

# Chapter 13

## Essential Fatty Acids in Aquatic Food Webs

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### 13.1 Introduction

Aquatic ecosystems occupy the largest part of the biosphere, and lipids in those systems provide the densest form of energy. Total lipid energy can be used to predict features of animal population dynamics such as egg production by fish stocks. Difficulties in determining the relationship between spawner biomass and the number of offspring produced (recruitment) have led researchers to look at lipids (Marshall et al. 1999). A positive association between recruitment and liver weights in cod prompted an investigation of total lipid energy as a proxy for total egg production by fish stocks. Marshall et al. (1999) found a highly significant linear relationship between total egg production and total lipid energy, and they suggested this approach should be used at other trophic levels too. Total lipid content of fish has also been connected to climate-induced community changes (Litzow et al. 2006). It is hypothesized that this relates to the dietary availability of just two fatty acids which were positively correlated with total lipid content.

The study of fatty acids in aquatic food webs has often focussed on their broad use as biomarkers in trophic transfer studies (e.g. Napolitano 1999; Dalsgaard et al. 2003; Iverson et al. 2004). By contrast the study of fatty acids in aquaculture has usually centred on only two or three fatty acids and their importance as essential dietary nutrients. The focus has been on the long-chain fatty acids, docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and, to a lesser, extent arachidonic acid (ARA, 20:4n-6) which are required by organisms for optimal health. These polyunsaturated fatty acids (PUFA) maintain membrane structure and function and are precursors of bioactive compounds in vertebrates (Lands – Chap. 14), invertebrates, and plants. In finfish, they are required for normal somatic growth, survival, neural development, pigmentation, and reproduction (e.g. Sargent et al. 1999a). Based on a detailed examination of lipid biochemistry, this

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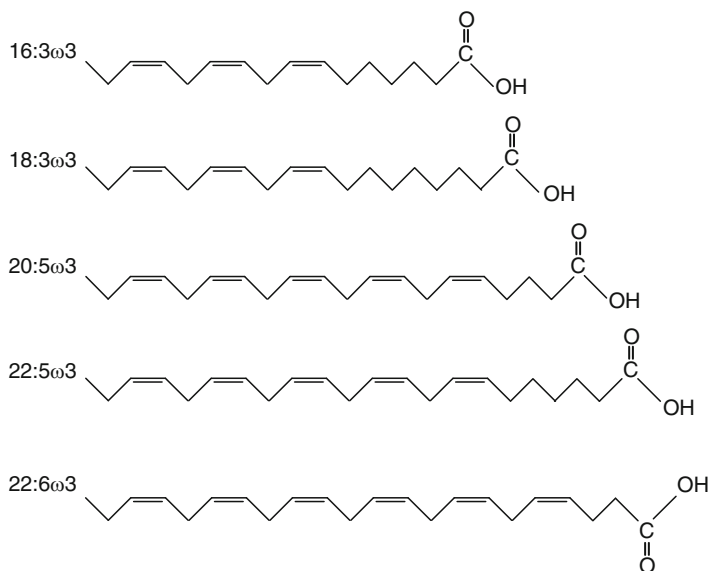
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chapter argues for a crossover between these two approaches, i.e. a broader examination of nutritional fatty acids in aquaculture and a more thorough consideration of nutritional implications in trophic transfer studies.

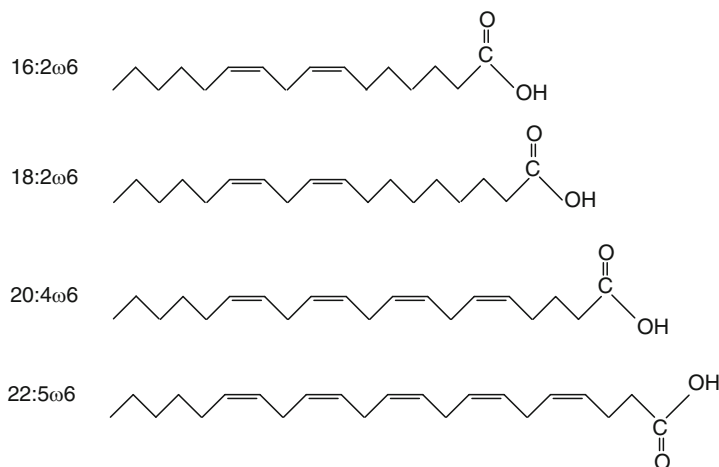
## 13.2 Definition of Essential Fatty Acids

