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Fatty acids fromlipids ofmarine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects

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Abstract: Because of their characteristic living environments, marine organisms produce a variety of lipids. Fatty acids constitute the essential part of triglycerides and wax esters, which are the major components of fats and oils. Nevertheless, phospholipids and glycolipids have considerable importance and will be taken into account, especially the latter compounds that excite increasing interest regarding their promising biological activities. Thus, in addition to the major polyunsaturated fatty acids (PUFA) such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, a great number of various fatty acids
occur in marine organisms, e.g. saturated, mono- and diunsaturated, branched, halogenated, hydroxylated, methoxylated, non-methylene-interrupted. Various unprecedented chemical structures of fatty acids, and lipid-containing fatty acids, have recently been discovered, especially from the most primitive animals such as sponges and gorgonians. This review of marine lipidology deals with recent advances in the field of fatty acids since the end of the 1990s. Different approaches will be followed, mainly developing biomarkers of trophic chains in marine ecosystems and of chemotaxonomic interest, reporting new structures, especially those with biological activities or biosynthetic interest. An important part of this review will be devoted to the major PUFA, their relevance to health and nutrition, their biosynthesis, their sources (usual and promising) and market.

Keywords: Lipids · Fatty acids · Marine organisms · Biomarkers · Nutrition

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Abbreviations

AA	arachidonic acid 20:4(n-6)
DHA	docosahexaenoic acid 22:6(n-3)
EPA	eicosapentaenoic acid 20:5(n-3)
FA	fatty acid
FATM	fatty-acid trophic markers
GC/MS	gas-chromatography mass spectrometry
PUFA	polyunsaturated fatty acids
LC-PUFA	long-chain polyunsaturated fatty acids
VLC-PUFA	Very-long-chain polyunsaturated fatty acids
MUFA	monounsaturated fatty acids
NMI	non-methylene-interrupted fatty acids
NMID	non-methylene-interrupted dienoic fatty acids
PL	phospholipids
SFA	saturated fatty acids
TL	total lipids
EFA	essential fatty acid
TAG	triacylglycerols
SCO	single-cell oil

1 Introduction

Lipids are major sources of metabolic energy and essential materials for the formation of cell and tissue membranes. They are very important in the physiology and reproductive processes of marine animals and reflect the special biochemical and ecological conditions of the marine environment [1–3]. The interest of chemists, biochemists and biotechnologists in lipids and fatty acids (FA) from marine animals and algae has been stimulated, in particular, by the recognition that polyunsaturated fatty acids (PUFA) are important to human health and nutrition. They are required for reproduction and growth. The relative proportion and composition of FA in marine organisms are characteristic for every species and genus, and also depend on environmental conditions. Several comprehensive reviews are available on marine FA, their occurrence, their roles and the methods used in their analysis [4–8].

The principal role of neutral lipids, which in marine organisms consist predominantly of triacylglycerols (TAG) and wax esters, is as an energetic reserve of FA that are destined either for oxidation to provide energy (ATP) or for incorporation into phospholipids. Phospholipids are the building blocks for the membrane lipid bilayer. FA provide the hydrophobic interior of all cell membranes, forming an impermeable barrier to water and polar molecules and separating the cell contents from the extracellular medium. The physical properties of the membrane are determined by the individual lipids

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within the FA components of the lipids and their interaction with sterols and proteins. While their function as structural lipids in membranes has been known for a long time, ceramides and glycosyl ceramides (glycosphingolipids) play an important role in many fields of cell biochemistry, such as molecular recognition. In addition, ceramides from marine organisms have excited great attention as signal transducers, and some of them have been recognized as possessing antimicrobial and cytotoxic activities. Marine glycosphingolipids, chiefly isolated from sponges, show interesting biological activities such as immunomodulation and antitumoral activity [9]. The fatty acyl chains linked to these classes of compounds are often commonly occurring but several new and original structures have been reported recently [10, 11]. Arising from alpha-glycosphingolipids isolated from marine sponges, the simplest (KRN 7000) is being considered as an anticancer agent ([12], see Bourguet-Kondracki & Kornprobst, this book).

Fat not only provides energy, it facilitates the absorption of fat-soluble vitamins (vitamins A, D, E, and K), and plays an important role in the production and regulation of eicosanoids. In addition, lipid class and FA compositions can be used to understand and identify food web interactions.

Different approaches can be applied in this field of marine lipidology, focused on FA, mainly:

- searching for new FA structures
- evaluating new sources of major PUFA of biological interest
- evaluating their role in cell membranes
- investigating biosynthetic pathways
- developing trophic and/or chemotaxonomic biomarkers in ecosystems.

The rapid development of excellent analytical methods, especially gas liquid chromatography coupled to mass spectrometry (GC/MS), which can deal with complex mixtures, has also been a major contributing factor to the progress in the chemistry and biochemistry of marine lipids [5, 6, 13].

As the principal producers in the marine environment, microalgae support both pelagic and benthic food webs, and their lipids and FA are been extensively studied. Microalgae are known to have different FA compositions depending on their taxonomic position [14, 15]. At the next trophic level, zooplankton form an essential link between primary producers and higher-order consumers. Very recently, a complete review was devoted to fatty-acid trophic markers (FATM) in the pelagic marine environment [8]. The FATM concept is based on the observation that marine primary producers lay down certain FA patterns that may be conservatively transferred through aquatic food webs. Thus, they can be recognized in their primary consumers. The next step is concerned with the dynamics of FA in fish, which catabolize and transform dietary FA.

Marine invertebrates, especially sponges, have proved to be a rich source of many unusual FA. Sponges are very ancient animals with special structural features, particularly the cell membrane, which have allowed their adaptation

to often precarious environments. These primitive organisms are difficult to classify due to the few available useful morphological characteristics. The use of taxonomic knowledge allows investigation to be focused on genera that offer the great potential in metabolites of biological interest. Recently, a comprehensive taxonomy was published, which provides the state of the art [16]. Representatives of the Coelenterate phylum have remarkable peculiarities in their FA composition. Some species contain unusual FA or unusual concentrations of common components.

The main purposes of this paper are to illustrate the molecular biodiversity in marine lipids (from the first planktonic marine producers to fish) and, in another part, to focus on the most important PUFA (sources, biosynthesis, economy). This work has been done by considering the most interesting advances in marine lipidology since the end of the 20th century. Thus, without covering the subject exhaustively, this review deals with FA from total lipids or those specifically linked to a particular lipid class such as TAG, wax esters, ceramides, polar lipids and some additional atypical secondary metabolites. Thus, unusual or novel FA, and FA that are interesting due to their applications in chemotaxonomy, biosynthesis and as biomarkers, will mainly be taken into account.

2
Nomenclature of fatty acids

According to the international nomenclature, the position of the first double bond is given by the (n-x) notation, counting the number of carbon atoms from the methyl end. For instance, 18:4(n-3) identifies an FA with 18 carbon atoms and four double bonds, the first double bond occurring after the third carbon atom [4, 7] (Fig. 1). An additional PUFA is depicted in Fig. 1 (middle), namely 24:6(n-3).

According to an alternative notation, the locations of the double bonds are counted from C-1 (the carboxyl group). Thus, 18:4(n-3) is designated 6,9,12,15-18:4. This latter notation will be used in particular in the case of non-methylene-interrupted fatty acids (NMI FA), such as 7,13,17-20:3 shown above, the most commonly encountered among these being the dienoic acids (NMID) (Fig. 1, bottom).

The configuration of double bonds, generally assumed as *cis* (*Z*) in natural compounds, must be indicated in other cases. The positions of a methyl branch or another group is indicated by the number of the carbon atom on which the chain is substituted (e.g. 10-methylhexadecanoic acid or simply 10-Me-16:0; 2-hydroxydocosanoic acid or 2-OH-22:0 ; 6-bromo-5,9-heptacosadienoic acid or 6-bromo-5,9-27:2).

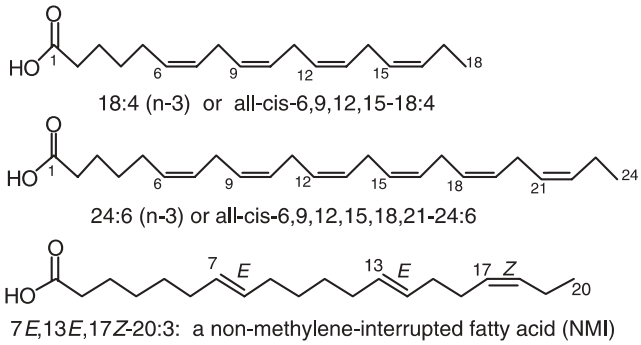

Fig. 1 Examples of numbering and designation of fatty acids (FA)

3
Fatty-acid biosynthetic pathways in primary producers

The basic processes of FA biosynthesis are summarized in Fig. 2. The de novo biosynthesis of FA follows the common pathway with major end products being mainly 16:0 and also 14:0, 18:0 and 20:0 (also produced by chain elongation) [8].

Then, an aerobic desaturation is catalyzed by the enzyme Δ9-desaturase to give rise to 16:1(n-7), 18:1(n-9) and 20:1(n-11). Generally, only plants are capable of biosynthesizing de novo (n-3) and (n-6) PUFA (Fig. 2A). Oleic acid 18:1(n-9) is the precursor of all (n-3) and (n-6) PUFA. The next double bonds are introduced to form 18:2(n-6) and 18:3(n-3). Through appropriate desaturations and chain elongations, 18:2(n-6) may be further converted to 20:4(n-6) (AA), and 18:3(n-3) to EPA. DHA is obtained via C_{24} PUFA intermediates rather than direct elongation of EPA, according to the so-called Sprecher pathway [21–23]. This biosynthetic scheme is typically observed in dinoflagellates, in which FA such as 18:4(n-3) and DHA are often dominant. Furthermore, the biosynthetic pathway producing 16:4(n-1) from 16:0 is characteristic of diatoms [8, 24]. The de novo biosynthesis of long-chain monounsaturated FA (MUFA) typically pronounced in calanoid copepods is showed in Fig. 2B. Biosynthetic considerations will be developed in detail in the last part of this review.

4
Marine bacteria and cyanobacteria

Marine bacteria are known for their role in nutrient cycling and the degradation of organic matter [8]. The roles of bacteria in marine food webs has two aspects, firstly as primary food sources, and secondly as components of

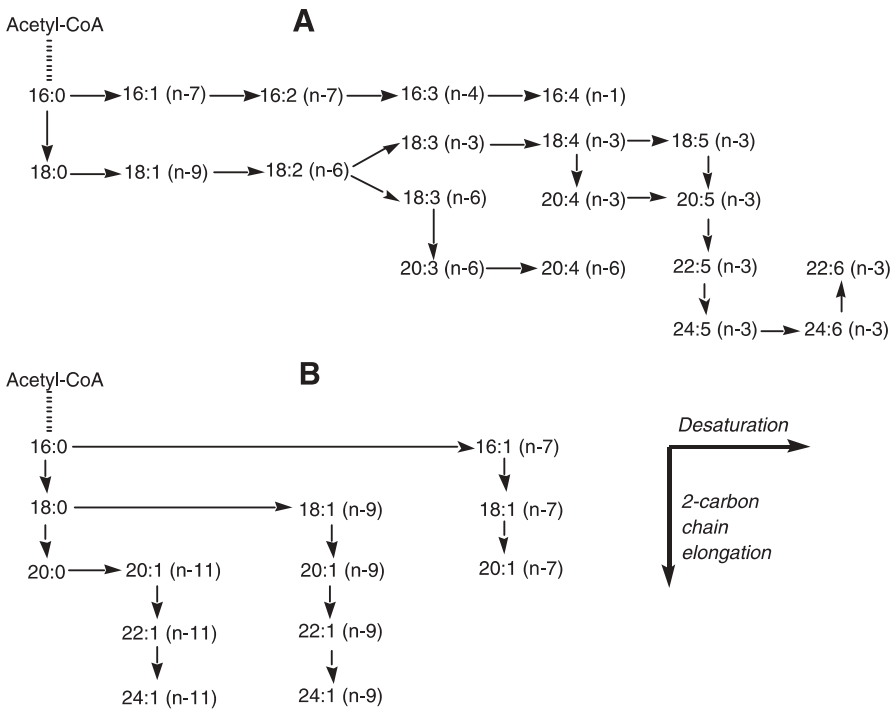

Fig. 2 Major pathways of FA biosynthesis in marine algae (**a**), modified after Gurr & Harwood [17] and Cook [18], and herbivorous calanoid copepods (**b**), modified after Sargent & Henderson [19] and Kattner & Hagen [20]. Extracted from Dalsgaard et al. [8]

the commensal microbial communities of marine animals [25]. Marine heterotrophic bacteria are abundant in sediments and as colonizers of settling particulate matter following plankton blooms [26]. That observation explains why the FA composition of marine bacteria has mainly been studied by geochemists [26–29]. Bacteria incorporate FA mainly in PL. Bacterial FA are commonly saturated (SFA) and monounsaturated (MUFA), ranging from C_{10} to C_{20}, whereas PUFA are quite rare. Bacterial FA biomarkers are typically odd-numbered, branched *trans*-unsaturated and cyclopropyl FA such as 15:0, 17:0, *iso*- and *anteiso*-branched SFA and MUFA, 10-methyl-16:0 ([8] and references therein).

Furthermore, deep-sea bacteria and several bacterial strains (*Pseudomonas*, *Vibrio*) have been shown to be capable of producing (n-3) PUFA, as recently reviewed [24, 25, 30–32]. Very little is known about the biosynthesis of PUFA in bacteria. EPA and DHA in bacteria are contained within phospholipids rather than TAG. Therefore, marine bacteria seem to be of limited use as a source of oils rich in (n-3) PUFA [24]. (see the chapter entitled “Promising sources of marine LC-PUFA”)

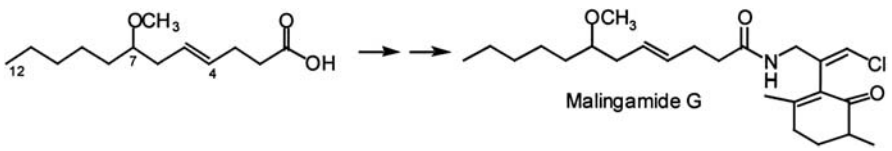

Fig. 3 Malingamide G and its potential precursor 7-methoxy-dodec-4-enoic acid

Cyanobacteria, a class of photosynthetic prokaryotes occurring in the phytoplankton, produce C_{18} PUFA esterified to polar lipids, but they do not biosynthesize EPA or DHA [24]. EPA production was obtained by a transgenic marine cyanobacterium carrying a plasmid containing the essential open reading frames for EPA synthesis [33].

Fatty-acid amides are widespread in nature [34]. They are incorporated into some lipid classes such as ceramides, glycosphingolipids and various *N*-acylated lipid molecules. A new diacylgalactolipid was isolated from the marine cyanobacterium *Oscillatoria* sp. comprised of 9,12-octadecadienoyl and 4-hexadecenoyl chains [35]. Cyanobacteria of the genus *Lyngbya* are a rich source of bioactive secondary metabolites including fatty-acid amides [36]. A series of biologically active malyngamides has been identified from marine cyanobacteria with a mid-chain methoxylated fatty acyl chain [37–40]. As an example, Malyngamide G and the new 7-methoxydodec-4(*E*)-enoic acid (its possible precursor) were isolated from *Lyngbya majuscula* collected off the French Mediterranean coast (Fig. 3).

Both these compounds are non-cytotoxic to KB cells and show immunosuppressive activity [39]. In several other malyngamides, the fatty-acid amid is 7-methoxy-tetradecen-4-enoic.

5 Phytoplankton

Primary producers provide the basic FA patterns in marine food webs. They consist of macroalgae and phytoplankton, which mainly comprise microalgae and photoautotrophic bacteria. Phytoplankton in the pelagic environment comprises mainly Bacillarophyceae (diatoms), Dinophyceae (dinoflagellates) and Prymnesiophyceae. Algal FA are biosynthesized in the chloroplasts comprising the thylakoid membranes, and are chiefly esterified to glycolipids rich in (n-3) PUFA. During the exponential growth phase of phytoplankton blooms, carbon fixed through photosynthesis is allocated to growth and cell division rather than lipid storage. Therefore, the level of glycolipids is particularly high in this phase, and the proportion of (n-3) PUFA can reach 50% of total lipids [8, 14, 41, 42]. It is well known that plants are usually the only organisms that can biosynthesize de novo the acids 18:2(n-6) and 18:3(n-3).

These FA and their principal derivatives (e.g. AA, EPA and DHA) are essential constituents of heterotrophic organisms. Thus, algae occupy a central position within marine food webs.

As shown in a recent review, FA patterns can be used as potential taxonomic markers regarding the presence and combinations of certain FA that can be characteristic of particular algal classes, whereas individual FA cannot be used as indicators of particular algal species [43]. This approach has been developed in the case of diatoms and dinoflagellates, two important classes in marine environments. Thus, high values of 16:1(n-7)/16:0 (typically > 1), and $\sum C_{16}/\sum C_{18}$ have been associated with a dominance of bacillariophytes [28, 29, 44]. Furthermore, high values of 18:5(n-3)/18:3(n-3) and $\sum(C_{18}$ PUFA, C_{22} PUFA) have been associated with dinophytes [45]. Bacillariophytes can be distinguished from dinophytes by means of high values of $\sum C_{16}/\sum C_{18}$together with low values of 18:5(n-3)/18:3(n-3) [45]. This criteria can be reinforced by the examination of the ratio 22:6(n-3)/20:5(n-3) [28]. A value ≥ 1 indicates a predominance of dinophytes, while a value < 1 indicates a predominance of bacillariophytes. The lipids of diatoms, characterized by high levels in EPA and the absence of DHA, are also rich in C_{16} PUFA [24].

If investigations of FA isolation and purification have principally been carried out on (n-3) PUFA, there are other potentially interesting FA commercially unavailable. Thus, the acids 16:3(n-4), 16:2(n-4) and 16:2(n-7) were isolated as methyl esters by means of liquid chromatography using a porous graphitic-carbon phase [46].

The taxonomy of the Raphidophyceae is still uncertain and needs the help of chemotaxonomic data, such as FA composition, in order to distinguish correctly the genera [45–49]. The FA compositions of twelve raphidophyte strains were established and were very similar to previous data [47]. The major PUFA were EPA (14.8–24.5%) and 18:4(n-3) (12.0–26.6%, with an exception at 0.3%) [49]. High levels of free FA were observed in lipids of *Fibrocapsa* species (23.6–37.9%). FA profiles allowed clear discrimination between the genera. By using a selected EPA-deficient mutant of *Porphyridium cruentum*, it has been demonstrated that TAG of the red microalga *P. cruentum* can contribute to the biosynthesis of eukaryotic galactolipids [50]. FA biomarkers were used to investigate the biogeochemistry of a former blue-mussel aquaculture site and the high levels of PUFA found indicated a substantial phytoplankton source (diatoms and dinoflagellates) [29]. FA composition of toxic microalgae have been determined to detect useful biomarkers in screening seawater seafood samples [51]. Two *Pseudo-nitzschia* species were studied and both displayed similar FA compositions typical of diatoms, including 16:1(n-7), 16:2(n-4) and EPA as major unsaturated FA. 16:4(n-1) occurred in both species and therefore could be used as a signature compound in differentiating toxic *Pseudo-nitzschia* from other diatoms [51]. Furthermore, the possibility of using boiling water to deactivate lipolytic enzymes, as previ-

ously found [52], was confirmed, and it was suggested that some mechanisms of PUFA degradation was also inhibited [51].

The FA compositions of microalgae have been shown to change in response to changes in salinity. Green unicellular microalgae of the genus *Dunaliella* are known for their capability to grow at high salinities up to salt-saturated water [53]. The major unsaturated FA in two *Dunaliella* isolates originating from Antarctic hypersaline lakes were 16:4(n-3), 18:3(n-3), 18:2(n-6), 18:1(n-9) and 16:1(n-7). The results suggest that the appropriate environment necessary for the growth of these halophilic species is a certain level of osmotic pressure in the medium [53].

High levels of DHA are noticeable in the lipids of the Dinophyceae, another major component of marine phytoplankton, together with significant amounts of EPA, 18:5(n-3) and 18:4(n-3) [24, 54]. Thus, it seems possible that dinoflagellates are capable of synthesizing DHA through the chain-shortening of 24:6(n-3), since a similar mechanism could produce 18:5(n-3) from EPA [24]. The presence of EPA and also DHA and 18:5(n-3) has been linked to potential toxicity in a raphidophyte *Heterosigma* as well as in a dinoflagellate *Gymnodinium* [55, 56]. It was suggested that the mode of action in the ichthyotoxicity of these harmful bloom-forming flagellates is correlated to oxygen radical formation [49]. Two *Gymnodinium* species and several other dinoflagellate species were found to contain unusual octacosapolyenoic FA, namely 28:7(n-6) and 28:8(n-3) at levels up to 2.2% of total FA [57] (Fig. 4).

VLC-PUFA have also been found in some autotrophic and heterotrophic lower organisms such as microalgae, fungi, sponges and bacteria [59], and most of them are either saturated or monounsaturated. Such FA were identified in Baltic herring, namely 28:7(n-3) and 28:7(n-6) [58]. Furthermore, the two *Prorocentrum* species studied were found to contain 18:4(n-3) (12.7–15.3%), 18:5(n-3) (36.4–37.6%) and DHA (18.3–22.0%) [57, 58]. 28:8 (n-3) was also identified in a commercially available oil, used as dietary supplements of PUFA for humans, from the heterotrophic dinoflagellate *Crypte-codinium cohnii* [60]. Furthermore, these unusual octacosapolyenoic FA also occurred as minor components in lipids of all five dinoflagellates studied for fatty acid and sterol compositions [57]. The major unsaturated FA were as followed: 18:4(n-3) (2.5–11.5%), 18:5(n-3) (7.0–43.1%), EPA (1.8–20.9%) and

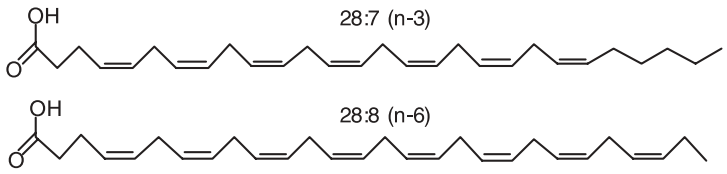

Fig. 4 Octacosapolyenoic FA from dinoflagellates

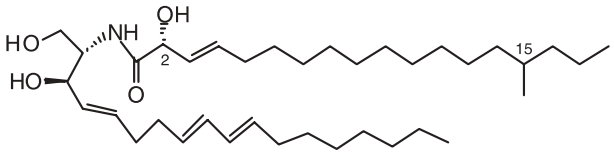

Fig. 5 New ceramide from the dinoflagellate *Coolia monolis*

DHA (9.9–26.3%). A new ceramide isolated from the epiphytic dinoflagellate *Coolia monotis* bears a novel fatty acyl moiety, namely 2-hydroxy-15-methyl-3-octadecenoyl [61] (Fig. 5).

The fatty acid composition of polar lipids and TAG was determined in different types of symbiotic dinoflagellates isolated from several hermatypic corals from a fringing reef in Japan [15]. 18:4(n-3) (10.0–26.2%) and DHA (10.6–17.8%) were found as major PUFA in polar lipids of symbiotic dinoflagellates isolated from all species studied. In addition, polar lipids of dinoflagellates from *Millepora intricata* were different from those originating from other corals in that they contained high amounts of 18:5(n-3) (8.7%) and 22:5(n-6) (10.3%). It was suggested that FA might provide useful information on possible taxonomic differences among symbiotic dinoflagellates [15].

6 Macroalgae

FA in marine algae have attracted considerable attention among researchers because they can produce significant amounts of interesting PUFA and provide useful distinguished features of chemotaxonomic value [62]. The data available on lipids from macroalgae have been reviewed recently [8]. Three classes are mainly concerned: Chlorophyceae, Rhodophyceae and Phaeophyceae. FA from 11 macroalgae from the French Brittany coast were studied [63]. More recently, Li et al. [62] reported the FA compositions of 22 species of marine macrophytes, collected from the coast of the Bohai Sea belonging to the three aforementioned algal classes. These algae have FA patterns typical of red, brown and green algae from other regions. In general, red algae from the Bohai Sea contained high levels of C_{20} PUFA, primarily that of EPA (up to 37.5%) and AA (up to 29.4%). The main difference in the FA compositions between red and brown algae was that the latter were richer in C_{18} PUFA, especially in 18:4(n-3) (up to 20.1%). Seven of the teen[CE b] brown algal species studied also contained EPA as a major component, accounting for 8.4–24.2%. Green algae studied had the highest level of C_{18} PUFA, mainly 18:3(n-3) (20.5–27.2% of total lipids) and 18:4(n-3), and the lowest level of C_{20} PUFA [62]. Interestingly, another study of FA composition of

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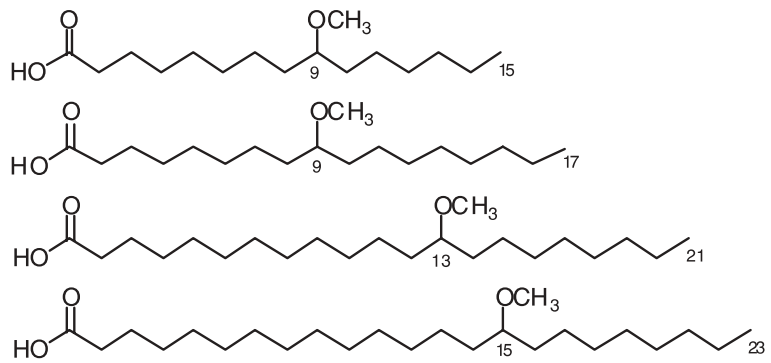

Fig. 6 New methoxylated FA from lipids of *Schyzimenia dubyi*

19 species of the same algal classes, collected on the Pacific coast of North California gave rise to very similar conclusions [64]. Red Californian algae contained AA (5.3–23.4%) and EPA (27.8–45.4%). Brown algae contained 18:4(n-3) (3.6–18.6%) and EPA (3.1–15.5%). Two of the three green algae studied contained 16:4(n-3) (13.6–16.2%) and 18:4(n-3) (12.1–22.1%). Both these studies show that red, brown and green algae have distinguished FA profiles that do not depend on the geographical location of the algae and that have a chemotaxonomic significance for seaweeds [62, 64]. A recent comparative study of FA composition of Arctic and Antarctic macroalgae considered their use as indicators of phylogenetic and trophic relationships [65]. Several eicosanoids, metabolites of AA, such as hydroxytetraenoic acids, associated with prostaglandins, were identified in a Japanese red alga *Gracilaria asiatica* [66].

