"Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution[1]

Michael T. Arts, Robert G. Ackman, and Bruce J. Holub

Abstract: Fatty acids (FA) are inextricably linked with key physiological and biochemical processes and are thus integral to proper ecosystem functioning. FA not biosynthesized effectively by animals are termed essential fatty acids (EFA). These EFA are important "drivers" of ecosystem health/stability and are therefore highly conserved in aquatic food chains. Aquatic organisms have been and continue to be our primary source of readily available EFA. However, overfishing and our burgeoning population may be acting in concert to threaten our access to this source of EFA. Here, we review the marine FA synthesis/transport cycle and traditional and nontraditional sources of EFA. Our review suggests that, while some traditional sources of marine oils (e.g., tuna) are in steady decline, other sources (e.g., krill) and technologies (e.g., heterotrophic fermentation) hold great promise for maintaining our access to EFA. We provide a minireview which illustrates that EFA contribute to our health and well-being. Finally, there is growing evidence that EFA have been an important force in our past evolution, leading us and others to speculate that an unbroken link exists between EFA, our present health, and, in all likelihood, our continuing evolution.

Résumé : Les acides gras (FA) sont intimement liés aux mécanismes physiologiques et biochimiques essentiels et sont donc nécessaires au bon fonctionnement des écosystèmes. Les FA non biosynthétisés efficacement par les animaux sont dits des acides gras essentiels (EFA). Ces EFA sont d'importants moteurs de la santé/stabilité des écosystèmes et c'est pourquoi ils sont fortement conservés dans les chaînes alimentaires aquatiques. Les organismes aquatiques ont été et continuent d'être notre source principale d'EFA facilement accessibles. Cependant, l'effet combiné de la surpêche et de l'accroissement de la population humaine pourrait mettre en péril notre accès à cette source d'EFA. On trouvera ici une revue des cycles marins de synthèse et de transport des EFA, ainsi que des sources traditionnelles et inusitées de ces acides gras. Bien que certaines sources traditionnelles d'huiles marines (e.g., le thon) soient en déclin continu, d'autres sources (e.g., le krill) et certaines technologies (e.g., la fermentation hétérotrophe) semblent prometteuses pour maintenir notre accès aux EFA. Les effets positifs des EFA sur notre santé et notre bien-être sont brièvement mis en lumière. Il y a, enfin, des indices de plus en plus convaincants que les EFA ont joué un rôle important au cours de notre évolution; cela nous amène, comme d'autres avant nous, à faire l'hypothèse qu'il existe un lien ininterrompu entre les EFA et notre santé actuelle et vraisemblablement aussi, avec la poursuite de notre évolution.

[Traduit par la Rédaction]

Introduction

As we enter this next millennium, we face profound, multifaceted challenges. One of the most urgent is ensuring that we can secure foodstuffs not only in adequate quantity, but also of high quality. Modern nutritional theory, for example, is focusing our attention on the numerous health benefits of maintaining sufficient levels of fatty acids (FA) and, in particular, preformed long-chain (i.e., C_{20} and C_{22}) polyunsaturated fatty acids (PUFA) in our diets. Marine fish/shellfish remain the principal source of these PUFA (Ackman 1988; Thomas and Holub 1994). Fish/shellfish stocks, however, are in decline worldwide (Williams 1998). Furthermore, new evidence suggests that we are, in effect, "fishing down" our marine food webs, i.e., the mean trophic levels of catches are in decline in several important fisheries (Pauly et al. 1998). Simultaneously, the United Nations estimates that the current world population is nearly 6 billion humans and could reach 8.9 billion by 2050 AD. The dilemma here is that an ever-increasing human population and technological advances in fish harvesting have merged to severely reduce some populations of the most desirable (in terms of FA composition) of the highest trophic level fish species. What are the implications of these two opposing trends in terms of our

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M.T. Arts.[2] National Water Research Institute, 867 Lakeshore Road, P.O. Box 5050, Burlington, ON L7R 4A6, Canada.
R.G. Ackman. Canadian Institute of Fisheries Technology, DalTech, Dalhousie University, P.O. Box 1000, 1360 Barrington Street, Halifax, NS B3J 2X4, Canada.
B.J. Holub. Human Biology & Nutritional Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada.

[1]Invited perpective for this 100th Anniversary Issue.
[2]Corresponding author (e-mail: michael.arts@ec.gc.ca).

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access to these PUFA? Are there alternative, viable sources of PUFA? How important are these compounds to human health and evolution (see Acknowledgments for an expanded definition of the term evolution)?

Here, we explore the fundamental link between ecology and human nutrition within the context of essential fatty acids (EFA). We briefly summarize the pros and cons involved with sourcing, production, and blending of various oils, highlighting some of the more viable alternative sources of EFA. We also provide new information on FA profiles in amphipods from prairie ponds and demonstrate that these organisms are relatively rich in two EFA. In order to clearly establish the requirements and benefits of these compounds, we provide a minireview of the effects of EFA on human health. We conclude with a discussion of the possible role of EFA in human evolution.

Terminology: essential fatty acids

Some FA are considered to be EFA in that they cannot be biosynthesized with an efficiency sufficient to meet our needs. The irony is that α-linolenic acid (ALA, C18:3ω3[3]) and linoleic acid (LA, C18:2ω6) are still called the only "essential" FA by most nutritionists and some biochemists. This term was coined in the 1930s in studies with rats and it has been a long and hard road to persuade scientists that it is the longer-chain C_{20} and C_{22} successor PUFA that are of vital concern to the functioning of most of the organs of our bodies (Holman 1998). Cunnane (1996) argued, on the basis of modern nutritional studies, that the term "essential fatty acid" is outdated when applied to ALA and LA, the precursors of eicosapentaenoic acid (EPA; C20:5ω3) + docosahexaenoic acid (DHA, C22:6ω3) and arachidonic acid (ARA, C20:4ω6), respectively. Instead, he recommended that the term "conditionally dispensable" be used in reference to LA and ALA. This recommendation is based on the observations that we have (*i*) an innate ability to synthesize ALA and LA from C_{16} PUFA precursors present in our diets, (*ii*) imprecise knowledge of true LA requirements, and (*iii*) an absence of clear symptoms of LA and ALA deficiency in healthy adults (Cunnane 1996).

Although some research (Carnielli et al. 1996; Salem et al. 1996) has demonstrated qualitatively that humans can convert the parent ALA to EPA and then to DHA, the most recent consensus is that the degree of conversion is "unreliable and restricted" (Gerster 1998). Furthermore, the rates of conversion (determined with ^{13}C-labeled precursors) suggest that, with a background diet high in saturated fat, conversion of ALA to long-chain metabolites is only ~6% for EPA and 3.8% for DHA in adults (see Gerster 1998 and references therein). In support of this, Emken et al. (1994) estimated a similar conversion efficiency of ALA to DHA in adults of ~5%. Furthermore, with a diet rich in ω6 PUFA, these conversion rates may be reduced by as much as 40–50% (Gerster 1998). On this basis and after examination of the available epidemiological studies showing an inverse association between cardiovascular disease (CVD) and fish consumption, the British Nutrition Foundation has officially recommended a diet including two or three portions of fatty fish per week (equivalent to 3–4 g standardized fish oil·day^{-1}) or, more specifically, 1.25 g EPA+DHA·day^{-1} (Gerster 1998). Similarly, Simopoulos et al. (1999) indicated that 0.65 g·day^{-1} corresponds to an adequate intake of EPA+DHA. We therefore here restrict our definition of the phrase "essential fatty acids" to mean preformed long-chain fatty acids (i.e., EPA and DHA) that cannot be synthesized in sufficient quantity to insure optimal physiological performance. That is, ideally, adequate amounts of EFA should be available for the best possible level of neural development (especially in infants) and, during adulthood, for the prevention and mitigation of cardiovascular, inflammatory, and other diseases (see below).

Marine fatty acid biosynthesis/transport cycle

In marine plants, the proportion of FA in lipids is only 40–50% by weight. This is because the total includes a large non-FA (carbohydrate) component composed of galactolipids (an often neglected part of total algal lipids; Parrish et al. 1992). If one assumes that the North Sea phytoplankton community structure is similar to that of the phytoplankton community off the coast of Nova Scotia, then their FA content would be 0.5–0.8% as wet weight (Ackman et al. 1968). The 530×10^6 t of North Sea photosynthetic biomass (Christensen 1977) could then accumulate only 0.25×10^6 t of FA.

The whole-body fat concentration of noncommercial Nova Scotian fish such as American sand lance (*Ammodytes americanus*) is 6–8% (Ackman and Eaton 1971). They are a major prey item for local Atlantic cod (*Gadus morhua*). The latter are identical to North Sea Atlantic cod and have a variable total fat content (Jangaard et al. 1967), but if it is taken as typically 5% of body weight, the 5×10^6 t of North Sea Atlantic cod would contain ~0.25×10^6 t of FA.

