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Algal polyunsaturated fatty acids and effects on plankton ecology and other organisms

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Abstract

A review and discussion of the effects of algal polyunsaturated fatty acids (PUFAs) on phytoplankton and zooplankton ecology and their effects on other animal systems. Topics discussed also include the relationship of cyanobacterial classification to PUFA occurrence, lipid concentrations occurring in the aqueous environment, the general effect of microalgae on zooplankton, and how algal PUFAs may be exerting their inhibitory and toxic effects.

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Introduction

A large number of factors have been implicated in the rise and fall of algal populations under natural conditions. These include physical factors (light, temperature, weather, water movements, flotation), inorganic chemical nutrients (nitrogen, phosphorus, silicon, calcium, magnesium, potassium, sulfate, chloride, iron, manganese, other trace elements), organic matter, and biological factors (perennation (resting stages), predation, parasitism) (Lund, 1965). This review is concerned primarily with organic matter and in particular with polyunsaturated fatty acids (PUFAs)[1] and related substances produced by algae[2] which may inhibit algal growth or have an inhibitory effect on zooplankton. There is much evidence that suggests that algae produce substances that are either autoinhibitory or that inhibit the growth of other algae. Table 1 lists examples of early phases of work which have been carried out with cell-free culture filtrates or with cultures separated by a membrane or fine filter, which indicate the presence of unspecified allelopathic substances which inhibit the growth of other algae or are autoinhibitory. In the table, algal sources of inhibitory substances include the cyanobacteria, green algae, diatoms, and dinoflagellates. Algae affected by the inhibitors include the cyanobacteria, green algae, diatoms, xanthophyceae, and cryptophyceae.

A large variety of toxic and biologically-active substances have been isolated and identified from cyanobacteria (Patterson *et al*., 1994; Moore, 1996; Carmichael & Falconer, 1993) and dinoflagellates (Baden & Trainer, 1993). Although their effects on animals have been extensively studied, their allelochemical significance in algal ecology have not been determined to any great extent. Cyanobacterin isolated from the cyanobacterium *Scytonema hofmanni* inhibits a number of green algae, the red alga *Porphyridium aerugineum*, and the euglenophyte *Euglena gracilis* at a concentration of 5 μM (Gleason & Baxa, 1986). Fischerellin from the cyanobacterium *Fischerella*

Footnotes

[1]Besides using common names, fatty acids are also indicated as C(number of carbon atoms: number of double bonds). Double bonds are cis(Z) unless otherwise specified as trans(E). Linoleic acid is $\Delta_{9,12}$-octadecadienoic acid, The common linolenic acid is α-linolenic acid ($\Delta_{9,12,15}$-octadecatrienoic acid). γ-Linolenic acid is $\Delta_{6,9,12}$-octadecatrienoic acid. Arachidonic acid is $\Delta_{5,8,11,14}$ – eicosatetraenoic acid. n indicates unsaturation counting from the methyl end of the acid.

[2]In the context of this review, the term "algae" will be considered to encompass all prokaryotic and eukaryotic photosynthetic aquatic organisms.

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musicola inhibits other cyanobacteria and green algae (Gross *et al*., 1991).

The toxic and ecological effects of fatty acids have been of considerable interest to us (Ikawa, 1989), and we have studied the activity of fatty acids in inhibiting the growth of the green alga *Chlorella pyrenoidosa* (McGrattan *et al*., 1976; Ikawa *et al*., 1984). There is increasing evidence in the literature that fatty acids, and PUFAs in particular, may be involved in algal dominance and may also be inhibitory to zooplankton and other animals in the aqueous environment. This is brought out by a review by Watson (2003) on cyanobacterial and eukaryotic odor compounds. This paper is concerned with the PUFAs and related substances produced by the various divisions of algae and what significance they may have in algal and plankton ecology and what effects they may also have on other animals.

Cyanobacteria (Blue-green algae)

Vincent & Silvester (1979) and Lam & Silvester (1979) showed that the growth of the green alga *Chlorella* was inhibited by cultures of *Microcystis* and *Anabaena*. The inhibition was still observed when the cultures were separated by a membrane. Ikawa *et al*. (1996) isolated the total lipids of *Microcystis aeruginosa* and found *Chlorella*-inhibition only in the free higher fatty acid fraction, which consisted mainly of palmitic acid but contained high amounts of linoleic (C18:2) and linolenic (C18:3) acids. It could be concluded that, if the inhibitory activity is due to lipoidal substances, it must be due to the PUFAs, since the higher saturated acids, including palmitic acid , are inactive (McGrattan *et al.*, 1976). Since it had been shown that the inhibition of *Chlorella* by the marine red alga *Chondrus crispus* was due to hydrogen peroxide generated by the action of a hexose oxidase in the alga on glucose or galactose (Sullivan & Ikawa, 1973), the question arose as to whether the inhibitory activity of the PUFAs could be due to PUFA hydroperoxides, the first step in the biodegradation of PUFAs. When linoleic and linolenic acids were treated with soybean lipoxygenase under aerobic conditions, a decrease in inhibitory activity was observed and the activity at the lower level was maintained over a 22 hour period (Ikawa *et al*., 1997). Soybean and other plant lipoxygenases under aerobic conditions produce both the 9 and 13 hydroperoxides of linoleic acid in varying amounts (Galliard and Chan, 1980; Vick and Zimmerman, 1987; Gardner, 1991). This indicated that although the PUFA hydroperoxides resulting from the reaction with lipoxygenase were active in inhibiting *Chlorella* growth, they were not the main reason for PUFA activity. If the hydroperoxides were the active impurities responsible for the activity of the PUFAs, a large increase in inhibition should have been observed after lipoxygenase treatment. Yamada *et al*. (1993) had also concluded that PUFA hydroperoxides were not responsible for the activity of PUFAs in autoinhibiting the cyanobacterium *Phormidium tenue*.

Murakami and coworkers have carried out an extensive series of studies with *Phormidium tenue*. Murakami *et al*. (1990a) and Yamada *et al*. (1993) investigating the autolytic and auto-inhibiting principle of *P. tenue*, extracted lyophilized cells with acetone and found activity in the free fatty acid fraction, the major components of which were linoleic (C18:2) and linolenic (C18:3) acids. These two acids were the most active of the ones tested. The monounsaturated acids oleic (C18:1) and palmitoleic (16:1) showed less activity, and the saturated acids myristic (C14:0) and palmitic (C16:0) were inactive. Yamada *et al*. (1993) also tested the hydroperoxyacids (Fig. 1: I, III) derived from air-oxidized linoleic acid and the corresponding hydroxy acids (Fig. 1: II, IV) and concluded that linoleic acid and not its hydroperoxides or their derived hydroxy acids was the actual inhibiting substance. Murakami *et al*. (1990b, 1991) have reported the isolation of a series of 1'-monogalactosyldiglycerides (Fig. 2: V) and 1'-digalactosyldiglycerides (Fig. 2: VI) from *P. tenue* where the 2'- and 3'- positions of the glycerol moiety were esterified with various combinations of myristic (C14:0), palmitic (C16:0), oleic (C18:1), palmitoleic (C16:1), palmitelaidic (C16:1(E)), linoleic (C18:2), and linolenic (C18:3) acids. The monogalactosyldiglycerides (Fig. 2: V) exhibited autolytic activity, but less than the free fatty acids themselves. Higher inhibitory activity was associated with the galactolipids containing the more highly unsaturated fatty acids. The

digalactosyldiglycerides (Fig. 2: VI) exhibited weaker activity than the monogalactosyl-diglycerides. Murakami *et al*. (1992) showed that *P. tenue* released monogalactosyldiglycerides (Fig. 2: V) into the growth medium, while little digalactosyldiglyceride (Fig. 2: VI) could be detected in the medium. Although it had been suggested that free fatty acids were released by an esterase to account for the activity of the monogalactosyldiglycerides, Yamada *et al*. (1994) concluded that cell lysis is actually initiated by other factors, such as lack of carbon dioxide, and that the lysis released free fatty acids hastened the death of more algal cells. They also stated that bacteria may degrade unsaturated fatty acids and prolong the life of the cultures.

Among other toxic and inhibitory effects of cyanobacterial PUFA derivatives, monogalacto-syldiglyceride (Fig. 2: V) has also been isolated by Matsui *et al*. (1989) as a hemolytic toxin from *Synechococcus* strain Miami BGII6S (S. Naegeli). Unsaturated fatty acids from *Microcystis aeruginosa* inhibited fish gill Na^+/K^+-ATPase activity of tilapia (*Oreochromis mossambicus*) (Bury *et al.* (1998). The unusual unsaturated C18 fatty acid cyclic lactone mueggelone (Fig. 3: VII) isolated from *Aphanizomenon flos-aquae* inhibited fish development (Papendorf *et al*., 1997).Harada *et al*. (2000) showed that the unsaturated fatty acids from *Oscillatoria agardhi* were toxic to mosquito larvae.

The activity of PUFAs as allelochemical substances produced by cyanobacteria raises an interesting point relating PUFAs to cyanobacterial classification. Table 2 shows the classification of cyanobacteria according to Rippka *et al*. (1979), and Table 3 lists the PUFA contents of the various orders. Also included in Table 3 are the prokaryotic prochlorophytes, which carry out oxygenic photosynthesis with chlorophylls a and b, characteristic of green plants and green algae, instead of by means of chlorophyll a and phycobiliproteins, characteristic of cyanobacteria (Palenik & Haselkorn, 1992; Urbach *et al*.,1992). It is seen that the prochlorphytes appear either to produce no PUFAs or to produce the unusual C16:2 instead of the usual C18:2 and C18:3 acids. This may reflect the polyphyletic nature of this group (Honda *et al*., 1999; Litvaitis, 2002). The coccoid Chroococcales appear to be divided between those that do not produce PUFAs and those that do. The non PUFA-producing Chroococcales, in this respect, resemble the eubacteria, which for all practical purposes do not produce PUFAs (Asselineau, 1966). Most of the Oscillatoriales and all of the Nostocales examined produce PUFAs. The PUFAs produced by the producing cyanobacteria are for the most part linoleic (C18:2) and α-linolenic (αC18:3) acids. Occasionally, however, γ-linolenic (γC18:3), palmitdienoic (C16:2), or octadecatetraenoic (C18:4) acids may partially or totally replace these acids. The polyphyletic nature of the Chroococcales and Oscillatoriales (Honda, 1999; Litvaitis, 2002) may also explain the existence of PUFA-producers and –nonproducers. The filamentous Stigonematales also appear to be divided between PUFA-producers and –nonproducers. If PUFAs are involved in algal allelopathy, it may be conjectured that many of the more primitive algal forms, especially among the Chroococcales, have not evolved this capacity.