In 1930, linoleic acid (LIN, 18:2n-6) was termed an ‘essential fatty acid’ because it could eliminate acute deficiency states in rats that had been fed fat-free diets (Burr and Burr 1930). The ensuing search for fatty acids with essential fatty acid activity revealed a variety of polyunsaturated fatty acids which had the first double bond in the n-6 position. Later it was also shown that  $\alpha$ -linolenic acid (ALA, 18:3n-3) could remove deficiency symptoms (Gurr and Harwood 1991), and now we know there to be a number of n-3 fatty acids with carbon numbers ranging from C<sub>14</sub> to C<sub>36</sub> that qualify as essential fatty acids (Cunnane 2000). In fact there are 23 PUFA in which the first double bond starts either 3 or 6 carbons from the methyl end that have essential fatty acid activity, some of which are shown in Figs. 13.1 and 13.2

In the synthesis of unsaturated fatty acids, the first double bond is usually inserted near the middle of the molecule in all organisms, for example in the n-9 position in stearic acid (18:0) to create oleic acid (18:1n-9). In animals, subsequent



**Fig. 13.1** Structures of some n-3 polyunsaturated fatty acids present in aquatic samples. Hexadecatrienoic acid (16:3n-3),  $\alpha$ -linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), n-3 docosapentaenoic acid (n-3DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) are all related biochemically because of the location of the first double bond 3 carbons from the methyl end of the chain



**Fig. 13.2** Structures of some n-6 polyunsaturated fatty acids present in aquatic samples. Hexadecadienoic acid (16:2n-6), linoleic acid (LIN, 18:2n-6), arachidonic acid (ARA, 20:4n-6), and n-6 docosapentaenoic acid (n-6DPA, 22:5n-6) are all related biochemically because of the location of the first double bond 6 carbons from the methyl end of the chain

double bonds are introduced between an existing double bond and the carboxyl end of the molecule, while plants normally introduce a second double bond between the existing position and the terminal methyl group. The inability of animals to insert a double bond between the first one and the methyl end, combined with a requirement for fatty acids with the first double bond in the n-3 or n-6 position for disease prevention, is the basis of the essentiality of n-3 and n-6 fatty acids. When provided with sufficient n-3 and n-6 fatty acids in the diet, most animals can make other n-3 and n-6 fatty acids by desaturation and elongation or by retroconversion to shorter-chain fatty acids, but the n-3 and n-6 series are not interconvertible in vertebrates and most other animals except in the case of transgenic animals (Kang et al. 2004). The extent to which a given species can convert one n-3 fatty acid to another or one n-6 fatty acid to another leads to degrees of essentiality.

It is generally believed that ARA, EPA, and DHA are the most important long-chain ( $C_{20} - C_{22}$ ) PUFA in mammals (Simopoulos 2002; Ruxton et al. 2004; Shahidi and Miraliakbari 2004; Wijendran and Hayes 2004) and fish (Sargent et al. 1999a, b; Montero et al. 2003, 2004). They have to be supplied to animals in their diet, although some animals can synthesize at least some of them when sufficient quantities of the LIN and ALA precursors are available. Many freshwater fish can synthesize these long-chain PUFA by a series of desaturations and elongations, although pollution decreases the ability of whitefish, *Coregonus lavaretus* to convert LIN to ARA by a factor of twofold to threefold (Toivonen et al. 2001). However, marine fish appear to have lost the ability to express a key elongase and/or desaturase gene (Sargent et al. 1999a, 2002; Tocher 2003). Gurr and Harwood (1991) make a distinction between 'essential metabolites' and 'essential nutrients', so that using this terminology, LIN and ALA would be considered to be essential nutrients in

freshwater fish, while the long-chain products would be essential metabolites. In marine fish, ARA, EPA, and DHA would be essential nutrients. Similarly, rats and humans have some ability to synthesize LIN and ALA from 16:2n-6 and 16:3n-3 in green vegetables (Cunnane and Likhodii 1996), so that the essential nature of LIN and ALA depends on availability of precursors as well as the amount of these fatty acids in storage. These two short-chain PUFA (Figs. 13.1 and 13.2) also commonly occur in microalgae (e.g. Dunstan et al. 1992; Viso and Marty 1993; Nanton and Castell 1998). Borrowing terminology used in the amino acid literature, Cunnane (1996) suggested essential fatty acids should be divided into 'indispensable' or 'conditionally dispensable' fatty acids where requirements may change according to amounts in storage, age, or availability of other precursors. He subsequently modified the two categories to 'conditionally indispensable' and 'conditionally dispensable' fatty acids on the basis that there is insufficient evidence that any single PUFA is absolutely indispensable through the lifespan (Cunnane 2000).

While the mammalian literature recognizes 23 fatty acids having essential fatty acid activity, for the most part, the aquatic literature recognizes only two: EPA and DHA (e.g. Klein Breteler et al. 1999; Anderson and Pond 2000; Muller-Navarra et al. 2000, 2004; Arts et al. 2001; Tang and Taal 2005; Litzow et al. 2006; but see Kainz et al. 2004; Ahlgren et al. – Chap. 7). This despite the fact that ARA is now known to be an essential fatty acid during early development of marine finfish, albeit at lower concentrations (reviewed by Sargent et al. 1999b; Izquierdo et al. 2000).