These figures create an apparent paradox. Removal of a small proportion of fish near or at the top of the food chain appears to remove the total year's production of FA. The explanation is, of course, that there is a tremendous buffer of lipid "tied up" in all the trophic levels in the oceans, but only the PUFA are conserved by animals at all trophic levels. They are a component of membrane phospholipids, transmit neural signals, are crucial for vision, and control ionic balance including gill function. Further, the very low melting points (approaching –50°C) of long-chain PUFA (LCPUFA) in their free form means that they will also remain highly fluid in situ and thus play an important role in biochemical adaptation to cold environments in aquatic ectotherms.

The transfer of FA from plants to higher animals is very uneven and must therefore be considered in terms of bioconcentration. For example, as noted above, primary productivity in the North Sea yielded ~530×10^6 t of biomass in 1970. Zooplankton and benthic invertebrates converted this to an estimated 41×10^6 and 55×10^6 t of biomass, respec-

[3] In the lipid shorthand commonly in use today, *m*:*p*ω*x* and *m*:*pn*–*x* are identical notations for a fatty acid with *m* carbon atoms, *p* ethylenic bonds (methylene interrupted if more than one), and *x* carbon atoms from and including the terminal methyl group to and including the carbon atom of the nearest ethylenic bond.

Table 1. Distribution of selected FA (% wet weight) in various marine organisms compared with Atlantic menhaden oil.

		Animal tissues (whole)			
Component	Atlantic menhaden oil[a]	Common oyster (*Crassostrea virginica*)[b]	Northern krill (*Meganyctiphanes norvegica*)[c]	Atlantic salmon[d]	Skipjack tuna[e]
C14:0	7.3	3.5	4.6	5.5	–8.2
C16:0	19	25.9	18.4	10.2	26.5
C18:0	4.2	3.6	2.2	2.7	11.9
ΣSAFA	30.5	36	25.2	18.4	>38.4
C16:1	9.1	4.2	3.5	8.1	–8.2
C18:1	13.2	8.2	14.7	16.9	6.8
C20:1	2	5	2.3	15.1	0.5
C22:1	0.6	0.3	0.9	14.4	—
ΣMUFA	24.9	17.7	21.4	54.5	>7.3
C18:2ω6	1.3	2	1.8	4.5	1.1
C18:3ω3	1.3	3.3	1.3	0.9	0
C18:4ω3	2.8	2.6	3.1	1.7	1.3
C20:4ω6	0.2	2.3	1	0.6	3.1
C20:5ω3	11	11.2	10.8	6.2	11.1
C22:5ω3	1.9	—	1	1.8	—
C22:6ω3	9.1	9.7	28.6	9.1	29.1
ΣPUFA	27.6	31.1	45.8	24.8	45.7
Other	17	15.2	7.6	2.3	—

[a]Ackman (1982).
[b]Watanabe and Ackman (1974).
[c]Mayzaud et al. (1999).
[d]Aursand et al. (1994).
[e]Tanabe et al. (1999). Data for juveniles (0.19–0.25 g). Analyses were by high-pressure liquid chromatography, therefore 14:0 and 16:1ω7 were not separated.

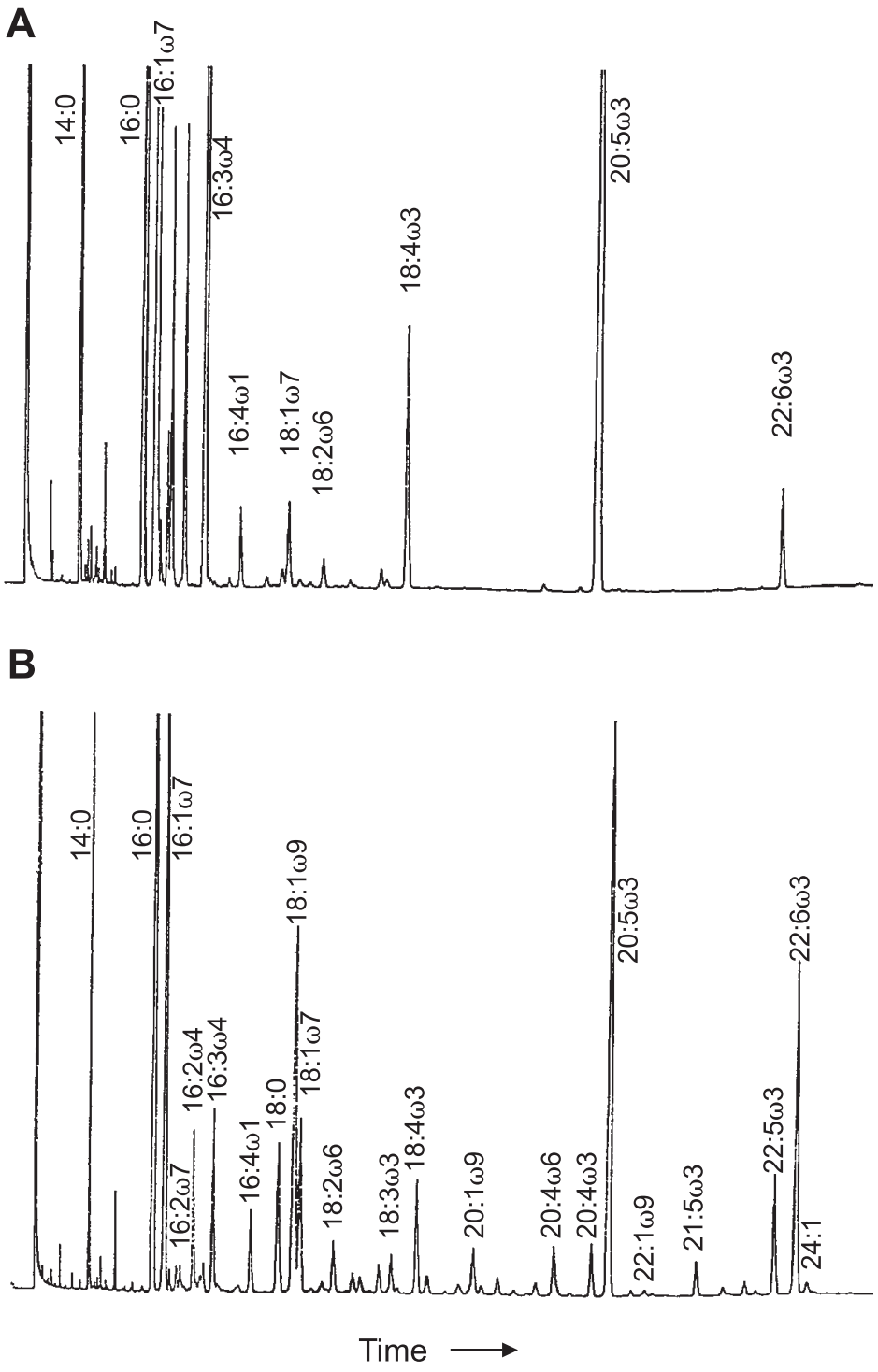
tively. Some was consumed by other successful and stable fish populations involved, such as sand lances (*Ammodytes* sp.), that contribute to the diet of Atlantic cod and other gadoids (Ackman et al. 1986), but only 5×10^6 t of Atlantic cod and similar commercial fish could live off this level. The crux of the matter is that, from an FA perspective, $\leq 3 \times 10^6$ t of Atlantic cod could be harvested annually in a sustainable fishery.

Saturated FA (SAFA) and the most common monounsaturated FA (MUFA) (C16:1ω7, C18:1ω9, C18:1ω7) can be biosynthesized de novo by most animals. Algal polysaccharides may be used as raw material for this purpose in lower trophic levels either through grazing or through direct intake of marine colloids (Liu et al. 1998). These polysaccharides are a basic part of detritus and seston layers (Parrish et al. 1995) and serve as bacterial substrates. As far as we know, this component of FA biosynthesis has never been seriously considered despite the vast quantities of available plant polysaccharides.

Atlantic menhaden (*Brevoortia tyrannus*) are a good example of direct transfer of FA from primary producers to fish. Adult Atlantic menhaden are filter-feeders that produce an oil with roughly one third each of SAFA, MUFA, and PUFA (Table 1; Fig. 1). The unicellular alga *Chaetoceros calcitrans* is a typical food item of Atlantic menhaden and is also widely used in shellfish nutrition research. It has a relatively simple FA composition, although the family of C16:2ω4, C16:3ω4, and C16:4ω1 FA (Fig. 1) is not commonly encountered by lipid chemists. Green algae tend to produce relatively simple FA mixtures based on C_{18} PUFAs (Volkman et al. 1989). Even so, the metabolism of FA by Atlantic menhaden, possibly aided by gut bacterial action, produces a variety of branched-chain FA, odd-numbered FA, and a few extra isomers of PUFA such as C20:4ω3 (Ackman 1989). This accounts for the numerous extra peaks in the chromatogram (Fig. 1).

