

Fish Farming and Aquaculture. Can We modify Fish Fat with More EPA?*

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Received: September 2, 1986

Outline of Fisheries in Japan

For the challenge of the present title, I would like to introduce first an outline of fisheries in Japan, not only from production, but also from demand and consumption. The world production of fish and shellfish totaled about 75 million metric tons per year. Of this total, the Japanese production accounted for approximately 11 million metric tons.

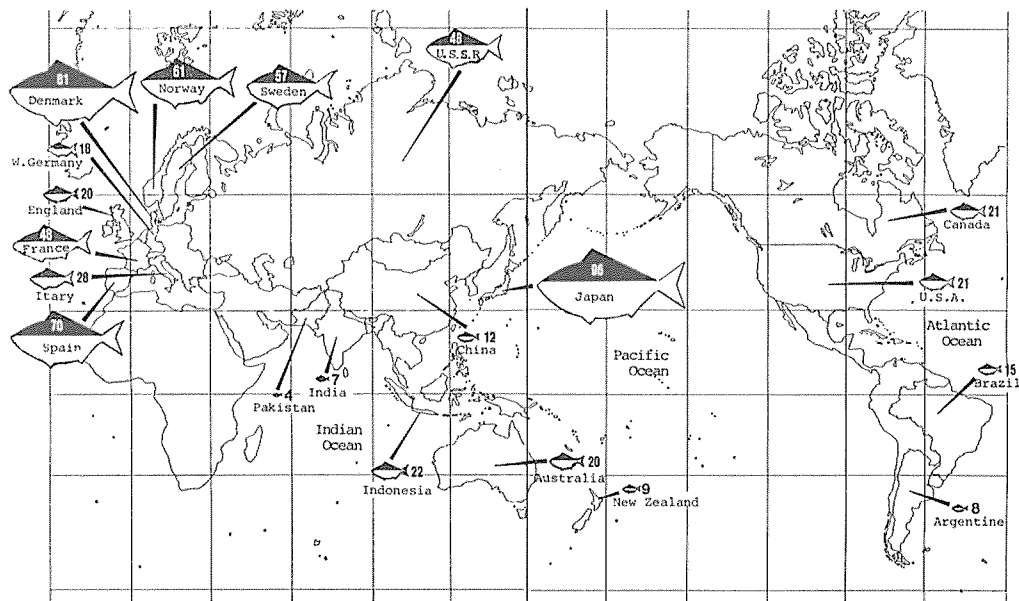


Fig. 1. Comparison of fish and shellfish consumption (g/person/day).
Data are cited from the tables of "Food Demand and Supply".³⁾

Moreover, comparing the human consumption of fish and shellfish per person per day in each country, a Japanese eats 96 grams per day, followed by northern Europeans as shown in Fig. 1.^{1a)} That's why the Japanese people are called "Fish-eating Nation" as to traditional living habits or customs.

To meet the Japanese demand for fish and shellfish Japan has increased its imports,

* The manuscript was orally presented in the Short Course of the American Oil Chemists' Society on "Marine Lipids and EPA" from May 11 to 14, 1986, at Kohala Coast, Island of Hawaii, U.S.A.

and also increased the production of coastal fisheries including marine aquaculture, under regulation of 200-nautical miles.

Japan is narrow and long islands surrounded by sea, where warm and cold currents meet. Favored by circumstances, fishery products were supplied to whole nation from long ago, and these products are essential for Japanese life style. Recently by increasing our national income, the consumption of animal proteins reached 40 g / person / day and increased year by year. Approximately a half of the protein consumption is from these fishery products. Compared with foreign countries, the consumption of fishery products in Japan is very high and the ratio of fishery products in animal proteins is also high.

Also in the trade of fishery products, Japan is the biggest import country and influences most of world trade in fishery products. The exports of canned fish, pearls etc. are decreasing slowly, while the imports are increasing of shrimp, tuna, salmon, squid, and octopus from year to year.