Methoxy FA are not very widespread in nature [67, 68]. Several mid-chain methoxy FA have been reported only in certain microorganisms and marine cyanobacteria (genus *Lyngbya*), as seen above. Four novel mid-chain methoxy FA (16% of total acid mixture) were identified in lipids from a red alga as 9-MeO-15:0, 9-MeO-17:0, 13-MeO-21:0 and 15-MeO-23:0 acids [69], as depicted in Fig. 6.

These algal lipids contained C_{14}-C_{28} SFA, accounting for 77%, and hydroxylated FA, but no PUFA. Furthermore, marine sponges have provided new 2-methoxy long-chain acids that will be presented in a following chapter.

7 Zooplankton

During the last decade, zooplanktonic organisms from Arctic and Antarctic waters have given rise to intense research, especially on lipids [70–90].

These investigations have highlighted the general lipid characteristics in high-latitude zooplankton communities, such as copepods and ctenophores, in terms of food webs and biomarkers. The Southern Ocean has a complex food web including planktivorous herbivores (krill, salps, copepods) that are fed upon by fish, squid, seals and whales [83, 87]. Krill (*Euphausia superba*) provide 30–90% of the diet for these carnivores and have an estimated standing stock biomass of 200–400 million metric tons. This high biomass reflects krill's ability to adapt to marked seasonality in food supply. Krill are primarily herbivores feeding on phytoplankton in the summer. In the winter krill feed mainly on ice algae [87, 89]. Krill lipids have been intensively studied because of their commercial interest and undisputed importance in the Southern Ocean, while the importance of gelatinous organisms, such as salps, ctenophores and medusae, is now recognized in marine pelagic ecosystems.

As the best-studied group of zooplankton with respect to the FA trophic-markers concept, herbivorous calanoid copepods have been mainly examined. They dominate the zooplankton biomass in high-latitude ecosystems and accumulate large lipid reserves. In addition, the FA characteristics of omnivorous and carnivorous copepods have been summarized, especially using FA as markers of carnivory [8]. Moreover, calanoid copepods are themselves important producers of specific FA and fatty alcohols (from wax esters). They play an important role in polar food webs and provide higher trophic levels with a lipid-rich high-energy diet [71]. However, similarities and differences between species emerge more clearly if the major lipid classes are analysed separately to determine their compositions. Phospholipids and the storage lipids (TAG and wax esters) are expected to exhibit strong compositional differences, which may provide additional information on their structural and energetic functions [71].

The compositions of wax esters, TAG and phospholipids in nine Arctic and Antarctic copepods have provided evidence of energetic adaptations with similarities and differences between the species [71]. The wax esters of the herbivorous species were clearly characterized by the long-chain monounsaturated FA 20:1(n-9) and 22:1(n-11), whereas the omnivorous and carnivorous species usually had high relative amounts of 18:1(n-9). The phospholipids contained very high levels of PUFA, especially 22:6(n-3). The phospholipid FA compositions in both Arctic and Antarctic species were found to be very similar. This extremely high degree of unsaturation (EPA and DHA together accounted for 46–60% of the total phospholipid FA) is quite unusual. In a recent investigation, the variation in the FA content was related to the spatial distribution of krill and the available diet as well as to maturity and sex [83]. *E. superba* are known as essentially herbivores when phytoplankton is abundant, but they can be omnivorous if algal biomass becomes relatively low. Three regionally groups of krills were considered. Krills from one group, almost exclusively juveniles, were surviving in the region characterized by

lowest algal biomass and had probably resorted to carnivory on PUFA-rich copepods [83]. The latter krill had unusually high level of PUFAs, mainly 18:4(n-3), EPA and DHA. Changes in lipid composition of the Antarctic *E. superba* were investigated regarding the influence of geographical location, the sexual maturity stage and the distribution among organs [73].

Lipid metabolism of *E. crystallorophias* and its ecological implications have been studied because this euphausiid is the dominant krill species in high-Antarctic waters. Thus, it has to cope with the most extreme environmental conditions of all euphausiids [76]. Wax esters were the primary storage lipids and accounted for up to 55.6% of total lipids, including 18:1(n-9) and 18:1(n-7) as major components (together up to 90% of total wax esters acids). 16:0, EPA, DHA and 18:1(n-9) were the major phospholipid FA components.

Little is known about lipids and FA of a member of gelatinous zooplankton, the salps (Tunicates). The pelagic tunicate *Dolioletta gegenbauri* is abundant in the North Atlantic and occurs in frequent blooms [74]. Polar lipids were the dominant lipid classes. The FA composition was largely dominated by EPA (13–14%) and DHA (27–30%).

8
Marine invertebrates

8.1
Sponges

Marine sponges are the most primitive multicellular animals and contain many new metabolites, including lipids, in particular glycolipids and phospholipids [91–97]. Thus, sponge lipids are one of the richest source of unusual FA. Sponges are very ancient animals with special structural features in their cell membranes, in particular phospholipid FA and sterols, since sterol–phospholipid interactions are assumed to play a major role in cell membranes [91–93]. Furthermore, sponge classification needs to be supported by chemotaxonomic criteria, in particular regarding FA. Recently, a comprehensive taxonomy was published, which provides the state of the art [16]. Marine invertebrates, e.g. sponges, are filter feeders and consequently they can be associated with microorganisms. Thus, particular FA appear as biomarkers for such organisms. It has been chosen here to focus on the most unusual recent data, such as unsaturated or branched patterns.

8.1.1
Δ5,9 fatty acids

Major sponge phospholipid FA include very low amounts of the usual methylene-interrupted PUFA, or none at all, but high levels of very-long-

chain acids (C_{23} to C_{34}, representing up to 80%); these are the so-called demospongic acids. Sponges (Demospongia) are a source of novel FA structures, especially unusual long-chain Δ5,9 FA with no counterpart in the terrestrial world, with sometimes a third double bond or a bromine atom [93, 96–102]. In each set of sponge phospholipids studied, about 50 to 70 FA were identified. Some sponges contain up to fifteen Δ5,9 FA in their phospholipids and, in several cases, one of them accounted for more than 50% of the total phospholipid FA mixture [99, 102]. Such NMI FA are showed in Fig. 7.

Approximately twenty Δ5,9 unsaturated FA were found in these sponges [102]. It is generally admitted that Δ5,9 demospongic acids are biosynthesized by Demospongiae through short-chain unsaturated FA, mainly from exogenous Δ9-16:1 [93]. The sponge *Geodinella robusta* was very interesting in that it contained an unusually high level of free FA, mainly with Δ5,9 unsaturation, including the rare *anteiso*-5,9-24:2 acid (19.5% of the total free FA), and the new *iso*-5,9-24:2 acid (30%) [103]. Mixtures of these latter compounds showed cytotoxic activity against mouse *Ehrlich carcinoma* cells and a hemolytic effect on mouse erythrocytes [103]. The FA of total lipids from *Halichondria panicea* included Δ5,9,19-26 a major component (33%), and six other Δ5,9 FA [104]. Several common PUFA, such as AA, EPA and DHA, occurred in this sponge. The 5,9,23-triacontatrienoic methyl ester, isolated as a natural compound by bioassay-guided fractionation from the marine sponge *Chondrilla nucula*, is an elastase inhibitor with the potential to be a therapeutic agent in some diseases such as pulmonary emphysema and chronic bronchitis [105]. The 5,9,21-30:3 sponge acid was reported as a DNA topoisomerase inhibitor [106].

Brominated FA from marine invertebrates have also been reviewed [100]. Recently, a comprehensive review of natural halogenated FA included those from marine algae and invertebrates [36]. *Cinachyrella* sponges from the Red Sea also contained three 6-brominated acids with the new 6-bromo-5,9-nonacosadienoic [102]. 6-Bromo-5,9,24-27:3 and 6-bromo-5,9,24-28:3 acids have been isolated from the sponge *Xestospongia* sp. [107, 108]. It should be noted that the latter FA, and the 6-bromo-5,9-heptacosadienoic acid, displayed moderate activity against murine leukaemia L1210 cells and against human carcinoma KB cells [108].

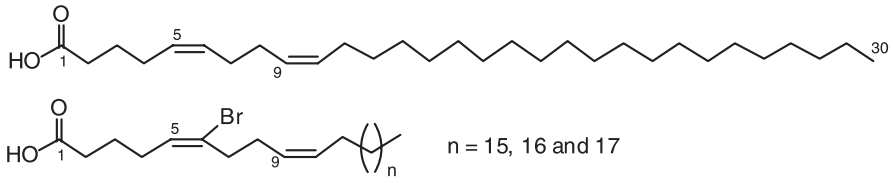

Fig. 7 Δ5,9 fatty acids from *Cinachyrella* sponges

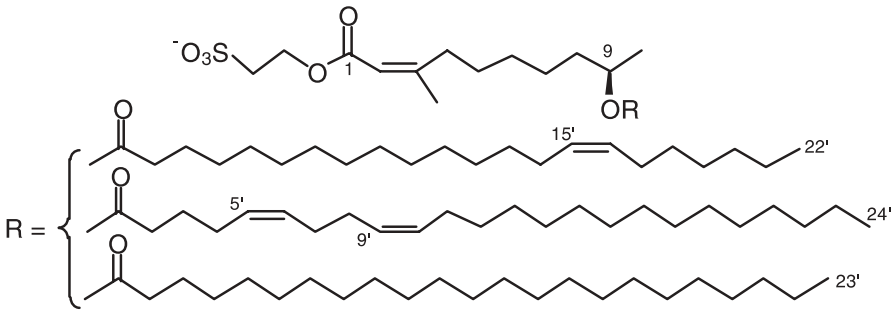

Fig. 8 Irciniasulfonic acid from *Ircinia* sp.

Nevertheless, it was demonstrated that sponges are not the only source of these unusual Δ5,9 FA since they have been observed in zoanthids and sea anemones [109, 110], gorgonians [100, 111]. Thus, the novel *iso*-5,9-pentadecadienoic acid was isolated from *Eunicea succinea* and prepared by synthesis [111]. In addition, new 6-bromo-5,9-eicosadienoic acid was identified from an anemone and a zoanthid [110].

A novel FA analogue, named irciniasulfonic acid, has been isolated from the Japanese marine sponge, *Ircinia* sp. Its structure consists of three different kinds of acids, i.e. common FA, a novel unsaturated branched C_{10} FA and an isethionic acid. Irciniasulfonic acid and deacyl irciniasulfonic acid reverse multidrug resistance in human carcinoma cells caused by overexpression of membrane glycoprotein [112] (Fig. 8).

8.1.2 Branched fatty acids

Branched FA including isoprenoid acids have often been found in marine sponges. The main isoprenoid FA are 4,8,12-trimethyltridecanoic and phytanic acids [95, 96, 101, 102]. As a recent example, phospholipid FA of two sponges of the genus *Cinachyrella* from the Red Sea were studied and compared with previous results for other *Cinachyrella* sponges [102]. Five SFA not hitherto found in nature were identified, namely 17-methyl-24:0, 18-methyl-24:0, 18-methyl-25:0, 18-methyl-26:0 and 18,24-dimethyl-26:0 acids [102] (Fig. 9).

The rare 10,13-dimethyl-14:0 and the new 9,13-dimethyl-14:0 were identified in marine sponges [102, 114]. The occurrence of bacteria in sponges is supported by the huge levels of phosphatidylglycerol and phosphatidylinositol that are characteristic of bacterial membranes [93]. In addition, several branched short-chain FA were identified, which are typically of bacterial origin, such as *iso*- and *anteiso*-15:0, *iso*- and *anteiso*-17:0, 10-methyl-16:0, 13-methyl-16:0, 10-methyl-18:0, and 11-methyl-18:0 acids. The new branched

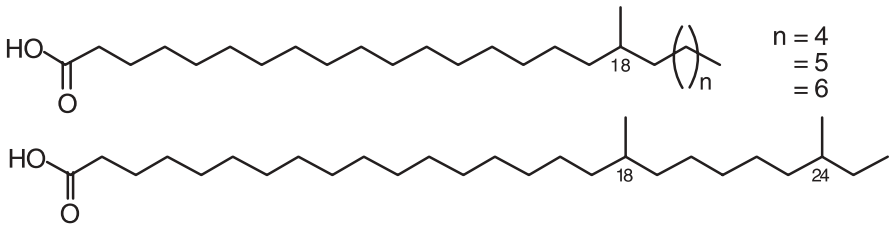

Fig. 9 New long-chain branched FA from *Cinachyrella* sp.

long-chain acids probably arise from shorter precursors of exogenic origin through a homologation process [102]. Other sponges were found to be rich in such branched FA, including additional branched FA, 3-methyl branched short-chain acids and the new 8,10-dimethyl-16:0 acid [101, 115–117]. The lipids of the sponge *Hymeniacidon sanguinea* from the Black Sea contained 73 FA, including the novel 13-methyl-20:0, 15-methyl-22:0 and 3,13-dimethyl-14:0 [116]. The compositions of lipids in this sponge collected from two locations with different ecological conditions (Canary Islands and Black Sea) were compared [94, 116]. The significant differences in the structures and relative concentrations of the typically bacterial FA, and the occurrence of cyclopropane-containing FA only in the Canary Islands sponge, suggests that the symbiotic bacteria in the two species are different [116].

A most interesting finding in *Callyspongia fallax* was a series of *iso* monoenoic branched-chain C_{15}-C_{17} and double bonds at either $\Delta 4$, $\Delta 5$, $\Delta 6$, $\Delta 7$, or $\Delta 9$ [117]. The lipids of the stromatoporoid *Astrosclera willeyana* (a "living fossil" sponge) and the demosponge *Agelas oroides* contained complex isomeric mixtures, at large amounts, of branched FA including *iso*-/*anteiso*-branched FA and abundant mid-chain branched acids present in the C_{15} to C_{25} range. These compounds are most likely derived from specific heterotrophic bacterial symbionts [118].

8.1.3 Methoxylated fatty acids

Methoxylated FA are relatively rare in nature and limited to primitive organisms such as cyanobacteria, bacteria and sponges [66, 101, 117, 122]. The first naturally occurring α-methoxylated FA were found in phospholipids from the sponge *Higginsia tethyoides*, which contained saturated, monounsaturated and diunsaturated α-methoxylated FA with chain lengths between 19 and 28 carbon atoms [121, 122]. Methoxylated lipids have been recently reviewed with an emphasis on the alkylglycerol ethers and FA bearing the methoxy group in the alkyl chain [119]. In that recent review, 29 α-methoxylated acids were listed [67]. These phospholipid α-methoxylated FA share the common molecular properties of possessing the *R* configuration

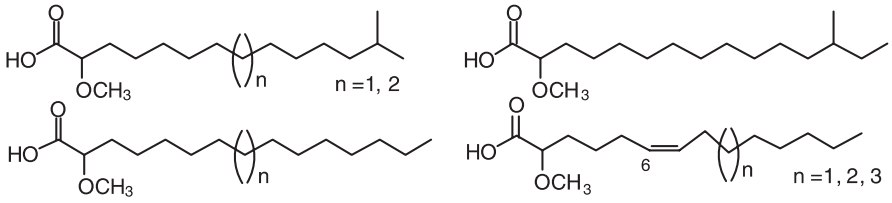

Fig. 10 Some α-methoxylated FA identified in Caribbean sponges

at the chiral center [123]. While the first very-long-chain α-methoxylated FA (C_{19}–C_{28}) could have arisen from sponge cells, recent examples of short-chain analogs (C_{14}-C_{18}) are postulated to originate from bacteria in symbiosis with sponges. A novel series of α-methoxylated FA have been reported from Caribbean sponges from the genera *Amphimedon*, *Callyspongia* and *Spheciospongia* (Fig. 10) [122, 123]. Various syntheses of methoxy FA have been performed [124].

8.1.4 Acetylenic fatty acids

Acetylenic FA have been found in sponges [125, 126]. Brominated C_{16}, C_{18}, and C_{20} acetylenic FA have been reported from sponges of the genera *Xestospongia* and *Oceanapia* [127]. An already known brominated acetylenic acid was isolated from the sponge *Xestospongia testudinaria* by bioassay fractionation (A_1 adenosine receptor affinity), as shown in Fig. 11 [128].

This FA was the active compound. Two novel steryl esters with bromoacetylenic chains that were also isolated were found to be inactive. A C_{14} acetylenic acid from the sponge *Oceania* sp. showed significant antimicrobial activity against various bacteria and fungi [129]. Recently, new acetylenic FA from the *Steletta* sponge species exhibited weak cytotoxicity against a human leukemia cell line [130] (Fig. 12). The second compound from *Stellata* is a symmetric dimer of the first, and is the first example of a sponge metabolite possessing an acid anhydride functionality.

In a recent work, a sulfated C_{24} acetylenic FA, namely callysponginol sulfate A, was isolated by bioassay-guided fractionation from the Japanese sponge *Callyspongia truncata* (Fig. 13) [131].

Callysponginol sulfate A is the first example of an acetylenic acid containing a sulfate group from marine organisms. These compounds inhib-

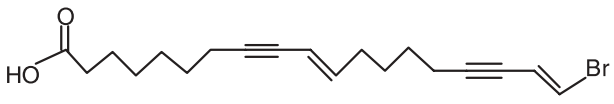

Fig. 11 Biologically active FA from *Xestospongia testudinaria*

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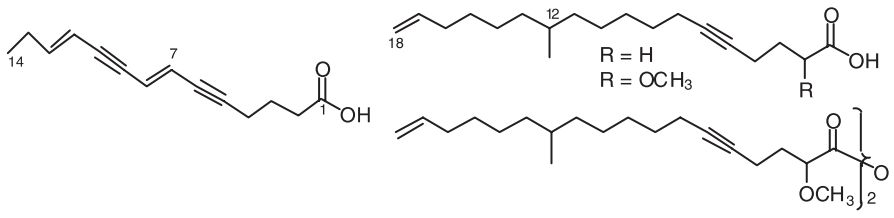

Fig. 12 New acetylenic FA from (**a**) *Oceanapia* and (**b**) *Stellata*[TS c]

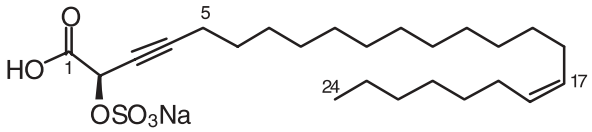

Fig. 13 Callysponginol sulfate A from *Callyspongia truncata*

ited membrane type 1 matrix metalloproteinase (MTP1-MMP), one of the key enzymes involved in tumor growth, migration, angiogenesis, invasion and metastasis [131]. Recently, new lysophosphatidylcholines and monoglycerides were reported from *Stelletta*, which include acetylenic fatty acyl chains and dimethyl branched chains [132].

Ceramides have received increasing interest because of their various properties, including antifungal and antimicrobial activities. Recently, several new ceramides have been isolated from various marine sponges including *Haliclona tenuiraniosa* (Fig. 14A) [133, 134]. They contain the usual saturated C_{20} to C_{26} fatty acyl chains. Biofouling organisms such as barnacles, mussels and macroalgae cause serious damage to ship's hulls and aquaculture nets. Thus, it is very important to identify nontoxic alternatives to the organotin compounds currently used. The sponge *H. koremella* provides a new ceramide with activity as an antifouling substance against macroalgae (Fig. 14B) [133]. This work showed that the length of the acyl residue seems to be important for the antifouling activity of ceramides. Recently, a mixture of ceramides has been isolated from the red alga *Ceratodictyon spongiosum* containing the symbiotic sponge *Sigmadocia symbiotica* [135]. Their non-hydroxylated acyl chains ranged from C_{22} to C_{24}. In addition, a unique 24-methylenecholesteryl ester (19:0) was characterized in these organisms.

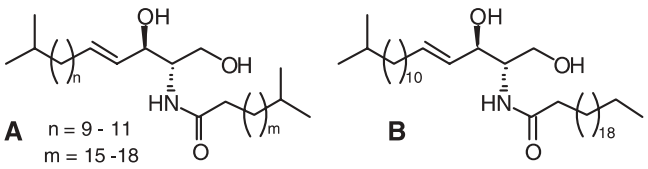

Fig. 14 Ceramides from the sponges *Haliclona tenuiraniosa* (**a**) and *H. koremella* (**b**)

Glycosphingolipids (GSL) are ubiquitous membrane constituents in animals that play fundamental roles in major phenomena such as cell–cell recognition and antigenic specificity. GSL from marine sponges possess interesting immunostimulatory and antitumor activities [9–11, 136–141]. Sponges are a very rich source of new glycolipid structures [9–11, 139–141]. Two glycolipids were isolated from the sponge *Pseudoceratina* sp.: a galactosyl diacylglyceryl, and an alkylacylglycerol linked to a five-membered cyclitol, with common fatty acyl chains ranged from 14:0 to 18:0 [142]. Ectyoceramide, the first natural hexofuranosylceramide has been isolated from *Ectyoplasia ferox* in a pure form, instead of a complex mixture as is usually found [11]. The FA attached to the amino group is a 2-hydroxylated *anteiso*-16:0. It has been demonstrated that a change in the stereochemistry of the glycosidic linkage from the usual β to the quite rare α configuration can affect their biological activity. Thus, α-glycosyl GSL, such as the natural Agelasphines and their synthetic derivative KRN7000 [143–145], are immunomodulating and antitumor compounds. Not all sponge glycolipids are GSL and a great variety of structures is observed.

These compounds stimulated the microtubule polymerization at 10 °C. The investigation of glycolipids from the Caribbean sponges allowed the isolation of Plakosides A and B from *Plakortis simplex* [10] (Fig. 15), and Plakosides C and D from *Ectyoplasia ferox* [140] (Fig. 15). These glycolipids are among the most fascinating lipids isolated from marine organisms. They have a unique structure with a prenylated galactose and two cyclopropanyl alkyl chains. The Plakosides had the same α-hydroxylated C_{22} fatty acid amid[CE d] with a cyclopropane at the C11–C12 positions. As the sponges are taxonomically distant, it is possible that these unusual glycolipids may originate from bacteria. Plakosides A and B are immunosuppressants that act through a non-cytotoxic mechanism [9, 10].

Crasserides, and their minor associated compounds isocrasserides, are widely distributed in marine sponges, and are considered to be glycolipids although their sugar unit is replaced by an unusual five-membered cyclitol [146] (Fig. 16).

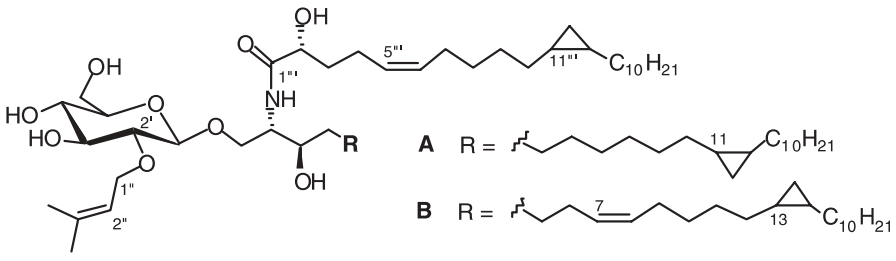

Fig. 15 Plakosides A and B from *Plakortis simplex*, and Plakosides C and D from *Ectoplasia ferox*

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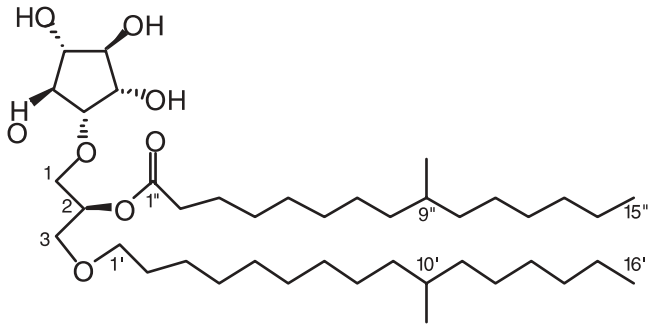

Fig. 16 An example of an isocrasseride isolated from *Plakortis simplex*

The cyclitol is linked to the glycerol with an *O*-3 ether bond. Also linked to the glycerol are an *O*-1 alkyl group and an *O*-2 acyl group. Furthermore, various fatty acyl chains are present including mid-chain branched chains. The Italian group has found crasserides in all sponges whose glycolipids have been studied.

8.2 Coelenterate – Cnidaria

Representatives of the Coelenterate phylum have remarkable peculiarities in their FA composition. Some species contain unusual FA or unusual concentrations of common components. Thus, large amounts of tetracosapolyenoic acids, namely 24:6(n-3) and 24:5(n-6), were found in different orders of the Octacorallia subclass of Anthozoa [147]. Gorgonian corals are of interest since some of these invertebrates are known sources of methylene-interrupted PUFA, in particular of the (n-3) and (n-6) series [148, 149]. The gorgonian specimens harvested in colder waters contained high amounts of methylene-interrupted PUFA, unlike specimens from warmer waters. This could have been due to the temperature or to the high content of wax esters. Nevertheless, arachidonic acid, a major component in all FA mixtures studied (14–21%), is a well-known precursor of prostanoid compounds. The high levels of tetracosapolyenoic FA in specimens from colder waters were of particular interest: 24:6(n-3) (5.1–5.3%), 24:5(n-6) (8.4–15.8%) and 24:5(n-3) (5.0–5.2%) [149]. An analysis of four gorgonians from the genus *Pseudopterogorgia* revealed that the main PUFA are 18:3(n-6), 18:4(n-3), AA, DHA, 24:5(n-6) and 24:6(n-3), with the (n-6) PUFA predominating [150]. All five gorgonians of the genus *Eunicea* presented a similar phospholipid FA composition with unsaturated acyl chains from C_{18} to C_{24}, as shown below in Table 1 [151]. 2-Hydroxy long-chain acids also occurred in these gorgonians. Several 2-hydroxy FA have been identified before in marine sponges [152, 153].

