

## LIPIDS AND FATTY ACIDS

### Introduction

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Lipids are a diverse group of organic compounds that are important components of plant, animal, and microbial membranes. They are insoluble in water but soluble in nonpolar, organic solvents such as ether and alcohol. Dietary lipid has two main functions: as source of metabolic energy and as source of essential fatty acids that have specific functions in the body such as for cellular structure and maintenance of the integrity of biomembranes.

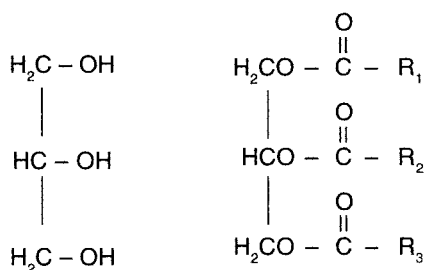
Lipids are important components of fish diets because they supply a concentrated source of energy that is well utilized by fish. They also supply essential fatty acids which cannot be synthesized by fish. As a source of dietary energy, lipids have been shown to spare some protein for growth. Lipids are also important sources of sterols, phospholipids, and fat-soluble vitamins. Fatty acids from dietary lipids may also serve as precursors of steroid hormones and prostaglandins.

The objective of this section is to acquaint the reader about common fatty acids, their nomenclature and formulas, and differentiate between saturated and unsaturated fatty acids; to know how environmental factors (temperature, salinity, diet) influence the fatty acid composition of fish; the mechanisms of fatty acid biosynthesis and oxidation, and factors that favor fatty acid biosynthesis and oxidation; the effects of lipid peroxidation and the function of antioxidants; and to understand the importance of fatty acid profiles in fish nutrition, and differences in the essential fatty acid requirements of warmwater and coldwater fishes.

### Types of Lipids

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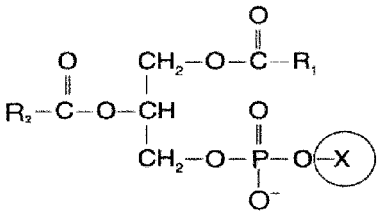
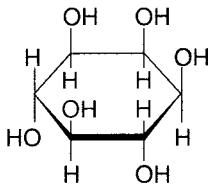
- **Triglycerides** or fats are formed by the reaction of glycerol with fatty acid molecules and hence called glycerides. Thus when a triglyceride is hydrolyzed, 3 molecules of fatty acid and one molecule of glycerol are formed. Triglycerides do not occur as components of biomembranes but they accumulate in the adipose or fat tissues. They are the primary means by which animals store energy.



glycerol

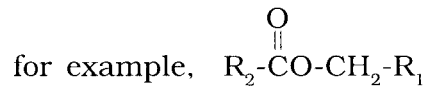
triglyceride

- **Phospholipids** are esters of fatty acids and phosphoric acid ( $\text{H}_3\text{PO}_4$ ) and a nitrogenous base. The resulting compound is called a phosphatidic acid. Some of the important phospholipids are phosphatidyl choline (lecithin), phosphatidyl ethanolamine (cephalin), phosphatidyl serine, and phosphatidyl inositol. They are the main components of biological membranes.

	Name of X-OH	Formula of -X
	choline	$\text{HO} - \text{CH}_2 - \text{CH}_2 - \text{N}^+(\text{CH}_3)_3$
	ethanolamine	$\text{HO} - \text{CH}_2 - \text{CH}_2 - \text{N}^+\text{H}_3$
 <p>phosphatidic acid</p>	inositol	
	serine	$\begin{array}{c} \text{N}^+\text{H}_3 \\   \\ \text{HO} - \text{CH}_2 - \text{C} - \text{COO}^- \\   \\ \text{H} \end{array}$

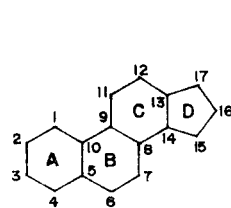
□ **Waxes** are esters of long-chain fatty acids and of high molecular weight monohydric alcohols. Like triglycerides, they are sources of energy stored in plants and animals and serve as protective coating. They are solids at ambient temperature.