By such habits Japanese life style provides fishery products with a good balance of proteins, fats and oils, and carbohydrates. In fishery products the characteristic components such as polyunsaturated fatty acids (PUFA) lowering the cholesterol level, calcium and other minerals and various vitamins necessary for human health are generous. Moreover, eicosapentaenoic acid (EPA) effective for the prevention of adult diseases like a heart attack is provided by mackerel, sardine and saury oils, and its role is evaluated. Since in the European and American countries people are suffering from an obesity problem, all excellent marine products are recommended highly for human nutrition, and increasing their consumption is the policy of life style.

Fisheries for Breeding and Culture²⁾

Fisheries in our country expanded fisheries grounds from coastal to offshore and from offshore to far seas fisheries by the progress of fisheries techniques, however, fisheries regulation within 200-nautical miles from 1977 reduced the far seas fisheries. On the other hand in the coastal fisheries grounds were diminished by the industrial water pollution owing to factory developments. In such circumstances fisheries by fishing boats and fixed nets are stagnant; however by aquaculture growth fish production actually increased. Since the offshore fisheries objectives of migrating fishes such as sardine and mackerel are changeable as the resources, and the restriction of new oceanic development of far seas fishing becomes harder and harder, and yet middle and high class fish and shellfish live abundantly in the coastal areas, the utilization of high productive coasts becomes more and more important. For these reasons, in addition to fisheries based on natural resources, fisheries for breeding and culture are positively becoming critical to keep and increase the useful resources through human management of the cycle of natural resources. The fish farming nowadays produces young larvae and liberates them, and also prepares the fisheries grounds. Moreover, the propagation is controlled by man from young larvae to harvest. Fisheries for breeding and culture in addition to

these methods are the managements of natural resources by the production of young larvae and the liberation to keep the resources in the optimal condition.

In general fish and shellfish spawn a large quantity of eggs, however, in many cases young larvae are diminished suddenly owing to the shortage of food plankton or a sudden change of environmental conditions. The production of seed and liberation of young larvae in fish farming is for the reproduction processes of fishes and shells. From few parents large amounts of young larvae are obtained. Furthermore, these larvae are liberated in addition to the natural stocks to maintain the maximum resources by keeping under the human control. Nowadays by improving the management of parent organisms, technique of maturing gonads, mass production of live feed for young larvae, feeding methods, increasing the density of organisms, and control of water quality, high level seed production of red sea bream, prawns, blue crabs, abalone, *etc.* is available. Besides these species the production of other species is becoming possible. In the liberation of seed, the survival rate from liberation to catching is the critical point. Moreover, the optimum size for liberation, place of liberation, time of liberation, *etc.*, are considered from the standpoints of liberation technique, management of fish farming grounds, and protection technique after liberating young larvae.

The production methods of seeds are different from species to species. Moreover now the produced seeds are utilized not only for fish farming but also for propagation. To ensure the fish and shell seeds, several methods, for example by natural spawning in an aquarium by raising water temperature, by stimulation of low tide, irradiation by ultra-violet ray or electric stimulation, the spawnings are accelerated. In shrimp and crab fertilized females are used to get the seeds.

As a diet in early developing stage, fish, shrimp, and crab are fed with live baits such as rotifers, *Artemia*, and copepods as zooplankters, and *Chlorella* and diatoms as phytoplankters. Both live feeds are small size organisms fitted to the mouth size of young larvae, and different kinds of baits are used according to the desired species and stages of growth. For growth, minced fish meat, short necked clam, *Euphausia* and proper artificial diet are fed, and they are liberated when they reach a suitable sizes in the aquaria.

Moreover, in the seaweeds like *Undaria* and *Laminaria*, *etc.*, swimming spores detached from parent seaweeds are put on artificial fibres, then they are grown and their young are reared in the aquarium or natural sea waters.