Table 1 Principal unsaturated FA in phospholipid from gorgonians of the genus *Eunicea* [151]

Fatty acid	*Eunicea sp.*	*E. fusca*	*E. laciniata*	*E. mammosa*	*E. succinea*
18:4(n-3)	24.6	16.6	7.5	7.6	15.2
18:3(n-6)	18.0	15.8	17.0	9.8	10.2
18:2(n-6)	0.7	1.5	4.9	1.3	1.5
18:1(n-9)	4.2	9.6	2.4	1.0	2.2
20:5(n-3)	1.3	1.5	3.1	1.3	1.5
20:4(n-6)	12.4	12.4	13.5	15.7	11.5
22:6(n-3)	5.1	6.3	4.2	3.5	6.2
24:5(n-6)	3.8	1.5	2.5	14.2	3.9
24:6(n-3)	0.6	0.4	1.4	2.9	2.7

Several species also contained 2-OH-20:0 and 2-OH-22:0 acids up to 5% of total FA PL.
All species contained the new (Z)-7-Me-16:1(n-10) and (E)-7-Me-16:1(n-10) (0.5–2%)

The phospholipid FA composition of the Caribbean gorgonians *Gorgonia mariae* and *Gorgonia ventalina* (Gorgoniidae) was investigated [154]. This study reports that the main FA were 14:0, 16:0, 18:3(n-6), 18:4(n-3), 18:2(n-6), AA, DHA and 24:5(n-6), as shown in Table 2. In both gorgonians (n-6) PUFA predominated over the (n-3) family. In addition, Table 2 gives data for other gorgonians of the family Gorgoniidae [150].

Table 2 Main phospholipid unsaturated fatty acids of gorgonians from the family Gorgoniidae

Fatty acids	*Gorgonia mariae*	*Gorgonia ventalina*	*Pseudopterogorgia**
18:3(n-6)	10.6	15.8	7.3
18:4(n-3)	10.8	16.4	12.0
18:2(n-6)	8.6	10.3	4.6
18:1(n-9)	5.0	4.0	3.0
20:4(n-6)	10.0	9.4	17.2
20:5(n-3)	6.4	1.2	0.2
22:6(n-3)	5.6	8.6	6.1
22:4(n-6)	2.2	0.3	–
24:5(n-6)	4.0	1.4	10.2
24:6(n-3)	2.0	0.4	3.7

* average of 4 *Pseudopterogorgia* species
(Z)-7-Me-16:1 n-10 and (E)-7-Me-16:1 n-10 occurred at 0.8–3.5%
2-OH-21:0 and 22:0 acids were identified at < 1%

Within the experimental errors, all of these gorgonians share a similar phospholipid FA profile, in as much as: 1) they all biosynthesize, in a similar ratio, the acids 24:5(n-6) and 24:6(n-3), with the former predominating, 2) the (n-6) family of FA predominates over the (n-3) family, and 3) the polyunsaturated FA DHA and AA are key FA in these gorgonians. Several new branched unsaturated FA occurred in gorgonians [149–151]. The New Caledonian gorgonian *Rumphella aggregata* also contained 24:5(n-6) (11%), NMID FA, and unusual short branched-chain unsaturated acids [155].

An interesting investigation on the FA composition of solar coral *Heliopora coerulea* (Octocorallia, Helioporacea) supported a chemotaxonomic significance of tetracosapolyenoic acids in Coelenterates [148]. 24:6(n-3), a tetracosahexaenoic acid, was found in *Heliopora coerulea* [148]. The major FA were 16:0 (40–42%) and 18:3(n-3) (15–16%), as shown below.

Table 3 Main fatty acids from total lipids of *Heliopora coerulea* [147]

Fatty acids	% for two samples	Fatty acids	% for two samples
14:0	4.8–4.8	18:4(n-3)	3.5–3.6
16:1(n-7)	3.1–3.3	18:0	7.3–5.0
16:0	40.9–41.6	20:5(n-3)	5.4–5.5
18:1(n-9)	3.1–3.5	22:6(n-3)	4.7–5.5
18:3(n-6)	15.1–15.8	24:6(n-3)	1.7–1.9

Despite a relatively low content of 24:6(n-3) (2% of total FA), it was concluded that PUFA of regular structure with 24 carbon atoms and five to six methylene-interrupted cis-double bonds are typical constituents of representatives of all orders of the Octacorallia, Alcyonaria, Gorgonaria, Helioporida and Pennatularia. Three novel 10-hydroxydocosapolyenoic acids were isolated from deep-water scleratinian corals, as shown below [156].

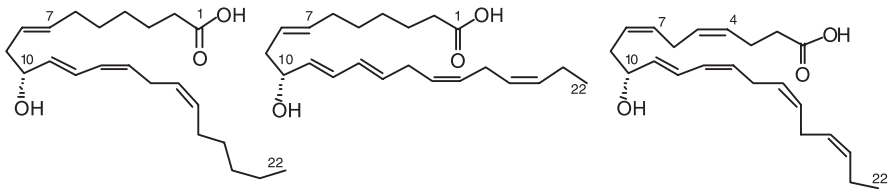

Fig. 17 10-Hydroxypolyenoic FA from *Madrepora oculata*

A work has recently been carried out aimed at elucidating the biosynthesis of docosahexaenoic acid in trout liver microsomes [157, 158]. This report conclude that the tetracosahexaenoic acids, such as 24:6(n-3), are intermediates in the biosynthesis of DHA. Therefore, it is very likely that gorgonians

utilize a similar biosynthetic route, thus providing yet another interesting system to study this type of biogenesis. However, work still remains to be done on the role of symbiotic zooxanthellae in the production of some of these unusual FA.

A survey of lipid and FA composition was made for 15 cnidarians from Okinawa, Japan [159]. Corals having symbiotic Zooxanthellate within their cells contain large amounts of lipid in their tissues (24–26% of dry weight). All specimens contained monoalkyldiacylglycerol and were rich in wax esters and triacylglycerol. Palmitic acid was the most abundant FA component of these lipid classes (more than 40% in each class), followed by stearic and oleic acids [159]. Four ceramides with the same hexadecyl acyl chain isolated from a gorgonian exhibited significant human cholesteryl ester transfer protein inhibitory activity [160].

8.3 Echinodermata

Significant amounts of tetracosapolyenoic acids such as 24:6(n-3) were found in different marine organisms, echinoderms (Ophiuroidea and Crinoidea) and coelenterates [147], and in their predators [161]. 24:6(n-3) occurred in symbiotic and non-symbiotic brittlestars [162]. Isolated from the brittlestar *Ophiura sarsi* [163], this FA had anti-inflammatory and anti-allergic properties similar to those of DHA [164]. Total lipids of this organism contained 14% of 24:6(n-3), 15% of EPA and 2.6% of DHA [163]. Furthermore, high levels of EPA and 24:6(n-3) were observed in phospholipids that accounted for more than 50% of total lipids from *O. sarsi*. Several novel NMI FA were further identified in *O. sarsi* [165], namely 7*E*,13*E*-20:2, 7*E*,13*E*,17*Z*-20:3 (13% of total FA), 9*E*,15*E*,19*Z*-22:3, and 4*Z*,9*E*,15*E*,19*Z*-22:4 acids, as shown below.

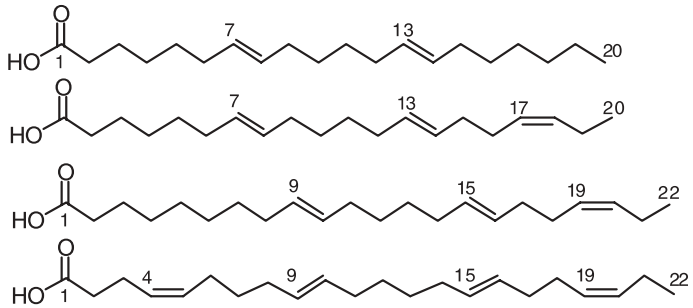

Fig. 18 New non-methylene-interrrupted FA from *Ophiura sarsi*

Other NMI FA also occurred in this brittlestar, namely 5,11-20:2, 5,13-20:2, 7,13-22:2 and 7,15-22:2. 24:6(n-3) Accounted for about 6%. Structural analyses of these fatty acids were performed after partial hydrogenation

with hydrazine sulfate and subsequent isolation of the monoenoate products by argentation thin-layer chromatography, followed by GC/MS analyses of dimethyl disulfide adducts [165]. A comparison of FA in symbiotic and non-symbiotic brittlestars from Oban Bay, Scotland, was performed in order to look for bacterial signals that might indicate contribution of subcuticular bacteria to their hosts' diet [162]. Odd-chained and branched FA were present in low amounts but palmitoleic and cis-vaccenic acids, also considered as good markers for bacteria, occurred at much higher amounts in symbiotic species studied than in the non-symbiotic species. EPA occurred at high levels in all species studied (11–23%) but DHA was present at low amounts. The unusually concentrations of 24:6(n-3) (up to 15%) may indicate that DHA derived from dietary phytoplankton is being elongated to the former [162]. Furthermore, three other tetracosapolyenoic acids and 26:6(n-3) were found in some species [162].

Three new ceramides from the starfish *Acanthaster planci* have been reported [166]. Several glycosphingolipids have been identified recently in sea cucumbers possessing α-hydroxylated or non-hydroxylated, saturated or monoenoic fatty acyl chains [167–169].

8.4 Tunicates

Few works have been published on the lipid composition of tunicates [147, 157, 170, 171]. Lipids of edible ascidian *Halocynthia roretzi*, very popular in Japan and Korea, have also been studied [170]. Several studies of phospholipids of pelagic tunicates (belonging to gelatinous zooplankton) have also been undertaken [171]. PUFA represent the most important class, accounting for around 50%. The tunicates *Eudistoma bituminis* and *Cystodytes violatinctus* from the Indian Ocean were investigated for their phospholipid FA content [172]. In both cases, the most abundant FA were the saturated ones (C_{10} to C_{18}). *Cystodytes violatinctus* contained high amounts of oleic acid (20%). Both *E. bituminis* and *C. violatinctus* contained phytanic acid and Δ10 FA, which had not previously been found in such organisms. These tunicates contained only trace amounts of PUFA, which are usually predominant in this phylum [172].

8.5 Molluscs

8.5.1 Introduction

Lipids are a very important food reserve, in particular in the oocytes of molluscs, which assures viability of the larvae. Lipids also provide energy for

growth during conditions of limited food supply when carbohydrate levels (the main energetic reserve in molluscs) are low. Phytoplankton represent the largest food source for bivalve molluscs and contain a high proportion of PUFA. The intensive rearing of bivalves still relies on the massive production of unicellular algae especially for growing young spat, which represent the largest biomass in a commercial hatchery. The high cost and unpredictable culture success of algae has inspired the development of artificial diets such as microcapsules, mixed diets, yeast based diets, lipid microspheres and liposomes to substitute or supplement live algal diets. The importance of lipids in bivalve nutrition is now well known [173]. The (n-3) PUFA, EPA and DHA, have been reported to be essential for optimal growth for at least some species of juvenile bivalves.

The biochemical composition of the intertidal rocky-shore bivalves, e.g. mussels, is greatly affected by periods of air exposure, at which times the bivalves are denied a food source. Consequently, the resulting effect would be similar to starvation [174].

In coastal environments, detritus, bacteria and zooplankton may greatly affect available food composition. It is known that organic detritus areas an energy source for bivalves during periods of scarce primary productivity. Additionally, detrital material is a source of saturated and monounsaturated C_{14}–C_{18} FA. A high proportion of SFA, such as 20:0, has been observed in bivalves distributed in environments rich in organic material with an abundant bacterial load [175], compared with those mainly nourished by marine phytoplankton, which are dominated by (n-3) PUFA of 18, 20 and 22 carbons. The lipid composition of molluscs can be affected by external factors, such as fluctuations in the environmental conditions and qualitative and or quantitative changes in food availability, or by internal factors, such as sexual maturation [175]. Recently, some very interesting investigations have dealt with the chemical composition and chemotaxonomic of cardiolipids from some marine bivalves and led to evidence of a tetradocosahexaenoic cardiolipin [176].

8.5.2 Mussels

FA profiles of seeds of the mussel *Mytilus galloprovincialis* originating from two habitats (rocky shore and subtidal) were compared after transfer to the same habitat (subtidal), in order to study the initial levels of different FA of metabolic importance and their variability [177]. According to previous studies, PUFA were found to be the group with highest percentage (42–49% of total FA), including EPA and DHA as major components [177]. Moreover, these findings concur with recent studies of numerous bivalve species distributed in other regions of Europe and America, such as *Argopecten purpuratus* [178] and *Crassostrea gigas* [179]. Additionally, the mussels of subtidal origin presented higher initial levels than the rocky-shore mussels with re-

gard to FA characterized by energetic-type functions, such as 14:0, 16:0 and EPA. High initial levels of some PUFA observed in the subtidal mussels, such as 18:3(n-3), 18:4(n-3) and EPA, are presumably due to the fact that these mussels had greater access to phytoplanktonic food. Initial levels of 14:0, 16:0 and 18:0 in the rocky-shore mussels were significantly greater than in the mussels of subtidal origin. FA characterized by structural-type functions, e.g. 18:0, DHA and NMID FA with 20 and 22 carbons, in rocky-shore mussels presented higher levels than those of the subtidal mussels. These NMID FA have been observed in greater proportions in phospholipids, thus implying a structural-type function [177].

In addition, it is also known that these NMI FA are distributed in greater quantities in the organs more exposed to the immediate environment, such as the gills, mantle and foot. In another study, minor NMI FA were characterized in *Mytilus galloprovincialis* by GC/MS of their 2-alkenyl-4,4-dimethyloxazoline derivatives, namely 7,13–16:2, 5,11–20:2, 5,13–20:2, 7,15–22:2, and the new trienoic FA 5,11,14-20:3 and 7,13,16-22:3 [180]. This discovery supports a biosynthetic route implying the desaturation and subsequent elongation of 20:2(n-6) [180]. Lipid, FA and sterol composition of the New Zealand green-lipped mussel (*Perna canaliculus*) and the Tasmanian blue mussel (*Mytilus edulis*) have also been reported [181].

8.5.3 Oysters

Seasonal variations of lipid classes and FA in the flat oyster *Ostrea edulis* have been studied [182]. The dynamics of FA in the larval development, metamorphosis and post-metamorphosis of this oyster have been investigated [183]. The lipid composition of *Crassostrea gigas* was analysed during the reproductive phase in natural as well as under artificial conditions [179]. A specific accumulation of DHA and EPA in the polar lipids was observed under both conditioning diets. The proportions of DHA and EPA from neutral and polar lipids of oysters conditioned artificially were significantly lower than of those that were naturally conditioned. A useful comparison of the lipid class and FA composition between a reproductive cycle in nature and a standard hatchery conditioning of the Pacific oyster *Crassostrea gigas* was performed [179].

8.5.4 Patella

The effects of season and spatial distribution on the FA composition of four *Patella* species' gonads and soft body tissue were evaluated [185]. The results show that the quantitatively most important FA were 14:0, 16:0 and 18:0; the MUFA 18:1(n-7), 18:1(n-9), 16:1(n-7) and 20:1(n-9), EPA and AA. *P. depressa* and *P. ulyssiponensis* soft-body FA profiles revealed significant differences be-

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tween sexes, with males showing significantly higher percentages of PUFA, mainly EPA and AA, while in females significantly higher proportions of MUFA were found. The fatty-acid composition of *P. depressa* gonads revealed significant differences between sexes, CE^{e} which were more acids from the (n-3) and (n-6) series (AA and EPA), while females were seen to have higher proportions of SFA [185].

8.5.5 Clams

Recent studies on clam lipids were concerned with their aquaculture. Thus, the possible use of emulsions rich in EPA and DHA as an artificial lipid supplement to live algae was investigated for seed of the Manila clam *Tapes philippinarum* [173]. In addition, the influence of the lipid composition of microalgal diets and cornstarch on the lipid classes and FA of the *Ruditapes decussatus* spat was studied [186]. This clam species is of commercial interest in Spanish aquaculture. In order to increase its production, experiments have been carried out with alternative foods to live microalgae, such as freeze-dried microalgae or cornstarch. The main FA present in the spat of *R. decussatus* were 16:0, 18:1(n-9) and DHA, followed by lower contents of 18:4(n-3) and 18:1(n-7). The essential FA EPA is present in small amounts. The content of (n-3) PUFA, (n-9), (n-6), and 20:2 and 22:2 NMID FA differed significantly according to the diet supplied. Spat fed on a microalgal diet show the significantly highest content in (n-3) PUFA and (n-9) FA [186].

8.5.6 Scallops

Overfishing has often resulted in a decline of natural beds of *Pecten maximus* in several areas. Thus, several experiments have been performed on *P. maximus* culture, growth and reproduction to improve the biological knowledge of this species [187, 188]. Lipids are a very important food reserve in the oocytes, which assures viability of the larvae. It has been shown that success of *Pecten maximus* hatching is related to the lipid status of the eggs when spawned. Lipids in the female gonad were analysed for lipid class composition and FA composition of TAG and phospholipids, and their variations in relation to gametogenic cycle were studied [184–188]. PUFA were more abundant in the TAG and the series (n-3) was clearly predominant. The principal (n-3) FA (mainly in phospholipids) showed a seasonal variation clearly related to the reproductive cycle [188]. Soudant et al [184] have analysed the composition of polar lipid classes in male gonads of *Pecten maximus* and the effect of nutrition. Seasonal digestive-gland dynamics of the scallop *Pecten maximus* have been reported [189].

The distribution of lipids and FA in different organs of the Chilean scallop *Argopecten purpuratus* broodstock, namely female and male gonads, digestive gland, gills, mantle and adductor muscle, have been investigated [178]. The highest level of (n-3) PUFA (mainly EPA and DHA) was found in the adductor. The major FA in all parts studied were 16:0, EPA (8.1–20.3%) and DHA 9.2–25.6%). Similarities between the FA composition of the triglyceride fraction of the female gonad and the digestive gland (e.g. the high level of 14:0 and 18:4(n-3)) indicated the transfer of lipids from the lipid-rich digestive gland to the female gonad. A special feature of the gills and mantle was the presence of high levels of plasmalogens (phosphoglycerides with 1-alkenyl chains) recognized by the presence of dimethylacetals, which are formed simultaneously with FAME by acid-catalyzed transmethylation [178]. In another investigation, dietary supplementation with lipid emulsions during broodstock conditioning of *A. purpuratus* was used to manipulate the fatty-acid composition of the eggs [187, 190]. The scallops were fed a mixed algal diet either alone or supplemented with an emulsion rich in ethyl esters of DHA or EPA. Lipid supplementation resulted in a significant increase of the total lipid content of the eggs. The EPA and DHA levels in the total and neutral lipids of eggs from broodstock supplemented with diets including the corresponding emulsions were significantly higher than in eggs from scallops fed solely algae [190, 191]. NMID FA, which are not present in the phytoplankton, have been reported in many species of molluscs. Thus, they are presumably synthesized by molluscs. Interestingly, the identification and occurrence of a novel *cis*-4,7,10,*trans*-13-docosatetraenoic acid in the female gonads of the scallop *Pecten maximus* was described [192].

Lipid deterioration during frozen storage at – 20 °C of the adductor muscle, the major edible part of the giant scallop, was examined by determination of fatty chain compositions in the sn-1 and/or sn-2 positions of ether and ester glycerophospholipids [193]. During storage, the contents of total lipid and polar lipid decreased but that of non-polar lipid increased. The percentages of PUFA such as EPA and DHA in the total lipid and polar lipid fractions decreased during storage, but those of the PUFA in the non-polar lipid increased. Changes during storage in alkenyl and alkyl chain compositions of ether glycerophospholipids and in fatty acyl chain compositions of ether glycerophospholipids were determined [193]. The 20:2 and 22:2 NMID FA also occurred.

8.5.7 Squids

Biochemical composition changes and FA in several tissues of the squid *Illex argentinus* from the South Atlantic Ocean at different sexual stages were studied. All tissues contained high concentrations of PUFA followed by SFA and MUFA, e.g.16:0, 18:0, 18:1(n-9), 20:1, 22:1, EPA and DHA. The lipids

also contained unusually high levels of FA of the linoleic family (2–3%). The digestive gland of the squid is rich in polyenoic FA, EPA and DHA. This tissue could be a cheap raw material, presently discarded, for production of PUFA [194].

The lipid content and composition of phosphatidylcholine and FA were determined in tissues of 70 species of teleosts, and in muscle of six species of squid. Amount and composition of diacyl glyceryl ethers were recently analysed in various tissue lipids of the deep-sea squid *Berryteuthis magister* [195].

9 Crustacea

The mud crab *Scylla serrata* is a commercially important species in the Indo-Pacific region and has been cited as a target species for a stock-enhancement program in Japan and also as a target species for aquaculture in many Asian countries [196]. A recent study evaluated the requirements of linoleic acid (18:2(n-6)), linolenic acid (18:3(n-3)), EPA and DHA during rotifer, as well as *Artemia* feeding on survival and larval development of mud-crab larvae [196]. Decreased natural seed availability and the low survival rate in crab hatcheries [197–199] have been major problems in increasing aquaculture production. Moreover, Takeuchi et al [197] described the requirement of EPA and DHA for larval development, where EPA is effective in maintaining survival while DHA plays an important role in accelerating the intermolt period and produces a wider carapace width in swimming crab larvae. A previous study showed that the *Artemia* feeding schedule (in combination with rotifers) for larval mud crab and their essential FA composition affected the survival of larvae [199–201].

If the conditions of thermal adaptation are well documented in fish, little information is available for marine invertebrates. A comparative study of the phospholipid FA compositions was conducted with the Baltic Sea amphipod crustacean *Gammarus* spp. collected from different thermal environments [202]. It was reported that environmental temperature had little effect on FA composition. In fact, *Gammarus* was shown to use the same strategy to control membrane fluidity in the cold as fish species investigated so far [203], namely the crustacean accumulates sn-1 monoenoic and sn-2 polyenoic phospholipid FA at reduced temperatures.

10
Polyunsaturated FA (n-3) of commercial interest

10.1
Introduction

An "essential" nutrient is one that is needed for normal development and function of mammalian cells throughout the life cycle. In its active or precursor form there is a minimum amount of such a nutrient that must regularly be provided in the diet. This dietary requirement generally varies with species, gender, age and the presence of physiological and pathological challenges (pregnancy, lactation, infancy, aging, infection, disease, etc.).

The term "essential fatty acid" is ambiguous and inappropriately inclusive or exclusive of many polyunsaturated FA. When applied most rigidly to linoleate and a linolenate, this term excludes the now well-accepted but conditional dietary need for two long-chain polyunsaturates (arachidonate and docosahexaenoate) during infancy. The metabolism of essential and nonessential FA is clearly much more interconnected than previously understood. Replacing the term "essential fatty acid" by existing but less-biased terminology, i.e. polyunsaturates, (n-3) or (n-6) polyunsaturates, or naming the individual fatty acid(s) in question, would improve clarity and would potentially promote broader exploration of the functional and health attributes of polyunsaturated FA [204].

10.2
Health benefits

Initially, it should be noted that the chemical form of the PUFA supplementation used for clinical assays and up to the final consumers will not be detailed. Indeed, sometimes they take TAG forms while occasionally they are ethyl esters or free fatty acids, depending on the target.

Polyunsaturated FA (PUFA) are essential components in higher eukaryotes that confer fluidity, flexibility and selective permeability to cellular membranes. PUFA affect many cellular and physiological processes in both plants and animals, including cold adaptation and survival [205, 206], modulation of ion channels [207, 208], endocytosis/exocytosis [209], pollen formation, pathogen defense, chloroplast development in plants [210], and activities of membrane-associated enzymes that are sensitive to the biophysical properties of lipid membranes [211–213].

In mammals, metabolism of LC-PUFA by oxygenases yields a range of important short-lived molecules (generically known as the eicosanoids), such as prostaglandins, leukotrienes and thromboxanes (Fig. 19). These resulting metabolites bind to specific G-protein-coupled receptors and signal cellu-

lar responses and modulate many biological processes (see below). Because the production of various classes of these molecules depends in part upon the availability of their PUFA precursors in membrane phospholipids, modulation of PUFA is a potential target of pharmaceuticals and nutraceuticals [213, 214].

The (n-3) LC-PUFA, particularly EPA and DHA, are thought to display a variety of beneficial effects in areas ranging from foetal development to cancer prevention [215]. Some of those health effects are presented below.

10.2.1 Heart health

It is well established that populations with a high consumption of oily fish have a lower incidence of heart disease and several studies have confirmed that EPA and DHA are the protective components [216–224].

Recent research concludes that perhaps the most important effect of (n-3) LC-PUFA, when it comes to preventing cardiovascular disease, is their ability to stabilize atherosclerotic plaque by reducing the infiltration of inflammatory and immune cells (lymphocytes and macrophages) into the plaque [225]. This could explain the reduction in fatal and nonfatal heart attacks and strokes associated with an increased intake of fish oils [226]. In addition, the anti-inflammatory effect of DHA by reducing the C-reactive protein (CRP) level in blood may decrease the risk of coronary heart disease such as atherosclerosis [227].

Several large clinical trials have confirmed the ability of fish oils to prevent sudden cardiac death in both presumably healthy subjects as well as in patients having suffered a heart attack [228–231]. Although most research so far has focused on the effect of (n-3) on life-threatening ventricular arrhythmias it is likely than many of the findings may also be applicable to atrial fibrillation [232]. It has also been shown that a high (n-3) content of blood cells and serum cholesterol esters is associated with increased heart rate variability and leads to a decreased risk of cardiac disease and a longer lifespan [233]. In addition, EPA and DHA seem to reduce the mortality among not only patients who have survived a first heart attack [234–236] but also among old people [237, 238].

Numerous studies have confirmed that (n-3) LC-PUFA included in fish oils significantly combat hypertension by a notable reduction of blood pressure and benefit heart transplant patients [239–247].

EPA and DHA are also effective in lowering the blood level of triglycerides [248, 249], in improving large artery dilation in patients with high cholesterol levels [250], and they possess antithrombotic effect [251]. Such positive actions also contribute to reduce the risk of cardiovascular disease.

10.2.2 Cancer

Several studies have shown the effect of LC-PUFA against cancer such as an inverse relationship between blood levels of EPA and DHA and the risk of prostate cancer [252, 253] or fish and fish oil consumption and adenocarcinomas [254]. In addition, (n-3) PUFA can also act positively against cancer effects like cachexia (abnormal weight loss) or survival rate in end-stage cancer [255, 256].

10.2.3 Arthritis

The (n-3) PUFA are also known to decrease rheumatoid arthritis; notably by lowering interleukin-1*beta* production which results in a significant reduction in morning stiffness and the number of painful joints [257–261].

10.2.4 Psoriasis

Psoriasis is a fairly common skin disease characterized by high concentrations of AA in the plaques and profound changes in the metabolism of eicosanoids leading to an increase in proinflammatory agents. Some studies have shown that n 3 PUFA[CE f], notably EPA, can counteract the formation of these proinflammatory agents and that oral supplementation with fish oils benefit psoriasis patients [262, 263].