- a. some are esters of long-chain alcohols,  $\text{R}_1 - \text{CH}_2\text{OH}$  and long chain fatty acids,  $\text{R}_2 - \text{COOH}$

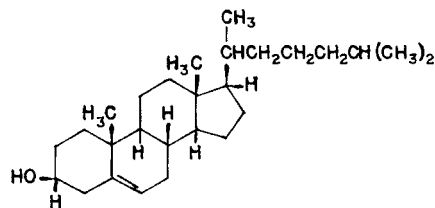


- b. some are ethers,  $\text{R}_2 - \text{CH}_2 - \text{O} - \text{CH}_2 - \text{R}_1$

□ **Steroids** are usually polycyclic long-chain alcohols. They are precursor of sex or other hormones in fish and shrimp and are biologically important in the reproductive processes. Steroids have the same general structure consisting of a fused-ring system. Cholesterol is a physiologically important sterol and is widespread in biological membranes, especially in animals.

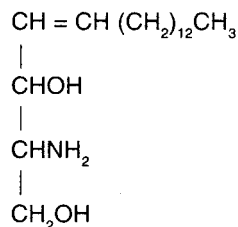


steroid nucleus

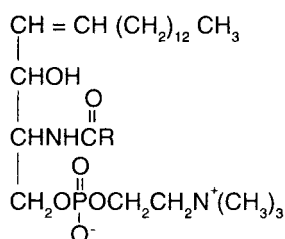


cholesterol

- **Sphingomyelins** do not contain glycerol, but are fatty acid esters of long-chain amino alcohol sphingosine. They are lipid components of the brain and nerve tissue of plants and animals.



sphingosine



sphingomyelin

## General Function of Lipids

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The general functions are:

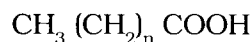
1. Sources of metabolic energy, adenosine triphosphate (ATP). They contain approximately twice the energy of proteins and carbohydrates.
2. Sources of essential fatty acids (EFA) which are important for growth and survival. EFA cannot be synthesized by the animal itself or are synthesized in insufficient amounts for good growth and has to be provided for in the diet. Examples: linolenic, linoleic, arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are essential fatty acids in fish and crustaceans.
3. Essential components of cellular and sub-cellular membranes. Examples: phospholipids and polyunsaturated fatty acids.
4. Sources of steroids which perform important biological functions such as maintenance of membrane systems, lipid transport, and precursors of steroid hormones.

## Fatty Acids

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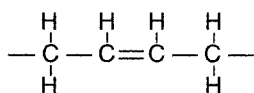
### Structure and Classification

Fatty acids are an important constituent of lipids. Over 40 fatty acids are known to occur naturally. They can be represented by the general formula:

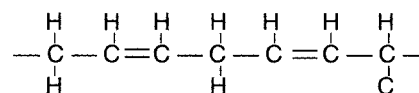


where: n varies from 0 to 24 and is usually an even number

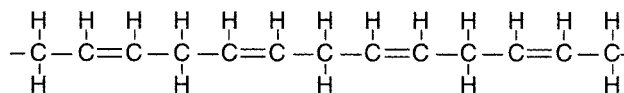
Most naturally occurring fatty acids contain a single carboxyl (COOH) group at one end and a straight unbranched hydrocarbon (C) chain. Fatty acids may be saturated (no double bond), where all carbon atoms are filled with hydrogen, or unsaturated, where one or more carbon atom lacks a hydrogen atom. Unsaturated fatty acids may either be monounsaturated (one double, bond), or polyunsaturated (PUFA) with two or more double bonds or highly unsaturated fatty acids (HUFA) containing four double bonds or more.



monounsaturated fatty acid



polyunsaturated fatty acid



highly unsaturated fatty acid

Polyunsaturated fatty acids normally have a methylene (-CH<sub>2</sub>-) interrupted system of double bonds. The common fatty acids are shown in Table 2.4.

**Table 2.4** The common fatty acids

Common Name	Chemical Name	Shorthand Notation*
<b>Saturated</b>		
Formic, Acetic, Propionic		1:0, 2:0, 3:0
Butyric, Valeric	Butanoic acid, Pentanoic acid	4:0, 5:0
Caproic	Hexanoic acid	6:0
Caprylic	Octanoic acid	8:0
Capric	Decanoic acid	10:0
Lauric	Dodecanoic acid	12:0
Myristic	Tetradecanoic acid	14:0
Palmitic	Hexadecanoic acid	16:0
Stearic	Octadecanoic acid	18:0
Arachidic		20:0
Behemic		22:0
Lignoceric		24:0
<b>Unsaturated</b>		
Palmitoleic acid	Hexadecenoic acid	16:1 <i>n</i> -7
Oleic acid	Octadecenoic acid	18:1 <i>n</i> -9
<b>Polyunsaturated</b>		
Linoleic acid	Octadecadienoic acid	18:2 <i>n</i> -6
Linolenic acid	Octadecatrienoic acid	18:3 <i>n</i> -3
<b>Highly unsaturated</b>		
Arachidonic acid	Eicosatetraenoic acid	20:4 <i>n</i> -6
Eicosapentaenoic acid		20:5 <i>n</i> -3
Docosahexaenoic acid		22:6 <i>n</i> -3