Chlorophyceae (Green algae)

Proctor (1957) found that cell-free supernates from 4-6 day old cultures of *Chlamydomonas reinhardi* strongly inhibited the growth of another green alga *Haematococcus pluvialis*. On further study he showed that the condensate from a boiled acidified 4-6 day old culture inhibited the growth of *H. pluvialis*. He reasoned that the inhibition was due to long chain fatty acids, and when a number of available fatty acids were tested, inhibitory activity was found in both saturated and unsaturated fatty acids. McCracken *et al*. (1980), examining the same system, identified the fatty acid composition in the toxic *C. reinhardi* culture distillate as a mixture of saturated, monounsaturated and polyunsaturated fatty acids. Since it was found that linoleic and linolenic acids were particularly toxic to *H. pluvialis*, it was concluded that the toxicity of the fatty acids in the distillate was due mainly to the PUFAs. Kroes (1972) fractionated the culture filtrate of *Chlorococcum ellipsoideum* into (1) a steam volatile fraction, (2) a lipophilic fraction, (3) a water-soluble pigment fraction, and (4) a high molecular weight fraction. Only the lipophilic fraction had an initial inhibiting effect on the green

alga *Chlamydomonas globosa*, and the high molecular weight fraction had an initial promoting effect which was later followed by an inhibiting effect. Since the effects were small, Kroes concluded that the inhibitory effects were probably not caused by inhibitory substances, but that other factors such as pH may play a more important role. Work by Chiang *et al.* (2004) on the toxic effects of *Botryoccus braunii* on various phytoplankton and zooplankton indicated that the allelochemical involved was a mixture of free fatty acids, including α-linolenic, oleic, linoleic, and palmitic acids.

Pratt & Fong (1940) reported that the filtrates from 15 day old cultures of *Chlorella vulgaris* contained an autoinhibitor. The inhibitory substance was shown to have antibacterial properties and was named "chlorellin" (Pratt *et al*., 1944). From further studies by Spoehr *et al*. (1949) it was concluded that the antibacterial activity was due to products resulting from the photooxidation of unsaturated fatty acids. Scutt (1964) reported that fresh filtrates of *Chlorella vulgaris* did not contain autoinhibitors and that only after storage for several days did autoinhibitory activity appear. This was attributed to peroxide formation during standing. Inhibition was observed when *Chlorella* was cultured in media containing a mixture of peroxides from photooxidized linoleic and oleic acids or containing t-butyl hydroperoxide.

Among other toxic and inhibitory effects of green algal PUFA derivatives, Murakami *et al*. (1989) isolated the conjugated unsaturated fatty acid 4(Z), 7(Z), 10(Z), 13(Z)-hexadecatetraenoic acid (Fig. 3: VIII) from freshwater *Pediastrum,*. The substance was cytotoxic and also inhibited the development of fertilized echinoderm eggs. From female gametes of *Chlamydomonas allensworthii,* Starr *et al*. (1995) isolated the pheromone lurlene (Fig. 3: IX). It was identified as a β-D-xylopyranoside of an unusual aromatic polyunsaturated fatty acid. From the marine alga *Ulva pertusa,* Fusetani and Hashimoto (1975) isolated the lysoglycerides 1'-O-palmitoyl-3'-O-(6-O-α-D-galactopyranosyl-β-D-galactopyranosyl)-glycerol (Fig 4: X) and 1'-O-palmitoyl-3'-O-(6-sulfo-O-α-D-quinovopyranosyl)-glycerol (Fig 4: XI), which hemolyzed rabbit blood cells and inhibited the development of sea urchin eggs.

Bacillariophyceae (Diatoms)

In 1997 in a cooperative study involving 12 nations, 16 of 17 diatom species, when fed to 16 copepod species, significantly reduced egg production and egg viability in the copepods (Ban *et al.*, 1997). Shortly thereafter, Miralto *et al.* (1999) described the isolation of 3 polyunsaturated C10 aldehydes from the diatoms *Thalassiosira rotula, Skeletonema costatum,* and *Pseudo-nitzschia delicatissima* which impaired egg development in copepods and sea urchins. (These aldehydes are described in further detail later in the paper when PUFA oxidation products are considered.)

Dinophyceae (Dinoflagellates)

The dinoflagellates have been very extensively studied because of the serious consequences their blooms have caused in relation to human toxicity. The most studied of their toxins have been the guanidine-related paralytic shellfish poisons (such as saxitoxin and neosaxitoxin), and the vast array of toxic cyclic polyethers, including brevetoxins, diarrhetic shellfish poisons, and the very potent ciguatoxins. Polyunsaturated fatty acids and their derivatives are adding an extra dimension to toxic products from dinoflagellates.

Arzul *et al*. (1993) reported that high density blooms of *Gyrodinium cf. aureolum* repressed the growth of the diatom *Chaetoceros gracile*. Later Arzul *et al*. (1995) showed high concentrations of octadecapentaenoic acid (C18:5n3) (OPA) (Fig.5: XII) (25%) and docosahexaenoic acid (C22:6n3) (DHA) (Fig. 5: XIII) (15%) in the fatty acids of *Gymnodinium cf. nagasakiense*. These acids inhibited the growth of the diatom *C. gracile* and had hemolytic activity. Uchida *et al*. (1988) investigating the antibacterial and antialgal substances produced by *Peridinium bipes*, isolated the fatty acids and found large amounts of a C(20:2) acid, of eicosapentaenoic acid (C20:5n3)(EPA) (Fig. 5: XIV) and of DHA (Fig.5: XIII) and small amounts of C(18:2) and C(18:3) acids. The EPA, DHA, C(18:2), and C(18:3) acids inhibited the growth of the cyanobacterium

Anabaena cylindrica and the chlorophyte *Chlamydomonas reinhardii.*

With regard to toxic affects of dinoflagellate-produced PUFAs on other systems, Sellum *et al*. (2000) found that, of the PUFAs of *Gymnodinium cp. mikimotoi,* the major PUFA OPA (Fig.5: XII) inhibited or delayed the first cleavage stage of the sea urchin *Peracentrotus lividus* eggs and provoked abnormalities in embryonic development. Of four acids tested (OPA (Fig.5: XII), EPA (Fig. 5: XIV), DHA (Fig.5: XIII), and octadecatetraenoic acid (C18:4n3) (ODTA) (Fig. 5: XV)) ODTA was the most toxic, while EPA and DHA were the least toxic. Yasumoto *et al*. (1987) identified two lysoglycerides from *Amphidinium carteri* which had hemolytic properties. Hemolysin I (Fig. 6: XVII) was identified as a galactosyl monoglyceride of OTA and hemolysin II (Fig. 6: XVIII) as a digalactosyl monoglyceride also of OTA. Yasumoto *et al*. (1990) identified the hemolytic principles of *Gyrodinium aureolum* from Norwegian waters as the highly unsaturated fatty acid 3(Z), 6(Z), 9(Z), 12(Z), 15(Z)-octadecapentaenoic acid (OPA) (Fig. 5: XII), and also as digalactosyl monoglycerides (Fig. 6: XIX) of the highly unsaturated fatty acids OPA and EPA. Most of the hemolytic activity of the dinoflagellate appeared to reside in the free fatty acid. These authors found the same hemolysins in the chrysophyte *Chrysochromulina polyepsis* (see below). Hemolytic activities have also been reported for *Heterocapsa circularisquama* from various sources in Japan (Kim *et al*., 2002). Recently Hiraga *et al.* (2002) have isolated a digalactosyldiacylglycerol and two monogalacto-syldiacylglycerols with cytolytic properties from *Heterocapsa circularisquama.* The diglycerides had structures similar to (Fig. 2: V) and (Fig. 2: VI) isolated from the cyanobacterium *Phormidium tenue* , except that the glycerol was esterified with the more highly unsaturated fatty acids ODTA and OPA.

Onodera *et al.* (2004) have isolated a novel C22-γ-lactone zooxanthallactone (Fig. 5: XVI) from *Symbiodinium* sp., which is believed to result from oxidation and subsequent lactonization from DHA. The lactone showed some toxicity to two human tumor cell lines.

Haptophyceae (Chrysophyceae)

Prymnesium parvum has long been known to contain a potent toxin which has caused extensive fish kills in brackish waters in Europe and Israel. It is a hemolytic toxin, and early efforts at purification indicated it was of a complex nature (Ulitzer & Shilo, 1970). Kozakai *et al*. (1982) isolated a series of 6 hemolytic principles from *P. parvum*, two of which were identified as di-D-galactosyl monoglycerides (Fig. 6: XX) of ODTA and OPA. However, these digalactosides differed from other digalactosides in that the linkage between the galactose units was β rather than the usual α linkage. From the chrysophyte *Chrysochromulina polyepsis* from Norwegian waters, however, Yasumoto *et al*. (1990) isolated the same hemolysins (Fig. 6: XIX) as they found in the dinoflagellate *Gyrodinium aureolum* also from Norwegian waters (see under Dinophyceae), in which the linkage between the galactose units was the usual α.

Kamiya *et al*. (1979) investigating *Uroglena volvox* concluded that the ichthyotoxicity was due to free fatty acids. The principal fatty acids found were C(14:0) (37.6%), C(16:0)(10.1%), C(18:1) (7.7%), C(16:2) (3.2%), C(18:2) (5.0%), C(18:3) (2.5%), C(22:2) (7.7%), and C(22:4) (9.6%).

Radiophyceae (Chloromonads)

The toxins produced by *Chattonella antiqua* and *Chattonella marina*, which have long been implicated in widespread toxicity to cultured yellowtail in the Seto Inland Sea (Setonaikai) of Japan, as well as by other Raphidophytes *Fibrocapsa japonica* and *Heterosigma akashiwo,* have been identified as brevetoxins (see Haque & Onoue, 2002). However, the possible involvement of PUFAs and their oxidation products as causing toxicity to fish and to *Chattonella* itself has also been suggested. Okaichi (1989) reported that C(16:4) and C(18:4) acids were causative agents in the death of cultured yellowtail by blooms of *Chattonella antiqua* in Seto Inland Sea. Death was by damage to the mucus coat of the gill lamellae of the fish, which resulted in edema formation in the

lamellae and impairment of gas exchange across the gills resulting in oxygen deficiency and death (Toyoshima *et al.*, 1989). Fu *et al.* (2004) isolated three hemolytic compounds from *Fibrocapsa japonica* collected from the German coast. These were identified as OTA (Fig. 5: XV), EPA (Fig. 5: XIV), and arachidonic acid. There was no indication of the presence of brevetoxins. With regard to any autoinhibition which may be taking place with *Chattonella marina*, Murata *et al*. (1989) indicated that free radicals derived from hydrogen peroxide or eicosapentaenoic acid are probably responsible for the destruction of *C. marina* cells themselves. Cells were not destroyed by saturated fatty acids or PUFA methyl esters. Oda *et al*. (1992) investigating the autotoxic effects of *C. marina* showed that superoxide radical is released into the medium followed by production of the hydroxyl radical. These are toxic species in biochemical reactions, and it is surmised that they may have a role in the catabolic processes of the organism itself as well as toxic effects on fish, although evidence for the latter is not presented.