In the aquatic literature, EPA and DHA are termed 'essential fatty acids' without qualification, but generally appear to be assumed to be essential nutrients, essential metabolites and indispensable, using the terminology of Gurr and Harwood (1991) and Cunnane (1996). It is clearly time for an examination of whether two fatty acids are enough to describe essential fatty acid status in aquatic food webs and to start to examine the degree of essentiality of these and other PUFA.

### 13.3 Effects of Essential Fatty Acids

Public awareness of n-3 fatty acids has increased dramatically over recent years (Lands – Chap. 14). Consumption of EPA and DHA has beneficial effects on plasma lipids and lipoproteins (Harris 1997a, b), cardiovascular disease (Kris-Etherton et al. 2002), cancer (Shahidi and Miraliakbari 2004), inflammatory and autoimmune diseases (Simopoulos 2002), brain development and function (Ruxton et al. 2004), and even adipose tissue hypertrophy (Parrish et al. 1990). For the most part, meta-analyses have confirmed these observations (He et al. 2004; Balk et al. 2006; Mozaffarian and Rimm 2006), although one recent one has not (Hooper et al. 2006). Nonetheless, Hooper et al. state that their findings 'do not rule out an important effect of omega 3 fats on total mortality' and they advise consumption of more oily fish.

There are marked differences in the effects of dietary fish oils on plasma lipid and lipoprotein concentrations in experimental animals and in humans. Harris (1997a) reviewed studies of seven species of experimental animals and compared them with

human trials (Harris 1997b). The n-3 fatty acids consistently lowered serum triacylglycerol concentrations in humans but not in most species of experimental animals. Conversely there was a marked reduction in high-density lipoprotein-cholesterol concentrations in experimental animals, which is almost never seen in humans. Harris suggests these differences result not only from species differences but also from feeding experimental animals much larger concentrations of n-3 acids.

Many people know that EPA and DHA are recommended by the American Heart Association, for example, and that they are derived from fish. However, few people realize that the main source of these fatty acids is the aquatic food web, and they have to be acquired directly or indirectly by fish themselves through their diets (Gladyshev et al. - Chap. 8; Lands - Chap. 14). Even fewer know that EPA and DHA are actually very important at various trophic levels in the food web. In finfish, EPA and DHA are important in growth, immunity, and stress resistance. In sea bream, a 75% reduction in EPA + DHA resulted in a 13% reduction in growth, an increase in erythrocyte fragility, and alterations in cellular immunity and in renal morphology including renal tube degeneration (Montero et al. 2004). In another experiment a 50% reduction in dietary EPA + DHA, but with replacement this time with ALA, significantly affected stress response in sea bream (Montero et al. 2003). Plasma cortisol concentrations were significantly higher after net chasing or overcrowding.

DHA alone, which is especially rich in vertebrate neural tissue, is needed for finfish eye development and schooling behaviour. Phospholipids in the excitable membranes of the central nervous system are uniquely enriched in DHA. This n-3 fatty acid is contained in the phospholipids of the membrane bilayer, where it may produce an optimal acyl-chain packing array for the functioning of transmembrane proteins involved in the excitatory response. Animals deprived of n-3 fatty acids show reduced levels of DHA in the brain and retina and concomitant impairments in retinal function and learning ability (e.g. Moriguchi et al. 2000). DHA-deficient yellowtail, *Seriola quinqueradiata*, do not show schooling behaviour (Masuda and Tsukamoto 1999), and dietary DHA was important in terms of survival, eye development, and pigmentation in halibut larvae (Shields et al. 1999). Pigmentation may require signal transmission via the visual system to the brain which controls melanin synthesis.

Dietary DHA is also needed in invertebrates, for example for successful hatching in copepods (Arendt et al. 2005) and for somatic growth in shellfish. The growth of zebra mussel larvae was enhanced by DHA supplementation and not by EPA supplementation (Wacker et al. 2002), while in postlarval sea scallops the lowest growth rate occurred with the diet containing the lowest DHA concentrations (Milke et al. 2004).

In marine organisms, ARA, is another equally important fatty acid even though it is present in low proportions, except in echinoderms (Copeman and Parrish 2003). This n-6 acid has received extensive attention in the mammalian literature, and it is well established that ARA acts in concert with EPA to control physiological properties. Much attention has been paid to the role of these C<sub>20</sub> compounds as precursors of a wide variety of short-lived hormone-like substances called eicosanoids (Lands – Chap. 14), which are a large group of cyclized compounds found in protozoa and all major animal phyla (Stanley and Howard 1998). ARA is important in sea urchin

and finfish eggs (Ciapa et al. 1995; Pickova et al. 1997) and is needed for finfish growth, survival, and stress resistance (Bell and Sargent 2003).