### Nomenclature

Fatty acids are given a common name besides their chemical formula and shorthand abbreviation. In fatty acid nomenclature, a fatty acid is identified by the formula: A:B *n*-3, A:B *n*-6, A:B *n*-9, sometimes *n* is designated as ω (omega) where, A is the number of carbon atoms, B is the number of double bonds, *n*-3, *n*-6, *n*-9 is the position of the first double bond from the methyl end of the fatty acid. For example, the



## Fatty Acid Composition of Fish

The composition of fatty acids in fish is affected by a number of environmental factors like salinity, temperature, and diet.

### 1. Salinity

Fish live in environments of varying salinity. The major fatty acids in lipids of marine and freshwater fishes are shown in Table 2.6. The fatty acid composition of freshwater and marine fishes differ from each other:

- a. marine species have higher content of long-chain (20 and 22 carbon) monoenoic acids while freshwater species have higher levels of medium-chain (16 and 18 carbons) monoenoic acids;
- b. marine species contain more highly unsaturated fatty acids than freshwater species;
- c. the ratio of *n*-3 fatty acids to *n*-6 fatty acids is greater for marine than freshwater species.

Differences in fatty acid composition is reflected also in fish that migrate from freshwater to marine environment. As fish migrate from freshwater to seawater, their fatty acid composition changes. The *n*-3/*n*-6 ratio is higher for migratory fishes like smelts and salmon.

**Table 2.6 Major fatty acids in lipids of marine and freshwater fishes\***

Fatty Acid	Percent Fatty Acids			
	Marine		Freshwater	
	A	B	C	D
14:0	3.7	2.2	2.8	6.7
14:1	0.1	0.2	1.0	0.7
16:0	12.6	17.0	16.6	14.6
16:1	9.3	4.1	17.7	14.7
18:0	2.3	3.2	3.3	1.5
18:1	22.7	21.4	26.1	18.2
18:2 <i>n</i> -6	1.5	2.0	4.3	3.7
18:2 <i>n</i> -3	0.6	1.0	3.6	3.6
20:1	7.5	5.4	2.4	1.6
20:4 <i>n</i> -6	1.4	0.9	2.6	2.4
20:5 <i>n</i> -3	12.9	6.7	2.7	8.2
22:1	6.2	9.4	0.3	0.4
22:4 <i>n</i> -6	0.1	0.6	0.4	0.4
22:5 <i>n</i> -3	1.7	2.3	2.0	1.5
22:6 <i>n</i> -3	12.7	16.1	2.0	6.0
Total saturated	18.6	22.4	22.7	22.8
Total monoenes				
medium	32.2	25.7	44.8	33.6
long-chain	13.7	14.8	2.7	2.0
Total <i>n</i> -3	27.9	26.1	10.3	19.3
Total <i>n</i> -6	4.1	3.5	7.3	6.5
Ratio $\frac{n-3}{n-6}$	6.8	7.5	1.4	3.0

\* A = Atlantic cod  
B = Chinook salmon  
Source: Ackman 1976

C = Sheepshead  
D = Alewife

## 2. Temperature

Temperature is a major factor that causes differences in fatty acid composition. Fish that live in warmwaters contain more saturated fatty acids than fish that live in colder waters. Fish reared at higher temperatures accumulate more saturated fatty acids than the same fish acclimated at lower temperatures:

Fish and crustaceans are able to adjust their levels of polyunsaturated fatty acids to maintain membrane integrity and function in the cold. Most PUFAs remain in the liquid state even at low temperatures while saturated fatty acids congeal and solidify at colder temperatures, therefore, high levels of unsaturated fatty acids in coldwater fish is necessary for maintenance of membrane fluidity.