10.2.5 Lung disease

A few years ago it was shown that children who regularly eat fresh, oily fish have a four times lower risk of developing asthma than children who rarely eat such fish. EPA was suspected to be responsible by reducing airway inflammation and responsiveness. Later, studies on supplementation by (n-3) LC-PUFA have confirmed their benefit in the reduction of breathing difficulties and other symptoms in asthma patients. More recently, it has been demonstrated that those PUFA are also beneficial in the treatment of other lung diseases such as cystic fibrosis and emphysema [264–267].

10.2.6 Attention-deficit disorder

Attention-deficit hyperactivity disorder (ADHD) is characterized by hyperactivity, emotional instability, poor coordination, short attention span, poor

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concentration, impulsiveness, and learning disorders. It is very common among school-age children with an incidence of 4–20%. Initial studies have linked ADHD to a deficiency of certain long-chain FA, notably AA, EPA and DHA [268]. More recently Burgess et al. [269] have found that children with ADHD were breastfed less often as infants than children without ADHD (breast milk is an excellent source of DHA). In addition to ADHD, other disorders such as dyslexia (difficulties in learning to read and write) and dyspraxia (problems with coordination and muscle control) also have deficiency in LC-PUFA as a common denominator that may be avoided by oral supplementation of concentrated fish oils [270].

10.2.7
Mental health

Several epidemiological studies have shown that a high dietary intake of linoleic acid and a low intake of EPA and DHA are associated with cognitive impairment and an increased risk of dementia. In a recent study, it was that EPA and especially DHA help keep the membranes of brain cells more fluid while saturated and (n-6) FA tend to "harden" them. Authors believe this and the anti-inflammatory effects of EPA and DHA are what help preserve cognitive function [271].

In addition to preventing dementia, (n-3) PUFA help in combating depression, schizophrenia, Alzheimer's disease and other mental illnesses [272–283].

10.2.8
Pregnancy and infancy

An adequate intake of DHA and EPA is particularly important during pregnancy and lactation. During this time the mother must supply all the baby's needs for DHA and EPA because it is unable to synthesize these essential FA itself. DHA makes up 15–20% of the cerebral cortex (a normal adult human brain contains more than 20 g of DHA) and 30–60% of the retina (it is also concentrated in the testes and sperm), so it is absolutely necessary for normal development of the foetus and baby, which implies optimal levels in pregnant and lactating mothers. There is some evidence that an insufficient intake of (n-3) FA may increase the risk of premature birth and an abnormally low birth weight. There is also emerging evidence that low levels of (n-3) acids are associated with hyperactivity in children [268, 284–292].

The constant drain on the mother's DHA reserves can easily lead to a deficiency that may be linked to preeclampsia (pregnancy-related high blood pressure) and postpartum depression [288–290]

10.3 Nutrition: importance of the ratio of (n-6) and (n-3) essential FA

There are good fats and there are bad fats. Artificially produced *trans*-FA are bad in any amount and saturated fats from animal products should be kept to a minimum. The best fats, or oils rather since they are liquid at room temperature, are those that contain the essential FA that are so named because without them we die (see chapter introduction). Essential FA are polyunsaturated and grouped into two families, the (n-6) EFA and the (n-3) EFA.

Seemingly minor differences in their molecular structure make the two EFA families act very differently in the body. 18:2(n-6) and 18:3(n-3) are not interconvertible and compete for the rate-limiting 6-desaturase in the synthesis of LC-PUFA (see biosynthesis). AA and EPA are the parent compounds for the production of eicosanoids with opposite properties, see Fig. 19. Many scientists believe that a major reason for many diseases (see below) is the profound imbalance between our intake of (n-6) and (n-3) FA. Our ancestors evolved on a diet with a ratio (n-6)/(n-3) of about 1 : 1. A massive change in dietary habits over the last few centuries (modern agriculture) has changed this ratio to something closer to 20:1[1] [293–295].

An increase in the dietary intake of (n-6) EFA changes the physiological state to a prothrombotic, proconstrictive, and proinflammatory state. Many of the chronic conditions, cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, rheumatoid arthritis, asthma and depression, are associated with increased production of thromboxane A_2 (TXA_2), leukotriene B_4 (LTB_4), IL-1β, IL-6, tumor necrosis factor (TNF), and C-reactive protein [295]. All these factors increase with (n-6) fatty-acid intake and decrease with (n-3) fatty-acid intake, whether 18:3(n-3), 20:5(n-3) or 22:6(n-3). EPA and DHA are more potent[2], and most studies have been carried out using EPA and DHA (see above).

The optimal dose or ratio of (n-6)/(n-3) varies from 1/1 to 4/1 depending on the disease under consideration. In the secondary prevention of cardiovascular disease, a ratio of 4/1 was associated with a 70% decrease in total mortality [296]. A ratio of 2.5/1 reduced rectal cell proliferation in patients with colorectal cancer, whereas a ratio of 4/1 with the same amount of (n-3) PUFA had no effect [297]. The lower (n-6)/(n-3) ratio in women with breast cancer was associated with decreased risk [298]. A ratio of 2–3/1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5/1 had a beneficial effect on patients with asthma, whereas a ratio of 10/1 had adverse consequences [265, 299]. These studies indicate that the optimal ratio may vary with the disease under consideration. This is consistent with the fact

[1] Lipid consumption in Europe nowadays: vegetal (65%) > land animals (17%) > butter (16%) > marine animals (2%).

[2] Alpha-linolenic acid can be converted to EPA and DHA in the body, but the conversion is quite inefficient, especially in older people [294].

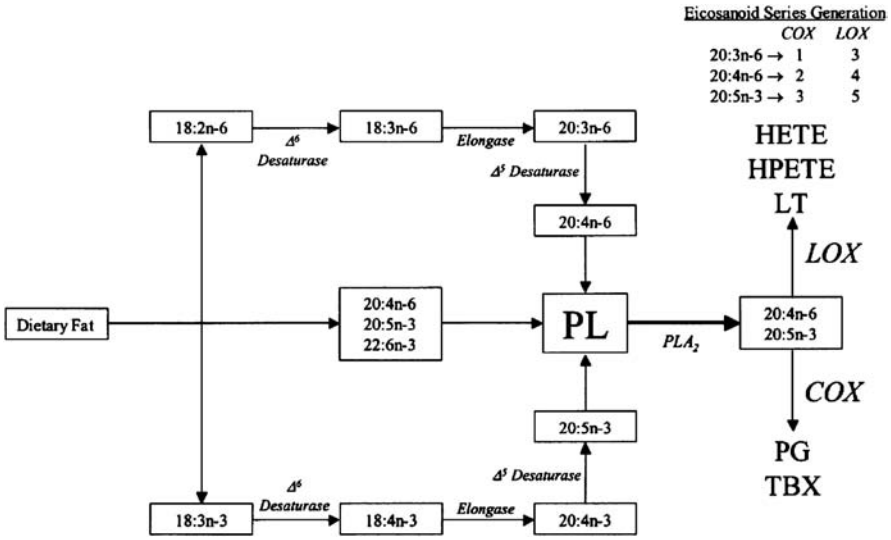

Fig. 19 Biosynthesis of eicosanoids from the essential FA [304]. Abbreviations: PL, phospholipids; PLA2, phospholipase A2; COX, cyclooxygenase; LOX, lipoxygenase; PG, prostaglandins; TBX, thromboxanes; HETE, hydroxy-eicosatetraenoic acids; HPETE, hydroperoxyeicosatetraenoic acids; LT, leukotrienes. Dietary lipids provide EFA and preformed substrates for the COX/LOX pathways. Dietary FA such as 20:3(n-6), 20:4(n-6), and 20:5(n-3) are direct precursors, while 18:2(n-6) and 18:3(n-3) must be elongated and desaturated prior to their conversions to eicosanoids

that chronic diseases are multigenic and multifactorial. Therefore it appears important to restore the balance between (n-6) and (n-3) for homeostasis and normal development. On this basis and by recognizing the unique benefits of EPA and DHA and the serious consequences of a deficiency, recommendations for daily intake of (n-3) PUFA has been published by several international scientific authorities [300–303].

10.4 Routes for biosynthesis

Despite extensive screenings, no angiosperm plants have been detected that accumulate in reserve triacylglycerols or in membrane lipids cis-polyunsaturated FA with carbon chains longer than C18 [305]. However, it is well known that polyunsaturated FA have crucial roles in membrane biology and signalling processes in most living organisms (see the section on health benefits). As indicated by Abbadi et al. [306], the daily requirement of such PUFA may vary and depend on the availability of linoleic and linolenic acid for conversion by elongation and desaturation, but a more regular consumption and an accordingly sustainable source of PUFA would be highly

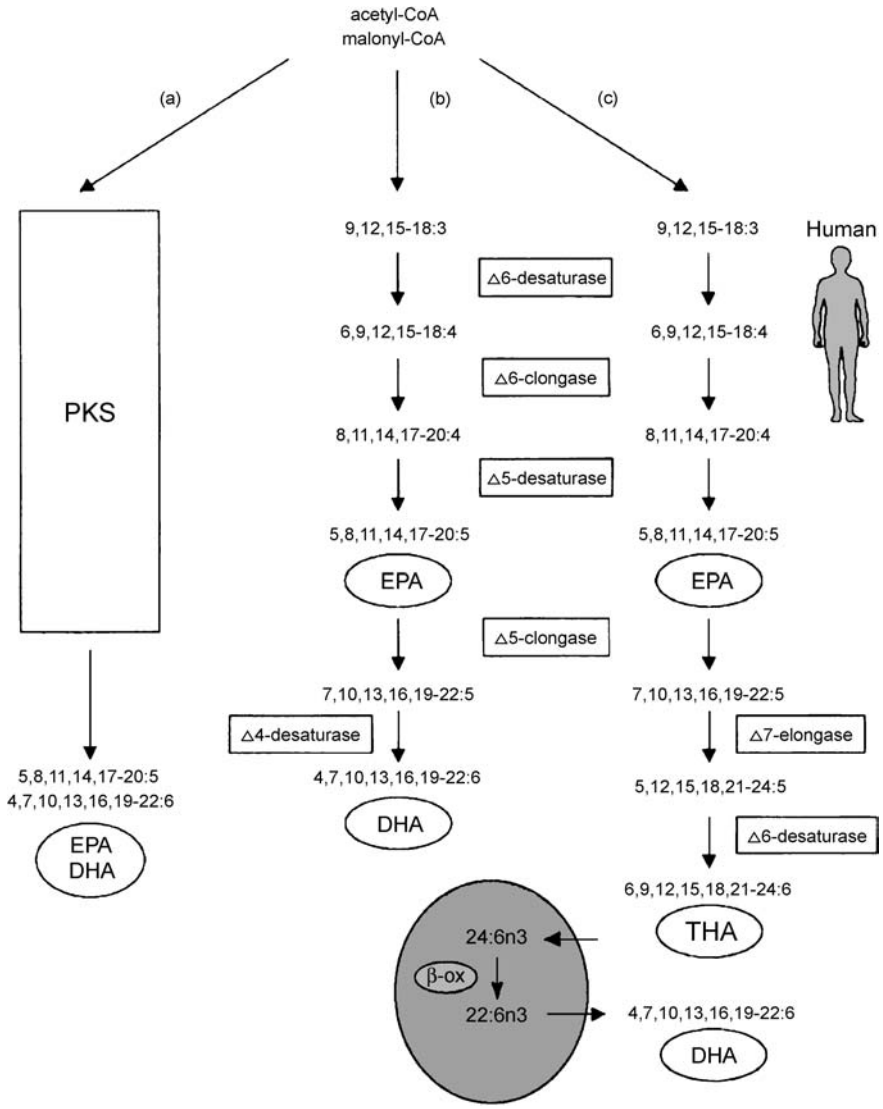

Fig. 20 Pathways of very-long-chain polyunsaturated synthesis in different organisms. Major products are indicated in ovals while enzymes are boxed. The anaerobic route (a) makes use a polyketide-like system (PKS), whereas aerobic routes (b, c) use different enzymes (desaturases and elongases). If routes (b, c) are started with linoleic acid (9,12–18:2), arachidonic acid (AA) = 5,8,11,14–20:4 is obtained. Route (b) is a direct pathway that may operate in marine primary producers and initiate the food chain of "oceanic" polyunsaturated FA ending in large carnivorous fish. Route (c) represents the Sprecher [22] pathway as typical for mammalian cells. Mammals lacks Δ12 and n3 desaturase activities and obtain linoleic acid and α-linolenic acid (9,12,15:18:3) from their diets. In this route, the synthesis of DHA is now known to consist of two succeeding elongation cycles, a Δ6 desaturation and a β-oxidation chain-shortening

desirable. At present, these FA enter the human diet mainly in the form of marine and freshwater fish. But in view of the increasing human population, the overfishing of marine resources, the dependence of fish farming on PUFA from fish oil and the environmental impact of aquaculture systems, neither farmed nor captured fish can be considered as a sustainable source of PUFA (see section on market) [307]. Thus alternatives have to be found such as new sources or by modifying existing ones by genetic approaches. For example, transgenic oilseeds could be a way out of the forthcoming shortage, particularly in view of the fact that a low percentage of PUFA in daily-consumed plant oils would satisfy nutritional requirements [306].

So, over the last few years, the biosynthetic LC-PUFA pathway has been the subject of much interest and it is only recently that molecular genetic approaches have allowed detailed studies of the enzymes involved in their synthesis. Thus, at this time, three distinct routes for LC-PUFA biosynthesis have been identified; two are aerobic and one is anaerobic (Fig. 20).

10.4.1 Aerobic pathways

In these routes, LC-PUFA biosynthesis is catalysed by sequential desaturation and fatty acyl elongation reactions. Known pathways involve the processing of the saturated 16:0 or 18:0 products of fatty acid synthase (FA) by elongation and aerobic desaturation reactions. The desaturase enzymes insert double bonds at specific carbon atoms in the fatty acid chain and the fatty acid elongation system elongates the precursors in two-carbon increments [308, 309]. Significant progress has been made in the identification of the enzymes required for PUFA synthesis; in particular, the fatty acid desaturases which are central to this pathway have now all been identified [308, 310]. The most relevant desaturases required for PUFA biosynthesis are the so-called "front-end" desaturases [311] that introduce a new double bond between an existing one and the carboxyl end of the acyl group. These "front-end" desaturases are all members of the cytochrome b_5 fusion desaturase superfamily, since they contain an N-terminal domain that is orthologous to the microsomal cytochrome b_5 [312].

Interestingly, there is a division of labor in higher eukaryotes for the synthesis of 20:4(n-6), 20:5(n-3) and other PUFA. Angiosperm plants convert oleic acid (18:1) to linoleic acid (18:2) and linolenic acid (18:3(n-3)), which are essential FA for mammals as substrates for the synthesis of C20, but these plants are unable to elongate the FA further. Mammals also convert 18:0 to 18:1(n-9) using a membrane-bound 18:0-CoA desaturase, however, they lack both Δ12 and (n-3) desaturase activities. Therefore, they require 18:2(n-6) and 18:3(n-3), the essential FA (EFA), in their diet [213]. These EFA are converted to long-chain PUFA via a series of desaturation and elongation reactions in the ER[CE g] [308]. The Δ6 desaturase uses 18:2(n-6) and 18:3(n-3)

CE g please define abbreviation ER used here for the first time

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as a substrates and inserts a double bond to produce 18:3ω6 and 18:4ω3. PUFA with a double bond at Δ6 are substrates for the elongation machinery, which uses malonyl-CoA to add two carbons to the C-terminal end of the FA, producing 20:3(n-6) and 20:4(n-3). These FA are substrates for a Δ5 desaturase, which produces 20:4(n-6) and 20:5(n-3) [213]. Those pathways for the synthesis of arachidonic acid (AA) and eicosapentaenoic acid (EPA) have been characterized biochemically and are supported by the recent cloning and characterization of desaturase and elongase genes.

In addition, some notable variations exist such as, for example, the free-living nematode *Caenorhabditis elegans* [313–316], the fungus *Mortierella alpina* [317] the moss *Physcomitrella patens* [318] the red algae *Porphyridium cruentu*m [319], the freshwater protist *Euglena* [320] and the microalga *Isochrysis galbana* [321]. All these organisms posses particular enzymatic pathways.

At this stage of LC-PUFA biosynthesis a split occur. The force of logic suggested that the steps succeeding AA and EPA synthesis would be another two-carbon elongation step and countervailing desaturation by Δ4 desaturase to produce the C22 PUFA (elongation of EPA and AA to C22:5(n-3) and DTA, respectively, and the desaturation of the latter to form DHA and DPA). The cloning of a Δ4 desaturase from the DHA-producing marine protist, *Thraustochytrium* sp., suggests that this pathway (route b, Fig. 22) is valid in some organisms [322]. However, mammals lack Δ4 desaturase activity, and evidence has accumulated for an alternative pathway for C22 PUFA biosynthesis.

The sequence involving Δ4 desaturase is the simplest one. It operates in various unicellular, eucaryotic algae belonging to different systematic groups and contributing to marine primary production. These and other photosynthetically active organisms are considered to be the primary sources of EPA and DHA entering marine feeding webs with large carnivorous fish and finally humans at the end [306, 307].

The other route, the so-called Sprecher pathway (route c, Fig. 20), remained controversial but recent experiments have indicated that it is the predominant route to DHA in mammals [22, 23, 323–328]. It now seems clear that the human C22 PUFA synthesis pathway consists of two succeeding elongation cycles (leading to 24:5(n-3)) followed by a Δ6 desaturation, all of which occur in the endoplasmic reticulum. After transfer of the fatty acid to peroxisomes, there is a specific β-oxidation chain-shortening to the C22 product. By this route, the synthesis of DHA from acetyl–coenzyme A (acetyl-CoA) requires approximately 30 distinct enzyme activities and nearly 70 reactions, including the four repetitive steps of the fatty acid synthesis cycle [329].

Over the past few years, sequences encoding virtually all the enzyme activities involved in microsomal PUFA biosynthesis have been isolated, identified, and expressed in a variety of heterologous hosts (Table 4) [330]. Results from these studies help to increase our understanding of the biochemistry of elongases and desaturases and the regulation of PUFA biosynthesis. Then, the

Table 4 Origin of presently available genes for cDNAs encoding desaturases and elongases involved in the biosynthesis of LC-PUFA. The encoded enzymes have been characterized by functional expression. The numerous sequences published for the ubiquitous Δ9-, Δ12- and Δ15-desaturases are not included [308]

Enzyme	Organism	Reference
Δ4 desaturase	Thraustochytrium sp	[322]
Δ5 desaturase	Homo sapiens	[331, 332]
	Caenorhabditis elegans	[333, 334]
	Mortierella alpina	[335, 336]
Δ6 desaturase	Homo sapiens	[337]
	Caenorhabditis elegans	[338]
	Borago officinalis	[339]
	Ceratodon purpureus	[340]
	Physcomitrella patens	[341]
	Mortierella alpina	[342, 343]
Δ8 desaturase	Euglena gracilis	[320]
(n-3) desaturase	Caenorhabditis elegans	[344]
Δ6 elongase	Caenorhabditis elegans	[314]
	Mortierella alpina	[317]
	Physcomitrella patens	[345]

next challenge will be the selection of a set of suitable copies of the available genes, which after transformation and expression in a heterologous host (such as oilseed crop) should result in a cooperating ensemble of enzymes and production of PUFA.

10.4.2 Anaerobic pathway

The diversity of PUFA synthesis described above relies on variations on desaturase and elongase biochemistry. This aerobic biosynthetic pathway was thought to be taxonomically conserved, but nature has also solved the problem of PUFA synthesis using a fundamentally different anaerobic pathway [329]. Indeed, numerous PUFA-producing bacterial strains are capable of producing PUFA under strictly anaerobic conditions, thus precluding the participation of an oxygen-dependent mechanism. The system involved here does not require the multiple desaturase and elongase enzymes outlined above, but instead uses a polyketide synthase-like (PKS) gene cluster, found in both prokaryotic and eukaryotic marine microbes, to synthesize PUFA [214].

Several marine bacteria contain EPA and DHA at levels as high as 25% of total membrane FA [346]. A genomic library prepared from one of these marine bacteria (*Shewanella sp*. Strain SCRC2738) was used to identify a 38 kb DNA fragment that resulted in EPA synthesis when expressed in *E. coli*. [347].

Experimental results [329, 348–350] indicated that these genes expressed in *E. coli* encode a protein complex that is capable of EPA synthesis without any reliance on enzymes of the *E. coli* FA or any long-chain intermediate such as 16:0-ACP. Apparently, the genes encode a PKS that acts independently of FA elongase and desaturase activities to synthesize EPA directly [213]. Genes with high homology to the *Shewanella* gene cluster have been identified in *Photobacterium profundum* [351] and in *Moritella marina*, which accumulates DHA rather than EPA [352]. Thus it is likely that the PKS pathway for PUFA synthesis is widespread in marine bacteria [213].

The thraustochytrid *Schizochytrium sp* accumulates large quantities of triacylglycerol[CE h] rich in DHA (see chapter on thraustochytrids). Biochemical experiments have revealed the involvement of a PKS protein complex [329], which was further confirmed by molecular genetic analysis. The *Schizochytrium* genome encodes three proteins with domains highly similar to those encoded by genes from *Shewanella*, raising the possibility that the PUFA PKS has undergone lateral gene transfer [213].

Several "front-end" PUFA aerobic desaturases from *Thraustochytrium* have been identified recently [322, 353], but the prevalence of this newly discovered PKS-like biosynthetic pathway is not known. Because *Schizochytrium* is also a member of the Thraustochytriidae, it is perhaps surprising to find these two distinct biosynthetic pathways represented in the same family, but molecular characterization of PUFA biosynthesis in the Thraustochytriidae might provide insights into the evolution of this important pathway.

The primary structure of *Shewanella* PKS (and its relatives) does not conform to any of the previously described classes of PKS proteins. Instead, it suggests the assembly of several multifunctional proteins into a complex. PUFA PKS carry out some of the same reactions as FA and use the same small protein (or domain), acyl carrier protein (ACP), as a covalent attachment site for the growing carbon chain. However, in these enzyme systems, the complete cycle of reduction, dehydration, and reduction seen in FA is often abbreviated, so that a highly derivatized carbon chain is produced, typically containing many keto and hydroxy groups as well as carbon–carbon double bonds in the trans configuration (Fig. 21). The linear products of PKSs are often cyclized to form complex biochemicals that include antibiotics, aflatoxins, and many other secondary products [329]. Since the double bond on the PUFA molecules were formed by dehydration and isomerization of keto groups in cycles of polyketide-forming chain elongation, reaction to the C20 PUFA may not be the direct precursor of C22 PUFA [354].

The relative simplicity of this PKS-like system makes it attractive in terms of transgenic production of LC-PUFA. For example, introducing and regulating the three *Schizochytrium* PKS-like open reading frames in a transgenic plant is relatively simple compared with the more than five desaturase and elongase genes required for the aerobic pathway [306]. In addition, the identification of new (PUFA-specific) PKS activities such as double-bond isomer-

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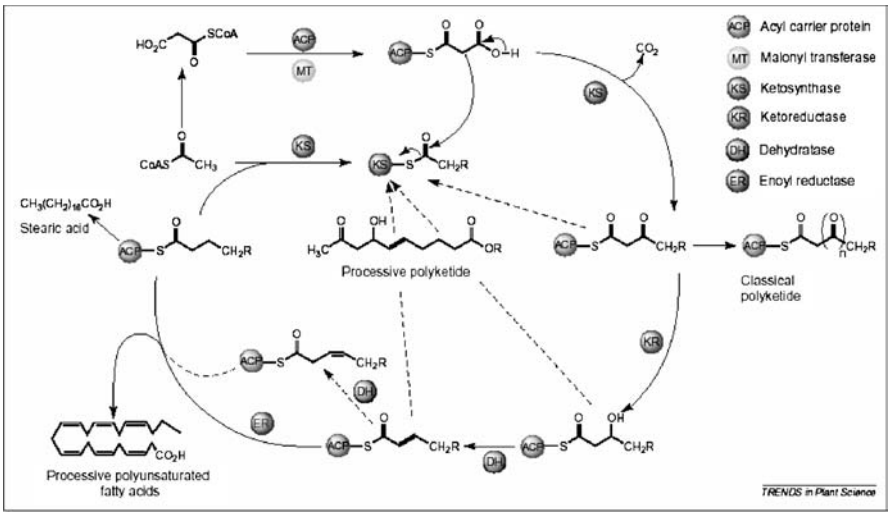

Fig. 21 Generalized scheme for the processive synthesis of polyunsaturated FA (PUFA) by a polyketide synthase (PKS) system [214]. In the case of PUFA, it is envisaged that a primer molecule (in the form of acetyl-CoA) undergoes several rounds of sequential reactions (keto-synthase, keto-reductase, dehydratase and enoyl reductase), resulting in repeated synthesis and fatty acyl chain (esterified to the acyl carrier protein) elongation by two carbons per cycle. Because PUFA contain methylene-interrupted double bonds (i.e. at the third carbon), it is likely that a dehydratase (FabA-like) module in the PKS also simultaneously carries out a trans–cis isomerization to generate this configuration

ization might help in the bioengineering of additional families of polyketide antibiotics [214].

Furthermore, our improved knowledge of PUFA synthesis in *Shewanella*, *Schizochytrium* and their relatives has implications for understanding food-web dynamics in marine ecosystems. Because these organisms are significant primary producers of 20- and 22-carbon PUFA in cold-water oceans [346], the PKS pathway may be an important source of PUFA for fish and mammals and thus also for human diets. The importance of 20:5 in food-web dynamics of freshwater ecosystems has recently been discussed [355]. Finally, the identification of these PKS systems in ancient lineages raises intriguing questions about the evolutionary relationship of this newly discovered pathway to 20:5 and 22:6 FA relative to the enzymatically more complex desaturase/elongase route found in higher eukaryotes [213].

10.5 Some promising sources of marine LC-PUFA

10.5.1 PUFA from nonphotosynthetic microorganisms

If nowadays conventional fish oils are the main industrial sources of PUFA they may be not suitable to meet the increasing markets, notably for DHA owing to their limited supply (see section on sources and market), lower content of DHA in comparison to that of EPA, and peculiar taste and odour. Thus, the production of (n-3) PUFA by microbial fermentation of oleaginous microorganisms has attracted considerable attention in relation to industrial application of single-cell oil (SCO) [354, 356, 357]. For instance, a range of autotrophic and heterotrophic microbes has been assessed for potential commercial sources of EPA and DHA by various workers including Barclay et al. [358], Lewis et al. [359] and Vazhappilly and Chen [360], and the results of many studies in this area have been reviewed by Singh and Ward [361] and Ratledge [362].