Rhodophyceae (Red algae)

Suzuki *et al.* (1996) identified EPA (Fig. 5: XIV) from *Neodilsea yendoana* and showed that it was autoinhibitory and suppressed spore settlement. The same acid was also found in other red algae examined (*Palmeria palmata, Chondrus yendoi, Ptilota filicina*). EPA (Fig. 5: XIV), arachidonic acid, and ODTA (Fig. 5: XV) inhibited growth at a minimum concentration of 1 μg/ml, while linolenic acid inhibited at 10 μg/ml. Kitamura *et al.* (1993) studying the lipophilic inducers of larval settlement and metamorphosis of the sea urchins *Pseudocentrotus depressus* and *Anthocidaris crassispina* from the red alga *Corallina piluliphera* showed that the active agents were EPA (Fig. 5: XIV) and arachidonic acid. Palmitic and palmitoleic (C16:1) acids were inactive. Lopez & Gerwick (1988) reported a fatty acid, 11-hydroxy-16-oxo-5(Z), 8(Z), 12(E), 14(E), 17(E)-icosapentaenoic acid (ptilodene) (Fig. 7: XXI) from *Ptilota filicina*, which had antibacterial activity and inhibited 5-lipoxygenase and Na^+/K^+-ATPase. Lopez & Gerwick (1987) had also previously isolated from this alga two other highly unsaturated fatty acids, 5(Z), 7(E), 9(E), 14(Z), 17(Z)-icosapentaenoic acid (Fig. 7: XXII) and 5(E), 7(E), 9(E), 14(Z), 17(Z)-icosapentaenoic acid (Fig. 7: XXIII) from antibacterial extracts, but properties are not reported. McPhail *et al.* (2004) have isolated peyssonenynes A (Fig. 7: XXIV) and B (Fig. 7: XXIV) and peyssopyrone (Fig. 7: XXV) from the red alga *Peyssonnelia caulifera.* The peyssonenynes are glycerol esters of a polyunsaturated C18 acid containing a diyne structure and peyssopyrone the methyl ester of a C18 polyunsaturated acid with an oxygen bridge between carbons 4 and 8 to form a γ-pyrone structure. Peyssonenynes A and B inhibited the action of DNA methyl transferase.

There have been incidents of human intoxications in Japan from eating the red alga *Gracilaria verrucosa* (ogonori). Patients have suffered from nausea, vomiting, and diarrhea, and deaths have also been reported. The causative substances have been identified from water-treated samples as prostaglandins PGE_2 (Fig. 8: XXVI) and PGA_2 (Fig 8: XXVII) (Fusetani & Hashimoto, 1984; Noguchi *et al*., 1994). Since raw ogonori does not appear to contain prostaglandins, it is believed that water treatment of the alga probably liberates a cyclooxygenase which converts arachidonic acid or other highly unsaturated fatty acids of the alga or of the patient into prostaglandins (Noguchi *et al*., 1994). Addition of arachidonic acid to ogonori extract resulted in an increased concentration of PGE_2.

Phaeophyceae (Brown algae)

Kakisawa *et al*. (1988) observed that seawater containing fronds of *Cladosiphon okamuranus* remained clear of other seaweeds, but water free of the fronds became turbid due to the growth of other seaweeds and microalgae. The active agent was isolated and identified as 6(Z), 9(Z), 12(Z), 15(Z)-octadeca tetraenoic acid (ODTA) (Fig. 5: XV). It was not active, at a concentration of 5 ppm, against two species of cyanobacteria tested (*Microcystis wesenbergii, Oscillatoria raciborskii*), but active against a wide range of algae, including members of Bacillariophyceae, Dinophyceae, Raphidophyceae, Haptophyceae, Cryptophyceae, Prasinophyceae, Euglenophyceae, and

Chlorophyceae. Against the Raphidophyte *Heterosigma akashiwo*, ODTA (Fig. 5: XV), arachidonic acid, and EPA (Fig 5: XIV) were the most active, and more active than γ-linolenic acid by an order of magnitude.

Effect of microalgae on zooplankton

Since the zooplankton derive a great part of their nourishment from the microalgae, a large number of studies have been devoted to the effect of algae on zooplankton. There is a great deal of evidence that cyanobacteria in general are a poor food source for zooplankton as opposed to the green algae (Lampert, 1981; Arnold, 1971). Reasons for this situation have been given as (1) the presence of toxic substances, (2) nutritional deficiencies of the cyanobacteria as a food source, and (3) mechanical obstruction to assimilation due to unsuitable size or shape. Because certain commonly occurring cyanobacteria produce highly toxic compounds, it is appropriate to consider the effects on zooplankton of some of the more widely known compounds first.

Because of the widespread occurrence of the cyanobacterium *Microcystis aeruginosa* and of the toxins microcystins, considerable attention has been paid to the effects of *Microcystis* on zooplankton. Nizen *et al*. (1986) looked at the effects of ten strains of *M. aeruginosa* on the food uptake of the cladoceran *Daphnia magna*. They found a general blocking effect but no correlation with the "mouse-killing" factors (microcystins), indicating that factors other than microcystins may be involved. Jungmann & Benndorf (1994) also did not find any correlation between microcystin concentration and toxicity to *Daphnia pulicaria* and concluded that DTC (*Daphnia*-toxic compound) was independent of microcystin. Sensitivity to *Microcystis* and microcystins appears to depend to a large extent on the zooplankton involved. Fulton (1988) feeding with *M. aeruginosa* strain PCC 7820, a strain toxic to mice (Nizen *et al*., 1986), found that the cladoceran *Bosmina longirostris* showed higher survivals than unfed controls, whereas the cladocerans *Daphnia parvula* and *Moina micrura* showed lower survival rates. Vasconcelos (1990) found that the copepod *Acanthocyclops robustus* and the cladoceran *Ceriodaphnia pulchella* were able to utilize both toxic and nontoxic strains of *M. aeruginosa*. However, the cladocerans *Daphnia longispina* and *Simocephalus vetulus* did better on the nontoxic strain than the toxic strain, the latter causing death in two days. DeMott *et al*. (1991) found a wide variation in the sensitivity of zooplankton to microcystin-LR. The copepod *Diaptomus birgei* was the most sensitive, but three species of the cladoceran *Daphnia* were less sensitive by an order of magnitude. Although the numbers indicated that the primary cause of the toxicity of *Microcystis aeruginosa* strain PCC7820 to *Diaptomus bergei* could be microcystin-LR, the contributions to the toxicity of *Microcystis* to *Daphnia* by toxins other than microcystin-LR could not be excluded. A similar conclusion was reached by Reinikainen *et al*. (1994) between *M. aeruginosa* PCC7820 and *Daphnia pulex*.

The paralytic shellfish poison saxitoxin is produced by certain strains of *Aphanizomenon* and *Anabaena*. Haney *et al*. (1995) showed that cultures of toxic *Aphanizomenon flos-aquae,* and also saxitoxin itself, interfere with the feeding rate of the cladoceran *Daphnia carinata* by reducing the thoracic appendage beat rate and increasing the post abdominal rejection rate.

The possible involvement of PUFAs in algal ecology opens up their possible involvement also in zooplankton ecology. Curtis *et al*. (1974) tested a number of fatty acids against the brine shrimp *Artemia salina* and found highest toxicity among the polyunsaturated fatty acids linoleic, linolenic and arachidonic acids (LD_{50} = 1.5-3.3 μg/ml). Of the saturated acids lauric acid (C12:0) showed the highest toxicity (LD_{50} = 5 μg/ml. *Artemia salina* does not exhibit Na^+/K^+-ATPase activity in early stages of development due to the presence of inhibitors of the enzyme. The inhibitor has been identified as a long chain fatty acid (Morohashi *et al*., 1991). Unsaturated fatty acids with the cis-configuration were more effective than saturated acids. Juttner (2001) found that EPA (Fig. 5: XIV) liberated from freshwater diatom biofilms was toxic to the anostracan (fairy shrimp) grazer *Thamnocephalus platyurus*. He found only low levels of free fatty acids in unstressed cells and concluded that their release under stress afforded protection against grazing pressures. Reinikainen *et* al. (2001) found γ-linolenic acid toxic to *Daphnia*

magna at a concentration of 9 µg/ml. Although microcystin-LR did not significantly affect the survival time of the *Daphnia* at 3 µg/ml, the combination of γ-linolenic acid with microcystin appeared to have an additive effect.

As opposed to negative effects, the highly unsaturated fatty acid EPA (Fig. 5: XIV) in the seston has been found to enhance the growth of the cladoceran *Daphnia* (Muller-Navarra, 1995; Sundborn & Vrede, 1997; Weers & Gulati, 1997; DeMott & Muller-Navarra, 1997). Muller-Navarra *et al*. (2000) link the presence of this fatty acid to carbon transfer between primary producers and consumers, which would result in increased growth of the zooplankton. Since cyanobacteria do not produce the highly unsaturated pentaenoic acid, this would be one reason why they are a poor food source for the grazing zooplankton. Highly unsaturated fatty acids in the form of triglyceride emulsions also stimulated the growth of the rotifer *Keratella* (Boersma & Stelzer, 2000).

Concentration considerations

One question which arises is whether the concentrations of fatty acids used under controlled conditions in the laboratory are in any way consistent with the concentrations which occur in the field under natural conditions. The matter of soluble and particulate concentration should first be clarified. Generally water samples are filtered through glass fiber filters (e.g. Whatman GF/F or GF/C) , which may have pore sizes of 0.7-1.2 µm (Parrish, 1988). The filtrate is generally designated as the soluble fraction, and anything retained by the filter as the particulate fraction or seston. Concentrations of substances are given for both fractions. Although lipids are hydrophobic substances with a very limited solubility in water, it is suggested that 100 µg/L would be a conservative estimate for the solubility of various lipid classes in distilled water at 20^{o} C (Parrish, 1988). Table 4 lists some soluble and sestonic concentrations of lipids in freshwater lakes. The values appear to be in the 1-2 mg/L range. Kattner *et al* (1983) studying the lipid concentration during a spring phytoplankton bloom in the northern North Sea in Europe estimated that fatty acid constituted about 3% of the total dissolved organic matter. The total fatty acid concentration amounted to about 1.15 µmoles C/L before the bloom and increased to a maximum of 5 µmoles C/L during a bloom. The main fraction of fatty acid was free fatty acid and the most abundant component was palmitic acid. The 5 µmoles C/L of fatty acid would translate to about 80 µg of palmitic acid /L, a value consistent with the solubility of fatty acids in water. McCracken *et al* (1980) observed toxicity to the green alga *Haematococcus* by linoleic and linolenic acids at 12.5 mg/L. Yamada *et al* (1993) reported inhibition of the cyanobacterium *Phormidium tenue* by linoleic and linolenic acids at a concentration of ~0.5 mg/L. This value is approaching the low levels of fatty acids which one might expect under natural bloom conditions and indicates that linoleic acid or other PUFAs may indeed be natural inhibitors. `Furthermore , effective levels of toxins may vary widely depending upon whether they are exerting their action under acute or chronic conditions. It is generally recognized that long term exposure to sublethal doses of toxic materials can cause a build up and lead to devastating effects. This may also apply to PUFAs, where long term exposure to low levels under natural conditions can have effects which are only observed with higher level acute doses.