## 3. Diet

Diet is the largest single factor that affects the fatty acid composition. Under normal conditions, the fatty acid composition of fish comes from these sources:

- a. fatty acids derived from the diet;
- b. fatty acids derived from non-lipid sources by biosynthesis and
- c. fatty acids derived from lipid sources by biosynthesis

The effect of diet on the fatty acid composition of fish and shrimp lipids has been demonstrated. When the shrimp *Penaeus setiferus* is fed a diet high in linoleic acid (18:2 *n*-6) and low in C20 and C22 PUFA for one month, the shrimp's content of *n*-3 and *n*-6 fatty acids is modified (increase in *n*-6 and decrease in *n*-3), hence the *n*-6/*n*-3 ratio increases. After three months, the *n*-6/*n*-3 ratio of shrimp continues to reflect that of the dietary lipid (Table 2.7). The seasonal variation of the fatty acid composition of fish may also be influenced by the changes in the composition of their natural food.

**Table 2.7** Effect of diet on fatty acid composition of shrimp *Penaeus setiferus*

Composition of <i>Penaeus setiferus</i> lipid				
Fatty acid	0 month	1 month	3 months	Diet
14:0	0.6	0.5	0.5	1.6
16:0	14.8	13.4	15.0	15.5
16:1	11.2	8.7	10.0	7.9
18:0	5.1	2.3	2.2	1.7
18:1	13.1	22.9	20.0	28.4
18:2 <i>n</i> -6	2.3	18.1	14.1	32.3
18:3 <i>n</i> -3	2.8	2.1	1.3	4.4
20:4 <i>n</i> -6	11.6	9.4	10.3	0.7
20:5 <i>n</i> -3	10.4	8.7	9.7	2.6
22:6 <i>n</i> -3	11.3	6.3	6.9	0.3
Total saturated	26.6	22.6	25.6	25.0
Total monoenes	18.2	25.2	22.2	30.1
Total <i>n</i> -6	13.9	27.5	24.4	33.0
Total <i>n</i> -3	24.5	17.1	17.9	7.3
Ratio $\frac{n-6}{n-3}$	0.57	1.61	1.36	4.5

Source: Castell 1981

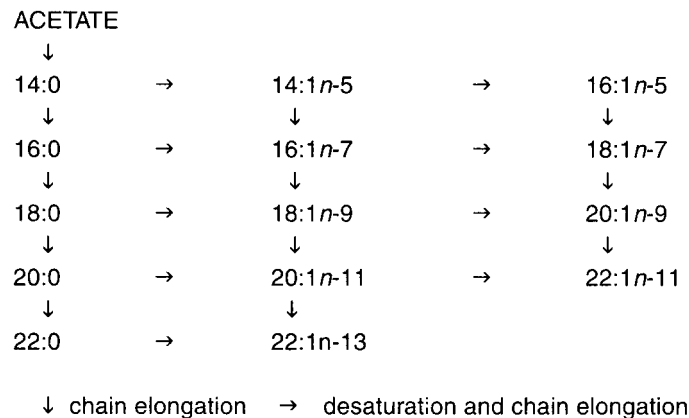
## Biosynthesis of Fatty Acids

Fish can biosynthesize fatty acids from a non-lipid source (acetate). The process begins from acetyl CoA and undergoes chain elongation by adding two carbon units at a time as it progresses through the biochemical pathway. The enzymes that catalyze the synthesis of fatty acids are: acetyl CoA carboxylase and FA synthetase. Acetate fragments are added to a fatty acid by the process of chain elongation. The reaction occurs in both the mitochondria and microsomes. Because fatty acids are synthesized from two-carbon acetyl units, they usually have an even number of carbon atoms. Palmitate is the major fatty acid produced by *de novo* biosynthesis.

*De novo* synthesis of saturated fatty acids

Acetate → C4 → C6 → C8 → C10 → C12 → C14 → C16:0 (chain elongation)  
**palmitic acid**

In the synthesis of unsaturated fatty acids, desaturation or removal of a hydrogen molecule is an additional step that is required to make a double bond (-C=C-). Desaturation of fatty acids occurs in the microsomes. Of the monounsaturated fatty acids however, only those of the *n*-5, *n*-7, *n*-9, *n*-11 and *n*-13 series are formed from non-lipid sources. *De novo* biosynthesis of saturated and monoenoic fatty acids is further illustrated in Figure 2.4. The main sites for *de novo* fatty acid synthesis are the liver and the adipocytes. The process occurs when the cells have abundance of energy and have high ATP content.