The FA selected for presentation are usually rather limited, even though, since the early 1970s, over 60 FA can be found with capillary (open-tubular) gas–liquid chromatography. Figure 2 shows some well-known ones, but the choice of FA presented in Tables 1 and 2 is based on nine that are likely to be quantitatively important and three (C18:2ω6, C18:3ω3, C20:4ω6) that are of nutritional interest in fish oils or meals used in animal feeds. If one discounts C20:1 and C22:1, there is only one generic cold-water marine fish oil (based on triacylglycerols) similar in composition to Atlantic menhaden oil (Ackman et al. 1988). The phytoplankton in the diet of Atlantic menhaden do not contain C20:1 and C22:1, and consequently, they are barely present (1–2%) in that fish's oil. Those two FA are derived principally from fatty alcohols of copepod wax esters where the dominant C22:1 alcohol isomer is C22:1ω11 (Sargent 1989). This C22:1 structure transfers to most common North Atlantic fish body oils such as capelin (*Mallotus villosus*), Atlantic herring (*Clupea harengus*), and Atlantic mackerel (*Scomber scombrus*) where low levels of the free fatty alcohols C16:0, C18:1, C20:1, and C22:1 can all be found, obviously having escaped the hydrolysis and oxidation processes in the gut wall that convert most of the alcohol of wax esters to the corresponding FA (Ratnayake and Ackman 1979*a*, 1979*b*). The copepods may produce this unusual C22:1ω11 isomer to maximize the buoyancy effect of their wax esters. Copepods

Fig. 1. Gas–liquid chromatograms showing representative FA of (A) the marine phytoplankter *C. calcitrans* and (B) the typical fish oil of Atlantic menhaden. Adapted from a figure first presented in Ackman and Kean-Howie (1995).



are the main component of the trophic level that converts plant material to animal material. This large-scale production of copepod-derived C22:1ω11 results in the dominance of this isomer in the C22:1 group of fish depot fats right up to seals and even circulates in humans eating seal fat (Ackman et al. 1980), although usually accompanied by C22:1ω13, C22:1ω9, and C22:1ω7.

In fish, depot triacylglycerols and flesh phospholipids typically contain ~95 and ~75% FA by weight, respectively. A review of fish triacylglycerols demonstrates that only a few of these molecules from any marine fish oil will contain EPA and DHA (Moffat 1995). This occurs because, in the triacylglycerol depot fats of fish, there is a fair degree of specificity in FA assembly (Brockerhoff et al. 1963, 1964).

Fig. 2. FA likely to be observed in aquatic organisms, including phytoplankton and macrophytes. The notation clearly shows the family relationships between those of different chain lengths (Ackman 1999).

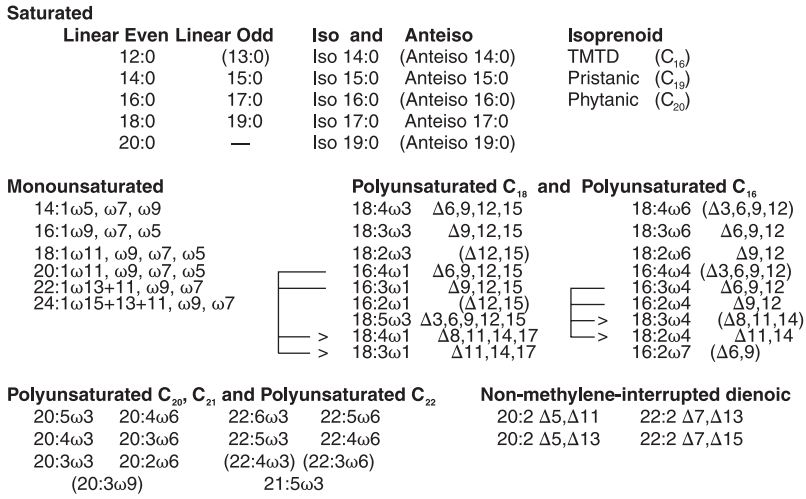


Table 2. FA concentrations (% wet weight) in different body tissues of a North Atlantic squid (*Illex illecebrosus*) compared with two cephalopods and a fish common in South African waters.

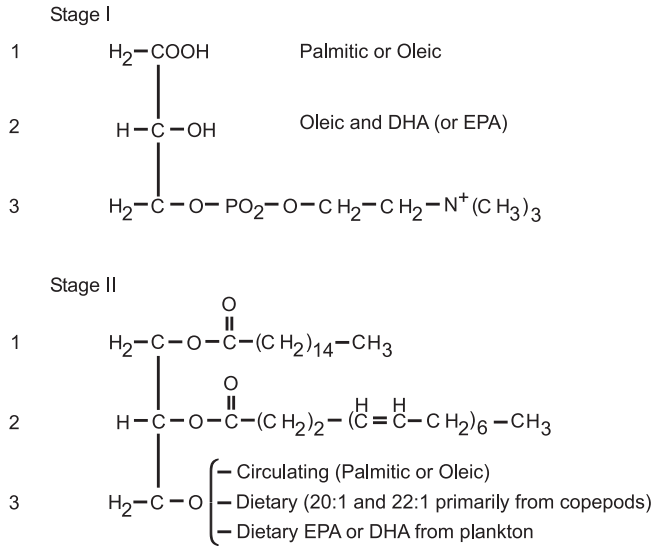
Component	Sole (*Solea vulgaris*) flesh[a]	Octopus (*Octopus vulgaris*) flesh[a]	Squid (*Loligo vulgaris*) flesh	*I. illecebrosus*[a]	
				Liver	Flesh
C14:0	4.3	2.7	2.5	4.4	2.2
C16:0	16.2	23.8	24.6	13.7	27.6
C18:0	6.9	10	2.8	1.8	4.4
ΣSAFA	29.2	38.4	30.4	19.9	34.2
C16:1	6.2	0.7	1.5	9	0.4
C18:1ω9	10.7	7.2	4.1	16.3	4.9
C18:1ω7	4	—	1.4	—	—
C20:1	3	3.8	4.6	12.4	4.9
C22:1	1.3	1.7	0.4	8.2	0.5
ΣMUFA	25.7	13.4	12.5	45.9	10.7
C18:2ω6	1.4	—	0.7	0.8	0.3
C18:3ω3	—	—	—	0.4	0.1
C18:4ω3	0.1	—	0.9	0.8	0.1
C20:4ω6	1.7	6.3	1.3	0.4	0.8
C20:4ω3	—	—	0.2	0.4	—
C20:5ω3	7.3	16.1	14.3	13.9	15.8
C22:5ω3	8.7	1.8	0.4	1.3	0.3
C22:6ω3	14.4	20.6	31.6	16.9	37.1
ΣPUFA	36.9	44.8	49.7	34.9	54.5
Other	8.2	3.4	6.5	—	—

[a]Riley (1999).
[b]Jangaard and Ackman (1965).

Specifically, biosynthesis via a phospholipid first stage is directed by enzymes that put a PUFA in the center (*sn*–2) position (Fig. 3). In the last (*sn*–3) position, all FA, including C22:1 and C20:1, derive from whatever FA are in the diet and are circulating in the blood when the triacylglycerol molecules are assembled. Thus, most of the DHA will be located in the central position of phospholipids, with most of the remaining EPA and DHA circulating in the fish's blood (especially after a meal).

There is less C22:1 in Atlantic cod and Atlantic salmon (*Salmo salar*) oils as compared with Atlantic herring, for example, which may contain up to 30% of their FA as C22:1. This reinforces the conclusion that these two fish are at a higher trophic level. Atlantic cod and Atlantic salmon, of course, do not, as adults, eat the C:22:1-rich copepods. Thus, it follows that C20:1 and C22:1 are generally absent in the phospholipid precursors of Atlantic salmon triacyglycerols and therefore that these two FA will also rarely be found in the *sn*–2 and *sn*–3 positions of Atlantic salmon triacylglycerols (Fig. 3). In fact, with the exception of C20:1 and C22:1, the FA composition of several freshwater fish does not differ radically from that of marine fish (Henderson and Tocher 1987). Other freshwater fish such as carp (mostly common carp (*Cyprinus carpio*)), tilapia (Mozambique tilapia

Fig. 3. Biosynthesis of a fish oil triacylglycerol molecule. The first stage is a phospholipid that may have any one of four common FA (myristic, C14:0; palmitic, C16:0; palmitoleic, C16:1ω7; or oleic, C18:1ω9) in the *sn*–1 position. The phospholipids would prefer a highly unsaturated FA (e.g., EPA or DHA) in the *sn*–2 position, but depending on whether the fish is in either the biosynthesis or the catabolism status for energy, the assembly process may have to again use a common FA. Finally, in the last stage of assembly, any of the currently surplus FA (e.g., C20:1, C22:1) from the diet or from the normal blood circulation are inserted.



(*Tilapia mossambica*) or Nile tilapia (*Tilapia nilotica*)), channel catfish (*Ictalurus punctatus*), and similar herbivorous fish are now the subject of intense nutrition research by aquaculturalists.