Marine aquaculture was represented by the traditional "Nori" laver (*Porphyra tenera*, *P. yezoensis*), oyster (*Crassostrea gigas*) and pearl. Recently yellow tail fish (*Seriola quinqueradiata*), scallop (*Pecten yessoensis*), tunica (*Cynthia roretzi*), "Kombu" *Laminaria*, "Wakame" *Undaria* cultures were developed, and more recently horse-mackerel (*Trachurus japonicus*), red sea bream (*Pagrus major*), prawn (*Penaeus japonicus*) cultures increased. Moreover, in addition to those coastal cultures the offshore cultures like coho salmon (*Oncorhynchus kisutch*) and blue fin tuna (*Thunnus thunnus*) are going to be developed.

On the other hand, in fresh water aquaculture, eel (*Anguilla japonica*), carp (*Cyprinus carpio*), rainbow trout (*Salmo gairdneri*), "Ayu" *Plecoglossus altivelis*, fresh water pearl, fancy carp (*Cyprinus carpio*) and gold-fish (*Carassius auratus*) are pointed out.

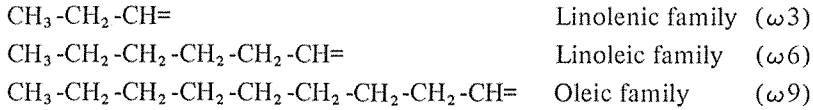
Can We modify Fish Fat with More EPA?

At early studies on the origin of marine lipids KELLY *et al.*⁴⁾ reported the biogenesis of PUFA by the alkali-isomerization technique. Later REISER and his coworkers⁵⁾ investigated the conversion of PUFA by using gas-liquid chromatography in young mullet, *Fundulus* and gold fish by feeding compounded diets, and the effect of temperature was tested. Following these fish raising experiments with test artificial diets containing various oils and fats or fatty acids, MEAD, KAYAMA and REISER⁶⁾ proved by using ¹⁴C labeled compounds that even in fish linoleic acid 18:2 ω 6 acts as an essential fatty acid, which is converted to arachidonic acid 20:4 ω 6 in *Tilapia* (*Tilapia mossambica*) just as shown in mammals. Moreover, KAYAMA *et al.*⁷⁾ presented the pathway from linolenic acid 18:3 ω 3 to docosahexaenoic acid 22:6 ω 3 via eicosapentaenoic acid 20:5 ω 3 in kelp bass (*Paralabrax clathratus*). Furthermore, there are obviously profound differences in the conversion ability. This might be due partly to the difference in salinity, or in species, or the nature of organisms, but to a large extent also to the diet which will influence the rate of synthesis and deposition of fatty acid.

Table 1. Characterized polyunsaturated fatty acids from fish.

Chain length	No. of double bond	Position of double bond	
		Counted from Δ carboxyl carbon	Counted from ω methyl carbon
C ₁₆ Hexadeca-	dienoic	9, 12 7, 10 6, 9	4, 7 6, 9 7, 10
	trienoic	9, 12, 15 7, 10, 13 6, 9, 12 4, 7, 10	1, 4, 7 3, 6, 9 4, 7, 10 6, 9, 12
	tetraenoic	6, 9, 12, 15 4, 7, 10, 13	1, 4, 7, 10 3, 6, 9, 12
C ₁₈ Octadeca-	dienoic	9, 12 6, 9	6, 9 9, 12
	trienoic	9, 12, 15 6, 9, 12	3, 6, 9 6, 9, 12
	tetraenoic	6, 9, 12, 15	3, 6, 9, 12
C ₂₀ Eicosa-	dienoic	11, 14 8, 11	6, 9 9, 12
	trienoic	8, 11, 14 7, 10, 13 5, 8, 11	6, 9, 12 7, 10, 13 9, 12, 15
	tetraenoic	8, 11, 14, 17 5, 8, 11, 14	3, 6, 9, 12 6, 9, 12, 15
	pentaenoic	5, 8, 11, 14, 17	3, 6, 9, 12, 15
C ₂₂ Docosa-	trienoic	7, 10, 13	9, 12, 15
	tetraenoic	7, 10, 13, 16	6, 9, 12, 15
	pentaenoic	7, 10, 13, 16, 19 4, 7, 10, 13, 16	3, 6, 9, 12, 15 6, 9, 12, 15, 18
	hexaenoic	4, 7, 10, 13, 16, 19	3, 6, 9, 12, 15, 18