Looking of a rationale

In looking for a rationale to explain the inhibitory effects of PUFAs on phytoplankton, we might consider some of the roles that PUFAs and their derived products have in biological systems: (A) In eukaryotic systems they are an integral part of the lipid membrane bilayer; (B) Through their degradation by oxidative reactions they lead to a variety of products, such as, hydroperoxides, prostaglandins, unsaturated aldehydes; (C) PUFAs may act as second messengers to modulate functionally active proteins (Kogteva & Bezuglov, 1998).

(A) Effect on membranes

A common feature of eukaryotic cells is the phospholipid bilayer surrounding the cells. Many studies have been carried out on the effects of fatty acids on vesicles of phospholipid bilayers, and there seems to be general agreement that saturated

fatty acids increase the phase transition temperature from gel to liquid-crystalline and that unsaturated fatty acids decrease the phase transition temperature(see Andreasen and McNamee, 1980). Castaing *et al* (1993) studied the leakage of tris(2,2'-bipyridyl)ruthenium (II) cations across the lipid bilayer membranes of dihexylphosphate vesicles and found that the unsaturated fatty acids palmitoleic, linoleic, and arachidonic acids induced permeation at concentrations an order of magnitude less than the saturated acids myristic, palmitic, stearic, and arachidic acids.

Working with natural membrane systems, Hoover *et al* (1977) observed that linoleic acid at 10 μg/ml markedly decreased the adhesion of baby hamster kidney (BHK) and Chinese hamster ovary (CHO) cells to their homotypic monolayers whereas stearic acid was not much different from controls. Klausner *et al* (1980) studied the effects of free fatty acids on splenic lymphocyte membranes by monitoring polarization changes of the fluorescent probes 1,6-diphenyl-1,3,5-hexatriene (DPH), which appears to localize in the lipid interior, and 8-anilino-1-naphthalene sulfonate (ANS), which, due to its charge, is localized in the polar domain. Oleic, linoleic, and arachidonic acids (Group A, cis unsaturated acids) reduced DPH polarization, and elaidic, nonadecanoic, and stearic acids (Group B, saturated and trans unsaturated acids) had no effect on DPH polarization. Both groups increased ANS polarization. This was interpreted as the intercalation of the free fatty acids into the lymphocyte membrane with the Group A acids packing into the more disordered lipid domains and further disrupting the lipid packing, and the Group B acids partitioning into the ordered region with minimal disturbance of the packing. These various studies indicate that unsaturated fatty acids, and polyunsaturated fatty acids in particular, can have a disrupting influence on the lipid bilayer of natural biological membranes of eukaryotic cells.

The differential effects of PUFAs on the one hand and the saturated fatty acids on the other may provide some rationale for the inhibitory effects of PUFAs on eukaryotic phytoplankton. It should be noted that PUFAs may also exert an effect as membrane constituents. In ectothermic animals it is known that the content of unsaturated fatty acids in the membranes generally increases with colder temperatures, resulting in more fluidity. Hall *et al.*(2002) showed a strong relationship between cold-induced membrane fluidity and an increase in EPA (Fig. 5: XIV) in the gill membranes of the sea scallop *Placopectin magellanicus*. Adaptation to cold, however, through the incorporation of PUFAs into their structure may be disrupting the normal functioning of membranes to some extent.

(B) PUFA oxidation products

A number of studies have indicated that the toxic activity of PUFAs may be due to oxidation products) derived through photooxidation or metabolic processes (Spoehr *et al.*, 1949; Murata *et al.*, 1989; Oda *et al.*, 1992). PUFA hydroperoxides, such as (Fig. 1: I) and (Fig 1: III) from linoleic acid, are formed from air oxidation (Yamada *et al.*, 1993) or from the action of lipoxygenases (Hamburg & Sammuelson, 1967). Although the hydroperoxides, the first products from the oxidation of PUFAs by lipoxygenases, show inhibitory properties, it is unlikely that they are primarily responsible for the inhibitory activity of PUFAs (Ikawa *et al.*, 1997; Yamada *et al.*, 1993). However, through the action of hydroperoxide lyases, hydroperoxides are cleaved heterolytically into aldehydes or homolytically into alcohols and hydrocarbons, the other product being an ω-oxo acid (Gardner, 1991). This paper does not cover these hydrocarbon products, some of which have been shown to have pheromonal activity in marine brown algae (see Watson, 2003). Boonprab *et al.* (2003) showed that in the brown alga *Laminaria angustata,* (Z)-3-nonenal and (E)-2-nonenal and hexanal arose from the action of lysase on the 12(S)- and 15(S)- hydroperoxides of arachidonic acid (12-HETE and 15-HETE) and that hexanal also arose from the action of lyase on C(18) PUFA hydroperoxides. Ikawa *et al.*(1997) tested a number of aldehydes for activity in inhibiting *Chlorella* growth. Although unsaturated aldehydes were more inhibitory than saturated aldehydes, and the inhibitory activity appeared to peak at around 9 carbon atoms, their activity was significantly no greater than the PUFAs themselves, and therefore they did not appear to be the main reason for the inhibitory activity of PUFAs on *Chlorella.* Miralto *et al.* (1999) isolated the three aldehydes 2(E)-4(Z)-

7(Z)-decatrienal (Fig. 8: XXVIII), 2(E)-4(E)-7(Z)-decatrienal (Fig. 8: XXIX), and 2(E)-4(E)-decadienal (Fig. 8: XXX) from diatoms, which impaired egg development in copepods and sea urchins. The inhibitory effects of blooms of the diatom *Skeletonema* on populations of the copepod *Calanus helgolandicus* was explained by impairment of the egg development of the copepod feeding on the diatom bloom (Ianora *et al.*, 2004). The aldehydes released from enzymatic cleavage of fatty acids immediately after cell damage to the diatom by the copepod are cited as the responsible agents. Pohnert (2000) showed that these aldehydes are rapidly formed by *T. rotula* on cell damage from arachidonic acid and that this represents a fast wound-activated chemical defense mechanism. Further studies by Pohnert *et al.* (2002) showed that inhibition of copepod egg development is probably not restricted to decatrienal or decadienal , but that probably other compounds resulting from the action of lipoxygenases and hydroperoxide lyases on PUFAs such as arachidonic acid and EPA (Fig. 5: XIV) may also be involved.

The toxic principles of the red alga *Gracilaria verrucosa* have been identified as prostaglandin E_2 (PGE_2) (Fig. 8: XXVI) and prostaglandin A_2 (PGA_2) (Fig. 8: XXVII) (Fusetani & Hashimoto, 1984; Noguchi *et al.*, 1994). These compounds arise from prostaglandin G_2 (PGG_2), a hydroperoxide arising from the action of cyclooxygenase on arrachidonic acid.

(C) Bioregulators

There is increasing realization that PUFAs may perform a regulatory in metabolism (Kogteva & Bezuglov, 1998).

Phospholipases A_1 and A_2 (PLA_1, PLA_2) cleave the fatty acids from the 1 and 2 positions of the glycerol moiety of phospholipids, respectively, to produce fatty acids and lysophospholipids. They act to release PUFAs which are in bound form in the phospholipids. The wound-activated triggering of phospholipase A_2 in the diatom *Thalassiosira rotula* results in a rapid increase in PUFAs and the formation of polyunsaturated C10-aldehydes which inhibit egg cleavage in copepods (Pohnert, 2002). As evidence of a modulating role, PUFAs also tend to inhibit phospholipase A_2 activity. Macrophage PLA_2 is most strongly inhibited by PUFAs, less inhibited by oleic acid, and not inhibited by palmitic acid (Lister *et al.*, 1988). Raghupathi & Franson (1992) showed marked inhibition of Ca^{++}-dependent snake venom PLA_2 by *cis*-unsaturated fatty acids, but were not potent inhibitors of non-Ca^{++}-dependent PLA_2 of adrenal medulla. Of the three principal types of PLA_2 in animal systems (secretory low molecular weight PLA_2 ($sPLA_2$); cytosolic Ca^{++}-dependent PLA_2 ($cPLA_2$); intracellular Ca^{++}-independent PLA_2 ($iPLA_2$)), $sPLA_2$-like PLA_2 and $iPLA_2$-like PLA_2 have been reported in plants. No $cPLA_2$-like sequence has yet been found in plants (Wang, 2001). Whatever the nature of the PLA_2 in diatoms, Pohnert (2002) found that quinacrine, an inhibitor of animal PLA_2, also inhibited lipase activity in the *Thalassiosira rotula* system, thus indicating that a feedback inhibition system might modulate the system.

Na^+/K^+-ATPase is involved in maintaining high intracellular K^+ concentrations and low intracellular Na^+ concentrations against extracellular concentration gradients in these ions. In the brine shrimp *Artemia salina* the α and β protein subunits of Na^+/K^+-ATPase are present in dormant cysts and in the early stages of development, but ATPase activity is not detectable in these stages. Morohashi *et al.* (1991), suspecting the presence of endogenous inhibitors, extracted dormant cysts of *Artemia* and found that most of the Na^+/K^+-ATPase-inhibitory activity was present in the non-esterified fatty acid fraction, and found that unsaturated fatty acids were more inhibitory than their saturated or *trans*-unsaturated forms. Bury *et al.* (1998) isolated the fatty acids of the cyanobacterium *Microcystis aeruginosa* and showed that they inhibited fish gill Na^+/Na^+-ATPase of *Tilapia* and concluded that the release of fatty acids following lysis of the cyanobacterium may explain fish kills during algal blooms. Lopez & Gerwick (1988) showed that the PUFA ptilodene (Fig. 7: XXI) from the red alga *Ptilota filicina* inhibited dog kidney Na^+/K^+-ATPase. Andreasen & McNamee (1980) measured the inhibition of carbamylcholine-induced increase in Na^+ ion permeability of membrane vesicles prepared from *Torpedo californica* electroplax, and found that unsaturated fatty acids completely blocked carb-induced increase while stearic acid showed no

blockage. These various results indicate the inhibitory activity of unsaturated fatty acids on the function of Na^+/K^+-ATPase.