The Atlantic salmon FA of Table 1 are relatively high in C22:1 because these were farmed fish fed on capelin or Atlantic herring meal and oil. However, Atlantic cod and Atlantic salmon employ a selective deposition or catabolism process so that their oils will contain a little less C22:1 than C20:1 relative to the ratio in the diet. For example, migrating Pacific salmon preferentially use up C22:1 for energy. Other salmonids such as rainbow trout (*Oncorhynchus mykiss*) also selectively use C22:1 as an energy source (Henderson et al. 1982). However, Jezierska et al. (1982) reported that MUFA are utilized first on starvation in rainbow trout.

Sources of essential fatty acids

Aquatic sources

Marine sources of essential fatty acids

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, shark (liver), and tunas. A recent addition to commercially available marine-sourced oils is seal oil. Seal oil differs from fish oils in that it contains appreciable amounts of docosapentaenoic acid (*n*–3 DPA, C22:5ω3). This form of DPA is valuable in that it is reputed to be at least 10 times more effective than EPA in inducing endothelial cell migration (Kanayasu-Toyoda et al. 1996), a process critical in wound repair and in keeping arteries soft, supple, and clear of deposits. Marine 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.

There are, however, potential problems associated with fish oils as a source of PUFA. These may include taste, odor, and stability problems as well as the presence of coextracted contaminants. Refining insures that fish oils conform to edible-food standards, and, to a great extent, gelatin encapsulation and deodorization negate the taste and odor issue. Deodorization also reduces organic contaminant levels in fish oils (Hilbert et al. 1998). Volatile components are among the most effectively removed contaminants; however, highly lipophilic contaminants (e.g., dieldrin and some 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) congeners) may remain at low levels. Clearly, a focus on obtaining source fish from a pristine environment is prudent. Another potential problem with fish oils is that the term is sometimes used generically. That is, "fish oil" can be obtained from a variety of fish species, locations, and seasons, and each of these variables influences the ultimate FA composition of the oil.

Beyond these issues, the concern that fish/shellfish stocks are in worldwide decline signals us to both protect existing EFA sources and be receptive to new sources of EFA. Furthermore, it is not yet cost effective to biochemically synthesize EFA on a commercial scale and we may therefore have to utilize "nontraditional" sources of EFA such as those presented in the following sections.

There is a gradation of desirability for food use among fish resources in the ocean. For example, a plentiful species such as capelin could be further exploited, as there already is a sound meal and oil industry in Iceland and Norway and a limited food industry based on roe only. Capelin is a circumpolar species that, like American sand lance, is an intermediary between zooplankton and the commercially valuable gadoids. There are two North Atlantic populations of special interest. One is heavily fished in Iceland and migrates thence to Norway. There, together with the Barents Sea stock, capelin support a vigorous Atlantic cod stock and are heavily fished for meal and oil. The second population spawns in June–July in Newfoundland and can have a fat content as high as 18% when feeding in waters off Labrador (Eaton et al. 1975). The Japanese consume large amounts of roe, and although some attempts to develop food products for North American tastes have been made (Jangaard 1974), capelin is a typical example of a pelagic fish with high edible potential that is rarely used directly for food. This is unfortunate, as, for unknown reasons, the lipids of frozen capelin are remarkably resistant to oxidation (Botta et al. 1983).

The capelin situation must be contrasted with the vast numbers of Myctophidae, or lanternfishes. These attracted attention among biologists several decades ago because of their diel migrations, midwater habitat, and large numbers (Neighbors and Nafpaktitis 1982). Possibly, diel migrations would allow commercial exploitation, but so far, utilization

has not been seen as practical because of wax esters discovered in the bodies of several species (Lee and Patton 1989). This, together with their small size, will likely preclude their use as food by humans. Another midwater family, the lightfishes or bristlemouths (Gonostomatidae), have lipids similarly rich in wax esters.

Wax esters are not necessarily all bad, as for example in the case of the orange roughy (*Hoplostethus atlanticus*) from New Zealand. The tissues of this large, deep-dwelling (1000 m) teleost fish were shown to be rich in wax esters (Hayashi and Takagi 1980). Subsequently, a deep skinning technique was developed and the delicious white muscle fillets were a market success in North America. Unfortunately, the fishery soon virtually collapsed, mainly because it was not appreciated that reproduction and replacement rates of this fish are extremely slow. Hopefully the development of fisheries for other edible deepwater fish species with wax esters (e.g., off Australia's coasts Bakes et al. 1995) will learn from this experience.

Tuna are grossly overfished and yet little is known about their early life stages. Juvenile tuna are known to have very high total body contents of EPA and DHA. The total lipid concentrations of skipjack tuna (*Katsuwonus pelamis*) tuna range from 2.24 to 4.0% (wet weight) for a weight range of 0.2–62.8 g (Tanabe et al. 1999). Adult tuna also contain high concentrations of DHA (~20–25% in body and viscera oils and ~25–29% in the orbital oil behind the eye socket) and 5–8% EPA. Fish phospholipids typically have a total of 35–40% of these two FA (Polvi and Ackman 1992; Ackman 2000), but generally, phospholipids are <1% of muscle lipid. The balance of the EPA and especially DHA exists therefore in the triacylglycerols of tuna. These high proportions of EPA and DHA are the result of the tuna's natural diet and may therefore be hard to match in fish farming operations. However, the adult frigate mackerel (*Auxis rochei*) fished off Japan shares the same triacylglycerol FA pattern with very high DHA in the orbital oil and a little less in total muscle lipid that ranges from a few percent in dorsal meat to nearly 20% in ventral meat (Morioka et al. 1999). Mackerel species are fairly plentiful in various parts of the world, but fishing *A. rochei* and other species for aquaculture food would likely not be welcomed because there already exists an extensive fishery for direct human use. This leaves aquatic invertebrates as the remaining consideration. Bivalves and shrimp are already farmed on a commercial level, leaving two other groups that can be considered for harvest.

Squid possess a fatty liver or hepatopancreas, the source of an oil in Japan that is sometimes used in aquaculture, as it has essentially the same composition as most fish oils (Table 2). A large part of the world catch is consumed in Japan, but local varieties have been overfished, forcing the fishery onto the high seas for deepwater species. Other squid fisheries (e.g., in Newfoundland), however, have declined because of unknown factors. The Canadian species *Illex illecebrosus* overlaps with *Loligo pealei*, common off the south Atlantic coast of North America. Mantle lipid of both species is between 1 and 2% (Krzynowek et al. 1989) with ~5% EPA and 30–40% DHA, reflecting the tissue's high phospholipid content. Deepwater species tend to have wax esters or diacylglyceryl ethers in their liver lipids (Hayashi 1989), but the extent of these deep-sea resources is unknown. Nearshore European exploitation of squid and octopus for food, however, seems to be sustainable (Sinanoglou and Miniadis-Meimaroglou 1998). Although large-scale culture is unlikely, a recent study has already established the FA needs of five octopus species and suggested that LCPUFA are necessary for larval survival and development (Navarro and Villanueva 2000).

Table 3. Comparisons among phytoplankton FA from three stations (A1, C1, and C5) near South Georgia Island with those of corresponding catches of *E. superba* (Cripps et al. 1999).

	Phytoplankton FA ($\mu g \cdot L^{-1}$)			*E. superba* fatty acids ($mg \cdot kg^{-1}$)		
Component	A1	C1	C5	A1	C1	C5
C14:0	1.3	1.5	1.1	512	24	54
C15:0	0.3	0.3	0.1	14	—	3
C16:0	5	8.3	2.7	765	31	71
C18:0	11.1	22.9	4.9	37	1	3
ΣSAFA	17.7	33	8.8	1328	56	131
C16:1ω7	1.9	2.7	0.5	274	32	70
C18:1ω9	7.2	11.7	2.9	269	22	42
C18:1ω7	0.6	4.1	0.8	126	11	32
C20:1ω9	1.4	2.1	11.7	14	1	0
C22:1ω11	0.3	0.7	0	4	—	0
ΣMUFA	11.4	21.3	15.9	687	66	144
C16:2ω6	0.2	1	0	23	8	15
C18:2ω6	0.9	2.1	1.1	52	6	37
C18:3ω3	0	0.5	1.5	13	2	27
C16:4ω3	0.4	0.5	0	5	11	22
C18:4ω3	0.4	1.7	8.3	23	13	85
C20:4ω6	0	0.4	0	6	1	8
C20:4ω3	0	0	0	6	1	15
C20:5ω3	4.8	11.4	4.7	128[a]	65[b]	260[c]
C22:5ω3	0	0.6	0	—	1	3
C22:6ω3	2.4	3.6	7.1	14	6	39
ΣPUFA	9.1	21.8	22.7	270	114	511
Total	38	76.1	47.3	2282	237	782

Note: Rough volumetric proportions of the main phytoplankton taxa are as follows: A1, one half small diatoms, one quarter pamnesiophytes, and one quarter large diatoms; C1, small diatoms; C5, one tenth dinoflagellates, one quarter chrysophytes, and two thirds small diatoms.