### **13.4 Mechanisms of the Effects of Dietary Essential Fatty Acids**

The competition among dietary fatty acids for key enzymes is central to our understanding of the importance of essential fatty acids in aquatic food webs. There is a fundamental biological difference between lipid nutrition and carbohydrate and protein nutrition (Sargent et al. 2002). The enzyme-substrate interaction in carbohydrate and protein metabolism depends mainly on strong ionic and hydrogen bond interactions resulting in high specificities in enzyme-catalysed reactions. Enzyme-substrate interaction in lipid metabolism depends much more on weak hydrophobic interactions (such as van der Waals and dispersion forces) resulting in lower specificities in enzyme-catalysed reactions. Thus, the fatty acid composition in animals depends on the low specificity of incorporation of dietary fatty acids into lipids (Sargent et al. 2002). It is for this reason that recent work in aquaculture nutrition has focussed on dietary fatty acid ratios, especially the DHA to EPA ratio (Ahlgren et al. – Chap. 7).

A multitude of studies have been undertaken on the mechanisms associated with the health effects of dietary fish oils in mammals. It appears that membrane phospholipids may be central to many of the mechanisms postulated for their effects. One way in which they exert their influence is through their effects on membrane structure. Phospholipids are by far the major lipid components of most membranes, and fatty acids are in most cases the principal determinants of a membrane's internal physical state (Thompson 1992). Highly unsaturated fatty acids have specific effects on the lipid bilayer and its dynamics, with potential influences on membrane protein functioning. Reduction in plasma triacylglycerol concentrations was one factor that early studies suggested might improve human health by reducing atherosclerosis. Now, however, the preferred mechanism for reduction in cardiovascular mortality is an antiarrhythmic effect arising from modulation of calcium ion flux (Hallaq and Leaf 1992; Goodnight 1996). High concentrations or high fluxes are believed to cause ventricular arrhythmias by producing a strong current, but in rat myocytes n-3 fatty acids reduce calcium flux into the cells. The reason for this effect is thought to be an alteration of calcium channel structure or function through inclusion of n-3 fatty acids in the plasma membrane.

Similarly, the mechanism for the dietary regulation of the activity of lipogenic enzymes (Herzberg 1989; Herzberg and Rogerson 1989) and of tumour development (Jiang et al. 1997) may relate to the fatty acid profile of the nuclear envelope phospholipids (Clandinin et al. 1992). Transport of RNA occurs only through nuclear pore complexes with lipid clustering modulating their opening and closing. Enzymes providing energy for translocation of RNA also occur near these complexes. In addition, the nuclear envelope possesses binding sites for hormones. These processes

may all be affected by diet-induced changes in membrane structure. Changes in adipocyte size and function following dietary supplementation with fish oil may also relate to the modification of membrane lipid structure (Parrish et al. 1997). In rat adipocyte plasma membranes, the ratio of phosphatidylcholine to sphingomyelin was found to be increased as was the proportion of molecular species with PUFA. Changes in physicochemical properties of plasma membranes are known to affect hormone receptors and the specific activities of enzymes. As might be expected, there are studies where increased membrane fluidity can apparently be ruled out as the mechanism of the metabolic effect of fish oil feeding. For example, Dulloo et al. (1994) demonstrated suppression in calcium-dependent heat production in muscles by fish oil feeding, which is the opposite of that which would be expected if there was an increase in membrane fluidity. However, they did not refer to other ways in which membrane lipids can regulate cellular processes.

In addition to exerting their effects through their physicochemical properties, membrane phospholipids are also a source of bioactive compounds or messengers (Arts and Kohler - Chap. 10; Lands - Chap. 14). ARA at position *sn*-2 of phosphatidylcholine and phosphatidylethanolamine is released by phospholipase A<sub>2</sub> to generate eicosanoids. Minute quantities of eicosanoids have potent physiological effects (e.g. Gurr and Harwood 1991). One group of eicosanoids, the leucotrienes, are generated by the action of lipoxygenase and are powerful mediators of the inflammatory process (e.g. Goodnight 1996). Another group of eicosanoids, the prostaglandins and thromboxanes, are generated by the action of cyclooxygenase. They are involved in reproduction, blood clotting, the circulation, kidney function, pain and stress reactions, protection of the gut from self-digestion, and regulation of immunological responses (e.g. Urich 1994; Goodnight 1996). Competition between ARA and EPA for these enzymes results in the production of different types of prostaglandins, thromboxanes and leucotrienes in varying quantities. The action of cyclooxygenase on ARA gives rise to the series 2 cascade with two double bonds in the molecules; EPA gives the series 3. These compounds are involved in regulating many types of actions as well as inflammation. Aspirin is a cyclooxygenase inhibitor that decreases the synthesis of prostaglandins.