In comparison to autotrophic microorganisms, the de novo synthesis of (n-3) and (n-6) PUFA by heterotrophic microorganisms may provide an easier and less expensive means of producing PUFA-rich biomass and oils [363]. Thus, Ratledge [362] considered that, despite improvements in the efficiency of photobioreactors, it is doubtful whether the growth of microalgae in bioreactors could be scaled up to satisfy even a modest demand for SCO rich in (n-3) PUFA, and suggested that heterotrophic microbes might be a more productive source.

In recent years, interest in the use of microheterotrophs as a source of PUFA has increased [364]. Microheterotrophs do not require some of the elements necessary for the culture of autotrophs (e.g., light, carbon dioxide), and some see them as a potential alternative to traditional commercial sources of PUFA. Arachidonic acid has been produced in quantity by some fungi [365, 366]. Certain bacteria have been shown to produce EPA and DHA [367, 368]. The recognized need in aquaculture for alternative sources of PUFA for feeding both larvae and adults has seen PUFA-producing bacteria successfully demonstrated as a means to enrich rotifers *(Brachionus plicatilis,* a live-feed organism for finfish larvae) with these FA [369–371]. In addition an increasing body of research into microheterotrophic PUFA production has concentrated on the thraustochytrids [363].

10.5.1.1 Bacteria

In marine food webs, microalgae have long been considered as the only de novo source of EPA [372]. Indeed PUFA were once thought to be absent in

bacterial membranes [373] and the production of PUFA by bacteria is often ignored [374]. However, numerous bacterial species of marine origin have now been shown to produce LC-PUFA such as EPA and DHA and some authors correctly pointed to the potential role of prokaryotic PUFA production in marine food webs [25, 347, 375].

Phylogeny

The screening of PUFA-producing prokaryote has led to the identification of previously undescribed taxa within the genera *Shewanella* and *Colwellia* [376, 377]. Subsequently, the chemotaxonomy of PUFA-producing bacteria was reviewed [30]. Indeed, until recently the taxonomic status of PUFA-producing bacteria received little attention, with most research efforts focused on the description of PUFA production [31].

Figure 22 represents the division-level diversity of the bacterial domain based on 16S rDNA sequences. Several of the divisions (e.g. Proteobacte-

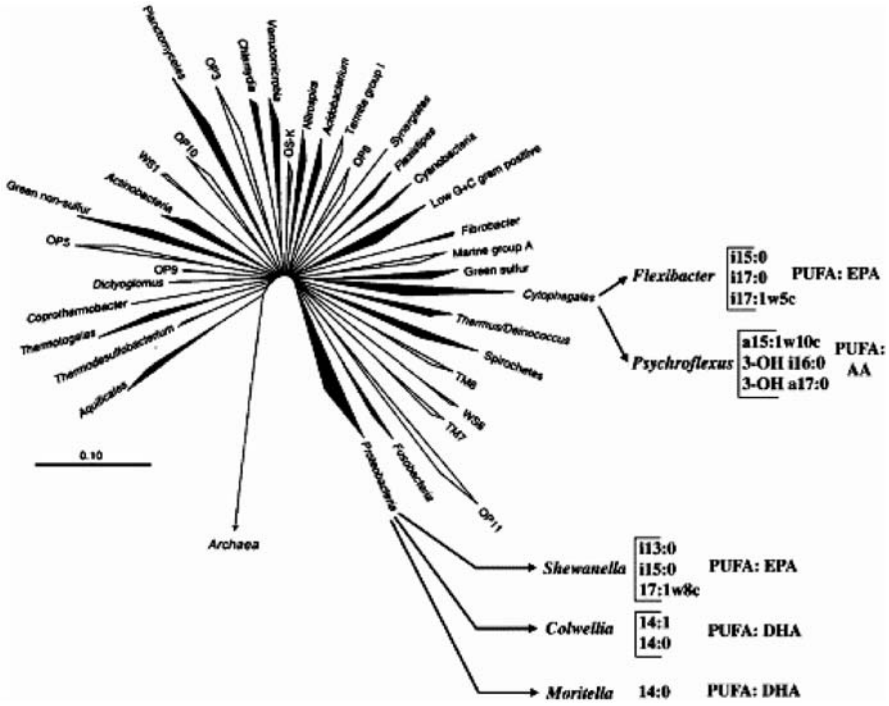

Fig. 22 Evolutionary distance tree of the bacterial domain [31]. The major PUFA-producing genera, *Shewanella*, *Colwellia* and *Moritella*, are expanded from the *Proteobacteria* division together with *Flexibacter* and *Psychroflexus* from the *Cytophagales* division. For each genus, biomarker FA and the types of PUFA are listed

ria, Actinobacteria) are well represented by cultivated strains. However the majority are poorly represented by cultured organisms. In fact 13 of the 36 divisions are defined by environmental sequences only [378]. The proportion of cultivated versus uncultivated sequences obtained from environmental molecular analyses may be used as an indication of the level of described biodiversity for a particular division. The occurrence of bacteria with the ability to produce PUFA is limited to five well-known marine genera which fall within two distinct domains of bacteria. Although separated by a significant evolutionary distance, the ability to produce EPA is apparent from both groupings. Species from each domain also possess the ability to produce further PUFA products, namely AA or DHA, respectively (Fig. 22) Thus, the ability to produce PUFA exhibits a phylogenetic linkage centred on two distinct lineages, the marine genera of the γ-Proteobacteria (*Shewanella*, *Colwellia*, *Moritella*, *Psychromonas*, *Photobacterium*) and more limited species within two genera of the *Cytophaga-Flavobacterium-Bacteroides* (CFB) grouping (Flexibacter, Psychroflexus). However, it is pertinent to note that not all species within these genera express the ability to produce PUFA (Figs. 22, 23). Evidence implies that PUFA production is associated with physiological adaptations within marine bacteria.

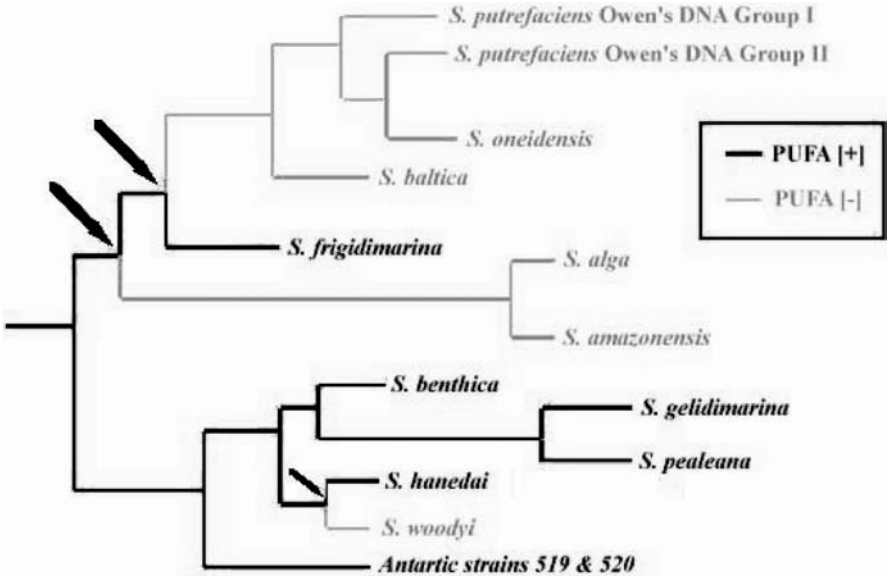

Fig. 23 Schematic of the phylogenetic relationship of the genus *Shewanella* based on 16S rDNA sequence. Species known to produce polyunsaturated FA (PUFA) and their lines of descent are highlighted in red. Species and lines of descent known not to produce PUFA are highlighted in blue CE[j]. Arrows indicate sites of divergence where the expression of PUFA synthesis has been lost. Adapted from Russell and Nichols [30]

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Marine ecology

Bacterial PUFA-producing isolates have been found to be particularly prevalent in high-pressure low-temperature deep-sea habitats and permanently cold marine environments [357, 367, 379]. Indeed, the majority of the γ-Proteobacteria PUFA producers are characterized as being psychrophilic[3], halophilic and predominantly piezophilic[4] or piezotolerant [30, 380]. These physiological traits have influenced the ecological distribution of PUFA-producing bacteria in the marine environment (Table 5). The enrichment of PUFA-producing strains from these environments has led to speculation that PUFA synthesis is an important adaptation for countering the effects of elevated hydrostatic pressure and low temperature on membrane fluidity or phase. In strains which have been analysed, PUFA synthesis undergoes temperature-dependent and, for deep-sea isolates, pressure-dependent regulation. Typically, as cultivation temperature is decreased, and or pressure increased, PUFA incorporation into membrane phospholipids is enhanced. This modulation is thought to maintain appropriate membrane physical structure. Indeed, in marine bacteria, PUFA are component FA of certain phospholipids which occur in the cell membrane. It is considered that the low melting temperature of these highly unsaturated membrane components, combined with their unique molecular geometry in the membrane, yields a particular advantage at low temperature in balancing the competing homeoviscous and homeophasic forces in the cell membrane [30].

However, if bacteria of the CFB grouping are similarly psychrophilic and halophilic, they lack the ability to grow at high pressure [380]. In addition, for at least one high-pressure-adapted deep-sea bacterium, *Photobacterium profundum* strain SS9, growth at high pressure and low temperature does not depend upon PUFA synthesis [351]. Hence while PUFA production appears as a phylogenetically linked genotypic strategy for such selective pressures, their presence may not be essential for the growth of bacteria in such environments.

While there is not necessarily a phylogenetic relationship between psychrophilic organisms, such relationships may exist through the evolution of adaptive strategies for low-temperature growth. This is supported by empirical observations such as in the genus *Shewanella* (Fig. 23). Here, there is a good correlation between those species which are cold-adapted and produce PUFA (*S. pealeana*, *S. hanedai*, *S. benthica*, *S. gelidimarina*, *S. frigidimarina*), in contrast to those which do not produce PUFA and grow at higher temperatures (mesophiles) (*S. putrefaciens*, *S. alga*, *S. baltica*, *S. woodyi, S. oneidensis*, *S. amazonensis*). However, as indicated by Nichols

[3] The term psychrophile (psychro = cold, phile = loving) is an operational definition, to describe the temperature-growth relationship of microorganisms [400].

[4] (Piezo = pressure, phile = loving). Same comments as above.

Table 5 Major bacterial genera responsible for the production of PUFA in the marine environment (adapted from Nichols [25])

Species	Ps	Ha	Pi	PUFA	Environmental source	References
Shewanella						
S. algae	–	–	–	–	Red algae, Japan	[376]
S. amazonensis	–	+	–	–	Water, Amazon river	[381]
S. baltica	–	–	–	–	Oil brine, Japan	[376]
S. benthica	+	+	+	+	Holourithan intestine	[376]
S. colwelliana	–	+	–	–	Aquaculture, USA	[31]
S. frigidimarina	±	–	–	+	Sea ice, Antarctica	[376]
S. Gelidimarina	+	+	±	+	Sea ice, Antarctica	[376]
S. hadenai	+	+	±	+	Sediment, Artic	[376]
S. japonica	±	–	–	+	Sediments, mussels	[382]
S. livingstonensis	±	–	–	nd	Sea water, Antarctica	[383]
S. oneidensis	–	–	–	–	Lake sediment, USA	[31]
S. pealeana	±	+	–	+	Squid gland	[31]
S. putrefaciens OG1	–	–	–	–	Butter, UK	[31]
S. putrefaciens OG3	–	–	–	–	Butter, UK	[31]
S. woodyi	±	+	–	–	Sea water, Hawaii	[31]
S. violacea	+	+	+	+	Deep-sea	[384]
Colwellia						
C. demingiae	+	+	nd	+	Sea ice, Antarctica	[377]
C. hadaliensis	+	+	+	nd	Deep-sea	[385]
C. hornerae	+	+	nd	+	Sea ice, Antarctica	[377]
C. maris	+	+	nd	+	Sea water, Japan	[386]
C. psychroerythraea	+	+	nd	+	Flounder eggs	[377]
C. psychrotropica	+	+	nd	+	Burton lake, Antarctica	[377]
C. rossensis	+	+	Nd	+	Sea ice, Antartica	[377]
Moritella						
M. japonica	+	+	±	+	Deep-sea	[384]
M. marina	+	+	±	+	Sea water	[387]
M. vavanosii	+	+	+	+	Deep-sea	[388]
M. viscosus	+	+	±	nd	Fish	[389]
Psychromonas						
P. antarticus	+	+	+	nd	Sea-ice, Antarctica	[390]
P. kaikoae	+	+	+	+	Deep-sea	[391]
P. marina	+	+	+	+	Seawater	[392]
Psychroflexus						
Ps. gondwanense	–	–	nd	–	Burton lake, Antarctica	[393]
Ps. torquis	+	+	nd	+	Sea ice, Antarctica	[393]
Photobacterium						
Ph. profundum	+	+	+	+	Deep-sea	[394]

Ps: Psychrophilic, Ha: Halophilic, Pi: Piezophilic

et al. [31], *S. frigidimarina* and *S. pealana* are rather psychotolerant than psychrophilic and in addition *S. frigidimarina* also grows without the presence of salt. Thus these authors point out that the correlation among psychrophily, halophily and PUFA production is therefore not exclusive. They suggest that PUFA production may be a common physiological strategy for coping with the combined constraints of low temperature and marine salinity.

In addition to cold environment (sea ice) and deep sea water, PUFA-producing bacteria have also been isolated in the intestinal contents of marine fish and invertebrates [347, 368, 379, 395–397] Moreover, the transfer of bacterially derived FA and specifically PUFA, between marine bacteria and higher trophic levels has been demonstrated [370, 398]. For example, recent studies have demonstrated that PUFA-producing bacteria can be used to enrich rotifers in EPA or DHA [370, 371, 399]. The greatest level of EPA enrichment in rotifers was 5.8% dry weight [370], whereas for DHA it was 0.3% dry weight [371]. These studies provide an important step in the development of novel sources of PUFA for aquaculture [346].

All theses facts uphold the idea presented by Nichols [25] that the assumption that microalgae provide the bulk of de novo PUFA production for all marine food webs must now be actively reviewed to determine the role and potential importance of PUFA-producing prokaryotes in marine microbial niches such as sea ice, marine animals and abyssal communities.

Biotechnology

Interest in the production of PUFA from alternative sources for use in aquaculture feeds and human nutraceuticals (see section on heath benefit) has fuelled recent research into the molecular biology of PUFA production in prokaryotes. A key advantage of bacterial PUFA production (as for thraustochytrid PUFA production, see below) is that only a single PUFA is produced, rather than the complex mixture yielded from fish or algal oils [30]. Thus bacterial sources of PUFA remove the expense of preparative purification in the production of high-purity PUFA oils.

In addition to their potential use as "cell factories", bacteria in particular offer the biotechnological opportunity to investigate the genes and enzymes responsible for PUFA production. A variety of bacterial fatty-acid biosynthetic mechanisms exist, which vary with taxonomic identity and class of fatty acid product [401–403]. Some reports have suggested that bacterial (n-3) PUFA production is mediated by undefined desaturases [30, 348, 352]. However, as indicated by Allen et al. [351], sequence studies of bacterial genes required for PUFA biosynthesis have gradually led to a reappraisal of this view (see section on biosynthesis).

Initial insight into the genetics of bacterial PUFA synthesis was gained by the transfer of a gene cluster from *Shewanella putrefaciens* SCRC-2738 into *Escherichia coli* and a *Synechococcus sp.* resulted in the successful expression

of EPA in these organisms [347]. However, the level of expression achieved was low. The gene cluster used in both cases consisted of a 38 kb fragment containing eight open reading frames with three of these possessing homology with genes that encode for enzymes involved in fatty-acid biosynthesis. Further characterization using these organisms has identified five genes responsible for PUFA biosynthesis, designated ORFs 2, 5, 6, 7 and 8. A subsequent analysis of the predicted amino acid sequences of the products of these genes indicated that they are most related to microbial polyketide synthase (PKS) complexes and fatty acid synthase (FA) enzymes (see section on biosynthesis). In addition to the *Shewanella* sp. SCRC-2738 sequences, related genes partially responsible for PUFA production have been analysed from the DHA-producing bacterium *Moritella marina* strain MP-1 (formerly *Vibrio marinus*) [352] and from a DHA-producing thraustochytrid marine protist belonging to the genus *Schizochytrium* [329]. Recently, Metz et al. [329] reported biochemical analyses of PUFA production in *E. coli* strains harbouring *Shewanella* sp. SCRC-2738 DNA and in the *Schizochytrium* species. Consistent with the examination of enzyme domains, isotopic-labelling studies provided compelling support for a PKS-like pathway of PUFA synthesis in both systems studied [329].

After spending significant effort on understanding the mechanistic microbial production of PUFA, research now has to focus on the regulation of such biosynthesis to maximize the transgenic potential of bacterial LC-PUFA genes.

10.5.1.2 Thraustochytrids

Thraustochytrids are microheterotrophs generally considered necessarily marine with a specific requirement for Na^+ ions [404] that feed as saprobes or occasionally as parasites [405]. Thraustochytrids have been characterized by the presence of sagenogenetosome, an ectoplasmic net, a cell wall with noncellulosic scales, and a life cycle consisting of vegetative cells, zoosporangium, and biflagellate zoospores [354]. They have a wide geographic distribution in estuarine and marine habitats, with strains isolated from Antarctica [406], the North Sea [407], India [408], Micronesia [409], Japan [410], Australia [359] and Fiji [354]. They have been reported frequently from seawater, sediments, algae, and invertebrates, both as saprotrophs and parasites. Their ubiquitousness and physiological capabilities to utilize a wide variety CE[k] suggest that they play a definite role in the marine ecosystem.

Originally thought to be primitive fungi, thraustochytrids have more recently been assigned to the subclass Thraustochytridae (Chromista, Heterokonta), aligning them more closely with the heterokont algae (e.g., brown algae and diatoms) [411].

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In research on microheterotrophic PUFA production, particular attention has been given to the thraustochytrids since their lipids contains proportionately large quantities of ω3 PUFA and particularly DHA [357].

Bowles et al. [386] have screened 57 thraustochytrids isolates from three different locations. Although a common fatty-acid profile for the thraustochytrid isolates emerged ((n-3) PUFA as a significant component, as previously found [**?**]), there was considerable difference in the DHA content of the oil. This large variation in DHA proportion can also be extended to biomass and lipid yields depending on the thraustochytrid strains (Table 6). Thus, in some isolates from a cold-temperate environment, DHA can represent almost 50% of the total FA present while those from a sub-tropical environment produce higher levels of biomass, with up to 37 (w/w) % oil but with a lower DHA content [423].

As indicated by Lewis et al. [363], most reports concerning the production of PUFA by thraustochytrids have dealt almost exclusively with DHA production (Table 6), as this compound is often the most abundant PUFA produced by strains of thraustochytrids reported to date. However, it is evident that some thraustochytrid strains also produce other PUFA. Thus, Huang et al. [354] have shown that the fatty acid profiles of DHA-producing thraustochytrids could be used to classify them into five separate categories:

- DHA/DPA (docosapentaenoic acid; C22:5 (n-6)),
- DHA/DPA/EPA,
- DHA/EPA,
- DHA/DPA/EPA/AA
- DHA/DPA/EPA/AA/DTA (docosatetraenoic acid, C22:4 (n-6)).

Their seven isolates from Japan and Fiji were proved to be new thraustochytrids by their specific insertion sequences in the 18S rRNA genes. The phylogenetic tree constructed by molecular analysis of 18S rRNA genes from those isolates and typical thraustochytrids shows that strains with the same PUFA profile form each monophyletic cluster. These results suggest that the C20–22 PUFA profile may be applicable as an effective characteristic for grouping thraustochytrids. Moreover, Bowles et al. [423] have shown that, among their 57 thraustochytrids, all synthesized the ω6 PUFA arachidonic acid in varying amounts, mainly as a minor component of the PUFA and that EPA was present in the oil produced by all the isolates except two (however EPA content was generally low, varying from 0.2 to [–(%w/w)]0.6 of the dried thraustochytrid cells). So, although about 15 strains of thraustochytrids, including *Thraustochytrium aureum*, *T. roseum*, *T aggregatum*, *Schiizochytrium limacinum* and *S. aggregatum*, have been reported to produce significant amounts of DHA (Table 6), there are many potential strains yet to be explored [363, 424].

As indicated by Hammond et al. [425], there are no reports in the literature of direct human consumption of thraustochytrids. This is due to the fact that, prior to the late 1980s, thraustochytrids had never been cultured

Table 6 Docosahexaenoic acid (DHA) production by thraustochytrids [350]

	Age (d)	Temp (°C)	Vessel	Other	Biomass (g/L)	Lipid (% dw)	(% TFA)	(mg/g)	(mg/L)	Refs.
Shizochytrium sp. SR21	2.5	28	Fermenter	PH. 4	21	50	35	224	4700	[412]
Shizochytrium sp. SR21	4		Fermenter 300 rpm	–	48	77	36	277	13 300	[413]
S. Limacium SR21	5	25	Flask	–	38	37	33	110	4200	[357]
S. aggregatum ATCC 28209	10	25	Flask 200 rpm	Dark	0.9	–	1.7	30	0.4	[360]
Thraustochytrium aureum ATCC 34304	6	25	Flask 300 rpm	Light	3.8	16.5	49	70	270	[414]

Table 6 (continued)

	Age (d)	Temp (°C)	Vessel	Other	Biomass (g/L)	Lipid (% dw)	(% TFA)	(mg/g)	(mg/L)	Refs.
T. aureum ATCC 34304	6	25	Flask 300 rpm	Light	4.9 20.3 51 104 511 1					[415]
T. aureum ATCC 34304	2.5	25	Flask	Light	5.7	8.1	40	–	–	[356]
T. aureum ATCC 28211	6	25	Flask 200 rpm	Dark	0.8	–	3.7	50	4.0	[360]
T. roseum ATCC 28210	5	25	Flask 250 rpm	Light	7.6	18.2	50	87	650	[416]
T. roseum ATCC 28210	12	25	Flask 250 rpm	Fed batch	17.1	25	49	115	2100	[417]
Thraustochytrium sp. ATCC 20892	4	25	Flask 200 rpm	–	2.7	7.3	35	25	68	[418]
Thraustochytrium sp. ATCC 20892	6	28	Flask 120 rpm	Light	7.5	32	25	–+	–	[419]

on a scale larger than a laboratory shake flask. Barclay [422] and Bajpai et al. [414, 415] were the first to successfully cultivate *Schizochytrium* sp. and *Thraustochytrium* sp., respectively, in fermenters (two patents have been filed detailing the cultivation of thraustochytrid strains to produce lipids containing EPA and DHA [422, 426]). However, thraustochytrids are primarily consumed by filter-feeding invertebrates in the marine ecosystem, including mussels and clams, and by fish that are consumed directly by humans. Thus, in the last few years, thraustochytrids have been successfully used for commercial production of PUFA-rich products notably in aquaculture applications. This is the case for a *Schizochytrium* strain, which is the basis for two products marketed for enriching rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.) with PUFA, prior to feeding these organisms to cultured finfish larvae ([427]; www.aquafauna.com; www.sandersbshrimp.com). OmegaTech commercialized a product for aquaculture applications (HUFA 2000, a spray-dried form of *Schizochytrium* sp. dried microalgae), which has been successfully utilized for over seven years as an excellent stable dietary source of DHA in shrimp larvaculture and finfish (red seabream, Japanese flounder) culture with no adverse effects. Use of *Schizochytrium* sp. in these applications has been found to promote larvae survival and growth [428]. Other uses of thraustochytrid oil are being actively explored. Monsanto (www.monsanto.com) is producing *Schizochytrium* sp.–derived oil under a cooperative technology agreement with OmegaTech (www.omegadha.com). Moreover, dried *Schizochytrium* microalgae (DRM, OmegaTech) have also been generally recognized as safe (GRAS) for use as a DHA-rich ingredient in broiler chicken and laying-hen feed at levels up to 2.8–4.3%, respectively [429]. Since 1997, DHA-enriched eggs from hens fed a diet containing approximately 1% DRM are now commercially marketed in the United States, Mexico, Germany, Spain, Portugal, the Benelux countries, Italy, Norway, and Israel [430].

These products have entered the market in direct competition with microalgal and fish-oil products. It is possible, however, that thraustochytrids will offer some advantage over other oils as sources of PUFA for aquaculture. Many aquaculture species require proportionally more DHA than EPA in their diet [431]. The PUFA profiles of many thraustochytrids fit this criterion, while most oils from the fish-meal industry contain more EPA than DHA. However it has to be noticed that a recent study [432] has revealed that the replacement of fish oil with a dried product made from a thraustochytrid culture in canola-oil-based diets for Atlantic salmon could affect the disease resistance of fishes. Indeed, if the authors didn't observe significance differences in final weight, weight gain, feed consumption, feed efficient ration or protein value between the diets, nor in whole-body chemical composition, organ somatic indices or measures of immune function, they have noticed that, following transfer to seawater and two challenges with *Vibrio anguillarum*, cumulative mortality was significantly lower in fish fed some

fish oils than the others. They concluded that their thraustochytrid strain had no detrimental effects on the performance of salmon although, at the current inclusion of 10%, it failed to confer the same effects as fish oil under challenging conditions.