**Figure 2.4**

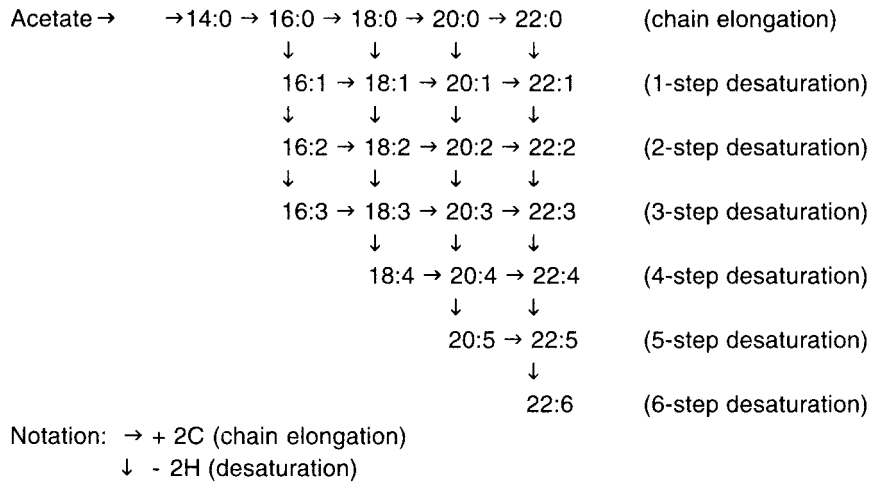
*De novo* synthesis of saturated monoenoic fatty acids

PUFAs are made by a series of chain elongation and desaturation steps. The chain elongation and desaturation of the *n*-3 and *n*-6 fatty acids are shown in Figure 2.5.

In common with other vertebrates, fish cannot directly synthesize *n*-3 and *n*-6 fatty acids *de novo* from non-lipid sources. Dietary precursors such as oleic, linoleic, and linolenic acids should be supplied in their diet.



Biosynthesis of fatty acids may be controlled by the competition between the different series of fatty acids for enzymes that desaturate and enzymes that chain elongate. High levels of *n*-3 inhibit the elongation and desaturation of the *n*-6 series. The potency of inhibition is ranked as follows: *n*-3 > *n*-6 > *n*-9. Thus, a proper balance of *n*-3 and *n*-6 essential fatty acids is needed in formulating the diet. After the biosynthesis of fatty acids, two major types of lipids are formed: the triglycerides and the phospholipids. The triglycerides are ultimately stored in fat deposits while the phospholipids are incorporated in the biomembranes.



**Figure 2.5**  
Biosynthesis of unsaturated fatty acids

## Oxidation of Fatty Acids

Long chain fatty acids, combined as triglycerides, provide the long term storage form of energy in the fat or adipose tissue of the animal. When energy demands are great, fatty acids are broken down to yield energy. The most important mechanism by which these storage fatty acids are degraded in a step-wise manner to yield energy is known as  $\beta$ -oxidation.

According to the  $\beta$ -oxidation mechanism, the  $\beta$ - or 3<sup>rd</sup> carbon atom from the carboxyl end of the fatty acid chain is the site at which oxygen is introduced during oxidation. Catabolism begins with the carbonyl group by converting long-chain fatty acids to acetyl CoA, which is subsequently oxidized in the citric acid or Krebs cycle. In this process, two-carbon fragments are successively removed from the fatty acid in the form of acetyl CoA. Fatty acid oxidation occurs mainly in the mitochondria and this is followed by complete oxidation of the acetyl CoA in the citric acid cycle. It is one of the pathways through which cells derive energy for synthesis of ATP and ultimately produce energy.

For example, the  $\beta$ -oxidation of palmitic acid, C16:0, requires seven (7) cycles of  $\beta$ -oxidations and gives rise to eight (8) molecules of acetyl CoA which enters the citric acid cycle and produces ATP. The ATP yield for the complete oxidation of one molecule of palmitic acid is 106 ATP. The liver is the chief organ concerned with  $\beta$ -oxidation of fatty acids.

The oxidation of fatty acids is the chief source of energy in the catabolism of lipids. The pathways of lipid synthesis (anabolism) and catabolism occurs simultaneously but in the different parts of the cell.