A regulatory role for the oxylipins peyssonenynes (Fig. 7: XXIV) from the marine red alga *Peyssonnelia caulifera* is suggested by its inhibitory action on DNA methyl transferase.

If we consider the unsaturated fatty acids of very primitive origin, as evidenced by their presence in the cyanobacteria, the PUFAs could have functioned from early in evolution as regulators in metabolic processes as opposed to saturated fatty acids which primarily function as structural and storage components.

Concluding remarks

There is ample evidence that algae can show autoinhibitory activity or can inhibit the growth of other phytoplankton or of zooplankton. Although many factors may be involved, it appears certain that one of the factors is polyunsaturated fatty acid (PUFA) of algal origin. They may act *per se* as second messengers to modulate enzymatic activity as some data suggests. This may turn out to be a very important role. PUFAs are also subject to the action of oxygenases and lyases, which are involved in a widely occurring and important pathway of PUFA metabolism. These reactions result in the production of a number of oxidation products, including the prostaglandins, unsaturated and saturated aldehydes and alcohols, hydrocarbons, and acids. Some of the polyunsaturated aldehydes have been shown to inhibit the development of zooplankton. In summary, an inhibitory role of algal PUFAs in phytoplankton and zooplankton ecology seems real.

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Table 1: Inhibition of algal growth by algae

Reference	Source of inhibitor[a]	Removal of cells	Alga inhibited
1940-Pratt & Fong	G:*Chlorella pyrenoidosa*	Culture filtrate(15 day)	G:Autoinhibitory
1950-Lefevre *et al.*	BG:*Aphanizomenon gracile*	Canal bloom water filtrate	G:*Pediastrum boryanum* G:*P. clathratum v. punctulatum* G:*Cosmarium lundellii* G:*Micrasterias papillifera* BG:*Phormidium insinatum*
	BG:*Oscillatoria planktonica*	Canal bloom water filtrate	G:*Pediastrum boryanum* G:*Scenedesmus quadricauda* G:*Chlorella pyrenoidosa* G:*Cosmarium lundellii* G:*C. obtusatum* G:*Micrasterias papillifera* BG:*Phormidium insinatum* BG:*P. autumnale*
1956-Jorgensen	DT:*Nitzschia palea*	Culture filtrate(8 day)	DT:Autoinhibitory DT:*Asterionella formosa*
	DT:*Asterionella formosa*	Culture filtrate(8 day)	DT:*Nitzschia palea*
	G:*Scenedesmus quadricauda*	Culture filtrate(4-8 day)	DT:*Nitzschia palea*
		Culture filtrate(21 day)	G:*Chlorella pyrenoidosa* G:Autoinhibitory
	G:*Chlorella pyrenoidosa*	Culture filtrate(13-17 day)	DT:*Nitzschia palea*
1957-Jakob	BG:*Nostoc muscorum*	Culture filtrate(5-6 wk)	G:*Cosmarium lundellii*
1957-Proctor	G:*Chlamydomonas reinhardi*	Centrifugation supernate (4-6 day culture)	G:*Haematococcus pluvialis*
1954-Rice	G:*Chlorella vulgaris*	Culture filtrate(7 day)	G:Autoinhibitory DT:*Nitzschia frustulum*
	G:*Pandorina*	Pond water filtrate (2 wk bloom)	G:*Chlorella vulgaris* DT:*Nitzschia frustulum*
	DT:*Nitzschia frustulum*	Culture filtrate(5 day)	G:*Chlorella vulgaris* DT:Autoinhibitory
1965-Vance	BG:*Microcystis aeruginosa*	Cultures separated by ultrafine glass filters	G:*Chlamydomonas moewusii* G:*Haematococcus lacustris* DT:*Navicula pelliculosa* CP:*Cryptomonas ovata*
1971-Harris	G:*Eudorina*(4 sp) G:*Pandorina*(2 sp) G:*Volvox*(2 sp) G:*Gonium pectorale* G:*Volvulina pringsheimii* G:*Platydorina caudata*	Culture filtrate Culture filtrate Culture filtrate Culture filtrate Culture filtrate Culture filtrate	(Generally inhibited most of other algae, incl. same genus. *V. pringsheimii* and *P. caudata* were autoinhibitory. *E. california* not inhibited by any of the algae.)
1972-Kroes	G:*Chlorococcum ellipsoideum*	Culture filtrate	G:*Chlamydomonas globosa*
1977-Keating	BG:*Anabaena holsaticum*	Culture filtrate	BG:*Oscillatoria agardhi*[b]
	BG:*Aphanizomenon elenkinii*	Culture filtrate	BG:*O. agardhi*[b]
	BG:*Oscillatoria* sp	Culture filtrate Culture filtrate	BG:*Oscillatoria rubescens* BG:*Pseudoanabaena galeata*[b]
	BG:*Aphanizomenon elenkii*	Culture filtrate	BG:*Anabaena* sp[b]
1978-Keating	BG:*Oscillatoria rubescens*	Culture filtrate	DT: 5/8 (Varieties of diatoms
	BG:*O. agardhii*	Cultue filtrate	DT: 6/6 inhibited out of number
	BG:*O. elegans*	Culture filtrate	DT: 5/6 tested. Most species
	BG:*Anabaene holsaticum*	Culture filtrate	DT: 29/29 were Fragilariaceae,
	BG:*A. elenkii*	Culture filtrate	DT: 2/3 some Naviculaceae.)
	BG:*Pseudoanabaena galeata*	Culture filtrate	DT: 7/8
	BG:*Aphanizomenon flos-aquae*	Culture filtrate	DT: 4/7
	BG:*Synechococcus* sp	Culture filtrate	DT: 9/9
	BG:*Nostoc muscorum*[c]	Culture filtrate	DT: 4/8
	BG:*Nostoc* sp[c]	Culture filtrate	DT: 3/8

Table 1: Inhibition of algal growth by algae (cont.)

Reference	Source of inhibitor[a]	Removal of cells	Alga inhibited
1979-Vincent & Sylvester	BG:*Anabaena* sp	Cultures separated by a	G:*Chlorella*
	BG:*Microcystis aeruginosa*	membrane	G:*Chlorella*
1979-Lam & Sylvester	BG:*Anabaena oscillarioides*	Cultures separated by a	G:*Chlorella*
	BG:*Microcystis aeruginosa*	membrane	G:*Chlorella*
1979-Wolfe & Rice	G:*Cosmarium vexatum*	Culture filtrate	G:*Chlorella ellipsoidea*
		Culture filtrate	G:*Pediastrum boryanum*
		Culture filtrate	G:*Scenedesmus incrassatulus*
		Culture filtrate	X:*Botrydium becherianum*
	G:*Pediastrum boryanum*	Culture filtrate	X:*B. becherianum*
	G:*Scenedesmus incrassatulus*	Culture filtrate	X:*B. becherianum*
	G:*Chlorella ellipsoidea*	Culture filtrate	X:*B. becherianum*
	G:*Pandorina morum*	Culture filtrate	X:*B. becherianum*
1980-Chan *et al.*	DT:*Skeletonema costatum*	Methanol extract of cells	DT:*Cylindrotheca fusiformis*
	DT:*Nitzschia longissima*	Methanol extract of cells	DT:*C. fusiformis*
	DT:*Phaeodactylum tricornutum*	Methanol extract of cells[d]	DT:*C. fusiformis*
	DN:*Scrippsiella sweeneyae*	[d]	DT:*C. fusiformis*
	DN:*Heterocapsa triquetra*	Methanol extract of cells[d]	DT:*C. fusiformis*
	DN:*Amphidinium carterae*	Methanol extract of cells[d]	DT:*C. fusiformis*
	DN:*Gonyaulax spinifera*	[d]	DT:*C. fusiformis*
2001-Schagerl *et al.*	BG:*Anabaena torulosa*	Methanol extract of cells	G:*Scenedesmus acutus*
2001-Rengefors & Legrand	DN:*Peridinium aciculiferum*	Culture filtrate	CP:*Rhodomonas lacustris*

[a] Algal class abbreviations: BG-Blue-green algae(Cyanobacteria), G-Green algae(Chlorophyceae), DT-Diatoms(Bacillariophyceae, DN-Dinoflagellates(Dinophyceae), X-Xanthophyceae, CP-Cryptophyceae.
[b] The bloom preceeding the source of the filtrate.
[c] The source was other than Linsley Pond, North Branford, CT, USA, which was the source of the other filtrates.
[d] Culture filtrates were also passed through XAD-2 resin, and the adsorbed organic matter eluted from the resin with methanol, dried and tested.

Table 2. Taxonomic scheme of Rippka *et al.* 1979 for the cyanobacteria.

Cell type	Reproduction Mode	Rippka Section	Conventional Order
Unicellular (single or aggregated)	Binary fusion or budding	I	Chroococcales
	Multiple fission	II	Pleurococcales
Filamentous	Trichomes contain vegetative cells only. Division in one plane only (no true branching).	III	Oscillatoriales
	Trichromes contain heterocysts in absence of combined nitrogen. Division in one plane only (no true branching).	IV	Nostocales
	Trichromes contain heterocysts in absence of combined nitrogen. Division in more than one plane (true branching).	V	Stigonematales