[a]5.6% of FA.

[b]28% of FA.

[c]33% of FA.

"Krill" is a term that was first popularly applied to the Antarctic euphausiid *Euphausia superba*. This organism is ≤6 cm in size and has a lipid system based primarily on glycerol esters (Clarke 1980). Therefore, it does not include wax esters as found in its shelf-dwelling neighbor *Euphausia crystallorophias* (Kattner and Hagen 1998). Other species of krill are smaller in size and the economics do not favor commercial utilization. Table 3 compares three sets of FA data from phytoplankton with those of *E. superba* collected at the same station. Most of the FA, with the exception of C14:0, C16:0, and C18:1 (which are easily biosynthesized), are similar in proportion to those of other marine animals, although euphausiid EPA to DHA ratios are extremely high. As noted by various authors, these LCPUFA are freely available to krill and need not be biosynthesized. Clarke (1980) reported that EPA to DHA ratios were ~2:1 for phosphatidyl

cholines and ~1:1 for phosphatidyl ethanolamines for *E. superba* from the same location. In the corresponding triacylglycerols, ratios of EPA to DHA were ~3:1. These must, however, be considered as general relationships in the overall FA composition of euphausiids (Table 3). As noted in Table 3, krill from stations A1, C1, and C5 (Cripps et al. 1999) had different diets. If the FA potentially synthesized de novo are C14:0, C16:0, C16:1, and C18:1, then these four (as percentages of the total) are 85% for A1, 50% for C1, and 34% for C5. The figures for EPA are inversely 5.6, 28, and 33% of the total. Samples C1 and C5 are similar in FA composition to those reported by Clarke (1980). In sample A1, an ample supply of phytoplankton carbohydrate prevailed, allowing de novo synthesis of additional C14:0, C16:0, C16:1, and C18:1 into triacylglycerols, thus diluting the EPA and DHA, which could be reserved for functional phospholipids.

In *E. crystallorophias*, wax esters are a complicating factor, but the same FA patterns in triacylglycerols are observed, although C14:0 is relatively less important and C18:1 possibly more important (Kattner and Hagen 1998). In fact, C16:1ω7 and C18:1ω7 should be regarded as one FA with C18:1ω9 as distinctly different. This is because C16:1ω7 is presumably formed from C16:0 by desaturation, so studying C16:0, C16:1ω7, and C18:1ω7 in isolation (Cripps et al. 1999) is rather unlikely to be meaningful. In some samples examined by Clarke (1980), C18:1ω7 exceeded C18:1ω9. In the other reports discussed above, the more common 2:1 ratio for C18:1ω9 and C18:1ω7 seems to have been the rule. This species is described as an omnivore, a further complication in explaining FA compositions.

Commercial interest in exploiting *E. superba* and other similar large euphausiids as sources of protein (11%; Clarke 1980) has waned because of problems of excessively rapid proteolysis (Kolakowski and Gajowiecki 1992). Lipolysis is equally a problem (Sugii and Kinumaki 1982), and even research scientists have to be careful, as Clarke (1980) found that 10–14% of total lipid in carefully preserved krill samples was free FA. This is a well-established phenomenon in fish muscle, although it occurs much more slowly in frozen tissues (Haard 1992). Free FA in amounts exceeding 1% of total lipid in biological or commercial samples should be regarded with suspicion.

Other aquatic sources of essential fatty acids

Pond aquaculture

Despite years of effort, *Spirulena* and *Dunaliella* remain among the few algae successfully harnessed for mass outdoor culture. Both algae require specific growth conditions such as high carbonate, high pH, high light intensity, and (or) high temperature. Such features make these algae well suited to large-scale outdoor aquaculture but only in selected locations. A great deal is now known about the physiology and genetics of these two species, and although these species do not, in known forms, produce appreciable amounts of EPA or DHA (Ben-Amotz et al. 1985), selection of mutants and (or) gene splicing could conceivably be implemented to alter their FA profiles.

Freshwater sources

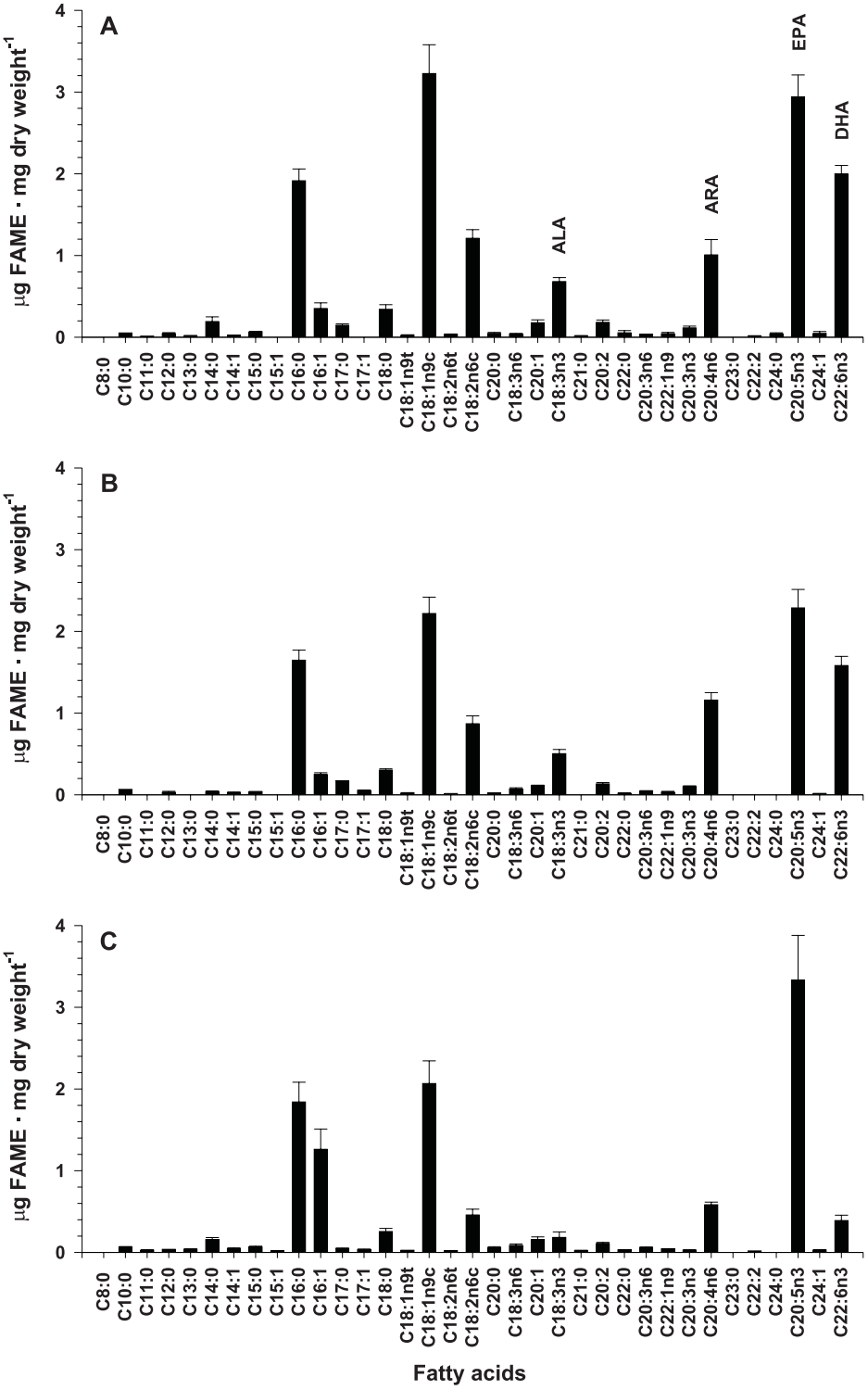
Historically, most research into FA composition has focused on marine fish/shellfish, reflecting, in part, the economic importance of these fisheries. However, less well studied are marine and freshwater benthic invertebrates and freshwater fish that can contain appreciable quantities of EFA of similar composition to FA found in marine fish (Ackman 1999).

There are undoubtedly many nontraditional freshwater sources of EFA yet to be discovered. We here provide two examples (the amphipods *Gammarus lacustris* and *Hyalella azteca*) of a novel freshwater source for EPA and DHA. The FA compositions of both amphipod species were broadly similar (Fig. 4). FA that were present in both species, at levels exceeding 0.5 μg FA·mg dry weight^{-1}, included palmitic acid (C16:0), oleic acid (C18:1ω9), LA, ALA, ARA, EPA, and DHA. ALA, ARA, EPA, and DHA represented 4.5, 6.7, 19.5, and 13.3%, 4.3, 9.8, 19.3, and 13.4%, and 1.6, 5.0, 28.7, and 3.4% of total FA analyzed in *G. lacustris* from pond 1 and Gursky's pond and *H. azteca* from Redberry Lake, respectively.