In Table 1 the polyunsaturated fatty acids isolated and identified so far from fish oils are listed. It was noticed that very characteristic regularity does exist in the double bond position of fish polyunsaturated acids counting from the methyl group end of the acids as shown in the table⁸⁾. The unsaturated acids, longer than octadecadienoic acid, will be classified into three groups with first double bond starting at 3, 6, and 9 positions respectively⁸⁾. The first group should be named linolenic ($\omega 3$), the second linoleic ($\omega 6$) and the third oleic ($\omega 9$) families as follows.



From the evidence presented by MEAD *et al.* it appears that the polyunsaturated acids are formed by alternative desaturation in divinyl methane relationship to the existing double bond and chain lengthening. It is logically expected that starting from the existing double bond of the parent acid (in this case, linolenic, linoleic, and oleic acids)

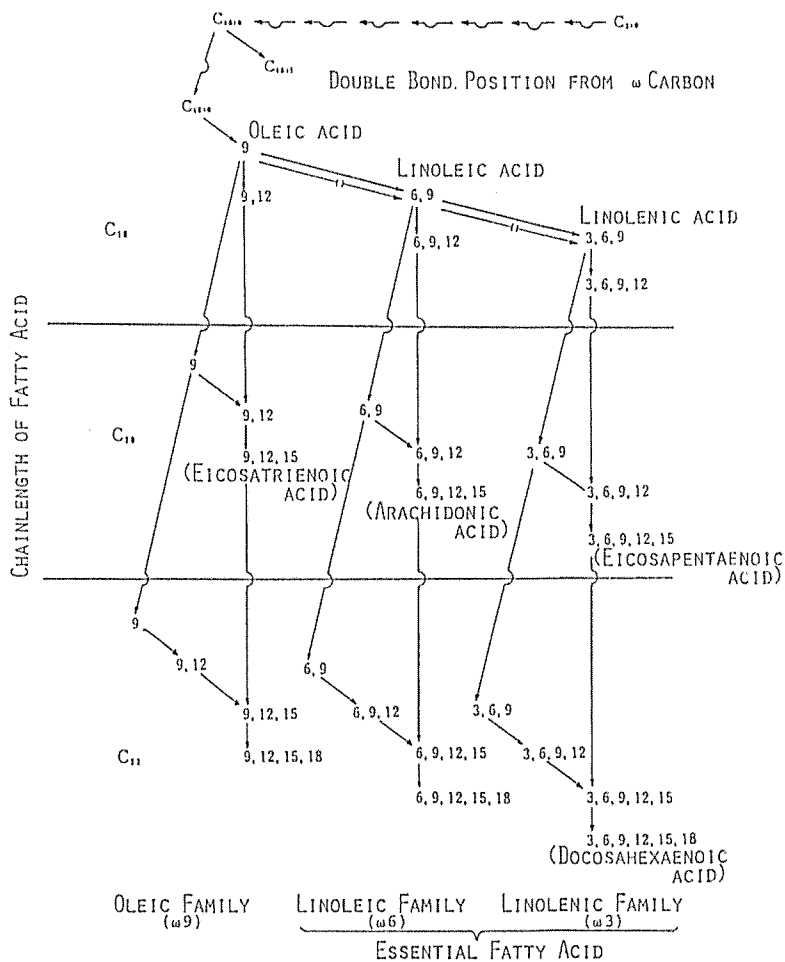


Fig. 2. Possible conversion pathways of main polyunsaturated fatty acids in fish.⁹⁾

the additional double bonds are introduced in the 1-4 relationship toward the carboxyl group until the next double bond would be in the $\alpha\beta$ or $\beta\gamma$ position, and the chain elongation then permits the more double bond addition.