Table 3. Fatty acids of prochlorophytes and cyanobacteria

Absence or traces of PUFAs	PUFAs present[a] (linoleic (18:2) and α-linolenic (α18:3) are major acids except where noted)	Reference
PROCHLOROPHYTES		
Prochloron		Johns *et al.*, 1981
		Murata & Sato, 1983
		Perry *et al.* , 1978
	Prochlorothrix hollandica(16:2, -18:2, -18:3)	Volkman *et al.*, 1988
		Gombos & Murata, 1991
CHROOCOCCALES		
Synechococcus 1		Kenyon, 1972
Synechococcus 3		Kenyon, 1972
Synechococcus		Murata *et al.*, 1992
		Fork *et al*., 1979
		Holton *et al*., 1968
		Kruger *et al*., 1995
Anacystis		Kenyon, 1972
		Kruger *et al*., 1995
		Parker *et al*., 1967
		Sato *et al*., 1979
		Holton *et al*., 1964
Synechococcus 4,5		Kenyon, 1972
Coccochloris		Kenyon, 1972
		Kruger *et al*., 1995
	Synechococcus	Murata *et al*., 1992
	Synechococcus 2 (*Anacystis*)(-18:3)	Kenyon, 1972
	Synochococcus 7(+16:2)	Kenyon, 1972
	Synechococcus 9	Kenyon, 1972
	Synechococcus 9(tr α18:3, +γ18:3)	Kenyon, 1972
	Agmenellum(+16:2)	Parker *et al*., 1967
		Kenyon, 1972
Aphanocapsa 2(*Gloeocapsa*)(tr 18:2)		Kenyon, 1972
Gloeocapsa		Kruger *et al*., 1995
Gloeocapsa(tr 18:2)		Kenyon, 1972
	Synechococcus 8(*Gloeocapsa*)(16:2, tr 18:2)	Kenyon, 1972
Aphanocapsa 3(tr 18:2)		Kenyon, 1972
Aphanocapsa 4(tr 18:2)		Kenyon, 1972
Aphanocapsa 5(tr 18:2)		Kenyon, 1972
	Aphanocapsa 1(γ18:3, tr α18:3)	Kenyon, 1972
	Synechococcus(γ18:3, -α18:3, +/-18:4)	Murata *et al*., 1992
	Synechocystis(γ18:3, tr α18:3)	Wada & Murata, 1989
	Microcystis(γ18:3, -α18:3)	Kenyon, 1972
Microcystis		Kruger *et al*., 1995
	Myxosarcina	Nichols & Wood, 1968
OSCILLATORIALES		
	Plectonema(-18:3)	Kenyon *et al*., 1972
	Plectonema	Murata *et al*., 1992
		Parker *et al.*, 1967
	Trichodesmium	Parker *et al*., 1967
Spirulina		Kenyon *et al*., 1972
	Spirulina	Kenyon *et al*., 1972
	Spirulina(γ18:3, -α18:3)	Nichols & Wood, 1968
		Murata *et al*., 1992
	Spirulina subsalsa I2(-α18:3)	Cohen *et al*., 1995
	Spirulina subsalsa 3F(tr 18:2)	Cohen *et al*., 1995
	Spirulina "pantelleria"(-18:3)	Romano *et al*., 2000
	Lyngbya	Kenyon *et al*., 1972
	Lyngbya(-α18:3)	Kenyon *et al*., 1972
		Parker *et al*., 1967

Table 3. Fatty acids of prochlorophytes and cyanobacteria (cont.)

Absence or traces of PUFAs	PUFAs present[a] (linoleic (18:2) and α-linolenic (α18:3) are major acids except where noted)	Reference
	Oscillatoria	Kenyon *et al*., 1972
		Schmitz, 1967
		Zepke *et al*., 1978
	Oscillatoria(16:2, Tr 18:2, -18:3)	Parker *et al*., 1967
	Oscillatoria(*Microcoleus*)(+18:4)	Kenyon *et al*., 1972
	Microcoleus	Parker *et al*., 1967
NOSTOCALES		
	Anabaena	Kenyon *et al*., 1972
		Parker *et al*., 1967
		Sato *et al*., 1979
		Murata *et al*., 1992
		Nichols & Wood, 1968
		Li & Watanabe, 2001
		Zepke *et al*., 1978
	Nostoc	Holton *et al*., 1968
		Parker *et al*., 1967
		Zepke *et al*., 1978
	Microchaete(γ18:3, -α18:3)	Kenyon *et al*., 1972
	Microchaete(*Nostoc*)(γ18:3, -α18:3)	Kenyon *et al*., 1972
	Calothrix(+18:4)	Kenyon *et al*., 1972
	Calothrix(*Nodularia*)(+18:4)	Kenyon *et al*., 1972
	Calothrix(*Tolypothrix*)(+18:4, +γ18:3)	Kenyon *et al*., 1972
	Tolypothrix(+γ18:3, +18:4)	Murata *et al*., 1992
	Tolypothrix(+γ18:3, +18:4)	Zepke *et al*., 1978
STIGONEMATALES		
	Chlorogloea fritschii	Kenyon *et al*., 1972
		Nichols & Wood, 1968
		Holton *et al*., 1968
Hapalosiphon laminosus		Holton *et al*., 1968
Mastigocladus laminosus		Murata *et al*., 1992
M. laminosus(*Fischerella*)(tr)		Nichols & Wood, 1968

[a] No notation when made when both the usual linoleic(18:2) and α-linolenic(α18:3) are present. Notation made when either one is absent, or when γ-linolenic(γ18:3), hexadecadienoic(16:2) , or octadecatetraenoic(18:4) acids are present.

Table 4: Lipid concentrations in freshwater lakes

			Lipid class (mg l^{-1}, maximum values)						
Lake	Type	Water fraction	Total	HC	DG,TG	FFA	TPL[a]	Other	Reference
Giles, Poconos,PA	Oligotrophic	Seston(1-53μm)	0.3-1.6	(Mostly triglycerides, polar lipids)					Kreeger *et al*.,1997
Lacawac, Poconos,PA	Mesotrophic	Seston(1-53μm)	~1-4.3	(Mostly triglycerides, polar lipids)					Kreeger *et al*.,1997
Waynewood, Poconos,PA	Eutrophic	Seston(1-53μm)	1.2-15.4	(Mostly triglycerides, polar lipids)					Kreeger *et al*.,1997
Humboldt Lake, Canada	Eutrophic	Soluble(<1.2μm)	~1	0.536	0.093	0.361	~0.2	[b]	Arts *et al*.,1997
		Sest(1.2-153μm)	~2	0.272	0.526	~0.03	~1.5	[c]	Arts *et al*.,1997

[a] Acetone mobile + acetone immobile polar lipid

[b] Ketones, ~0.025; free fatty alcohols, ~0.07; sterols, ~0.025; wax esters, 0.202

[c] Wax esters, ~0.07

HC = hydrocarbons, DG = diglycerides, TG = triglycerides, FFA = free fatty acid, TPL = total polar lipid

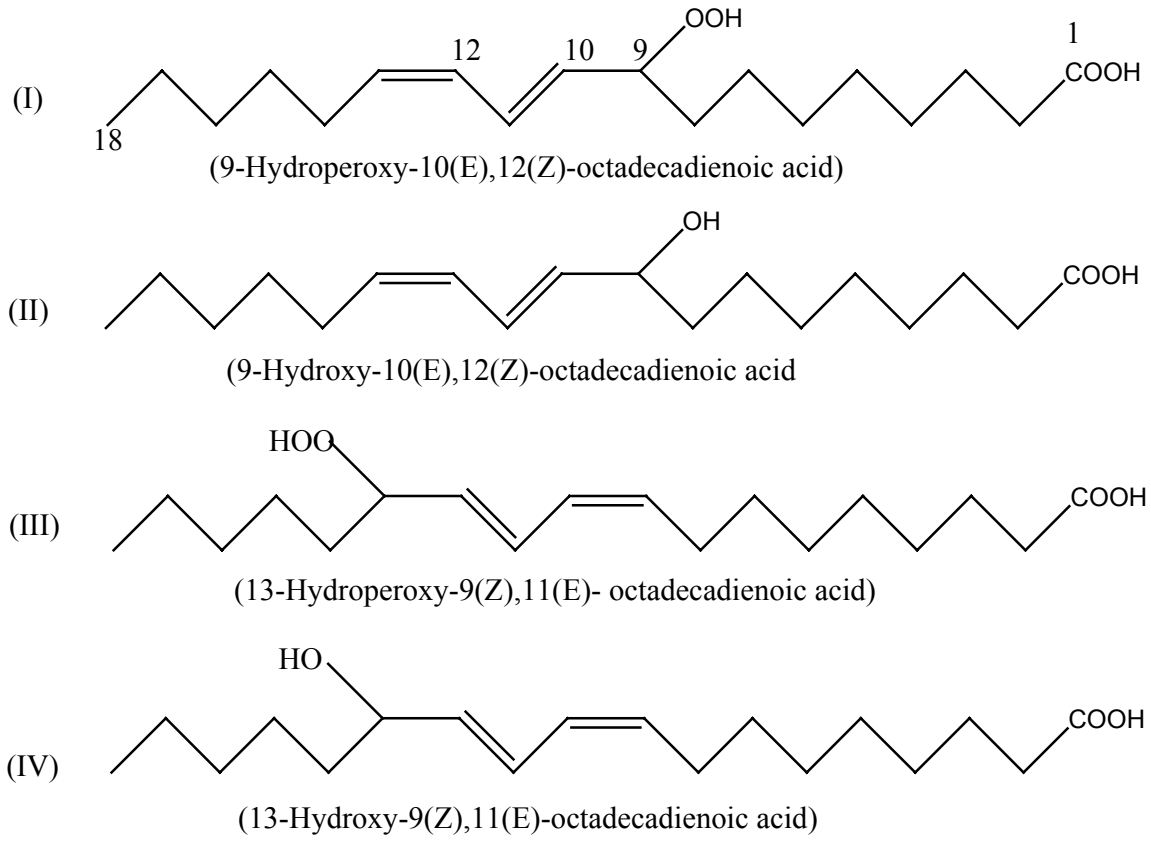
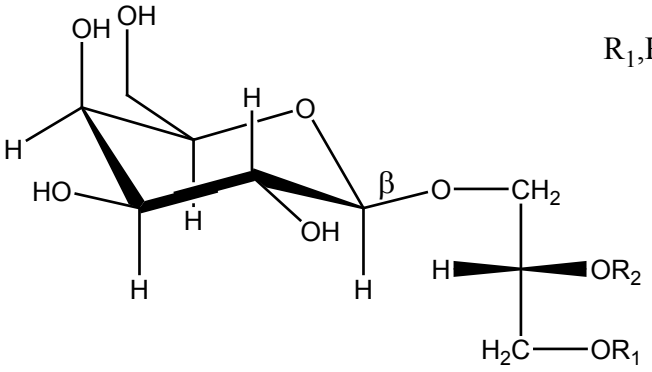


Figure 1: Hydroperoxy and hydroxy acids derived from linoleic acid..