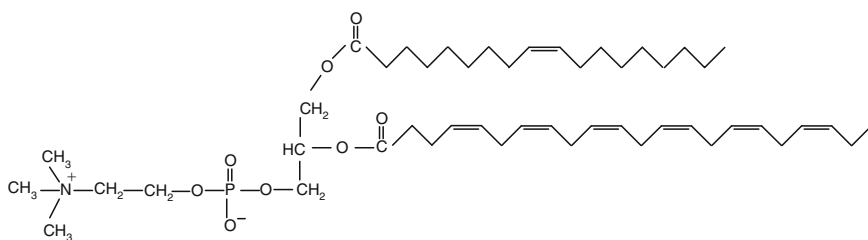
Together, prostaglandins and leucotrienes constitute a group of extracellular mediator molecules that are part of an organism's defence system (Arts and Kohler - Chap. 10); however, reports on the effect of n-3 and n-6 fatty acids on immune response in fish are not conclusive and often contradictory, probably because of other physiological and environmental factors (Lall 2000). For example, although the feeding of vegetable oil to salmon reduced nonspecific immune parameters, there was no increase in mortality when challenged by a pathogen (Bell and Sargent 2003). Nonetheless, given that eicosanoids are clearly involved in mammalian and invertebrate immunity (Stanley and Howard 1998), further research with fish should produce a direct connection. The importance of ARA for finfish growth, survival, and stress resistance is already much clearer (Bell and Sargent 2003).

Not only are the fatty acids important in messenger formation, but different types of phospholipids are involved in different ways as well. The enzymes known as protein kinase C require the phospholipid phosphatidylserine for their activity. Protein kinase

C play a central role in a system of signal transduction through the membrane that acts on another membrane lipid: phosphatidylinositol-4,5, biphosphate (e.g. Urich 1994). These enzymes become active on binding to a lipid bilayer and in turn are influenced by the level of polyunsaturation in that bilayer (Stubbs 1992).

Although it has long been known that dietary fatty acids can alter membrane phospholipid composition (e.g. Gurr and Harwood 1991), the fact that different types of membrane phospholipids are affected differently (e.g. Aukema and Holub 1989; Zsigmond et al. 1990; Parrish et al. 1997) is less well known. This is because it is analytically more challenging to perform molecular species analyses than a simple total fatty acid profile. The importance of studying molecular species for determination of physical properties is underlined by the work of Fodor et al. (1995) who showed that changes in overall membrane fluidity in carp livers could be related to just a few molecular species (Arts and Kohler – Chap. 10).

A membrane can contain well over 100 different molecular species. The glycerophospholipids among them consist of complex mixtures of diacyl- (e.g. Fig.13.3), alkenylacyl-, and alkylacyl-glycerol derivatives. Not only does fish oil feeding replace some of the ARA in these molecular species (Aukema and Holub 1989; Careaga-Houck and Sprecher 1989), but there is also a preference for incorporation of n-3 fatty acids in ethanolamine glycerophospholipids (Yeo et al. 1989; Parrish et al. 1997), especially alkenylacyl ethanolamine glycerophospholipids (Aukema and Holub 1989). For example, after feeding rats a diet supplemented with 21% EPA + DHA, the proportion of these fatty acids in phosphatidyl ethanolamine was also 21%, more than twice the proportion in other phospholipid classes (Parrish et al. 1997). It is also interesting that dietary fish oil can create several molecular species that do not exist without it, while for the most part preserving those that do exist without fish oil feeding (Parrish et al. 1997). As well, fish oil feeding can cause a dramatic increase in the C<sub>24</sub> monoenoic acid, nervonic acid, in sphingomyelin which contains no PUFA (Parrish et al. 1997). Bettger (1998) indicates that there is a nutraceutical benefit to this increase in nervonic acid in sphingomyelin which is involved in a cell signalling cascade leading to cell division or apoptosis (death). The increase in nervonic acid was not observed when an n-6 fatty acid diet was fed (Zsigmond et al. 1990).



**Fig. 13.3** Structure of an important phospholipid molecular species present in aquatic samples: 1-oleoyl, 2-docosahexaenoyl phosphatidyl choline



Thus, it is strongly recommended that molecular species analyses are performed in order to help define the mechanisms of the effects of essential fatty acids. However, simply cataloguing changes in molecular species distributions in membranes is also not enough: if the mechanism is believed to involve changes in physicochemical properties then fluidity determinations (Muriana and Ruiz-Gutierrez 1992; Hall et al. 2002) should be made concurrently, while phospholipase A<sub>2</sub> activity (Evans et al. 1998) should be determined if the effect involves eicosanoids. The rate-limiting step in the biosynthetic pathway of the eicosanoids is the release of a PUFA by phospholipase A<sub>2</sub> (e.g. Gurr and Harwood 1991; Urich 1994). This enzyme becomes active on binding to a membrane bilayer, and it may in turn be influenced by the level of polyunsaturation in that bilayer (Stubbs 1992). As well, it is interesting that phospholipase A<sub>2</sub> is also needed to produce another type of biologically active lipid: platelet-activating factor (Gurr and Harwood 1991).