It will be realized that the conversion steps from linoleic to arachidonic acid presented by MEAD *et al.* in rat are placed in the scheme just as their original. Moreover, this pathway has been shown to occur in the fish, *Tilapia mossambica*.⁶⁾ Also it can be assumed that the linoleic acid is essential in the true meaning even in fish, because its activity isolated after injection of [$1-^{14}\text{C}$] acetate was almost negligible.⁶⁾ For linolenic acid it can be seen that the similar process is formulated in the scheme. This conversion pathway from linolenic to eicosapentaenoic and docosahexaenoic acids has been demonstrated in the kelp bass, *Paralabrax clathratus*.⁷⁾ The similar processes are assumed to occur in oleic (palmitoleic) acid as illustrated with the above mentioned two pathways in Fig. 2.⁹⁾

Although fish cannot synthesize $18:2\omega 6$ ⁶⁾ and $18:3\omega 3$,¹⁰⁾ they can convert $18:2\omega 6$ to $20:4\omega 6$,⁶⁾ and $18:3\omega 3$ to $22:6\omega 3$ via $20:5\omega 3$.⁷⁾ OWEN *et al.*¹¹⁾ showed in rainbow trout that [^{14}C] $18:3\omega 3$ was easily converted to $\omega 3$ highly unsaturated fatty acids (HUFA) and about 70% of total radioactivity of fatty acids were detected in $22:6\omega 3$, while in turbot that $18:3\omega 3$ was chain-elongated to $20:3\omega 3$ but almost not to HUFA. KANAZAWA *et al.*¹²⁾ compared the conversion capability from $18:3\omega 3$ to $\omega 3$ HUFA, and presented conversion rates of 36 for "Ayu" *Plecoglossus altivelis*, 20 for prawn (*Penaeus japonicus*), 20 for eel (*Anguilla japonica*), 15 for red sea bream (*Pagrus major*), 13 for tiger puffer (*Takifugu rubripes*), and 7 for rockfish (*Sebastes marmoratus*), compared to 100 for rainbow trout (*Salmo gairdneri*). YAMADA *et al.*¹³⁾ reported that in the conversion ability from $18:3\omega 3$ to $\omega 3$ HUFA red sea bream, black sea bream (*Acanthopagrus schlegeli*), rudder fish (*Girella punctata*), and striped mullet (*Mugil cephalus*) were less active than trout.

Considering these conversion abilities of fishes, TESHIMA¹⁴⁾ grouped fishes by fresh water type (trout type and carp type), salt water type, and *Tilapia* type in the requirement of essential fatty acids. For trout in cold fresh water, there is no great differences in the essentiality between $18:3\omega 3$ and $\omega 3$ HUFA such as $20:5\omega 3$ and $22:6\omega 3$. On the other hand for carp,^{15a)} channel catfish (*Ictalurus punctatus*)^{15a)} eel,^{15b)} and Ayu¹⁶⁾ in warm fresh water, they cannot as well utilize $18:3\omega 3$ as trout, and $\omega 3$ HUFA has higher essential effect than $18:3\omega 3$. Moreover, since in the case of eel and carp $18:2\omega 6$ plus $18:3\omega 3$ in the level of 1% each in diets have the higher growth and feed effect than sole addition of both acids, they require both acids as essential fatty acids.^{15a,b)} TAKEUCHI *et al.*^{17,18)} indicated that chum salmon (*Oncorhynchus keta*) has similar need for essential fatty acids to carp in both habitats, in salt and fresh waters. In the cases of salt water fishes they are different from fresh water type, $18:3\omega 3$ has almost no effect and $\omega 3$ HUFA such as $20:5\omega 3$ and $22:6\omega 3$ are effective as essential fatty acids. In the case of *Tilapia*, *Tilapia zillii*¹⁹⁾ and *T. nilotica*,^{20,21)} $18:2\omega 6$ and linoleic family are effective as essential fatty acids like land animals. It is very interesting that $20:4\omega 6$ has no essential

effect in *T. nilotica*.²⁰⁾

In general, plants synthesize all of their fatty acids *de novo*. Animals not only synthesize most fatty acids, but they also reflect the diet components and alter exogenous and endogenous sources. In the aquatic field, phytoplankton is the basic food. It serves as food for zooplankton, which then serves as diet for small fish and other sea animals which, in turn, are eaten by larger species.