There are literally millions of wetlands in the North American central plains. Although less abundant than shallow, more ephemeral ponds, deeper wetlands (>1.0 m) are still very common. These range in surface area from small dugouts to large ponds and may also include extensive wetland complexes. Measured amphipod densities in these wetlands range from <100·m^{-2} (Lindeman and Clark 1999) to several thousands per square metre (Wen 1992) to a high of 57 245·m^{-2} (Hammer et al. 1990), but only in ponds that are deep enough to sustain populations over winter. For example, *G. lacustris* and *H. azteca* require a minimum fall water depth of ~1.5 and ~1.0 m, respectively (D. Lindeman, Canadian Wildlife Service, Saskatoon, SK S7N 0X4, Canada, personal communication).

In a previous study, Arts et al. (1995) obtained estimates of dry weight for adult *G. lacustris* (12.22 mg ± 0.42 SE, n = 96) and *H. azteca* (2.02 mg ± 0.03 SE, n = 416). We used these above data along with FA content measured here (Fig. 4) to produce hypothetical models of potential yields of EPA and DHA in prairie ponds of varying surface area. Mean EPA and DHA concentrations were 2.6 and 1.8 g·kg dry weight^{-1} (*H. azteca*) and 3.3 and 0.4 g·kg dry weight^{-1} (*G. lacustris*), respectively. Our extrapolations suggest that several tonnes of EPA and DHA could be present in a large pond (e.g., 100 × 100 m) where amphipods occur at high densities (Figs. 5 and 6). Further, we would expect concentrations of EPA and DHA within amphipod tissues to be highest in spring and fall, when food quality (in terms of EFA) of freshly sedimenting matter is highest because of the inclusion of diatoms, dinoflagellates, and cryptomonad algae. Amphipods, therefore, represent naturally occurring freshwater sources of EPA and DHA. Such resources may have commercial value, and this could be of particular importance in regions where agricultural diversification is desirable. We report here on naturally occurring levels of EPA and DHA. However, amphipods are readily amenable to aquaculture applications, and the high-productivity, high-light environments on the prairies would be "fertile ground" for such commercial endeavors. Indirect use of

Fig. 4. Fatty acid methyl ester (FAME) concentration in two amphipod species from three wetlands: *G. lacustris* from (A) pond 1 (52°13′N, 106°06′W) and (B) Gursky's Pond (52°09′N, 106°07′W) located in or near the St. Denis National Wildlife Refuge, Saskatchewan, and *H. azteca* from (C) Redberry Lake, Saskatchewan (52°43′N, 107°09′W). Error bars are standard errors.



gammarids and other zooplankton in pond-rearing of rainbow trout destined for human consumption looked appealing three decades ago (Johnson et al. 1970), but flavor problems in the rainbow trout ultimately put a stop to that form of commercialization. However, modern cost-effective refining methods, as well as different commercial interests (e.g., harvesting EPA and DHA), could instill new life into such endeavors. Furthermore, the high protein content "amphipod meal" by-product thus produced would be of potential use in other industries (e.g., livestock feed, petfood).

Fig. 5. Potential harvest of EPA and DHA in the amphipod *G. lacustris* in wetlands of varying surface area. A depth of at least 1.5 m is assumed.

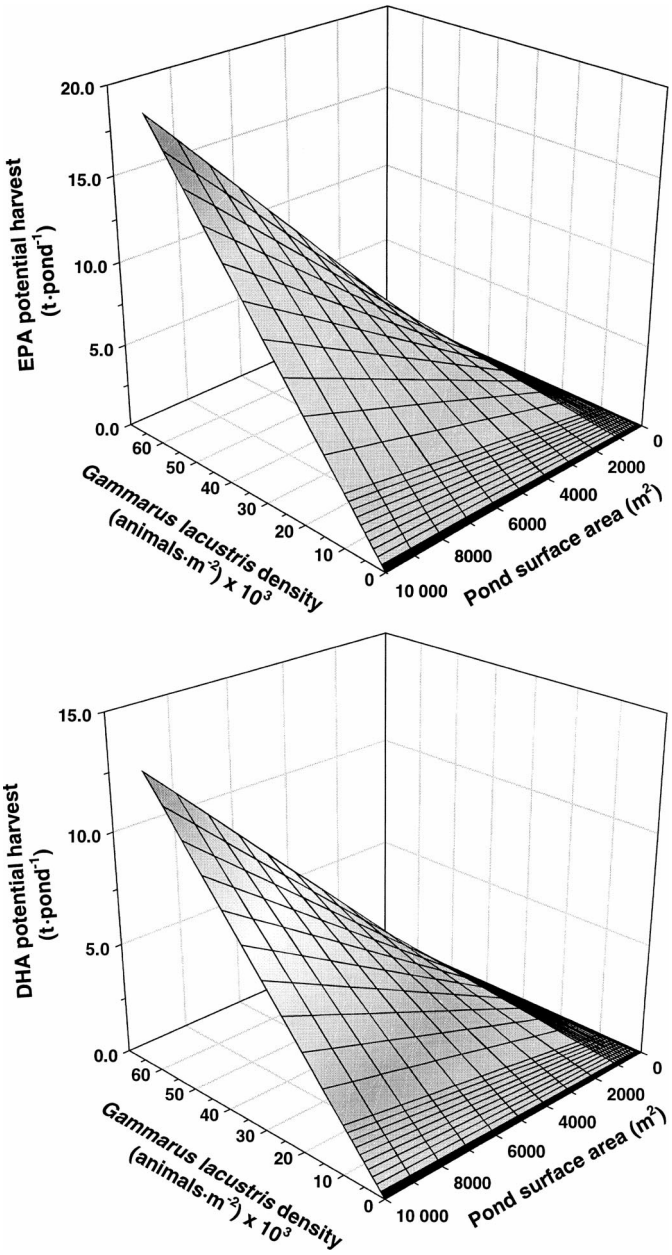
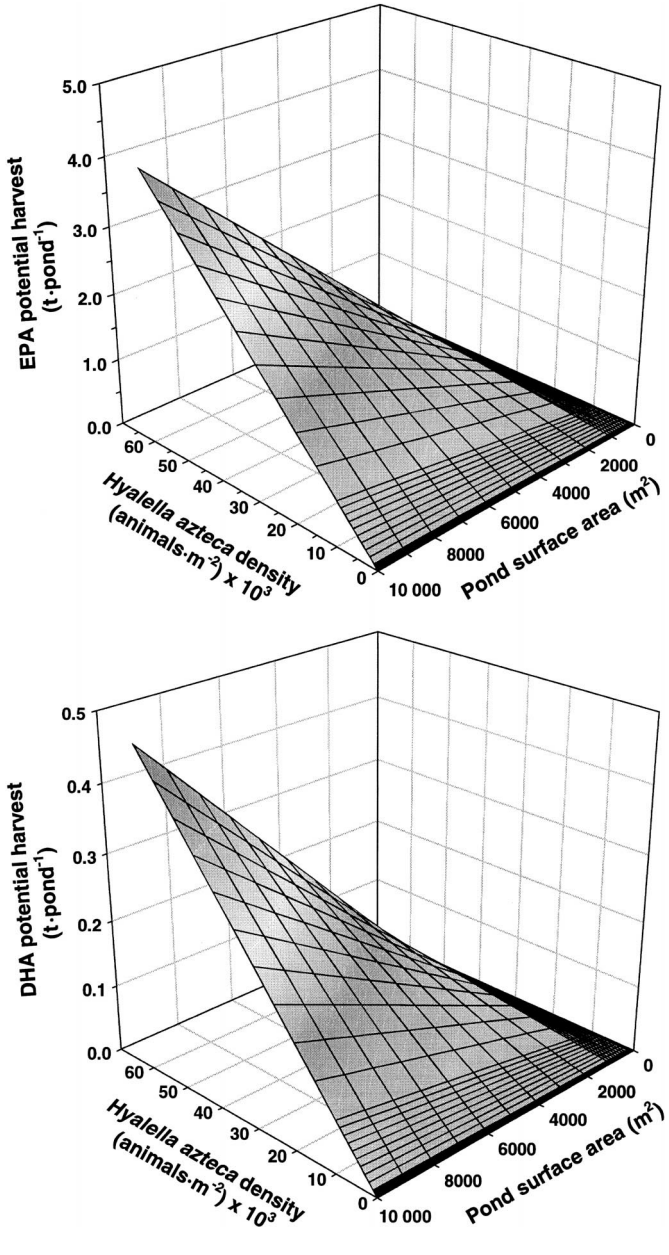


Fig. 6. Potential harvest of EPA and DHA in the amphipod *H. azteca* in wetlands of varying surface area. A depth of at least 1 m is assumed.