The influence of dietary fatty acids on an organism's lipid composition provides a challenge for membranes since their physical characteristics are a key determinant of membrane structure and function. Although the fatty acids in phospholipids respond readily to changes in dietary content, membrane lipid composition is regulated. In fact, organisms which inhabit variable environments exploit the considerable chemical diversity among membrane lipid constituents so that lipids of appropriate physical properties are matched to the prevailing environmental conditions (Hazel and Williams 1990). If essential fatty acids are missing, animals will modify their own metabolic pathways to try and compensate, but physiological abnormalities may result (Thomson 1992).

Ectothermic animals increase the membrane content of unsaturated fatty acids in response to cold (Arts and Kohler – Chap. 10) in order to counteract the ordering effects of reduced temperature. This defence of membrane fluidity in ectotherms following thermal challenge, termed homeoviscous adaptation, was first described by Sinensky (1974) for *Escherichia coli*. Finfish membranes have high levels of *sn-1* monoenoic, *sn-2* polyenoic phospholipid molecular species (e.g. 18:1n-9/DHA, Fig.13.3) with cold adaptation, rendering the membranes less packed (Farkas et al. 2001). During winter, this adaptation may have to be performed at a time when lipid levels are low. Lipid depletion during overwintering is quite common in finfish (e.g. Arctic charr: Jorgensen et al. 1997; Jobling et al. 1998; Atlantic salmon: Morgan et al. 2002; and perch: Eckmann 2004).

Although phospholipid molecular species containing DHA are important in controlling finfish membrane fluidity, a direct correlation with DHA has not been found. In contrast, Hall et al. (2002) found a simple but very strong relationship between fluidity and a single polyunsaturated fatty acid, EPA, in gill membranes from the scallop *Placopecten magellanicus*. This suggests a possible dual function for this fatty acid in scallops as EPA's biological importance is usually associated with its role as an eicosanoid precursor. DHA, with its exceptional flexibility resulting from the electronic structure of the polyunsaturated chain (Eldho et al. 2003) has been thought to have mainly a structural function in membranes. However, it too may play a dual role as it has recently been found to be the precursor of bioactive compounds in rainbow trout brain cells (Hong et al. 2005).

### 13.5 Are N-6 PUFA Essential Fatty Acids in Aquatic Food Webs?

The importance of ARA for marine finfish in culture is becoming well established, but there is also evidence for the importance of ARA and LIN in crustaceans, suggesting a wider ecological role for ARA as well as other n-6 fatty acids. ARA was the most efficiently accumulated PUFA in planktonic food webs in oligotrophic lakes (Kainz et al. 2004), and suboptimal levels of ARA were implicated in the decline of copepods in a Baltic Sea mesocosm experiment (Ahlgren et al. 2005). ARA and to a lesser extent, LIN, improved survival, moulting frequency, and growth rate in the Chinese prawn (*Penaeus chinensis*: Xu et al. 1994). On the other hand, it was LIN that was highly correlated with field estimates of egg viability in the copepod, *Calanus helgolandicus* (Pond et al. 1996). However, principal components analysis also indicated that ARA content in the food and in the eggs was a potential factor involved in hatching success.

While EPA, DHA, and to a lesser extent ARA are often quantitatively dominant among the long-chain PUFA, there are a number of n-3 and n-6 long-chain PUFA that are commonly found in lipid extracts, especially from aquatic food webs, which may be nutritionally important too. For this type of investigation, fish larvae are particularly attractive because they are small and grow very quickly. In fact, fish larvae can be among the smallest of all vertebrates and they can have the highest growth rates of all vertebrates. In addition, lipid nutrition of larvae is recognized as one of the bottlenecks for mass production of many species, and the effects of essential fatty acids on growth, survival, and stress resistance of marine larvae have received increasing attention in the last decade (Sargent et al. 1999b; Izquierdo et al. 2000).

Parrish et al. (2007) took advantage of an unusual molecular and isotopic composition of a thraustochytrid protist (*Schizochytrium*) to follow n-6 docosapentaenoic acid (n-6DPA, 22:5n-6) in a short food chain leading to cod larvae. Using mass spectrometry (MS) they showed that n-6DPA had the properties of an essential fatty acid allowing them to postulate that it acts in concert with DHA, and also to test the hypothesis put forward by Metz et al. (2001) that EFA synthesized by an unusual biochemical pathway in bacteria and protists may be transferred to fish.