In conclusion, as indicated by Lewis et al. [363], thraustochytrids are clearly a new and potentially competitive player in the PUFA market. Considerable work is required before the production of oil from these organisms significantly increases its share of the market for PUFA-rich products. To achieve this aim, the following key stages need to be negotiated. Firstly, the collection, screening, and maintenance of PUFA-producing strains. Several strains with potential for the commercial production of DHA-rich oils have been isolated already. However, if thraustochytrids that produce higher yields, more attractive PUFA profiles, or other less common but sought-after PUFA are isolated and optimized, then demand for these isolates and compounds may well increase. Secondly, the efficiency of PUFA production must be optimized. The types and amounts of PUFA produced by individual strains of thraustochytrids are susceptible to manipulation by varying culture conditions. Enhancement of PUFA profiles using molecular techniques may be also considered. Different markets will provide demand for strains that produce high levels of PUFA measured either in terms of biomass (i.e., PUFA production w/w cell mass) or volume (i.e., PUFA production w/vol fermentation medium). Thirdly, appropriate conditions for long-term storage of microbial cells and their products must be determined. The form and stability of thraustochytrid biomass and of oils will be major factors in determining the suitability of these products for use as food additives. Finally, oil extraction and refinement technologies must be developed to meet market demands for cost-effective and safe trophic transfer of PUFA to the target consumers. The bottom line for the biotechnological future of thraustochytrid oils will be their competitiveness against other PUFA-rich oils. Nevertheless, oils derived from fish and microalgae generally have a complex fatty acid (total and polyunsaturated) profile, and do not readily lend themselves to the isolation of high-purity (> 98%) FA. Conversely, oils produced by some thraustochytrids have relatively simple fatty-acid profiles and may well be more amenable to cost-effective refinement. DHA is a good illustration of the industrial potential of thraustochytrids. Thus, the largest potential market for microbial oils containing DHA is perceived to be as an additive to infant formulae as an essential fatty acid for brain and retinal development (see section on health benefit); Ratledge [362] considered that the presence of significant quantities of EPA in the thraustochytrid oils so far assessed precluded its use for this purpose. Indeed, eicosapentaenoic acid is considered contraindicatory in breast milk substitutes, but strain selection will easily allow this difficulty to be overcome [423].

10.5.1.3 Conclusions

Growing interest in PUFA applications in various fields coupled with their significance in health and dietary requirements (see section on health benefit) has encouraged "hunting" for more suitable sources of these compounds. The inadequacy of conventional agricultural and animal oils has put attention on developing new microbial technologies. Indeed, microorganisms represent the largest reservoir of undescribed biodiversity, and hence possess the greatest potential for the discovery of new natural products. It is estimated that the Earth currently supports 3–30 million species of organisms. Of these, approximately 1.4 million have been described by science. This includes virtually all the species of birds and mammals ($\sim$ 13 500). In contrast only around 200 000 of the estimated 1.0–1.5 million species of fungi have been characterized (i.e. 13–20%). For the bacteria this percentage is even lower with estimates ranging from only 1–10% of probable species being described in culture [433, 434].

However, the focus of biotechnology on highly valuable PUFA requires knowledge of how microorganisms control and regulate the fatty-acid biosynthetic machinery in order to obtain specific PUFA in high yield.

Elucidation of the signalling systems and mechanisms transmitting the signals from different membranes to the major sites of lipid biosynthetic machinery represents a challenging and potentially rewarding subject for further research [435]. At least, the extensive research and development of PUFA production carried out over the past few years will be aimed at improving the economic competitiveness of microbial lipids compared to plant- and animal-derived oils.

Nowadays, by using conventional stirred-tank fermenters, economically viable quantities of certain microorganisms that are rich in LC-PUFA can be produced [436]. The chief advantages of such techniques lie in the consistency and purity of the final fatty acid product. Further, unlike the scenario with fish oils, economies of scale have reduced the price of oils derived from organisms raised in fermenters by 10- to 30-fold [437].

10.5.2 PUFA from fish

Fish is a major source of food for mankind, providing a significant amount of the animal protein diet in many countries. Moreover, the consumption of fish has been linked to health benefits (see section on health benefit). Indeed, oils from fish are characterized by a large range of FA from 12–26 carbon atoms and 0–6 double bonds. The bulk of the fatty acid chains is contributed by saturated (15–25%), monoenes (35–60%) and polyenes (25–40%). In contrast with the other fats and oils, fish oils contain large amounts of EPA and

[TS 1] There is no caption for this table – please check.

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DHA, respectively, 14–19% and 5–8%. The proportion of polyunsaturated FA depends on many parameters (see below). Saturated FA include C12 up to C24:0 components, and some branched chains (iso C16, iso C17.) are also found. Among the monoenes, 16:1(n-7), 20:1(n-9) and 22:1(n-11) are present in various amounts, this last component being bioconverted from the corresponding fatty alcohol of copepod wax ester by the fish liver [438]. More than 50 different FA were described in marine fish oil, but eight species frequently represent more than 80% of the total amount (Table 7).[TS1]

In fish tissues, the composition of FA (mainly of triacylglycerols[CE1] and to a lesser extend of phospholipids), is determined by diet composition and lipid metabolism [439, 440]. Fish have the ability to synthesize de novo the saturated and monounsaturated FA and also to selectively absorb and metabolize dietary FA including LC-PUFA [440, 441] in order to obtain an optimal fatty acid composition [442]. This optimal composition seems to be a characteristic for each species and even each strain [443, 444]. Moreover, the PUFA conversion capacity in fish varies among species and even races [439]. Thus, freshwater fish are generally able to elongate and desaturate α-linolenic acid (18:3(n-3)) to EPA and DHA, whereas marine fish, which lack or have a very low activity of Δ5-desaturase, cannot and require LC-PUFA such as EPA and DHA in the diet [440].

In addition to food accessibility and lipid metabolism some environmental parameters also notably influence the proportion of PUFA [445]. Indeed, the colder the water, the higher the amount of these components. An inherent property of cells in poikilothermic animals is their capacity to adjust the physicochemical characteristics of their membranes to the prevailing temperatures. This phenomenon, known as homeoviscous adaptation of membrane fluidity, has been reported in poikilothermic fish [446]. In fish, during adaptation to reduced temperatures, unsaturation of the constituent FA increases, with the polar head group as well as the molecular species composi-

Table 7

	Menhaden	Herring
14:0	7–12	5–8
16:0	15–26	10–19
16:1(n-7)	9–16	6–12
18:0	2–4	1–2
18:1(n-9)	8–14	9–25
20:1(n-9)	–	7–20
20:5(n-3)	11–16	4–15
22:1(n-11)	< 1	7–30
22:6(n-3)	5–14	2–8

[CE1] Please confirm correction of spelling from triaclyglycerols to triacylglycerols

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tion of membrane phospholipids being reorganized [202]. Evidence suggests that the fatty acid distribution is very individual from species to species and depends on many factors like season, temperature, fishing ground, fish species, age, gender or nutritional habits [447–452].

The FA distribution in triacylglycerols is stereospecific [www.cyberlipid.org]. Indeed, results indicate that the stereospecific location of the PUFA is in position 2 (also 3 for EPA in cod) in fish but in position 3 for mammals (same in seal, whale and polar bear). This raises the question of the availability of these FA from food expecting a benefic effect on diverse human functions (see section on health benefit).

Familiar fish species used in the production of fish oil include among others, anchovies, capelin, Atlantic cod, Atlantic herring, Atlantic mackerel, Atlantic menhaden, salmonids, sardines (see section on market and sources). Of the world's fish oil production, 90% is produced from fatty fish where lipids are localized mainly under the skin, around the intestines or in the white muscle. In such fishes, the oil content varies (see above) but it can reach 21% (herring) and 18% (sardines). Such oils are still the least-expensive natural source of preformed long-chain PUFA, and several industries (e.g., Ocean Nutrition, Halifax, N.S., Canada, and Pronova Biocare, Sandefjord, Norway) now specialize in their production and purification through cold pressing, further concentration by winterization (i.e., chilling), and other technologies [431].

There are, however, potential problems associated with fish oils as a source of PUFA such as: taste, odor, stability problems as well as the presence of co-extracted contaminants. Some could be at least partially solved for example by microencapsulation [453] and deodorization [454]. Nevertheless, the crucial problem of those oils is their sustainability due to the worldwide decline of fish stocks (see section on market). A better use of raw material but as well of by-catch and by-products from fisheries may be one solution, another is to look for other sources (see above) including non- or little-exploited fish species.

Lipid content has been studied for a long time mainly in the liver and edible parts of fish such as muscles. Several investigations have evidenced that other parts i.e. liver and skin, often discarded for several reasons when fish are prepared for consumption, may have possible nutritional and therapeutic value mainly due to their EPA and DHA content [445, 455]. Indeed valuable fish oils can be obtained from trash fish and/or fish scraps or cannery wastes, which are often called "gurry" from filleting and canning operations. Fish scraps normally consist of the head, skeleton, and adhering proteinaceous tissues.

Surprisingly, it can also be observed that often no detailed chemical studies of the lipid and fatty acid content of certain fish are available, even though the fish most often consumed or commercially exploited, such as for example the species Sardinella and Cephalopholis in Senegal waters [455].

Capelin, squid liver, krill (*Euphausia superba*) could constitute sustainable new sources [437].

10.6 Current utilization of marine oils and lipids

10.6.1 Market

10.6.1.1 Production

From a world production of oil and fat of about 20 million tons in 1939, about 77 million tons were produced in 1989 where 74% were of vegetal origin (soybean 19% > palm 13% > rapeseed 10.3% > sunflower 9.6%). In 2003–2004 the global production of fats and oils is expected to be 128.5 million tons with 82% of vegetal origin. The world average consumption of oils and fats in 2003 is about 20 kg per capita [www.cyberlipid.org].

Of the estimated 89 million tonnes of fish produced in 2000 in the world, excluding China, nearly 71 percent (63 million tonnes) was used for direct human consumption. The remainder (about 29 percent) was utilized for various nonfood products, mostly for reduction to meal and oil (the state of world fisheries and aquaculture, 2002 FAO). Indeed, nowadays, a third of the world's catch from the seas is going into manufacturing fish meal and fish oil. Thus, the world production of marine oils represent approximately 1% of the commodity world fats and oils production (Table 8).

Remark: the International Fishmeal and Fish Oil Organisation (IFFO) is the international nongovernmental trade organization representing fish meal and oil producers worldwide. It has more than 200 member companies in 38

Table 8 Production (million tonnes) for 17 commodity oils in the four-year period 1998/99 to 2001/02 [456]

	98/99	99/00	00/01	01/02
Total production	107.6	113.5	117.3	119.7
Soybean	24.60	25.30	27.10	29.40
Palm	19.40	21.30	23.70	24.30
Rapeseed	12.70	14.50	13.90	13.40
Sunflower	9.30	9.50	8.70	7.50
Other vegetal oils	19.70	20.50	21.70	22.40
Fish	0.86	1.38	1.42	1.12
Other animal fats and oils	21.04	21.02	20.78	21.58

countries. Two-thirds of the world's production of fish meal and fish oil are members of the IFFO and 95% of the exports of fish meal and oil are also part of the IFFO.

Chile, Peru, Scandinavia, USA and Japan are the main suppliers of fish oil (Fig. 24). The average world production between 1991 and 2001 was about

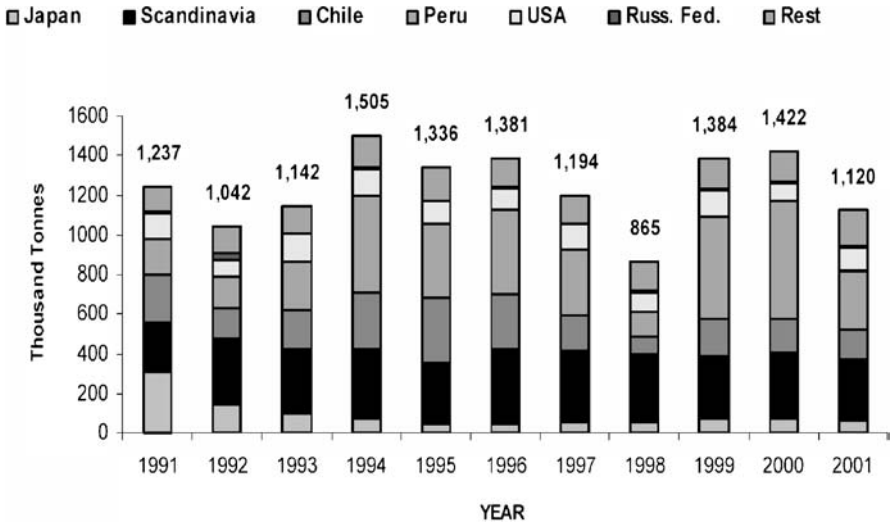

Fig. 24 World fish body oil production – major producers (IFFO)

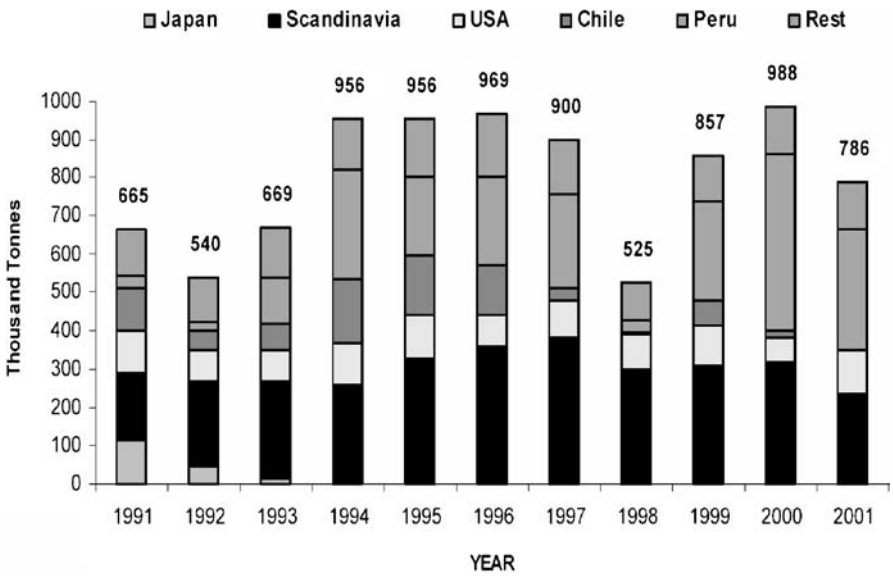

Fig. 25 World marine oils and fats exports by major exporters (IFFO)

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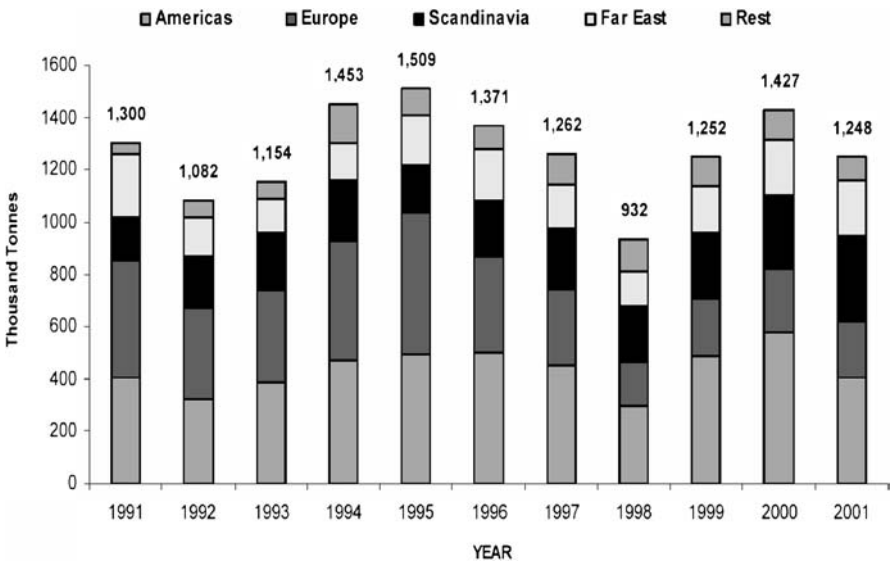

Fig. 26 World marine oil consumption and stocks – major consumers (IFFO)

1.25 million tonnes of fish oil produced annually. Important fluctuations in production can be observed, they were due to the El Niño phenomenon mostly in Chile and Peru. In 1998, which was the big El Niño year, the production of these two countries only reached 210 000 tonnes while the average over the last five years was 520 000 tonnes (the total catch volume by Peruvian fisheries was reduced to 3696 million tons[CE]^{n}, only 45% of the 2002 catch of 8238 million tons). However, due to better prediction of such climatic occurrence, the governments concerned are increasingly proactive in anticipating and taking precautionary measures for fishing. Thus, precautionary approach to fisheries management has been maintained (in Peru notably) to safeguard the viability and prevent depletion of stocks through overfishing. Careful management of the fishery and a return to normal environmental conditions allowed stocks to recover in 1999 and 2000.

10.6.1.2 Exportation

The major exporters are mainly the same countries, with the noticeable exception of Japan that is rather now a net importer (Fig. 25). Over the past decade fish oil exports by Peru (the main exporting country) have expanded by almost twelve times (US$ 91.1 million in 2001). However, Peru's exportation is variable; it can be enormous (up to 500 000 tonnes in 2000) or very small, notably during El Niño periods. The second main fish oil exporter is now the USA (US$ 41.7 million in 2001).

10.6.1.3 Consumption

Consumption of fish oils by countries is indicated in Fig. 26. Most of the fish oil goes into salmonid feed in Norway, Chile, Canada and various European countries, which is the reason for the predominance of these countries in terms of consumption.

Remark: fish oil is included in aquaculture feeds as a source of both dietary energy and PUFA. Considerable research is occurring worldwide in an effort to find alternatives to fishmeal and fish oil in aquaculture feeds. However, this research is tempered by the obligate dietary requirement of many marine finfish species for long-chain PUFA (LC-PUFA: e.g., EPA and DHA).

Aquaculture has been the world's fastest-growing food production over a decade [438]. The world aquaculture production has at least multiplied by a factor of two in the last ten years: 24 457 421 tonnes live weight in 1993 and 48 413 636 tonnes live weight in 2001 (Eurostat and FAO sources). The International Fishmeal and Oil Manufacturers Association [www.iffo.com] estimates that inclusion of fish oil in aquaculture feeds will rise from 380 000 tonnes in 1994 to 582 000 tonnes in 2001 and 1 133 000 tonnes in 2010 (Table 9). With aquafeed demand at about 1 million tonnes of fish oil in 2010, depending on the production of fish oil it could be around 80% or even close to 100%. This could well result in a worldwide undersupply of fish oil, leading to increased demand for fish oil alternatives. Moreover, this lack of fish oil will have an impact on aquafeed composition. It seems likely that cheap, plant- or animal-derived oils, which often contain low levels of LC-PUFA, will be used increasingly as alternative sources of energy in some aquaculture feeds. If such substitution does occur, sufficient LC-PUFA to meet the dietary requirements of cultured aquaculture species may be required from other sources. Typically, many cultured marine species require around 1% to 2 wt/wt % LC-PUFA in their diets [457, 458]. Pike and Barlow [459] estimated that marine aquaculture finfish species will require about 2×10^6 tonnes of feed in 2010.

Table 9 Fish oil use prediction based on an annual world production of 1.25 million tonnes for the period 2002–2010 [461]

	1990	2002	2010
Edible	76%	30%	14%
Industrial	8%	12%	5%
Aquafeed	16%	56%	79%
Pharmaceutical	–	2%	2%

Table 10 Predicted use of fish oil in fish feed (data from IFFO)

	% of fish oil inclusion in feed produced		Thousand tons of fish oil	
	2000	2010	2000	2010
Carp	–	0.5	–	103
Catfish	1.0	–	5	–
Tilapia	1.0	0.5	8	9
Milkfish	2.0	2.0	6	11
Shrimp	2.0	3.0	30	73
Eel	5.0	8.0	17	23
Marine fish[1]	10.0	12.0	23	156
Trout	15.0	15.0	95	121
Marine fish[2]	20.0	15.0	226	335
Salmon	25.0	20.0	307	379
TOTAL			716	1209

[1] Flat fish including flounder, turbot, halibut, sole and cod, hake
[2] Bass, bream, yellowtail, grouper, jacks, mullets

These figures point to a potential demand, for these species alone, for at least 10 000 tonnes of LC-PUFA per annum (Table 10).

In addition to aquafeed, the current and potential world market for fish oil products spans a number of sectors from unprocessed, oil-rich biomass for animal feeds, to high-quality food-grade oils for use as food additives and nutraceuticals, and to very-high-purity oils and even individual FA for use in the pharmaceutical industry (Table 9).

As indicated by Lewis et al. [363], the imprecise boundaries surrounding the nutraceutical market make estimating the size of this market sector more difficult. Sales of marine supplement oils were in the order of $55 million in the United States in 1996 [460], and represented 20% of sales from health food retail outlets. In the United Kingdom, fish oils account for approximately 29% (U.S. $140 million) of the total annual market for nutraceuticals [461]. Moreover, there is an increasing trend for infant formula manufacturers to include PUFA-rich oils in their products. Typical inclusion levels of PUFA-rich oils are designed to achieve a final DHA concentration in dry infant formula of 0.1% to 0.2 wt/wt %. Indeed, the Western European market for infant formula increased from 81 500 tonnes in 1988 to 103 933 tonnes in 1994. Extrapolating these figures suggests a potential annual demand in the European infant formula market for up to 100 to 200 tonnes of DHA. Several food and beverage products enriched with DHA or other PUFA are already on the market. Mukherjee [461] reported the availability of products such as enriched spreads, breads, eggs, and soft drinks in Europe and Japan. Bread

enriched with refined tuna oil as a source of LC-PUFA is achieving substantial market penetration in Australia. As awareness by both consumers and regulators of the importance of adequate levels of PUFA in our diet increases, it can be assumed that demand for a greater range of PUFA-enriched products will increase [363].

10.6.1.4 Prices

The biggest use of fish oils is by the aquaculture industry, where it is necessary to have an oil rich in the long-chain polyunsaturated FA characteristic of fish oils. For this purpose, therefore, fish oil cannot be adequately replaced by vegetable oils. To meet this demand there has been a reduction in stocks and an increase in price. In 2000 and 2001 the average monthly price for crude fish oil ranged from US$ 235–325/tonne and $ 323–598/tonne. In January 2002 it was $613/tonne. [Oil World, www.oilworld.org]. In August 2002, crude fish oil prices peaked (about 650 US$ per ton) and have started to decline ever since. In November 2002, finally soybean oil prices managed to overtake those of crude fish oil due to the shorter supply than initially forecast, which make the latter competitive once more on the hardening market. For 2002, the average price of crude fish oil from any origin was about US $587/tonne; in 2003 it was about US$ 562/tonne (data from Oilworld). Peru's enormous fish oil production capacity sets this product's international prices.

10.6.2 Common resources

Fish oil is a by-product of industrial fishing and the fish meal industry. Fish oils are produced almost exclusively from small, bony species of pelagic fish (living in the surface waters or middle depths of the sea), for which there is little or no demand for human consumption [Fishmeal Information Network, www.fin.org.uk]:

South America (three species)

In Peru, anchovy is by far the most important species for fishmeal and fish oil production, with sardine largely making up the difference. The Chilean fishmeal industry uses anchovy, sardine and jack mackerel.

Europe (seven species)

Seven key species are used to produce fishmeal and fish oil in Europe. These can be divided into three groups:

a) No use for human consumption (inedible feed-grade fish – sandeel, capelin, Norway pout).
b) Potential use for human consumption but mainly used for fishmeal because of limited outlets for human consumption (blue whiting, sprat).

c) Primary use is human consumption but surplus may be used for fishmeal (herring, horse mackerel).

Fishmeal production also provides a major outlet to recycle trimmings from the food-fish processing sector which would otherwise be dumped at extra cost to the environment and the consumer. In the EU, Spain, France, Germany, Ireland and the UK produce fishmeal and fish oil primarily from trimmings.

Global capture fisheries, i.e. catches of wild fish as distinct from farmed fish, are valuable and finite resources which, although renewable, are highly vulnerable. Moreover, overfishing has caused the collapse or near collapse of some valuable fisheries. Overexploiting one fish species can affect other species, not least birds and mammals, in the marine ecosystem. This situation has generated understandable and justifiable pressure for environmentalists to reduce fishing effort and catches further by introducing tighter regulatory measures [438].