Nonaquatic sources

Flax (linseed) and other vegetable oils

Flax oil is currently a popular source of ALA, the precursor to EPA and, ultimately, DHA (but see the section above on terminology for conversion rate estimates). Vegetable oils are normally rich in LA, but a few may also contain γ-linolenic acid (GLA, C18:3ω6). Oils that contain GLA include black currant seed oil, borage oil, and primrose oil. While GLA is the metabolic precursor to ARA, the process of conversion is very slow, requiring participation of the enzyme Δ5-desaturase. This enzyme and the complementary Δ6-desaturase are present in humans at very low levels (Bourre et al. 1990), hindering endogenous biosynthesis of ARA, EPA, and DHA.

Egg yolk lipids

ARA, EPA, DPA, and DHA can be obtained from egg yolk lipids, especially when chickens are flax fed. For example, BurnBrae Farms Ltd. (Lyn, Ont.), which markets Omega-3 chicken eggs (flax-fed chickens), lists the ALA, ARA, EPA, DPA, and DHA content of their eggs as ~331, 45, 6, 10, and 70 mg, respectively, per average 50-g egg. Egg yolks of chickens fed a special diet containing fish meal have especially high EPA+DHA levels (Farrell 1998).

Heterotrophic fermentation

Using conventional stirred-tank fermenters, economically viable quantities of certain microorganisms that are rich in oils containing desirable EFA such as DHA or ARA can be produced (Becker and Kyle 1998). Martek Biosciences Corp. (Columbia, Md.), for example, has successfully used such mass culture techniques to produce oils rich in DHA (with the marine dinoflagellate *Crypthecodinium cohnii*) and ARA (with fungi such as *Pythium insidiosum* or *Mortierella alpina*). The chief advantages of such techniques lie in consistency and purity of the final FA 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 (D. Kyle, Martek Biosciences Corp., Columbia, MD 21045, U.S.A., personal communication).

Fatty acids and human health

In humans, one of the bases for the heightened interest in EFA includes epidemiological/population studies suggesting that increased fish consumption as a source of ω3 PUFA is often associated with decreased mortality (as well as morbidity) from cardiovascular disease (CVD) (Holub 1988; Leaf 1990). For example, several years ago, it was recognized that Greenland Inuit, when compared with Danes (Bang and Dyerberg 1980), had a significantly lower death rate from acute myocardial infarction despite a high-fat diet (marine based) and only moderate differences in blood cholesterol levels. Furthermore, the higher fish (including EPA+DHA) intakes of the Japanese population relative to North America have been associated with considerably lower rates of acute myocardial infarctions, other ischemic heart disease, and atherosclerosis despite only moderately lower blood cholesterol levels in the Japanese population (Menotti et al. 1999).

The mean current daily intake of EPA+DHA in a typical North American diet is ~0.13 $g \cdot day^{-1}$, which is severalfold lower than Japanese intakes and only a small fraction of that consumed by the Inuit. Depending on the species eaten, fish consumed two and a half to three times per week would provide an equivalent intake of ~500–600 mg EPA+DHA$\cdot day^{-1}$ (when normalized). These latter intakes are about 4-fold higher than current North American consumption rates. The population data reported by Dolecek (1992) indicated that progressively higher intakes of fish-derived ω3 FA (up to ~650–700 $mg \cdot day^{-1}$) were associated with a progressive reduction in coronary heart disease related mortality as well as total mortality with no associated increase in total cancer related mortality.

Dietary supplementation and controlled intervention trials with encapsulated ω3 fish oil concentrates can reduce both the progression of CVD and cardiovascular-related mortality including sudden cardiac death in humans, but these have been mostly short-term studies. Various long-term studies (e.g., Daviglus et al. 1997) have also indicated that consumption of fish (two or three servings per week) is associated with lower primary heart attack rates and death from CVD. Fatty acid analyses of serum phospholipid, a biomarker for EPA+DHA intake and status, indicated that DHA levels were inversely correlated with coronary heart disease in men (Simon et al. 1995). Furthermore, intervention studies using fish oil concentrates providing EPA+DHA (combined ω3 FA) at intakes of 2–4 $g \cdot day^{-1}$ over several weeks have shown the ability of these FA to favorably attenuate various risk factors for CVD (independent of any blood cholesterol lowering effect). For example, ω3 FA accumulate to a considerable extent within circulating blood platelets, which is associated with decreased platelet adhesiveness and aggregation and an overall reduction in thrombogenicity (Harris 1997).

Human studies have also revealed the potent ability of EPA+DHA to significantly reduce circulating levels of blood triglyceride (Harris 1997). Moderate elevations in triglyceride (above 100 mg % or 1.1 $mmol \cdot L^{-1}$) have been associated with a progressively increased risk of coronary heart disease in both men and women (Criqui et al. 1993). Within 2–3 weeks of supplementation, EPA+DHA significantly reduced blood triglyceride levels by ~6–8% per gram EPA+DHA consumed (Harris 1997). In addition, the antiarrhythmic potential of EPA plus DHA (upon accumulation in cardiac tissue) has been considered as yet another important mechanism by which consumption of these FA can reduce cardiovascular-related mortality, particularly sudden cardiac death (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico 1999).

Recent studies have focused on the potential for fish oil supplements (EPA+DHA enriched) to modify clinical endpoints in patients with respect to coronary atherosclerosis and myocardial infarctions. Recently, von Schacky et al. (1999) studied the effect of dietary ω3 FA on coronary atherosclerosis in cardiovascular patients using a randomized, double-blind, placebo-controlled trial. This study revealed that patients with coronary artery disease given ω3 therapy (at levels of ~1–1.5 $g \cdot day^{-1}$) over 2 years had less progression and more regression of coronary artery disease on coronary angiography (discernible, modest mitigation of atherosclerosis) than did patients on placebo. Fewer clinical cardiovascular events were noted in the ω3 groups. The ω3 supplementation was considered safe and well tolerated. Very recently, the GISSI-Prevenzione trial was reported by Marchioli and colleagues (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardio 1999). In this study, 11 324 patients, who had experienced a myocardial infarction, were assigned to supplemental interventions following introduction of a Mediterranean-type diet (which included moderate fish consumption) as well as aggressive treatment with various pharmaceutical agents for cardiovascular care. Approximately half of the patients received an ω3 fish oil supplementation (providing ~0.9 g EPA+DHA$\cdot day^{-1}$). Over the subsequent interval (up to 3.5 years), the ω3-supplemented subjects were found to exhibit a significant reduction in overall cardiovascular deaths and an approximate 45% reduction in sudden cardiac death. These results support the concept that, independent of blood cholesterol lowering, EPA+DHA intakes (including supplementation) can favorably influence cardiovascular-related mortality (particularly sudden cardiac death) via various mechanisms (a combination of antiarrhythmic, antithrombogenic, lipid-lowering, etc.).

DHA is found at very high levels in the *sn*–2 position of membrane phospholipids in the brain and retina as well as in other tissues and cells (e.g., sperm cells, heart tissue). DHA

is required at optimal levels in the brain and retina to provide for maximal brain functioning and visual acuity, respectively (Cunnane et al. 2000), especially in infants. Consequently, some expert groups have recommended that DHA should represent at least 0.4% of the total FA in breast milk and infant formula. In order to maintain a minimal level of DHA in breast milk (at 0.4% or more), ~300 mg of DHA needs to be consumed daily. This contrasts with the current mean intake in pregnant and lactating women of only ~80 mg·day^{-1} (Conquer and Holub 1997). The level of DHA in breast milk has been found to be positively correlated (as expected) with dietary fish intake.

Both EPA and DHA, in their fatty acyl-CoA forms, are also esterified to cellular lipid (both phospholipid and nonpolar lipid) and undergo betaoxidation and other metabolic transformations including oxygenation reactions to eicosanoids (prostaglandins, thromboxanes, leukotrienes, etc.). The eicosanoids mediate the potentially beneficial effects of ω3 FA, in part, by replacing ω6 PUFA in cells and tissues, thereby reducing the overall ω6 PUFA to ω3 PUFA ratio and reducing the formation of ARA-derived eicosanoids (Thomas and Holub 1994). The eicosanoids may also affect certain cardiovascular disorders (e.g., thrombogenicity) and proinflammatory conditions (e.g., rheumatoid arthritis; Thomas and Holub 1994).

A number of neurological conditions have also been associated with reduced levels of circulating DHA levels (such as some depressive illnesses, manias, etc.). In many cases, however, controlled studies have not been performed to evaluate whether enhancing DHA intakes and corresponding levels in the circulation and target tissues/cells can improve clinically measured neurological performance. Recently, Stoll et al. (1999) have shown that supplementary ω3 fish oil (containing EPA+DHA) can significantly improve the clinical management of patients with bipolar disorder. A number of studies are now ongoing that utilize DHA alone, EPA alone, or combinations of EPA+DHA with respect to various neurological conditions (depressive illnesses and anxiety disorders, schizophrenia, attention deficit disorder, etc.).

Fish oil concentrates containing EPA+DHA, when given in supplementary form at levels of 3–5 g ω3 PUFA daily over a few months, have also been found to provide significant benefit in patients with various conditions including rheumatoid arthritis and inflammatory bowel disease, including Crohn's Disease (Connor 2000). The mechanisms for these latter effects appear to involve altered eicosanoid synthesis (suppression of ARA-derived eicosanoids including suppressed formations of neutrophil-derived leukotriene B_4, a proinflammatory eicosanoid).