Known pathways of PUFA synthesis involve the processing of the saturated 16:0 or 18:0 by elongation and desaturation so that the synthesis of DHA from acetyl-CoA requires nearly 70 reactions. Polyketide synthase systems conduct the same reactions but in an abbreviated sequence. This route is found in some marine bacteria and primitive eucaryotes like the thraustochytrid protist *Schizochytrium* (Metz et al. 2001). This abbreviated pathway is thought to be responsible for providing an unusual fatty acid isotopic signature in Algamac® (Parrish et al. 2007) which consists entirely of spray-dried *Schizochytrium* sp. To determine this signature, fatty acid stable carbon isotope ratios ( $\delta^{13}\text{C}$ , ‰) were measured after combustion, by continuous flow – isotope ratio MS. Compound-specific carbon isotope determinations are a refinement of bulk isotope measurements commonly used in food web studies (e.g. Canuel et al. 1995; Stapp et al. 1999; Vander Zanden et al. 1999; Post et al. 2000).

Algamac is also unusual in that it has high proportions of n-6DPA (9%) which larval cod take up rapidly when it is made available in the diet (Parrish et al. 2007). This fatty acid is also present in a few species of microalgae (Nanton and Castell 1998; Milke et al. 2004, 2006) and especially in *Pavlova* sp. where it can reach levels similar to those found in Algamac (Pernet et al. 2005). Exceptionally high amounts can be found in labyrinthulids, another type of marine microorganism which is related to the thraustochytrids. One isolate produces n-6DPA as its sole PUFA, amounting to 48% of total fatty acids (Kumon et al. 2003). Extensive bioaccumulation of this C<sub>22</sub> fatty acid has been linked to improved growth in different scallop species and at different life stages (Milke et al. 2004, 2006; Pernet et al. 2005) as well as in cod larvae (Garcia et al. 2005, 2008). In sea scallop (*Placopecten magellanicus*) early veligers, the initial proportion of n-6DPA was well below dietary amounts (<2%: Pernet et al. 2005), but it increased markedly during ontogeny to become a major component (up to 20%). Marked accumulation of n-6DPA was observed in sea scallop larvae irrespective of diet and life stage: late veliger, pediveliger, or postlarvae. This fatty acid may be important in later stages as well. Alkanani et al. (2007) tested 140 variables to predict the growth of mussels starting at 2-cm length in the field. N-6DPA was among only four that were found to be significant.

The fatty acid n-6DPA may play an important structural role in membranes and/or may be a precursor of bioactive compounds. Despite the similarity in chemical structure and some physical properties, DHA and n-6DPA chains do pack differently in bilayers (Eldho et al. 2003). The DHA chain, with one additional double bond, is more flexible which would likely have an impact on membrane proteins and their functions. Rats fed n-3-deficient diets appear to try and maintain an overall C<sub>22</sub> PUFA level in brain phospholipids by replacing DHA with n-6DPA, but their behaviour is affected (Moriguchi et al. 2000).

In animals, eicosanoid bioactive compounds are derived from 20-carbon precursors (eicosa is Greek for 20), but 18-carbon compounds are also used in algae, for which the term 'oxylipin' is applied (Gerwick 1994). Recently the C<sub>22</sub> DHA has been found to be a precursor of bioactive compounds termed 'docosanoids' (docosa is Greek for 22) generated via enzymatic oxygenations (Hong et al. 2003, 2005). The same enzymes could work on the C<sub>22</sub> n-6DPA to form a parallel series of competitive products as found with the C<sub>20</sub> eicosanoid precursors, EPA and ARA.

### 13.6 Ratios and Groups of Essential Fatty Acids in Food Webs

The balance required in dietary fatty acids and in membrane fatty acids has led to a focus on dietary fatty acid ratios in aquaculture nutrition (and see Ahlgren et al. – Chap. 7). A DHA:EPA ratio of 2:1 has been promoted, based on the ratio in finfish eggs (Sargent 1995; Sargent et al. 1999a; Bell et al. 2003). Most marine fish larvae feed on copepod eggs and nauplii during the first weeks after the onset of exogenous feeding and 2:1 is also the ratio found in early life stages of some copepod species (e.g. *Eurytemora velox*: Shields et al. 1999). However, Copeman et al. (2002)