References

1. Sargent JR (1995) Origins and functions of egg lipids: nutritional implications. In: Bromage NR, Roberts RJ (eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford, p 353
2. Sargent JR, Bell MV, Bell JG, Henderson RJ, Tocher DR (1995) Origins and functions of n-3 polyunsaturated fatty acids in marine organisms. In: Cevc G, Paltauf F (eds.), Phospholipids: Characterization, Metabolism and Novel Biological Applications. AOCS, Champaign, Illinois, p 248
3. Pazos AJ, Roman G, Acosta CP, Sanchez JL, Abad M (1997) Comp Biochem Physiol 117B:393
4. Ackman RG (ed.) (1989) Marine Biogenic Lipids Fats and Oils. Vol I II. CRC, Boca Raton, Florida
5. Rezanka T (1989) Progr Lipid Res 28:147
6. Christie WW (ed) (2003) Lipid Analysis, Isolation, Separation, Identification and Structural Analysis of Lipids. The Oily Press, PJ Barnes, Brigwater, UK
7. Hagen W, Auel H (2001) Zoology 104:313
8. Dalsgaard J, St John M, Kattner G, Müller-Navarra D, Hagen W (2003) Adv Mar Biol 46:227
9. Fattorusso E, Mangoni A (1997) Marine Glycolipids. In: Hertz W, Kirby GW, Moore RE, Steiglich W, Tamm W (eds), Progress in the Chemistry of Organic Natural Products. Springer, Wien, p 215
10. Costantino V, Fattorusso E, Mangoni A, Di Rosa M, Ianaro A (1997) J Am Chem Soc 119:12 465
11. Costantino V, Fattorusso E, Imperatore C, Mangoni A (2003) Eur J Org Chem 1433
12. Crul M, Mathot RAA, Giaccone G, Punt CJA, Rosing H, Hillebrand MJX, Ando Y, Nishi N, Tanaka H, Schellens J, Beijnen JH (2002) Cancer Chemotherap Pharmacol 49:287
13. Ackman RG (2002) Anal Chim Acta 465:175
14. Napolitano GE, Pollero RJ, Gayoso AN, MacDonald BA, Thompson RJ (1997)

Biochem Syst Ecol 25:739
15. Zhukova NV, Titlyanov EA (2003) Phytochemistry 62:191
16. Hooper JNA, Van Soest RWM (2002) Systema Porifera: A Guide to the Classification of Sponges, Kluwer Academic/Plenum, New York
17. Gurr MI, Hardwood JL (1991) Lipid Biochemistry: An Introduction. Chapman Hall, London, UK
18. Cook HW (1996) Fatty acid desaturation and chain elongation in eukaryotes. In: Biochemistry of Lipids, Lipoproteins and Membranes. Vance DE, Vance JE (eds), Elsevier Scientific, Amsterdam, p 129
19. Sargent JR, Henderson RJ (1986) Lipids. In: The biological chemistry of marine copepods, Vol 1, EDS Corner and SCM O'Hara (eds.), Clarendon, Oxford, UK, p 59
20. Kattner G, Hagen W (1995) ICES J Mar Sci 52:329
21. Sprecher H (1992) Omega-3 News 7:1
22. Sprecher H (2000) Biochim Biophys Acta 1486:219
23. Williard DE, Nwankwo JO, Kaduce TL, Harmon SD, Irons M, Moser HW, Raymond GV, Spector AA (2001) J Lipid Res 42:501
24. Henderson RJ (1999) The production of n-3 polyunsaturated faty acids in marine organisms. In: Lipid Technology, Barnes PJ, UK, p 5
25. Nichols DS (2003) FEMS Microbiol Lett 219:1
26. Nadjek M, Debobbis D, Miokovic D, Ivancic I (2002) J Plankton Res 24:494
27. Harvey HR, Macko SA (1997) Organic Geochem 26:531
28. Budge SM, Parrish CC (1998) Organic Geochem 29:1547
29. Budge SM, Parrish CC, McKenzie CH (2001) Mar Chem 73:285
30. Russel NJ, Nichols DS (1999) Microbiology 145:767
31. Nichols DS, McMeekin TA (2002) J Microbiol Meth 48:161
32. Pond DW, Allen CE, Bell MV, Van Dover CL, Fallik AE, Dixon DR, Sargent JR (2002) Mar Ecol Progr Ser 225:219
33. Yu R, Yamada A, Watanabe K, Yazawa K, Takeyama H, Matsunaga T, Kuranr R (2000) Lipids 35:1061
34. Hannun YA, Obeid LM, Dbaibo GS (1996) Handbook of Lipid Research, Vol 8, p 177
35. Wha Sona B, Chul Kima J, Dae Choib H (2001) Lipids 36:427
36. Dembitsky VM, Srebnik M (2002) Progr Lipid Res 41:315
37. Wu M, Milligan KE, Gerwick WH (1997) Tetrahedron 53:15 983
38. Kan Y, Sakamoto B, Fujita T, Nagai H (1998) J Nat Prod 61:152
39. Mesguiche V,Valls R, Piovetti L, Peiffer G (1999) Tetrahedron Lett 40:7473
40. Kan Y, Sakamoto B, Fujita T, Nagai H (2000) J Nat Prod 63:1599
41. Falk-Petersen S, Sargent JR, Henderson J, Hegseth EN, Hop H, Okolodkov YB (1998) Polar Biol 20:41
42. Henderson RJ, Hegesth EN, Park MT (1998) Polar Biol 20:48
43. Volkman JK, Barett SM, Blackburn SI, Mansour MP, Sikes EL, Gelin F (1998) Organic Geochem 29:1163
44. Reuss N, Poulsen LK (2002) Mar Biol 141:423
45. Viso AC, Marty JC (1993) Phytochemistry 34:1521
46. Viron C, Saunois A, André P, Perly B, Lafosse M (2000) Anal Chim Acta 409:257
47. Mostaert AS, Karsten U, Hara Y, Watanabe MM (1998) Phycol Res 46:213
48. Cho ES, Rhodes LL, Kim HK (1999) J Fish Sci Tech 2:58–65
49. Marshall JA, Nichols PD, Hallegraeff GM (2002) J Applied Phycol 14:255
50. Khozin-Goldberg I, Zheng Yu H, Adlerstein D, Didi-Cohen S, Heimer YM, Cohen Z (2000) Lipids 35:881
51. Budge SM, Parrish CC (1999) Phytochemistry 52:561

52. Bergé JP, Gouygou JP, Dubacq JP, Durand P (1995) Phytochemistry 39:1017
53. Xu XQ, Beardall J, Hallam ND (1998) Phytochemistry 49:1249
54. Bell MV, Dick JR, Pond DW (1997) Phytochemistry 45:303
55. Arzul G, Gentien P, Bodennec G, Toularastel F, Youenou A, Crassous MP (1998) Potential toxicity of microalgal polyunsaturated fatty acids. In: Baudimant G, Guezennec JH, Roy P, Samain JF (eds.), Marine Lipids, IFREMER, Plouzané, France, p 53
56. Bodennec G, Gentien P, Parrish CC, Crassous MP (1998) Lipid class and fatty acid compositions of toxic Gymnodinium and Heterosigma strains: haemolytic and signature compounds. In: Baudimant G, Guezennec JH, Roy P, Samain JF (eds.), Marine Lipids, IFREMER, Plouzané, France, p 66
57. Mansour MP, Volkman JK, Holdsworth DG, Jackson AE, Blackburn SI (1999a) Phytochemistry 50:541
58. Mansour MP, Volkman JK, Jackson AE, Blackburn SI (1999b) J Phycol 35:710
59. Rezanka T (1990) J Chromatogr 513:344
60. Van Pelt CK, Huang MC, Tschanz CL, Brenna JT (1999) J Lipid Res 40:1501
61. Tanaka I, Matsuoka S, Murata M, Tachibana K (1998) J Nat Prod 61:685
62. Li X, Fan L, Lou Q (2002) Phytochemistry 59:157
63. Fleurence J, Gutbier G, Mabeau S, Leray C (1994) J Applied Phycol 6:527
64. Khotimchenko SV, Vaskovsky VE, Titlyanova TV (2002) Botanica Marina 45:17
65. Graeve M, Kattner G, Wiencke C, Karsten U (2002) Mar Ecol Progr Ser 231:67
66. Sajiki J, Kakimi H (1998) J Chromatogr 795A:227
67. Kornprobst JM, Barnathan G (1998) Recent Res Dev Lipid Res 2:371
68. Carballeira NM (2002) Progr Lipd Res 41:433
69. Barnathan G, Bourgougnon N, Kornprobst JM (1998) Phytochemistry 47:761
70. Smith SL, Schnack-Schiel SB (1990) Polar zooplankton. In: Smith WO (ed), Polar Oceanography, Part B: Chemistry, Biology and Geology, Academic, San Diego, California, p 527
71. Albers CS, Kattner G, Hagen W (1996) Mar Chem 55:347
72. Atkinson A, Snyder R (1997) Mar Ecol Progr 160:63
73. Mayzaud P, Albessard E, Cuzin-Roudy J (1998) Mar Ecol Progr Ser 173:19
74. Pond DW, Sargent JR (1998) J Plankton Res 20:169
75. Kattner G, Hagen W, Graeve M, Albers C (1998) Mar Chem 61:219
76. Kattner G, Hagen W (1998) Mar Ecol Progr Ser 170:203
77. Falk-Petersen S, Sargent JR, Lonne OJ, Timofeev SF (1999) Polar Biol 21:37
78. Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Can J Fish Aquat Sci 57:178
79. Falk-Petersen S, Dahl TM, Scott CL, Sargent JR, Gulliksen B, Kwasniewski S, Hop H, Millar RM (2002) Mar Ecol Progr Ser 187:187
80. Scott CL, Falk-Petersen S, Dahl TM, Sargent JR, Hop H, Lonne AJ, Poltermann M (1999) Polar Biol 21:65
81. Scott CL, Kwasniewski S, Falk-Petersen S, Millar RM, Sargent JR, (2000) Polar Biol 23:510
82. Jones PJH, MacDougall DE, Ntanios P,Vanstone CA, Mayzaud P, Albessard E, Cuzin-Roudy J (1998) Mar Biol Progr Ser 173:149
83. Cripps GC, Watkins JL, Hill HJ, Atkinson A (1999) Mar Ecol Progr Ser 181:177
84. Cho KW, Shin J, Jung K (1999) Ocean Res 21:109
85. Nelson MM, Phleger CF, Mooney BD, Nichols PD (2000) Lipids 35:551
86. Nelson MM, Mooney B, Nichols PD, Phleger CF (2001) Mar Chem 73:53
87. Phleger CF, Nelson MM, Mooney B, Nichols PD (2000) Polar Biol 23:329
88. Phleger CF, Nichols PD, Virtue P (1998) Comp Biochem Physiol 120B:311

89. Phleger CF, Nelson MM, Mooney B, Nichols PD (1999) Comp Biochem Physiol 124B:295
90. Swadling KM, Nichols PD, Gibson JAE, Ritz DA (2000) Mar Ecol Prog Ser 208:171
91. Litchfield C, Morales RW (1976) Are Demospongiae membranes unique in living organisms? In: Harrison FW, Cowden RR (eds.), Aspects of Sponge Biology, Academic, New York, p 183
92. Bergquist P (ed) (1978) Sponges. Hutchinson University Library, London
93. Djerassi C, Lam WK (1991) Acc Chem Res 24:69
94. Christie WW, Brechany EY, Marekov IN, Stefanov KL, Andreev SN (1994) Comp Biochem Physiol 109B:245
95. Carballeira NM, Emiliano A, Hernandez-Alonso N, Gonzalez FA (1998) J Nat Prod 61:1543
96. Barnathan G, Kornprobst JM (1998) Fatty acids from marine organisms: recent research developments. In: Baudimant G, Guezennec J, Roy P, Samain JF (eds), Marine Lipids. IFREMER, Brest, France, p 35
97. Barnathan G, Kornprobst JM (1998) Recent Res Devel Lipids Res 2:235
98. Carballeira NM, Shalabi F (1993) J Nat Prod 56:739
99. Barnathan G, Kornprobst JM, Doumenq P, Mirallès J (1995) Lipids 31:193
100. Carballeira NM (1997) Recent Res Develop Lipid Res 1:9
101. Carballeira NM, Alicea J (2001) Lipids 36:83
102. Barnathan G, Genin E, Velosaotsy NE, Kornprobst JM, Al-Lihaibi S, Al-Sofyani, Nongonierma R (2003) Comp Biochem Physiol 135B:297
103. Makarieva TN, Santalova EA, Gorshkova IA, Dmitrenok AS, Guzii AG, Gorbach VI, Svetashev VI, Stonik VA (2002) Lipids 37:75
104. Rodkina SA, Latyshev NA, Imbs AB (2003) Russ J Bioorg Chem 29:382
105. Meyer M, Guyot M (2002) Lipids 37:1109
106. Nemoto T, Yoshino G, Ojika M, Sakagami Y (1997) Tetrahedron 53:166 699
107. Li Y, Ishibashi M, Sasaki T, Kabayashi J (1995) J Chem Res (S):126
108. Li Y, Ishibashi M, Sasaki T, Kabayashi J (1995) J Chem Res (M):901
109. Carballeira NM, Medina JR (1994) J Nat Prod 57:1688
110. Carballeira NM, Reyes M (1995) J Nat Prod 58:1689
111. Carballeira NM, Reyes ED, Sostre A, Rodriguez AD, Rodriguez JL, Gonzales FA (1997) J Nat Prod 60:502
112. Kawakami A, Miyamoto T, Higuchi R, Uchiumi T, Kuwano M, Van Soest RWM (2001) Tetrahedron Lett 42:3335
113. Carballeira NM, Pagan M (2000) J Nat Prod 63:666
114. Nechev J, Christie WW, Robaina R, de Diego F, Popov S, Stefanov K (2002) Eur J Lipid Sci Technol 104:800
115. Nechev J, Christie WW, Robaina R, de Diego FM, Ivanova A, Popov S, Stefanov K (2002) Hydrobiologia 489:91
116. Nechev J, Christie WW, Robaina R, de Diego F, Popov S, Stefanov K (2004) Comp Biochem Physiol 137A: 365
117. Carballeira NM, Pagan M (2001) J Nat Prod 64:620
118. Thiel V, Jenisch A, Worheide G, Lowenberg A, Reitner J, Michaelis W (1999) Org Geochem 30:1
119. Kornprobst JM, Barnathan G (2000) Recent Res Progr Lipid Res 2:371
120. Ayanoglu E, Kornprobst JM, Aboud-Bichara A, Djerassi C (1983) Tetrahedron Lett 24:1111
121. Ayanoglu E, Popov S, Kornprobst JM, Aboud-Bichara A, Djerassi C (1983) Lipids 18:830

122. Carballeira NM, Alicea J (2002) Lipids 37:305
123. Carballeira NM, Colon R (1999) Tetrahedron Asymmetry 10:3785
124. Soderquist JA, Rosado I, Marrero Y (1998) Tetrahedron Lett 39:3115
125. Quinn RJ, Tucker DJ (1985) Tetrahedron Lett 26:1671
126. Hirsch SC, Carmely S, Kashman Y (1987) Tetrahedron 43:3257
127. Van Soest RWM, Fusetani N, Andersen RJ (1997) Straight-chain acetylenes as chemotaxonomic markers of the marine Haplosclerida. In: Watanabe Y, Fusetani N (eds), Sponge Sciences–Multidisciplinary Perspectives, Springer-Verlag, Berlin Heidelberg New York, p 3
128. Pham NB, Butler MS, Hooper JNA, Moni RW, Quinn RJ (1999) J Nat Prod 62:1439
129. Matsunaga S, Okada Y, Fusetani N, Van Soest RWM (2000) J Nat Prod 63:690
130. Lee HS, Rho JR, Sim CJ, Shin J (2003) J Nat Prod 66:566
131. Fujita M, Nakao Y, Matsunaga S, Van Soest RWM, Itoh Y, Seiki M, Fusetani N (2003) J Nat Prod 66:569
132. Zhao QC, Lee SY, Hong JK, Lee CO, Im KS, Lee DS, Sim CJ, Jung JH (2003) J Nat Prod 66:408
133. Hattori T, Adachi K, Shizuri Y (1998) J Nat Prod 61:823
134. Venkateswarlu Y, Srinivasa Reddy N, Ramesh P, Rama Rao M, Siva Ram T (1998) Indian J Chem 37B:1264
135. Lo JM, Wang WL, Chiang YM, Chen CM (2001) J Chin Chem Soc 48:821
136. Natori T, Morita M, Akimoto K, Koezuka Y (1994) Tetrahedron 50:2771
137. Li HY, Matsunaga S, Fusetani N (1995) Tetrahedron 51:2273
138. Koezuka Y, Motoki K, Sakai T, Natori T (1999) Recent Res Dev Cancer 1:341
139. Costantino V, Fattorusso E, Mangoni A, Di Rosa M, Ianaro M (1997) J Am Chem Soc 119:12 465
140. Costantino V, Fattorusso E, Mangoni A (2000) Tetrahedron 56:5953
141. Costantino V, Fattorusso E, Mangoni A (2001) Glycolipids with immunomodulating activity from marine sponges. In: Tringali C (ed.), Bioactive Compounds from Natural Sources. Taylor Francis, London, New York, Chap 14:557
142. Meguro S, Namikoshi M, Kobayashi H (2002) J Antibiotics 55:256
143. Hayakawa Y, Rovero S, Forni G, Smyth MJ (2003) Proc Nat Acad Sci 100:9464
144. Kobayashi E, Motoki K, Uchida T, Fukushima H, Koezuka Y (1998) Oncol Res 7:529
145. Morita M, Motoki K, Natori T, Sakai T, Sawa E, Yamaji K, Koezuka K, Kobayashi E, Fukushima H (1995) J Med Chem 38:2176
146. Costantino V, Fattorusso E, Imperatore C, Mangoni A (2002) J Nat Prod 65:883
147. Vysotskii MV, Svetashev VI (1991) Biochim Biophys Acta 1083:161
148. Vysotskii MV, Svetashev VI (1998) Comp Biochem Physiol 119B:73
149. Mirallès J, Barnathan G, Galonnier R, Sall T, Samb A, Gaydou EM, Kornprobst JM (1995) Lipids 30:459
150. Carballeira NM, Sostre A, Rodriguez AD (1996) Comp Biochem Physiol 113B:781
151. Carballeira NM, Sostre A, Rodriguez AD (1997) Comp Biochem Physiol 118B:257
152. Carballeira NM, Emiliano A, Rodrıguez J, Reyes E (1992) Lipids 27:681
153. Barnathan G, Doumenq P, Mirallès J, Boury-Esnault N, Kornprobst JM (1993) J Nat Prod 56:2104
154. Carballeira NM, Miranda C, Rodrıguez AD (2002) Comp Biochem Physiol 131B:83
155. Urvois PA, Barnathan G, Biard JF, Débitus C, Verbist JF (1998) Fatty acid composition of the New Caledonian gorgonian *Rumphella aggregata*: identification of 9-methyl-6,9-heptadecadienoic acid. In: Marine Lipids, Baudimant G, Guezennec J, Roy P, Samain JF (eds.), IFREMER, Brest, p 44
156. Mancini I, Guerriero A, Bakken T, Zibrowius G, Pietra F (1999) Helv Chim Acta

82:677
157. Henderson RJ, Burkow IC, Buzzi M, Bayer A (1998) Biochim Biophys Acta 1392:309
158. Henderson RJ (2000) J Mar Biol Ass UK 80:311
159. Yamashiro H, Oku H, Higa H, Chinen I, Sakai K (1999) Comp Biochem Physiol 122B:397
160. Jeong TS, Ahn JA, Kim YK, Bok SH, Kwon BM, (1999) Bioorganic Medicinal Chem Lett 7:1481
161. Ota T, Chihara Y, Itabashi Y, Tagaki T (1994) Fisheries Sci 60:171
162. McKenzie JD, Black KD, Kelly MS, Newton LC, Handley LL, Scrimpgeour CM, Raven JA [TS o]
163. Kawasaki KI, Nabeshima YI, Ishihara K, Kaneniwa M, Ooizumi T (2000) Fisheries Sci 66:614
164. Ishihara K, Murata M, Kaneniwa M (1998) Lipids 33:1107
165. Sato D, Ando Y, Tsujimoto R, Kawasaki K (2001) Lipids 36:1371
166. Inagaki M, Isobe R, Kawano Y, Miyamoto T, Komori T, Higuchi R (1998) Eur J Org Chem 129
167. Yamada K, Matsubara R, Kaneko M, Miyamoto T, Higuchi R (2001) Chem Pham Bull 49:447
168. Yamada K, Sasaki K, Harada Y, Isobe R, Higuchi R (2002) Chem Pham Bull 50:1467
169. Yamada K, Hamada A, Kisa F, Miyamoto T, Higuchi R (2003) Chem Pham Bull 51:46
170. Jeong BY, Ohshima T, Koizumi C (1996) Lipids 31:9
171. Pond DW, Sargent JR (1998) J Plankton Res 20:169
172. Viracaoundin I, Barnathan G, Gaydou EM, Aknin M (2003) Lipids 38:85
173. Kraffe E, Soudant P, Marty Y, Kervarec N, Jehan P (2002) Lipids 37:507
174. Freites L, Fernandez-Reiriz MJ, Labarta U (2002a) Aquaculture 207: 97
175. Galap C, Netchitailo P, Leboulenger F, Grillot JP (1999) Comp Biochem Physiol 122A:241
176. Kraffe E, Marty Y ()[TS o]
177. Freites L, Fernandez-Reiriz MJ, Labarta U (2002) Comp Biochem Physiol 132B:453
178. Caers M, Coutteau P, Cure K, Morales V, Gajardo G, Sorgeloos P (1999) Comp Biochem Physiol 123B:97
179. Soudant P, Ryckeghem KV, Marty Y, Moal J, Samain JF, Sorgeloos P (1999) Comp Biochem Physiol 123B:209
180. Garrido JL, Medina I (2002) Analytica Chim Acta 465:409
181. Murphy KJ, Mooney BD, Mann NJ, Nichols PD, Sinclair AJ (2002) Lipids 37:587
182. Abad M, Ruiz, C, Martinez D, Mosquera G, Sanchez JL (1995) Comp Biochem Physiol 110C:109
183. Labarta U, Fernandez-Reiriz MJ, Perez-Camacho A (1999) Comp Biochem Physiol 123A:249
184. Soudant P, Moal J, Marty Y, Samain JF (1997) J Exp Mar Biol Ecol 215:103
185. Brazao S, Morais S, Boaventura D, Re P, Narciso L, Hawkins SJ (2003) Comp Biochem Physiol 136B:425
186. Fernandez-Reiriz MJ, Labarta U, Albentosa M, Perez-Camacho A (1999) Comp Biochem Physiol 124B:309
187. Le Pennec M, Beninger PG, Dorange G, Paulet YM (1991) J Mar Biol Assoc UK 71:451
188. Pazos AJ, Roman G, Acosta CP, Sanchez JL, Abad M (1997) Comp Biochem Physiol 117B:393
189. Le Pennec G, Le Pennec M, Beninger PG (2001) J Mar Biol Assoc UK 81:663
190. Caers M, Coutteau P, Cure K, Morales V, Gajardo G, Sorgeloos P (1999) Comp Biochem Physiol 123B:89

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191. Caers M, Coutteau P, Sorgeloos P (1999) Aquaculture 170:307
192. Marty Y, Soudant P, Perrotte S, Moal J, Dussauze J, Samain JF (1999) J Chromatogr A 839:119
193. Jeong BY, Ohshima T, Koizumi C (1999) Comp Biochem Physiol 122B:415
194. de Moreno JEA, Moreno VJ, Ricci L, Roldan M, Gerpe M (1998) Comp Biochem Physiol 119B:631
195. Hayashi K, Kishimura H (2002) J Oleo Sci 51:523
196. Suprayudi MA, Takeuchi T, Hamasaki K (2004) Aquaculture 231:403
197. Takeuchi T, Satoh N, Sekiya S, Shimizu T, Watanabe T (1999) Nippon Suisan Gakkaishi 65:988
198. Kobayashi T, Takeuchi T, Arai D, Sekiya S (2000) Nippon Suisan Gakkaishi 66:1006
199. Hamasaki K, Suprayudi MA, Takeuchi T (2002) Suisan Zoshoku 50:333
200. Suprayudi MA, Takeuchi T, Hamasaki K, Hirokawa J (2002) Suisan Zoshoku 50:205
201. Suprayudi MA, Takeuchi T, Hamasaki, K, Hirokawa, J (2002) Fish Sci 68:1295
202. Lahdes E, Balogh G, Fodor E, Farkas T (2000) Lipids 35:1093
203. Fodor E, Jones RH, Buda C, Kitajka K, Dey I (1995) Lipids 30:1119
204. Cunnane SC (2003) Prog Lipid Res 42:544
205. Wada H, Gombos Z, Murata N (1990) Nature 347:200
206. Miquel M, James D, Dooner H, Browse J (1993) Proc Natl Acad Sci USA 90:6208
207. Meves H (1994) Prog Neurobiol 43:175
208. Xiao YF, Ke Q, Wang SY, Auktor K, Yang Y, Wang GK, Morgan JP, Leaf A (2001) Proc Natl Acad Sci USA 98:3606
209. Schmidt A, Wolde M, Thiele C, Fest W, Kratzin H, Podtelejnikov AV, Witke W, Huttner WB, Söling HD (1999) Nature 401:133
210. Wallis JG, Browse J (2002) Prog Lipid Res 41:254
211. Goldberg EM, Zidovetzki R (1997) Biophys J 73:2603
212. Tsutsumi T, Yamauci E, Suzuki E, Watanbe S, Kobayashi T, Okuyama H (1995) Biol Pharm Bull 18:664
213. Wallis JG, Watts JL, Browse J (2002) Trends Biochem Sci 27:467
214. Napier JA (2002) Trends Plant Sci 7:51
215. Connor WE (2000) Am J Clin Nutr 71:171S
216. Albert, CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN, Manson JE (1998) JAMA 279:23
217. Kromhout D (1998) JAMA 279:65
218. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St Jeor S, Suttie J, Tribble DL, Bazzarre TL (2000) Circulation 102:2284
219. O'Keefe Jr, James H, Harris WS (2000) Am J Cardiol 85:1239
220. Oomen CM, Feskens EJ, Räsänen L, Fidanza F, Nissinen AM, Menotti A, Kok FJ, Kromhout D (2000) Am J Epidemiol 151:999
221. Torres IC, Mira L, Ornelas CP, Melim A (2000) Br J Nutr 83:371
222. Iso H, Rexrode KM, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Hennekens CH, Willett WC (2001) JAMA 285:304
223. Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, Hunter D, Manson JE (2002) JAMA 287:1815
224. He K, Rimm EB, Merchant A, Rosner BA, Stampfer MJ, Willett WC, Ascherio A (2002) JAMA 288:3130
225. Calder PC (2003) Ital Heart J 4:427
226. Thies F, Garry JMC, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ,

Calder PC, Grimble RF (2003) The Lancet 361:477
227. Madsen, T, Skou HA, Hansen VE, Fog L, Christensen JH, Toft E, Schmidt EB (2001) Am J Cardiol 88:1139
228. Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC, Ma J (2002) N Engl J Med 346:1113
229. Rosenberg IH (2002) New Engl J Med 346:1102
230. Christensen JH, Skou HA, Fog L, Hansen V, Vesterlund T, Dyerberg J, Toft E, Schmidt EB (2001) Circulation 103:651
231. Bigger JT, El-Sherif T (2001) Circulation 103:623
232. De Caterina R, Madonna R, Zucchi R, La Rovere MT (2003) Am Heart J 146:420
233. Dallongeville J, Yarnell J, Ducimetière P, Arveiler D, Ferrières J, Montaye M, Luc G, Evans A, Bingham A, Hass B, Ruidavets JB (2003) Circulation 108:820
234. Marchioli R, Schweiger C, Tavazzi L, Valagussa F (2001) Lipids 36:S119
235. Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, Franzosi MG, Geraci E, Levantesi G, Maggioni AP, Mantini L, Marfisi RM, Mastrogiuseppe G, Mininni N, Nicolosi GL, Santini M, Schweiger C, Tavazzi L, Tognoni G, Tucci C, Valagussa F, GISSI-Prevenzione Investigators (2002) Circulation 105:1897
236. Leaf A (2002) Circulation 105:1874
237. Lemaitre RN, King IB, (2003) n-3 polyunsaturated FA, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the cardiovascular health study. Am J Clin Nutr 77:319
238. Harris WS (2003) Am J Clin Nutr 77:279
239. Cobiac L, Nestel PJ, Wing LM, Howe PR (1991) Clin Exp Pharmacol Physiol 18:265
240. Radack K, Deck C, Huster G (1991) Arch Intern Med 151:1173
241. Appel LJ, Miller ER, Seidler AJ, Whelton PK (1993) Arch Intern Med 153:1429
242. Morris MC, Sacks F, Rosner B (1993) Circulation 88:523
243. Toft I, Bonaa KH, Ingebretsen OC, Nordoy A, Jenssen T (1995) Ann Intern Med 123:911
244. Connor WE (1995) Ann Intern Med 123:950
245. Andreassen AK, Hartmann A, Offstad J, Geiran O, Kvernebo K, Simonsen S (1997) J Am Coll Cardiol 29:1324
246. Bao DQ, Mori TA, Burke V, Puddey IB, Beilin LJ (1998) Hypertension 32:710
247. Mori TA, Bao DQ, Burke V, Puddey IB, Watts GF, Beilin LJ (1999) Am J Clin Nutr 70:817
248. Stark KD, Park EJ, Maines VA, Holub BJ (2000) Am J Clin Nutr 72:389
249. Laidlaw M, Holub BJ (2003) Am J Clin Nutr 77:37
250. Goodfellow J, Bellamy MF, Ramsey MW, Jones CJ, Lewis MJ (2000) J Am Coll Cardiol 35:265
251. Flaten H, Hostmark AT, Kierulf P, Lystad E, Trygg K, Bjerkedal T, Osland A (1990) Am J Clin Nutr 52:300
252. Norrish AE, Skeaff CM, Arribas GL, Sharpe SJ, Jackson RT (1999) Br J Cancer 81:1238
253. Terry P, Lichtenstein P, Feychting M, Ahlbom A, Wolk A (2001) The Lancet 357:1764
254. Takezaki T, Hirose K, Inoue M, Hamajima N, Yatabe Y, Mitsudomi T, Sugiura T, Kuroishi T, Tajima K (2001) Br J Cancer 84:1199
255. Barber MD, Ross JA, Voss AC, Tisdale MJ, Fearon KC (1999) Br J Cancer 81:80
256. Gogos CA, Ginopoulos P, Salsa B, Apostolidou E, Zoumbos NC, Kalfarentzos F (1998) Cancer 82:395
257. Kremer JM (2000) Am J Clin Nutr 71:349S
258. Volker D, Fitzgerald P, Major G, Garg M (2000) J Rheumatol 27:2343