As a model experiment of aquatic food chain with special reference to fatty acid conversion, KAYAMA *et al.*²²⁾ designed and studied the biological process: phytoplankton (*Chaetoceros simplex*) → zooplankton (*Artemia salina*) → small fish (*Lebistes reticulatus*). It is apparent that the lipid extracted from *Chaetoceros* represents its own synthetic fatty acids, but the lipids obtained from *Artemia* and guppy show the summation of both themselves and of each lower trophic level. In the step from *Chaetoceros* to *Artemia*, myristic (14:0) and palmitic (16:0) acids decreased, while stearic (18:0) and oleic (18:1 ω 9) acids increased, and a fairly large amount of 20:5 ω 3 was detected in *Artemia* lipid. In the step from *Artemia* to guppy, 14:0, palmitoleic (16:1 ω 7) and 20:5 ω 3 were much decreased, however, 16:0 and 18:0 were increased, and 22:5 ω 3 and 22:6 ω 3 were found in the guppy lipid. Comparing two groups of guppies raised at 17 \pm 1°C and 24 \pm 1.5°C, a larger proportion of 16:1 ω 7, 18:1 ω 9 and 22:6 ω 3 and a smaller proportion of 16:0 and 22:5 ω 3 were detected in the lower water temperature group. A further study of temperature influence on fish lipid has been reported by KNIPPRATH and MEAD^{2,3)} Increases in ω 3 HUFA such as 20:5 ω 3 and 22:6 ω 3 were observed in the colder water temperature so the α -linolenic acid conversion pathway is applicable as demonstrated by KAYAMA *et al.*⁷⁾ in fish.

The effect of water temperature on fatty acid composition, especially of 20:5 ω 3 content of algae such as *Porphyra*^{24a,b,c)} and marine *Chlorella*,^{25,26)} has been reported independently. The cellular fatty acid composition was found to be extremely sensitive to environmental temperature. The reasons for these temperature-associated changes have not been fully explained, but it is supposed that unsaturated fatty acids increase the thermal flexibility of cells to adapt to the environment. BROWN and ROSE²⁷⁾ postulated that because of increased solubility of O₂ at lower temperature, a greater amount of intracellular molecular oxygen is available, which is required by oxygen-dependent enzymes that catalyze the desaturation of long-chain fatty acids.

Rotifer (*Brachionus plicatilis*) is used for a living feed in the seed production of marine fishes. The relationship between the nutritional quality of rotifer and its culture media such as baker's yeast (*Saccharomyces cerevisiae*) and marine *Chlorella* (*Chlorella minutissima*) was investigated by WATANABE *et al.*²⁸⁾ from the viewpoint of essential fatty acids for fish. The rotifer cultured with yeast was quite low in content of ω 3 HUFA and high in content of monoenoic fatty acids, whereas those cultured with marine *Chlorella* were found to contain a high amount of 20:5 ω 3, which is one of the essential fatty acids for marine fish. The difference in the concentration of 20:5 ω 3 was also found to be attributable to the difference of fatty acid composition between yeast and marine

Chlorella. Judging from the influence of dietary fatty acids on polyunsaturated fatty acids of cultured and wild freshwater fishes, the ratio of $\omega 3$ to $\omega 6$ PUFA for dorsal muscle lipids was estimated in the following order: wild rainbow trout > cultured eel and rainbow trout > wild eel > wild carp > cultured carp.^{29,30)}

As you can see in Table 2, it would be recognized that the oils of land plants such as soybean, cottonseed, safflower and linseed oils do contain large amounts of linoleic or linolenic acids, and fats of terrestrial animals such as milk, tallow, and lard fats consist of largely palmitic, stearic and oleic acids, while the oils of most aquatic animals and algae hold highly unsaturated fatty acids such as eicosapentaenoic as well as docosahexaenoic acids with the exception of red alga *Porphyra yezoensis*, which has extraordinary high amounts of eicosapentaenoic acid.