demonstrated a relationship between unusually high DHA:EPA ratios and increased growth and survival in cultured yellowtail flounder, calling into question the assumption in aquaculture nutrition that 'nature knows best'. Using dietary DHA:EPA ratios ranging from 1 to 8, a strong positive correlation was obtained between the ratio in the diet and larval size and survival. High DHA:EPA ratios may be a more general requirement for cold water marine finfish larvae since Park et al. (2006) found cod larvae fed dietary ratios between 9 and 10 had the best growth and survival. It is interesting that high DHA:EPA ratios may be important for copepods themselves. Higher dietary ratios improve survival, time to maturity, maturation rate, egg production, hatching success, and female length in some copepod species (*Acartia tonsa*: Jonasdottir and Kiorboe 1996; *Gladioferens imparipes*: Payne and Rippingale 2000; *Temora longicornis*: Arendt et al. 2005) with values ranging from ~0.0 to 52.3. It is also interesting that the DHA:EPA ratio has been used as a bioindicator to distinguish dinoflagellate from diatom containing natural samples, with ratios  $\geq 1$  signalling the dominance of dinoflagellates (Budge and Parrish 1998; Dalsgaard et al. 2003). One implication from this could be that dinoflagellate-based food webs would provide more favourable essential fatty acid ratios; however, some marine protozoa (e.g. *Oxyrrhis marina*: Klein Breteler et al. 1999) and some copepods (e.g. *Eucyclops serrulatus*: Desvillettes et al. 1997; *Tisbe* sp.: Nanton and Castell 1998) can make DHA from short-chain precursors, e.g. ALA. In the first example, though, we are still talking about a dinoflagellate, albeit a heterotrophic dinoflagellate.

Other fatty acid ratios of interest include  $\Sigma n$ -3PUFA:  $\Sigma n$ -6PUFA (e.g. Sargent 1995; Milke et al. 2004), EPA:ARA (e.g. Sargent et al. 1999a; Milke et al. 2004), n-6DPA:DHA (e.g. Moriguchi et al. 2000), n-6DPA:ARA (e.g. Milke et al. 2004; Garcia et al. 2008), and DHA:ARA (Ahlgren et al. 2005). A statistical relationship with a ratio implies that the two fatty acids are not interchangeable and that they compete with each other biochemically. If a unique value of the ratio is important it indicates that the fatty acids within the organism should be present in a certain proportion for optimal structure and/or function.

Another approach to examining the importance of essential fatty acids is to treat them as groups and to look both at group correlations and variability. This is akin to assuming fatty acid interconversion by consumers before use, or an unlimited substitution in their functional roles (Anderson and Pond 2000). By taking groups of biochemically related fatty acids the possibilities of elongation and desaturation and/or retroconversion are implied. Egg-hatching success in the calanoid copepod *Acartia tonsa*, for example, was related to the sum of ALA, EPA, and DHA (Broglia et al. 2003). An examination of PUFA of the same chain length may reveal a mainly structural function. For example, looking at the stability in the sum of n-6DPA and DHA proportions is a way of seeing if these two C<sub>22</sub> PUFA are substituting for each other (Moriguchi et al. 2000; Pernet et al. 2005). It is also possible to broaden this approach, for example by investigating the sum of LIN, ALA, ARA, EPA, and DHA (Kainz et al. 2004) which may reveal the importance and interchangeability of n-3 and n-6 PUFA. In a field study of mussel growth, the sum of ARA, EPA, and DHA was significantly correlated with growth, while DHA and ARA individually

were not (Alkanani et al. 2007), suggesting interchangeability of C<sub>20</sub> and C<sub>22</sub> PUFA, presumably in a structural role.

## 13.7 Conclusions

Aquatic organisms need certain n-3 and n-6 fatty acids to support optimal health. An absolute requirement for these PUFA in the diet will occur only if there is not a sufficient amount already in storage, if they cannot be made from other compounds, or if they cannot be replaced by other compounds. There are probably several fatty acids in aquatic food webs with essential fatty acid activity, but these will depend on the organism, its life stage, and on environmental conditions. It is important to investigate all n-3 and n-6 PUFA, the relationships among them, and whether they may be essential nutrients or metabolites and conditionally dispensable or indispensable. Essential fatty acid activity is expressed physiologically (e.g. in terms of growth response) and biochemically (e.g. in terms of membrane fatty acid composition).

Membrane phospholipids may be central to many of the mechanisms of the health effects of essential fatty acids. Incorporation of n-3 and n-6 fatty acids in membranes affects their physicochemical characteristics altering cellular functions including the properties of certain membrane-bound enzymes and binding to receptors. Dietary PUFA also exert their influence by changing the availability of substrate fatty acids in the membrane that are used as sources of bioactive compounds or messengers. The importance of different fatty acids and phospholipids with different head groups in regulation of cellular processes, together with the fact that fluidity may be controlled by just a few compounds, suggests that molecular species analyses should be more widespread in this field. This type of analysis, together with determinations of membrane fluidity and phospholipase A<sub>2</sub> activity, might go a long way to defining the essentiality of individual fatty acids.

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