The above findings have led to a dramatic upsurge in intensity by the food industry towards development of nutraceutical and functional food products containing enhanced EFA levels. The goal is to provide products that will improve health in general and positively modify risk factors for chronic disorders (specifically cardiovascular) while simultaneously lowering overall health care costs. Furthermore, the traditional pharmaceutical industry is showing great interest in acquiring ω3 therapeutics for distribution on both a prescription- and a nonprescription-based release. In view of declining fish stocks and the interest among vegetarians to have animal-free sources of these long-chain ω3 FA, there is much recent interest in the development of commercially viable algal (plus other) sources of these FA for use as nutritional supplements and as ingredients for nutraceuticals/functional foods. In addition, these FA can be incorporated into processed food products and into diets/feeds of domestic animals for production of ω3-enriched products including eggs, dairy, and numerous meat products.

In the future, nutraceutical-type products plus numerous food products will be widely available with considerable quantities of EPA+DHA in oil form or as microencapsulated ingredients. These products will offer an overall rise in daily consumption of EPA+DHA, which is currently consumed only in moderate quantities in the form of fish/fish oils. It remains to be established whether the anticipated worldwide increase in EPA+DHA intakes will (*i*) result in significant benefits in reducing morbidity and mortality from CVD, (*ii*) attenuate inflammatory, neurological, and other disorders, and (*iii*) improve the overall quality of life in normal/healthy individuals. Based on published epidemiological, case-controlled, and intervention trials to date, the prospect of overall health improvement and better protection/management of chronic disorders is a reasonable expectation.

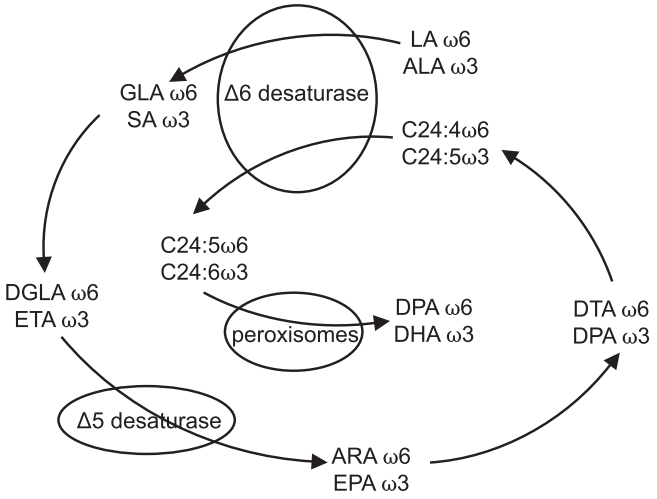
Fatty acids and human evolution

Students of the evolution of humans tend to focus on skulls and bones. With respect to the skulls, there are frequent comparisons made on the possible intelligence level of our remote ancestors based on likely cranial capacity. The possibility that the FA in the brain housed in that space might be factors in intelligence has received some attention (e.g., Crawford and Marsh 1989).The relationship between specific LCPUFA and brain intelligence now focuses on those of the ω family simply because DHA and ARA are known to be major FA in the brain lipids of primates. For example, ~60% (dry weight) of the human brain is made of lipids. PUFA make up ~20% (dry weight) of the brain, with ARA and DHA together representing roughly 75% of this mass (Crawford and Marsh 1989; Jamieson et al. 1999). These two FA are at the apices of a parallel system operated by a few enzymes for elongating and desaturating ALA and LA (Fig. 7).

Animal foods were probably important in hunter–gatherer diets, including fish and shellfish sources of long-chain ω3 FA (e.g., Boyd Eaton et al. 1998). Despite this probable long-term adaption over millions of years (Cordain et al. 2000), opposing views favoring plants are not uncommon (Milton 2000). Table 4 shows the probable excess of ALA over LA in the Late Paleolithic diet of largely vegetable origin. The switch to LA > ALA in our contemporary diet is of agribusiness origin and is of concern because of rapidly shifting dietary habits, even in advanced countries such as Japan (e.g., Sugano and Hirahara 2000), where marine foods have traditionally been important.

Unfortunately, aquatic organisms in the food supply have been rather neglected in evolution and diet considerations (Riley 1999). There are reasons for this. Sometimes the excuse is given that fish would be hard for primitive people to catch. Another is that fish bones are fragile and seldom recognized in archaeology. Although Riley's (1999) publication assesses the importance of the introduction of bronze fish-

Fig. 7. Metabolism of FA. LA, linoleic acid (C18:2ω6); ALA, α-linolenic acid (C18:3ω3); GLA, γ-linolenic acid (C18:3ω6); SA, stearidonic acid (C18:4ω3); DGLA, dihomo-γ-linolenic acid (C20:3ω6); ETA, eicosatetraenoic acid (C20:4ω3); ARA, arachidonic acid (C20:4ω6); EPA, eicosapentaenoic acid (C20:5ω3); DTA, docosatetraenoic acid (C22:4ω6); DPAω3, docosapentaenoic acid ω3 (C22:5ω3); DPAω6, docosapentaenoic acid ω6 (C22:5ω6); DHA, docosahexaenoic acid (C22:6ω3). Adapted from Yonekubo (1999).



hooks into Crete on diet and social development, that is a technological advance and the era in question is much too recent to show any impact of diet on our basic body FA biochemistry. One must go much further back, probably beyond 4 million years, to contemplate the diet and FA biochemistry of the evermore primitive hominids still being discovered by paleontologists. Unfortunately, our contemporary western fishing habits do not provide much useful reference material, although enormous shell middens in various parts of the world show that quite primitive people had a dietary base of such easily harvested foods. These are unlikely to be from the activities of hunter–gatherers but are evidence of more stable settlements, probably in the most recent stage (10 000 – 100 000 years?) of the evolution of *Homo sapiens*. Nevertheless, the middens suggest that one must look at specific types of FA in aquatic organisms (including invertebrates) for their availability for adaption by early hominids to become the specific types of long-chain FA that are the basis of our contemporary lipid body biochemistry. It should be remembered that "Lucy" (i.e., *Australopethicus*) and her progenitors lived on the edges of freshwater lakes in the African Rift Valley. Our problem is that inadequate attention has been paid to the freshwater milieu as sources of PUFA. Fish containing EPA and ARA (e.g., Kuusipalo and Käkelä 2000) were certainly present, but widely available freshwater invertebrates, such as shellfish and (or) the ubiquitous Amphipoda, likely contributed in establishing our basic FA biochemistry.

It is becoming increasingly clear that, beyond the proximate human health effects of EFA, there are important implications for learning, creativity, and evolution. For example, Horrobin (1998) argued that EFA such as DHA and ARA are major determinants of the richness of the brain's microconnections. He speculated that, in addition to effects on creativity and learning, even diseases such as schizophrenia might be linked to brain lipochemistry. Further, the evolution of the vascular system and, in particular, the placenta, which serves to focus energy and nutrients to one or a small number of progeny during the critical time of brain development has been associated with changes in our access to ARA and DHA (Cunnane et al. 1993; Broadhurst et al. 1998; Crawford et al. 1999). These three lead authors go on to conclude that "brain specific nutrition had and still has significant potential to affect hominid brain evolution" (Broadhurst et al. 1998). Given the intrinsic importance of these crucial biomolecules to our health (and to our environment), we clearly must not underestimate the convergent threats posed by our burgeoning population and the diminishing availability of the more traditional sources of EFA.

Table 4. Late Paleolithic sources of PUFA ($g \cdot day^{-1}$).

PUFA	Plant foods	Animal foods	Total intake
C18:2ω6	4.28	4.56	8.84
C18:3ω3	11.4	1.21	12.61
C20:4ω6	0.06	1.75	1.81
C20:5ω3	0.14	0.25	0.39
C22:4ω6	0	0.12	0.12
C22:5ω3	0	0.42	0.42
C22:6ω3	0	0.27	0.27

Note: Adapted from Boyd Eaton et al. (1998).

Conclusion

If we are to maintain/enhance our present-day health we must take a proactive approach to ensure our sustained access to EFA and, in particular, to EPA, DHA, and ARA. This process could involve some or all of the following steps. First, we should manage ecosystems with a view towards protecting organisms in the food chain that have high concentrations of EFA, principally the oil-rich fish. Second, we should strive to obtain better inventories of species with respect to their EFA content. This is important because it will help identify important natural stores of EFA and better inform us as to spatial and temporal "bottlenecks" in biosynthesis, supply, and (or) distribution of EFA. Finally, as traditional sources for EFA are diminished, we may increasingly have to turn to alternative sources of EFA.

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perspective as "the ongoing development in creation as initiated by God at creation and sustained in providence." B.J.H. wishes to thank Rev. Ed de Haan for this help in formulating the definition of "evolution" as inserted here.

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