## Lipid Peroxidation

One of the characteristic reaction of lipids which are exposed to oxygen is the formation of peroxides. This reaction is of great practical importance as it leads to the deterioration of tissues and also causes spoilage of foods. This is due to the production of free radicals such as,  $ROO\cdot$ ,  $RO\cdot$ ,  $OH\cdot$  during peroxide formation. These free radicals react by hydrogen removal and a variety of addition reactions that damage enzymes, proteins, vitamins and other lipids making other nutrients unavailable to fish. Antioxidants are substances that protect lipids from peroxidation. The use of lipids in fish diets requires the use of appropriate antioxidants (see Chapter 5).

## Importance of Fatty Acid Profiles in Fish Nutrition

Both fish and vegetable oils have been found useful in fish feeds. The fatty acid composition of commonly used oil sources in fish diets is shown in Table 2.8. In comparison with other vegetable oils or fats, fish oils contain a greater variety of unsaturated fatty acids of longer carbon chain (20 or 22 carbon chain length), most of which belong to the *n*-3 family of fatty acids. The long-chain *n*-3 fatty acids generally make up one-fourth to one-third of all fatty acids in fish oils, whereas, long chain fatty acids in most vegetable oils seldom exceed 5% and is frequently less than 1%. The dietary lipid requirement of fish can be derived from their fatty acid profiles.

**Table 2.8 Essential fatty acid composition of various lipid sources (g/100g fatty acid)**

Lipid source	18:2 $n$ 6	18:3 $n$ 3	20:5 $n$ 3	22:6 $n$ 3
<b>Plant sources</b>				
Corn oil	58	1	0	0
Coconut oil	2	0	0	0
Cottonseed oil	53	1	0	0
Linseed oil	17	56	0	0
Palm oil	10	1	0	0
Palm kernel oil	2	0	0	0
Rapeseed oil	15	8	0	0
Peanut oil	30	0	0	0
Soybean oil	50	10	0	0
Sunflower oil	70	1	0	0
<b>Marine animal sources</b>				
Capelin oil	5	0	7	5
Cod liver oil	5	1	16	14
Cuttlefish liver oil	1	2	12	18
Herring oil	1	1	8	5
Pollack liver oil	2	0	12	7
Salmon oil	3	0	10	10
Sardine oil	3	1	13	10
Short-neck clam oil	1	1	19	14
Skipjack oil	5	3	7	12
Squid liver oil	3	3	12	10

Source: Tacon 1987

## Essential Fatty Acid Requirements of Fish

Fish require  $n-3$  and  $n-6$  fatty acids in their diets. Linolenic acid (18:3 $n-3$ ), linoleic acid (18:2 $n-6$ ), eicosapentaenoic acid (EPA, 20:5 $n-3$ ) and docosahexaenoic acid (DHA, 22:6 $n-3$ ) are needed by fish. Failure to provide these fatty acids in the diet can cause impairment of growth and prolonged lack of these fatty acids in the diet lead to death.

The essential fatty acid (EFA) requirements have been established for most cultured fishes. A summary of these values is presented in Table 2.9. Fish species differ in their EFA requirements.

**Table 2.9** Essential fatty acid requirements of fish and shrimp

Fish species	Requirement	Reference
Asian sea bass	0.5% $n-3$ PUFA 0.5% $n-6$ PUFA	Borlongan and Parazo 1991
Ayu	1% 18:3 $n-3$ 1% 20:5 $n-3$	Kanazawa et al. 1982
Carp	1% 18:2 $n-6$ 1% 18:3 $n-3$	Watanabe et al. 1975 Takeuchi & Watanabe 1977
Chum salmon	1% 18:2 $n-6$ 1% 18:3 $n-3$ 0.5% $n-3$ HUFA	Takeuchi et al. 1979
Coho salmon	1-2.5% 18:3 $n-3$	Yu & Sinnhuber 1979
Eel	0.5% 18:2 $n-6$ 0.5% 18:3 $n-3$	Takeuchi et al. 1980
Grouper	1% $n-3$ HUFA	Millamena and Golez 1998
Milkfish	1-1.5% $n-3$ PUFA	Borlongan 1992
Nile tilapia	0.5% 18:2 $n-6$ or 20:4 $n-6$	Takeuchi et al. 1983
Rainbow trout	0.8% $n-3$ HUFA 0.8% $n-3$ HUFA 18:3 $n-3$ 20% of lipid $n-3$ HUFA 10% of lipid	Castell et al. 1972 Watanabe et al. 1974 Takeuchi & Watanabe 1977
Red seabream	0.5 $n-3$ HUFA 0.5% 20:5 $n-3$	Yone et al. 1978
Turbot	0.8% $n-3$ HUFA	Gatesoupe et al. 1977
Yellow tail	2% $n-3$ HUFA	Deshimaru et al. 1984
Tiger shrimp	2.6% $n-3$ PUFA <5% $n-6$ PUFA 0.5% $n-3$ PUFA	Catacutan 1991
Kuruma shrimp	0.5-1% $n-3$ PUFA	Kanazawa et al. 1980