259. Cleland LG, James MJ (2000) J Rheumatol 27:2305
260. Navarro E, Esteve M, Olivé A, Klaassen J, Cabré E, Tena X, Fernandez-Bañares F, Pastor C, Gassull MA (2000) J Rheumatol 27:298
261. Darlington LG, Stone TW (2001) Brit J Nutr 85:251
262. Mayser P, Mrowietz U, Arenberger P, Bartak P, Buchwald J, Christophers E, Jablonska S, Salmhofer W, Schill WB, Krämer HJ, Schlotzer E, Mayer K, Seeger W, Grimminger F (1998) J Am Acad Dermatol 38:539
263. Danno K, Sugie N (1998) J Dermat 25:703
264. Hodge L, Salome CM, Peat JK, Haby MM, Xuan W, Woolcock AJ (1996) Med J Aust 164:137
265. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM (1997) Am J Clin Nut 65:1011
266. Katz DP, Manner T, Furst P, Askanazi J (1996) Nutrition 12:334
267. Schwartz J (2000) Am J Clin Nutr 71:393S
268. Stevens LJ, Zentall SS, Deck JL, Abate ML, Watkins BA, Lipp SR, Burgess JR (1995) Am J Clin Nutr 62:761
269. Burgess JR, Stevens L, Zhang W, Peck L (2000) Am J Clin Nutr 71:327S
270. Stordy BJ (2000) Am J Clin Nutr 71:323S
271. Heude, B, Ducimetière P, Berr C, Study E (2003) Am J Clin Nutr 77:803
272. Edwards R, Peet M, Shay J, Horrobin D (1998) J Affect Disord 48:149
273. Calabrese, JR, Rapport DJ, Shelton MD (1999) Arch Gen Psychiatry 56:413
274. Kyle DJ, Schaefer E, Patton G, Beiser A (1999) Lipids 34:S245
275. Severus, WE, Ahrens B, Stoll AL (1999) Arch Gen Psychiatry 56:380
276. Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E, Cress KK, Marangell LB (1999) Arch Gen Psychiatry 56:407
277. Bruinsma KA, Taren DL (2000) Nut Rev 58:98
278. Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH (2000) Lipids 35:1305
279. Joy CB, Mumby-Croft R, Joy LA (2000) Cochrane Database Syst Rev 4
280. Richardson AJ, Easton T, Puri BK (2000) European Neuropsychopharmacology 10:189
281. Tanskanen A, Hibbeln JR, Hintikka J, Haatainen K, Honkalampi K, Viinamäki H (2001) Arch Gen Psychiatry 58:512
282. Peet M, Horrobin DF (2002) Arch Gen Psychiat 59:913
283. Small MF (2002) New Scientist, August 24:34
284. Levine BS (1997) Nutrition Today 32:248
285. Fidler N, Sauerwald T, Pohl A, Demmelmair H, Koletzko B (2000) J Lipid Res 41:1376–83
286. Olsen S,Secher NJ (2002) Brit Med J 324:1
287. Jensen CL, Maude M, Anderson RE, Heird WC (2000) Am J Clin Nutr 71:292S
288. Makrides M, Gibson RA (2000) Am J Clin Nutr 71:307S
289. Connor WE, Lowensohn R, Hatcher L (1996) Lipids 31:S183
290. Cunnane SC, Francescutti V, Brenna JT, Crawford MA (2000) Lipids 35:105
291. Carlson SE (1999) Acta Paediatr Suppl 88:72
292. Mitchell EA, Aman MG, Turbott SH, Manku M (1987) Clin Pediatr 26:406
293. Uauy-Dagach R, Valenzuela A (1996) Nutr Rev 54:S102
294. Pepping J (1999) Am J Health-Syst Ph 56:719
295. Simopoulos AP (2002) Biomed Pharmacother 56:365
296. de Lorgeril M RS, Mamelle N, Salen P, Martin JL, Mon-jaud I, Guidollet J, Touboul P, Delaye J (1994) Lancet 343:1454
297. Bartram HP, Gostner A, Reddy BS, Rao CV, Scheppach W, Dusal G, Richter A,

Richter F, Kasper H (1995) Eur J Cancer Prev 4:231
298. Maillard V, Bougnoux P, Ferrari P, Jourdan M-L, Pinault M, Lavillonnieree FF, Body G, Le Floch O, Chajes V (2002) Int J Cancer 98:78
299. James MJ, Cleland LG (1997) Semin Arthritis Rheum 27:85
300. Heath and Welfare Canada (1990). Table 19. Summary of examples of recommended nutrients based on energy expressed as daily rates. In: Nutrition Recommendations. The reports of the scientific review committee. Health and Welfare Canada, Scientific review committee, Ottawa, 203 (H49-42/1990E)
301. ISSFAL (1999) Adequate intakes. International Society for the Study of FA and Lipids (ISSFAL), Devon (http://www.issfal.org.uk/adequateintakes.htm)
302. BNF (2000) British Nutrition Foundation (BNF), London
303. IOM (2002) Dietary reference intakes for energy, carbohydrates, fiber, fat, protein and amino acids (macronutrients). National Academy of Sciences, food and nutrition board, Institute of Medicine (IOM). National Academy Press (NAP), Washington, DC, 335–432
304. Watkins BA, Lippman HE, Le Bouteiller L, Seifert MF (2001) Prog Lipid Res 40:125
305. Ucciani E (1995) Nouveau Dictionnaire des Huiles Végétales. Compositions en acides gras. Lavoisier TEC&DOC, Paris (France)
306. Abbadi A, Domergue F, Meyer A, Riedel K, Sperling P, Zank TK, Heinz E (2001) Eur J Lipid Sci Technol 103:106
307. Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MC, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Nature 405:1017
308. Tocher DR, Leaver MJ, Hodgson PA (1998) Prog Lipid Res 37:73
309. Shanklin J, Cahoon EB (1998) Annu Rev Plant Physiol Plant Mol Biol 49:611
310. Pereira SL, Leonard AE, Mukerji P (2003) Protaglandins, Leukotrienes and essential FA 68:97
311. Napier JA, Sayanova O, Sperling P, Heinz E (1999) Trends Plant Sci 4:2
312. Napier JA, Michaelson LV, Sayanova O (2003) Prostaglandins Leukot Essent Fatty Acids 68:135
313. Satouchi K, Hirano K, Sakaguchi M, Takehara H, Matsuura F (1993) Lipids 28:837
314. Beaudoin F, Michaelson LV, Hey SJ, Lewis, MJ, Shewry PR, Sayanova O, Napier J (2000) Proc Natl Acad Sci USA 97:6421
315. Napier JA, Michaelson LV (2001) Lipids 36:761
316. Watts JL, Browse J (2002) Proc Natl Acad Sci USA 99:5854
317. Parker-Barnes JM, Das T, Bobik E, Leonard AE, Thurmond JM, Chaung LT, Huang YS, Mukerji P (2000) Proc Natl Acad Sci USA 97:8284
318. Gunstone FD (1994) Prog Lipid Res 33:19
319. Khozin I, Adlerstein D, Bigongo C, Heimer YM, Cohen Z (1997) Plant Physiol 114:223
320. Wallis JG, Browse J (1999) Arch Biochem Biophys 365:307
321. Qi B, Beaudoin F, Fraser T, Stobart AK, Napier JA, Lazarus CM (2002) FEBS Lett 510:159
322. Qiu X, Hong H, Mc Kenzie SL (2001) J Biol Chem 276:31 561
323. Voss A, Reinhart M, Sankarappa S, Sprecher H (1991) J Biol Chem 266:19 995
324. Moore SA, Hurt E, Yoder E, Sprecher H, Spector AA (1995) J Lipid Res 36:2433
325. Ferdinandusse S, Denis S, Mooijer PA, Zhang Z, Reddy JK, Spector AA, Wanders RJ (2001) J Lipid Res 42:1987
326. Su HM, Moser AB, Moser HW, Watkins PA (2001) J Biol Chem 276:38 115
327. de Antueno RJ, Knickle, LC, Smith H, Elliot ML, Allen SJ, Nwaka S, Winther MD (2001) FEBS Lett 509:77

328. D'andrea S, Guillou H, Jan S, Catheline D, Thibault JN, Bouriel M, Rioux V, Legrand P (2002) Biochem J 364:49
329. Metz JG, Roessler P, Facciotti D, Levering C, Dittrich F, Lassner M, Valentine R, Lardizabval K, Domergue F, Yamada A, Yazawa K, Knauf V, Browse J (2001) Science 293:290
330. Napier JA, Michaelson LV, Stobart AK (1999) Curr Opin Plant Biol 2:123
331. Cho HP, Nakamura M, Clarke SD (1999) J Biol Chem 274:37 335
332. Leonard AE, Kelder B, Bobik EG, Chuang LT, Parker-Barnes JM, Thurmond JM, Kroeger PE, Kopchick JJ, Huang YS, Mukerji P (2000) Biochem J 347:719
333. Watts JL, Browse J (1999) Arch Biochem Biophys 362:175
334. Michaelson LV, Napier JA, Lewis M, Griffiths G, Lazarus CM, Stobart, AK (1998) FEBS Lett 439:215
335. Michaelson LV, Lazarus CM, Griffiths G, Napier JA, Stobart AK (1998) J Biol Chem 273:19 055
336. Knutzon DS, Thurmond JM, Huang YS, Chaudhary S, Bobik Jr EG, Chan GM, Kirchner SJ, Mukerji P (1998) J Biol Chem 273:29 360
337. Cho HP, Nakamura M, Clarke SD (1999) J Biol Chem 274:471
338. Napier JA, Hey SJ, Lacey DJ, Shewry PR (1998) Biochem J 330:611
339. Sayanova O, Smith MA, Lapinskas P, Stobart AK, Dobson G, Christie WW, Shewry PR, Napier JA (1997) Proc Natl Acad Sci USA 94:4211
340. Sperling P, Lee M, Girke T, Zähringer U, Stymne S, Heinz E (2000) Eur J Biochem 267:3801
341. Girke T, Schmidt H, Zähringer U, Reski R, Heinz E (1998) Plant J 15:39
342. Yuan L, Kirchner SJ, Mukerji P, Knutzon DS (1999) Lipids 34:649
343. Sakuradani E, Kobayashi M, Shimizu S (1999) Gene 238:445
344. Spychalla JP, Kinney AK, Browse J (1997) Proc Natl Acad Sci USA 94:1142
345. Zank TK, Zähringer U, Lerchl JE, Heinz E (2000) Biochem Soc Trans 28:654
346. Nichols D, Bowman J, Sanderson K, Mancuso-Nichols C, Lewis T, McMeekin, T, Nichols PD (1999) Curr Opin Biotechnol 10:240
347. Yazawa K (1996) Lipids 31:S297
348. Watanabe K, Yazawa K, Kondo K, Kawaguchi A (1997) J Biochem 122:467
349. Bisang C Long PF, Cortés J, Westcott J, Crosby J, Matharu AL, Cox RJ, Simpson TJ, Staunton J, Leadlay PF (1999) Nature 401:502
350. Heath RJ, Rock CO (2000) Nature 406:145
351. Allen EE, Facciotti D, Bartlett DH (1999) Appl Environ Microbiol 65:1710
352. Tanaka M, Ueno A, Kawasaki K, Yumoto I, Ohgiya S, Hoshino T, Ishizaki K, Okuyama H, Morita N (1999) Biotechnol Lett 21:939
353. Leonard AE, Bobik EG, Dorado J, Kroeger PE, Chuang LT, Thurmond JM, Parker-Barnes JM, Das T, Huang YS, Mukerji P (2000) Biochem J 350:765
354. Huang J, Aki T, Yocochi T, Nakahara T, Honda D, Kawamoto S, Shigeta S, Ono K, Suzuki O (2003) Mar Biotechnol 5:451
355. Müller-Navarra DC, Brett MT, Liston AM, Goldman CR (2000) Nature 403:74
356. Iida I, Nakahara T, Yocochi T, Kamisaka Y, Yagi H, Yamaoka M, Suzuki O (1996) Journal Ferment Bioeng 81:76
357. Yokochi T, Honda D, Higashihara T, Nakahara T (1998) Appl Microbiol Biotechnol 49:72
358. Barclay WR, Meager KM, Abril JR (1994) J Appl Phycol 6:123
359. Lewis TE, Mooney BD, McMeekin TA, Nichols PD (1998) Chem Aust 65:37
360. Vazhappilly R, Chen F (1998) Bot Mar 41:553
361. Singh A, Ward OP (1997) Microbial production of docosahexaenoic acid (DHA,

C22:6). In: Neidleman SL, Laskin AI (eds.), Advances in Applied Microbiology, Vol 45, pp 271–312
362. Ratledge C (1998) Opportunities for marine microorganisms for the production of polyunsaturated FA. In: Le Gal Y, Muller-Feuga A (eds.), Marine Microorganisms for Industry. Actes de colloque 21. IFREMER, Plouzane, France, pp 18–25
363. Lewis TE, Nichols PD, McMeekin TA (1999) Mar Biotechnol 1:580
364. Ratledge C (1993) Trends Biotechnol 11:278 *Circular* 886 (Rev 1) 1
365. Sajbidor J, Dobronova S, Certik M (1990) Biotechnol Lett 12:455
366. Gandhi SR, Weete JD (1991) J Gen Microbiol 137:1825
367. Nichols DS, Nichols PD, McMeekin TA (1993) Antarct Sci 5:149
368. Jøstensen, JP, Landfald B (1997) FEMS Microbiol Lett 151:95
369. Watanabe K, Sezaki K, Yazawa K, Hino A (1992) Nipp Suis Gakk 58:271
370. Nichols DS, Hart P, Nichols PD, McMeekin TA (1996) Aquaculture 147:115
371. Lewis TE, Nichols PD, Hart PR, Nichols DS, McMeekin TA (1998) J World Aquacult Soc 29:313
372. Gonzalezbaro MD, Pollero RJ (1998) Comp Biochem Physiol 119:747
373. Erwin J, Bloch K (1964) Science 143:1006
374. Zhukova NV, Kharlamenko VI, (1999) Aquat Microb Ecol 17:153
375. DeLong EF, Yayanos AA (1987) Appl Environ Microbiol 51:730
376. Bowman JP, McCammon SA, Nichols DS, Skerratt JS, Rea SM, Nichols PD, McMeekin TA (1997) Int J Syst Bacteriol 47:1040
377. Bowman JP, Gosink JJ, McCammon SA, Lewis TE, Nichols DS, Nichols PD, Skerratt JH, Staley JT, McMeekin TA (1998) Int J Syst Bacteriol 48:1171
378. Hugenholtz P, Goebel BM, Pace NR (1998) J Bacteriol 180:4765
379. Yano Y, Nakayama A, Yoshida K (1997) Appl Environ Microbiol 63:2572
380. Kato C (1999) Molecular analysis of the sediment and isolation of extreme barophiles from the deepest Mariana Trench. In: Extremophiles in Deep-Sea Environments, Tsujii K (Ed), Springer-Verlag, Tokyo, pp. 27–38
381. Venkateswaren K, Dollhopf ME, Aller R, Stackebrandt E, Nealson KH (1998) Int J Syst Bacteriol 48:965
382. Ivanova EP, Mikhailov VV (2001) Microbiology 70:10
383. Bozal N, Montes MJ, Tudela E, Jimenez F, Guinea J (2002) Int J Syst Evol Microbiol 52:195
384. Nogi, Y, Kato C, Horikoshi K (1998) J Gen Appl Microbiol 44:289
385. Deming JW, Somers LK, Sraube WL, Swartz DG, MacDonnell MT (1988) Syst Appl Microbiol 10:152
386. Yumoto I, Kawasaki K, Iwata H, Matsuyama H, Okuyama H (1998) Int J Syst Bacteriol 48:1357
387. Urakawa H, Kita-Tsukamoto K, Steven S, Ohwada K, Colwell RR (1998) FEMS Microbiol Lett 165:373
388. Nogi Y, Kato C (1999) Extremophiles 3:71
389. Benediktsdóttir E, Verdonck L, Sproër C, Helgason S, Swings J (2000) Int J Syst Evol Microbiol 50:479
390. Mountfort DO, Rainey FA, Burghardt J, Kaspar HF, Stackebrandt E (1998) Arch Microbiol 169:231
391. Nogi Y, Kato C, Horikoshi K (2002) Int J Syst Evol Microbiol 52:1527
392. Kawasaki K, Nogi Y, Hishinuma M, Nodasaka Y, Matsuyama H, Yumoto I (2002) Int J Syst Evol Microbiol 52:1455
393. Bowman JP, McCammon SA, Lewis TE, Skerratt JH, Brown JL, Nichols DS, McMeekin TA (1998) Microbiology 144:1601

394. Nogi Y, Masui N, Kato C (1998) Extremophiles 2:1
395. Iwanami H, Yamaguchi T, Takeuchi M (1995) Nipp Suis Gakk 61:205
396. Watanabe K, Ishikawa C, Ohtsuka I, Kamata M, Tomita M, Yazawa K, Muramatsu H (1997) Lipids 32:975
397. Barbieri E, Paster BJ, Hughes D, Zurek L, Moser, DP, Teske A, Sogin ML (2001) Environ Microbiol 3:151
398. Ederington MC, Mcmanus GB, Harvey HR (1995) Limnol Oceanogr 40:860
399. Nichols PD, Nichols DS, Lewis T, Bowman JP, Skerrat JH, McMeekin TA (1998) Novel bacteria as alternate sources of polyunsaturated FA for use in aquaculture and other industries. In: Marine microorganisms for industry. Le Gal Y, Muller-Feuga A (eds.), Plouzane: Editions IFREMER, 26–32
400. Russell NJ (1992) Physiology and molecular biology of psychrophilic microorganisms. In: Herbert RA, Sharpe RJ (eds.), Molecular Biology and Biotechnology of Extremeophiles, Blackie, Glasgow, pp 203–224
401. Cronan JE, Rock CO (1996) Biosynthesis of membrane lipids. In: *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, p 612. Edited by Neidhardt FC, and others. Washington, DC: American Society for Microbiology, 149–160
402. Fujii DK, Fulco AJ (1977) J Biol Chem 252:3660
403. Rawlings BJ (1998) Nat Prod Rep 15:275
404. Siegenthaler PA, Belsky MM, Goldstein S (1967) Science 155:93
405. Porter D (1990) Phylum Labyrinthulomycota. In: *Handbook of Protoctista*. Margulis L, Corliss JO, Melkonian M, Chapman DJ (eds). Boston, Jones and Bartlett, pp 388–398
406. Bahnweg G, Sparrow FK (1974) Am J Bot 61:754
407. Raghukumar S, Gaertner A (1980) Veroff Inst Meeresforsch Bremerhaven 18:289
408. Raghukumar S (1988) Transcr Br Mycol Soc 90:627
409. Honda D, Yokochi T, Nakahara T, Erata M, Higashihara T (1998) Mycol Res 102:439
410. Naganuma T, Takasugi H, Kimura H (1998) Mar Ecol Prog Ser 162:105
411. Cavalier-Smith T, Allsopp MTEP, Chao EE (1994) Philos Trans R Soc Lond B Biol Sci 346:387
412. Nakahara T, Yokochi T, Higashihara T, Tanaka S, Yaguchi T, Honda D (1996) J Am Oil Chem Soc CEP
413. Yaguchi T, Tanaka S, Yokochi T, Nakahara T, Higashihara T (1997) J Am Oil Chem Soc 74:1431
414. Bajpai P, Bajpai BP, Ward OP (1991) Appl Microbiol Biotechnol 35:706
415. Bajpai P, Bajpai BP, Ward OP (1991) J Am Oil Chem Soc 68:509
416. Li ZY, Ward OP (1994) J Indust Microbiol 13:238
417. Singh A, Ward OP (1996) J Indust Microbiol 16:370
418. Singh A, Wilson S, Ward OP (1996) World J Microbiol Biotechnol 12:76
419. Weete JD, Kim H, Ghandhi SR, Wang Y, Dute R (1997) Lipids 32:839
420. Ellenbogen BB, Aaronson S, Goldstein S, Belsky M, (1969) Comp Biochem Physiol 29:805
421. Findlay RH, Fell JW, Coleman NK, Vestal JR, (1986) Biochemical indicators of the role of fungi and thraus-tochytrids in mangrove detrital systems. In: Moss ST (ed.), The Biology of Marine Fungi. Cambridge University Press, Cambridge, pp 91–103
422. Barclay W (1992) US patent 5,130,242
423. Bowles RD, Hunt AE, Bremer GB, Duchars MG, Eaton RA (1999) J Biotechnol 70:193
424. Huang J, Aki T, Hachida K, Yokochi T, Kawamoto S, Shigeta S, Ono K, Suzuki O (2001) J Am Oil Chem Soc 78:605
425. Hammond BG, Mayhew DA, Naylor MW, Ruecker FA, Mast RW, Sander WJ (2001)

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Regul Toxicol Pharmacol 33:192
426. Barclay WR (1994) US Patent 5,340,742
427. Barclay W, Zeller S (1996) J World Aquacult Soc 27:314
428. Hammond BG, Mayhew DA, Robinson K, Mast RW, Sander WJ (2001c) Regul Toxicol Pharmacol 33:356
429. Hammond BG, Mayhew DA, Naylor MW, Holson JF, Nemec MD, Mast RW, Sander WJ (2001) Regul Toxicol Pharmacol 33:205
430. Fitch Haumann B (1999) INFORM 9:1108
431. Narciso I, Pousao-Ferreira P, Passos A, Luis O (1999) Aquacult Res 30:21
432. Carter CG, Bransden MP, Lewis TE, Nichols PD (2003) Mar Biotechnol 5:480
433. Bull AT, Goodfellow M, Slater JH (1992) Annu Rev Microbiol 46:219
434. Bull AT, Ward AC, Goodfellow M (2000) Microbiol Mol Biol R 46:573
435. Certik M, Shimizu S (1999) J Biosci Bioeng 87:1
436. Becker CC, Kyle DJ (1998) Food Technol 52:68
437. Arts MT, Ackman RG, Holub BJ (2001) Can J Fish Aquat Sci 58:122
438. Sargent JR, Tacon AGJ (1999) P Nutr Soc 58:377
439. Sargent JR (1995) (n-3) polyunsaturated FA and farmed fish. In: Hamilton, RJ, Rice, RD (eds.), Fish Oil: Technology, Nutrition and Marketing. Barnes and Associates, Bucks, pp 67–94
440. Peng J, Larondelle Y, Ackman RG, Rollin X (2003) Comp Biochem Physiol Pt B 134:335
441. Bell JG, Tocher DR, Farndale BM, Cox DI, McKinney RW, Sargent JR (1997) Lipids 32:515
442. Ackman RG (1980) Fish lipids. In: Connell, JJ (ed.), Advances in Fish Science and Technology. Fishing News, Farnham, p 86
443. Viga A, Grahl-Nielsen O (1990) Comp Biochem Physiol Pt B 96:721
444. Pickova J, Kiessling A, Petterson A, Dutta PC (1999) Fish Physiol Biochem 21:147
445. Ould El Kebir MV, Barnathan G, Siau Y, Miralles J, Gaydou EM (2003) J Agric Food Chem 51:1942
446. Fodor E, Jones RH, Buda C, Kitajka K, Dey I, Farkas T (1995) Lipids 30:1119
447. Lee KH, Jeong ICH, Suh JS, Jung WJ, Kim CG, Lee BH (1986) Han'guk Susan Hakhoechi 19:423
448. Tritar B, Attia-Chaouch S, Hammami M (1997) Ichtyophysiol Acta 20:67
449. Saito H, Ishihara K, Murase T (1997) J Sci Food Agric 73:53
450. Bandarra MN, Batista I, Nunes ML, Empis JM, Christie WW (1997) J Food Sci 62:40
451. Gamez-Meza N, Higuera-Ciapara I, Calderon de la Barca AM, Vazquez-Moreno L, Noriega-Rodriguez J, Angulo-Guerrero O (1999) Lipids 34:639
452. Tanakol R, Yazici Z, Sener E, Sencer, E (1999) Lipids 34:291
453. Marquez-Ruiz G, Velasco J, Dobarganes C (2000) Eur Food Res Technol 211:13
454. Hilbert G, Lillemark L, Balchen S, Højskov CS (1998) Chemosphere 37:1241
455. Njinkoué JM, Barnathan G, Mirallès J, Gaydou EM, Samb A (2002) Comp Biochem Physiol 131B:395
456. Gunstone FD (2002) Market report. Lipid Technology Newsletter 8:5
457. Rees JF, Cure K, Piyatiratitivorakul S, Sorgeloos P, Menasveta P (1994) Aquaculture 122:193
458. Salhi M, Izquierdo MS, Hernandezcruz CM, Gonzalez M, Fernandezpalacios H (1994) Aquaculture 124:275
459. Pike IH, Barlow SM (1999) Fish meal and oil to the year 2010: supplies for aquaculture. Presented at World Aquaculture 1999, Sydney, Australia, April 26–May 2 1999, Abstract, p 603

460. Molyneaux M, Chong ML (1998) Food Technol 52:56
461. Barlow SM (2002) 2nd Seafood By-Products Conference, Alaska, USA
462. Mukherjee KD (1999) INFORM 10:308