(V)



R_1,R_2= ester combinations with
myristic
palmitic
oleic
palmitoleic
palmitelaidic
linoleic
linolenic

(VI)

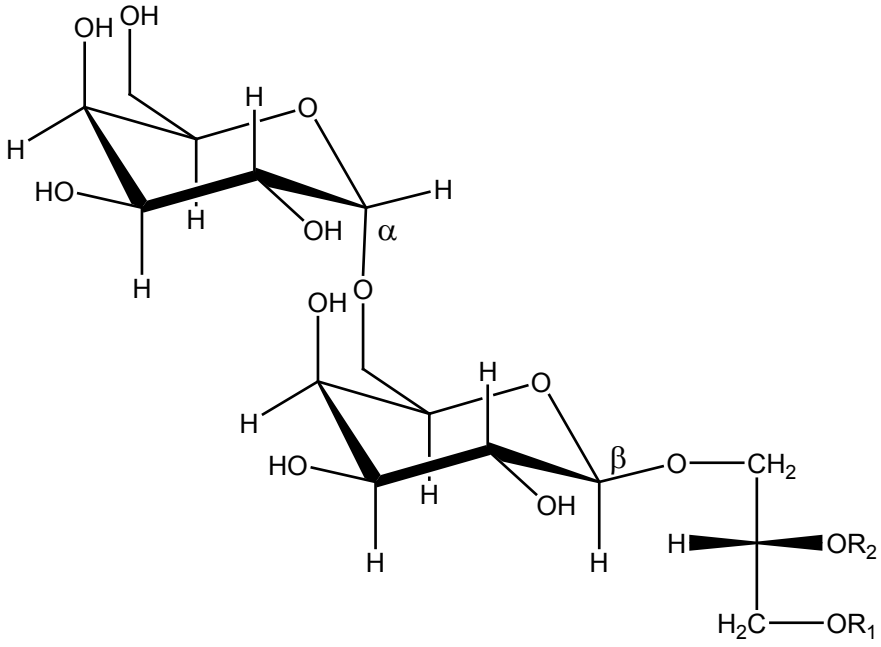
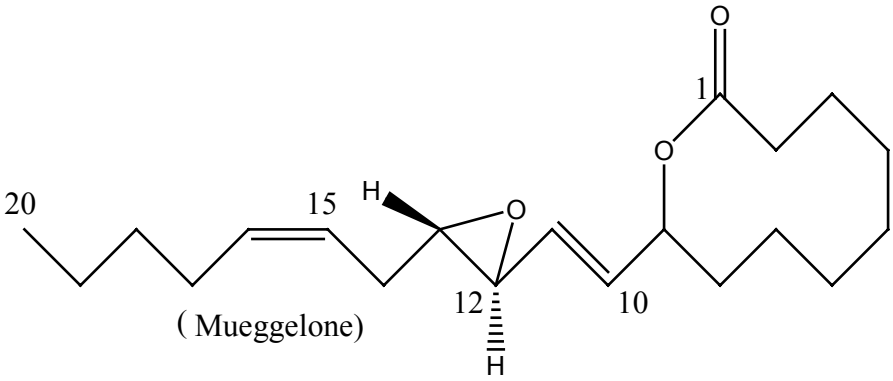


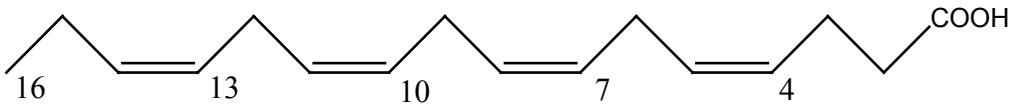
Figure 2: Mono- and di-D-galactosyl diglycerides from *Phormidium tenue*.

(VII)



(Mueggelone)

(VIII)



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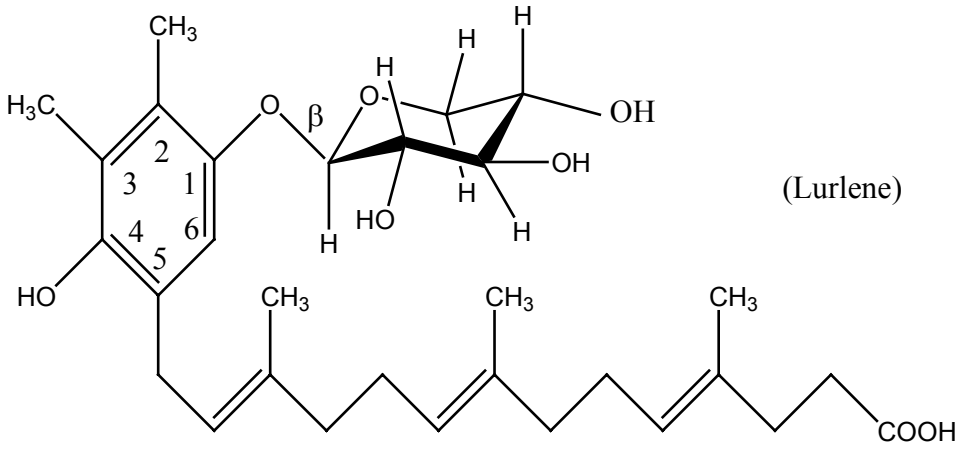
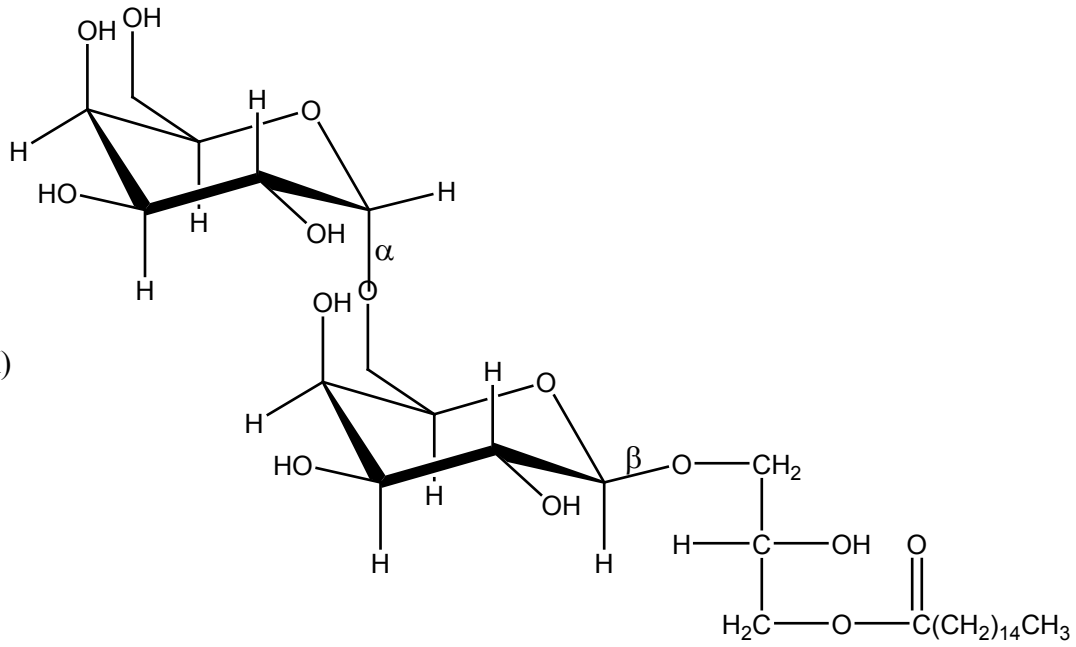


(Lurlene)

Figure 3: PUFA's and related compounds from Cyanobacteria and Chlorophyceae.

(X)



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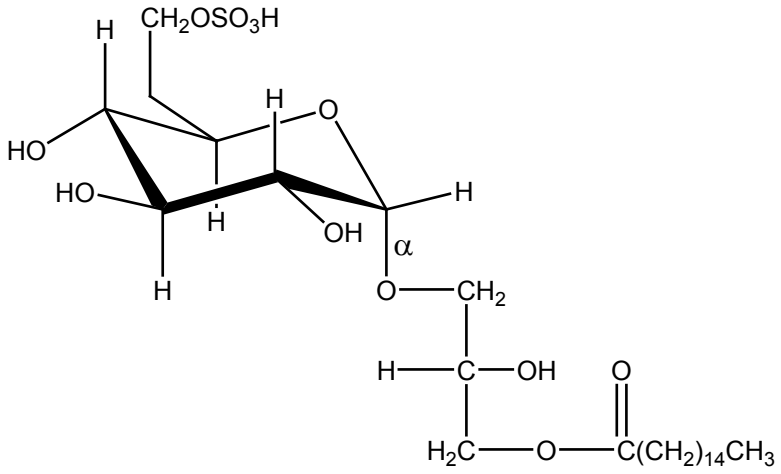


Figure 4: Di-D-galactosyl and 6-sulfo-D-quinovosyl lysoglycerides from Chlorophyceae.

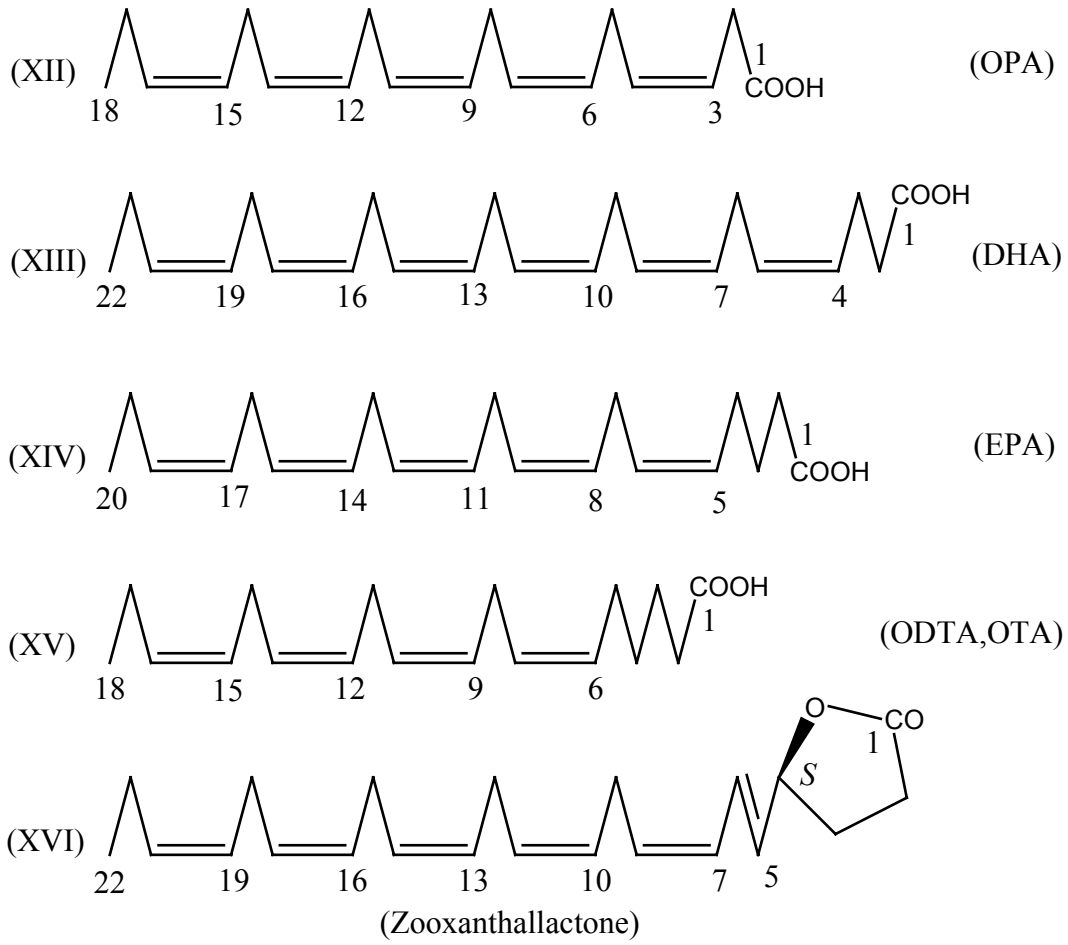


Figure 5: PUFA's from Dinophyceae and other eukaryotic algae.

