relationships between DHA, EPA and AA is as essential for optimising fish diets as for illuminating the complex interactions between nutrition, behaviour and environmental stress.

VI. References

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