Rainbow trout (*Oncorhynchus mykiss*) requires about 1% 18:3 $n-3$  in the diet. Combining 18:3 $n-3$  and 18:2 $n-6$  in various proportions did not improve growth rate or efficient feed conversion. In contrast, carp (*Cyprinus carpio*), one of the most important cultured fish in Japan requires both 18:2 $n-6$  and 18:3 $n-3$ . The best weight gain and feed conversion are obtained in fish given a diet with a mixture of 1% 18:2 $n-6$  and 1% 18:3 $n-3$ . The eel (*Anguilla*

*japonica*), another cultured warmwater fish, has a requirement for both 18:2 $n$ -6 and 18:3 $n$ -3, but at a level of 0.5%. A tropical herbivore, Nile tilapia (*Tilapia nilotica*) requires  $n$ -6 rather than  $n$ -3 fatty acids. Their dietary requirement for 18:2 $n$ -6 or 20:4 $n$ -6 is about 0.5% in the diet.

Highly unsaturated  $n$ -3 fatty acids ( $n$ -3 HUFA) are essential in the nutrition of some marine fish such as red sea bream (*Chrysophrys major*), and yellow tail (*Seriola quinquerodiata*). The requirement for long-chain polyunsaturated fatty acids is due to the limited ability of some marine fishes to add more carbon atoms or to remove hydrogen from dietary precursors. Thus, most coldwater and marine fish requires  $n$ -3 fatty acids.

Studies on the essential fatty acid requirements of warmwater fish and shrimp species in the Philippines show that some species require both  $n$ -3 and  $n$ -6 fatty acids while others only  $n$ -3. Growth inhibition at certain levels of  $n$ -3 and  $n$ -6 has also been observed in warmwater fishes. Milkfish (*Chanos chanos*) cultured in seawater requires  $n$ -3 PUFA. Good growth and survival were obtained using either linolenic (18:3 $n$ -3) or  $n$ -3 HUFA as lipid sources. Asian sea bass (*Lates calcarifer*) juveniles require both  $n$ -3 and  $n$ -6 PUFA at 0.5% in the diet or an  $n$ -3/ $n$ -6 ratio of 1.0. Grouper (*Epinephelus coioides*) requires about 1%  $n$ -3 HUFA. In juvenile tiger shrimp (*Penaeus monodon*), about 2.6% dietary PUFA enhances growth while levels of 18:2 $n$ -6 greater than 5% have a negative effect on growth. Thus, different species require different EFAs and the differences are more marked in warmwater than in coldwater fishes.

### Guide Questions

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1. Define the term "lipid". Name the two major functions of lipids.
2. What are the important types of lipids? Briefly define each component.
3. Show the general formula for fatty acids. Explain briefly.
4. What are the three major families of polyunsaturated fatty acids
5. Name the environmental factors that affect the fatty acid composition of fish.
6. What are the two major types of lipids formed after biosynthesis of fatty acid?
7. Where are the biosynthesized fatty acids or triglycerides ultimately stored?
8. Under what conditions or nutritional state of the animal is fatty acid oxidation favored? Under what conditions is it slowed down?
9. What is  $\beta$ -oxidation of fatty acids?
10. Describe the phenomenon of lipid peroxidation. Why is it of great practical importance?
11. Why does the type of lipid required for warm water fish differ from cold water fish?
12. Which among the fish species studied requires more  $n$ -6 rather than  $n$ -3 fatty acids?
13. Differentiate between: a) saturated vs. unsaturated fatty acids b) polyunsaturated vs. highly unsaturated fatty acid.
14. Name some oils that are used in fish diets and explain why they are useful.