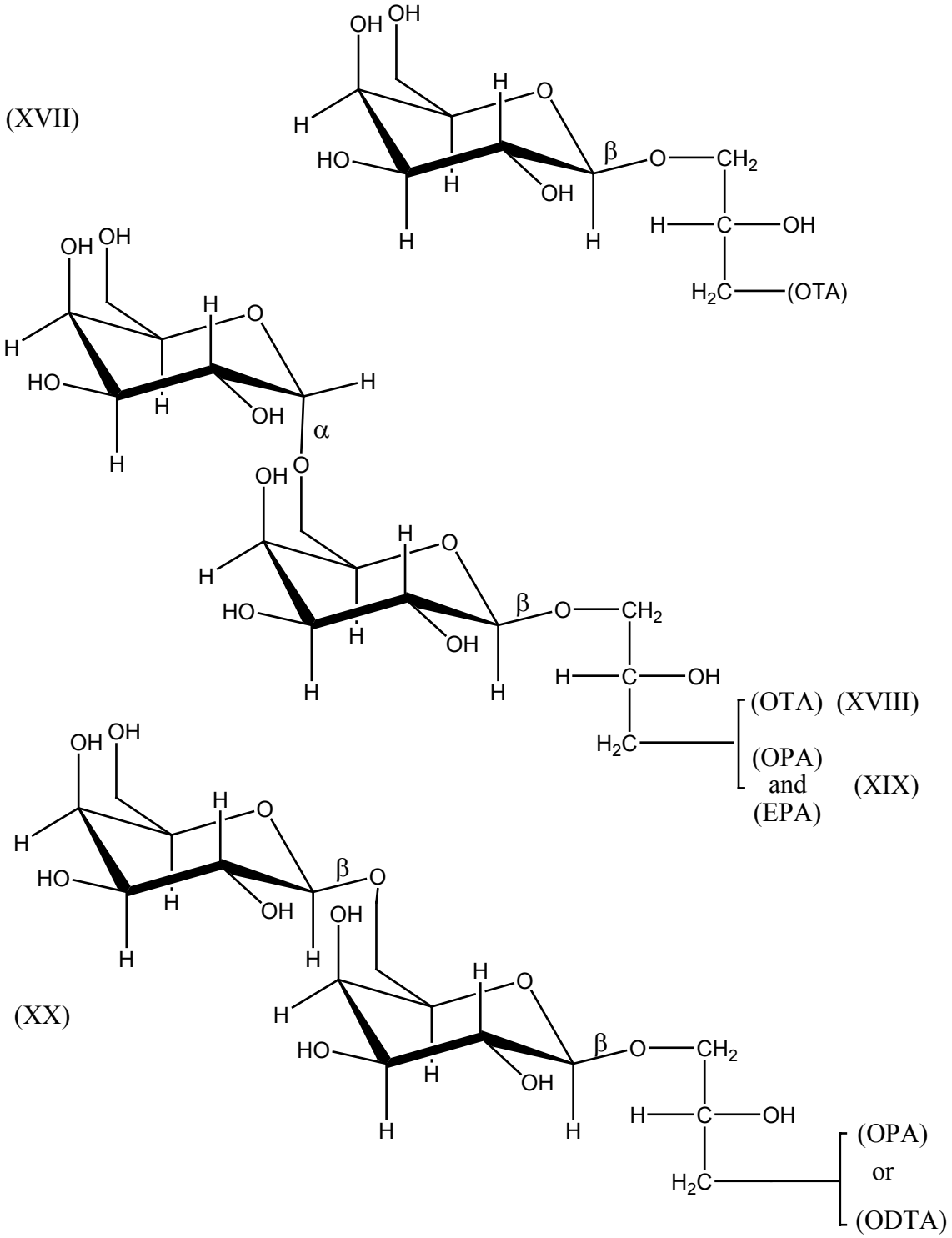


Figure 6: Mono- and di-D-galactosyl lysoglycerides from Dinophyceae and Haplophyceae.

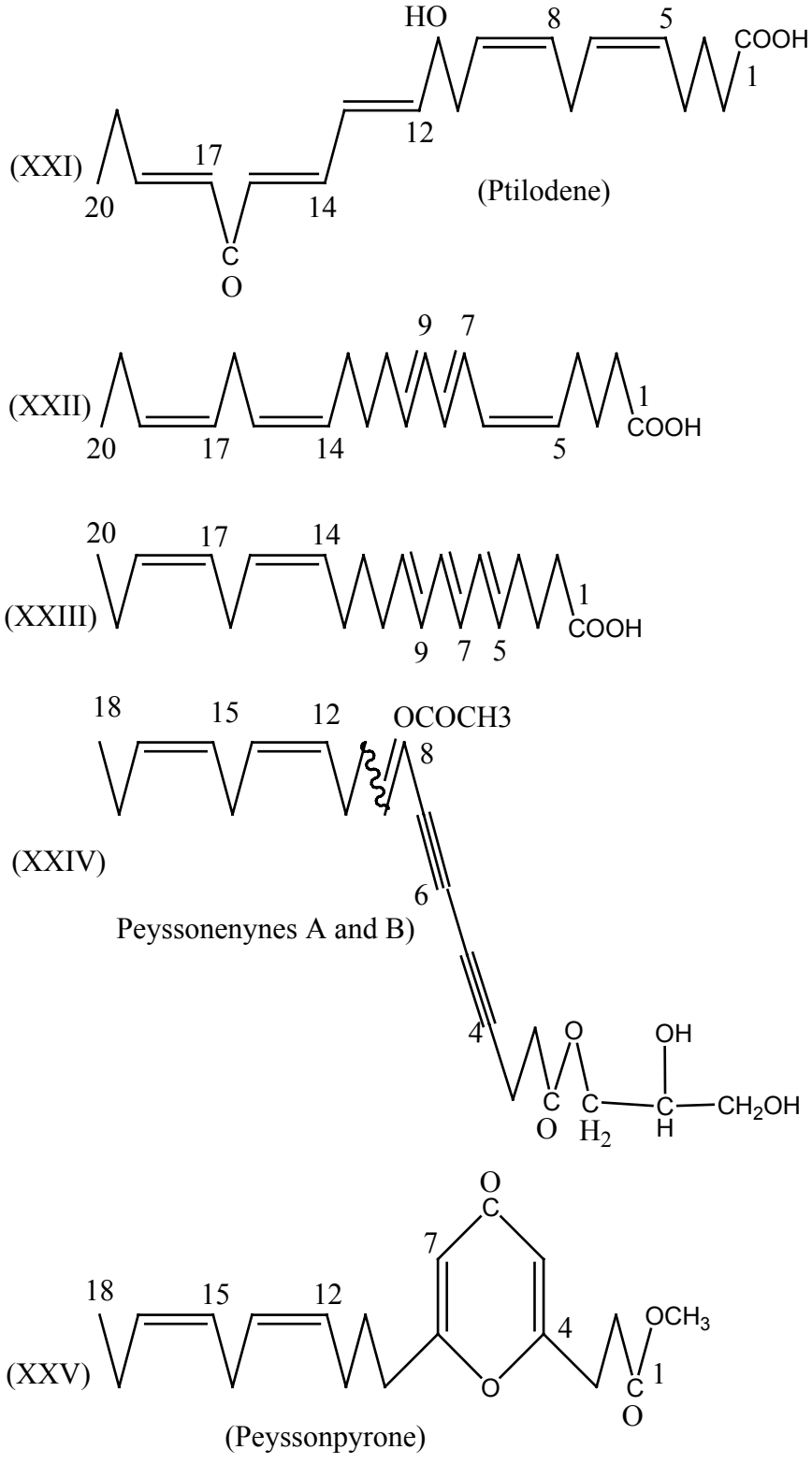
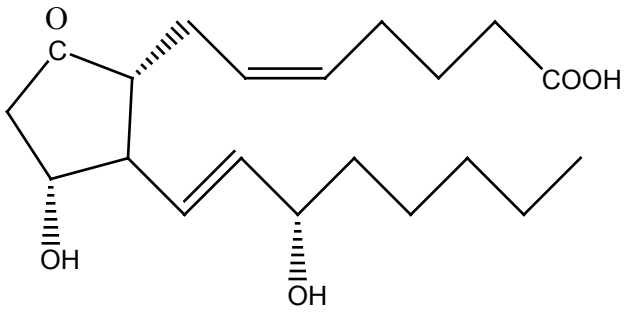


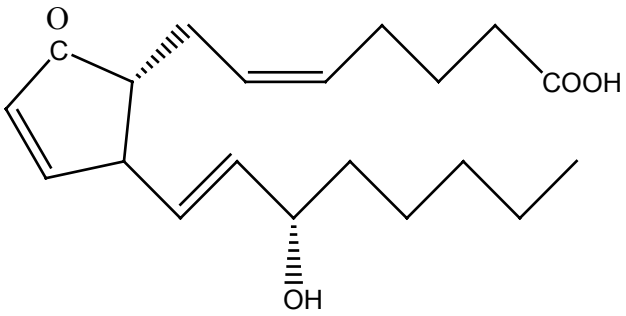
Figure 7: PUFA-related compounds from Rhodophyceae.

(XXVI)



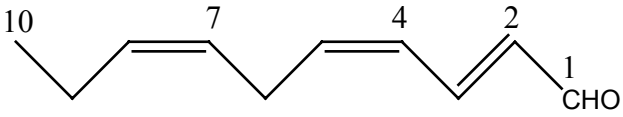
(Prostaglandin $PGE_{2)}$

(XXVII)

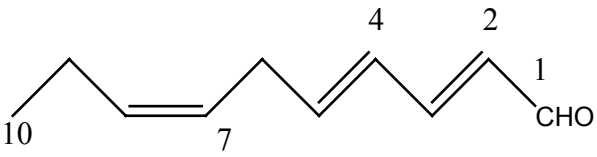


(Prostaglandin PGA_2)

(XXVIII)



(XXIX)



(XXX)

Figure 8: Oxidation products derived from PUFA's.