Table 2. Main fatty acid composition of fat and oil.*1

	Soybean oil	Cottonseed oil	Safflower oil	Linseed oil	Milk fat	Tallow	Lard	<i>Porphyra</i> oil	<i>Euphansia</i> oil	Minck Whale oil	Squid Liver oil	Saury oil	Mackerel oil	Sardine oil
<C10					10.4		0.1							
C12:0					3.3		0.1							0.1
C14:0		0.9		0.3	11.0	3.3	1.4	0.8	11.5	8.1	4.0	8.4	5.6	7.9
C16:0	11.3	23.4	8.5	7.1	28.7	26.6	20.4	24.0	18.4	11.9	13.3	10.7	15.4	21.0
C16:1		1.1		0.3	2.9	4.1	3.5	7.0	10.9	12.9	5.7	4.4	5.3	11.1
C18:0	3.4	1.9	2.8	4.4	10.9	18.2	10.1	1.0	1.6	1.8	3.8	1.7	3.1	5.4
C18:1	23.1	16.7	14.5	21.1	23.9	41.2	47.5	3.8	21.2	29.5	17.6	7.0	18.7	16.7
C18:2	55.8	56.0	74.2	13.9	1.5	3.3	12.1	1.2	1.8	2.5	1.6	1.3	1.1	3.1
C18:3	6.4			53.0	0.8*2		1.4	0.4	0.6	0.9	0.4			1.2*2
C20:1							2.7		2.5	3.1	12.1	19.7*5	10.8	2.4
C20:4								5.6	2.1*3	0.8*3			0.8	0.6
C20:5								47.5	18.7	10.7	10.2	4.9	8.1	15.8
C22:1										1.7	8.8*4	22.2*4	10.8	0.3
C22:6									9.3	8.8	15.2	10.5	10.6	8.4

*1 Gaschromatographic data by JOCS subcommittee except Linseed and *Porphyra* Oils^{24a)}

*2 Includes C20:0

*4 Includes C20:4

*3 Includes C20:3

*5 Includes C18:3

Finally I would like to comment on the difference of food culture between the American and the Japanese. Characteristically the former consumes a large amount of land animals, while the latter eats more aquatic animals as well as algae. To prevent adult onset diseases it is better to take more fish for the former and to preserve the customs of fish eating nation for the latter. Unfortunately the trade between two countries causes more friction. Now, however, in the culture of feeding marine products from the oceans, available to all countries of the world, our studies on marine lipids will not cause culture shock but contribute towards the welfare of human beings.

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栽培漁業，それによって高EPA水産油脂に 変えられるか？

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日本における水産業の現状を各種統計資料に基づいて、国内的に国際的に解説し、沖合漁業における回遊性の魚は資源の変動が大きく、また海洋新秩序が定着して遠洋漁業は厳しい状況下にあるが、需要の強い高中級魚介類は沿岸水域に多いことから、水産物の安定供給を図るためには生産力の高い沿岸水域の有効利用が従来以上に重視される。このため、従来の獲る漁業に加え、造り育てる漁業の推進が必要になっている。

水産物には人の健康保持に必要なバランスのよいタンパク質、各種ミネラル、ビタミン類を豊富に含むほか、最近になって明らかになった肥満、心筋・脳梗塞等の血栓症、高コレステロール・高中性脂肪血症等の成人病予防に効果のあるエイコサペンタエン酸（EPA）等高度不飽和脂肪酸が含有され、世界的にその評価が高まっている。その故に、増養殖される魚介類等水産物の脂質中EPA含有量を如何にして高めることができるか等について考察した。