ACTES DU PREMIER SYMPOSIUM FRANCO-JAPONAIS

AQUACULTURE

MONTPELLIER DECEMBRE 1983

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"Vie marine" publie des communications relatives à la biologie, l'écologie, l'aquaculture marines, la pollution des mers, l'aquariologie. Elle s'intéresse principalement au domaine méditerranéen mais également aux autres mers, aux lagunes, aux secteurs littoraux de même qu'aux agressions qui s'y manifestent.

La "Revue" annuelle et ses numéros hors série comportent des articles et mémoires originaux, des rapports techniques ainsi que des résumés de thèses et des analyses d'ouvrages. Les articles sont soumis à un comité de lecture dont les membres français et étrangers ont acquis une notoriété dans les domaines considérés.

"Vie marine" est éditée par la Fondation océanographique Ricard, qui se consacre à la recherche fondamentale et finalisée se rapportant au milieu marin et apporte sa contribution à la connaissance de ce dernier par l'information et par la formation. Toutes actions conduites en liaison avec les autres laboratoires marins, les universités, l'Institut français pour l'exploitation de la mer, le ministère de l'Environnement, l'agence de bassin Rhône-Méditerranée-Corse...

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PREMIER SYMPOSIUM FRANCO-JAPONAIS SUR L'AQUACULTURE

REUNIONS SCIENTIFIQUES
A L'UNIVERSITE DES SCIENCES ET TECHNIQUES
DU LANGUEDOC

MONTPELLIER 16 DECEMBRE 1983

Premier symposium franco-japonais sur l'Aquaculture

Ce premier symposium est une conséquence de plusieurs contacts établis depuis déjà plusieurs années entre des chercheurs français et leurs homologues japonais dans le domaine de l'Aquaculture. A la suite de plusieurs voyages de spécialistes aussi bien dans le sens France-Japon que dans le sens Japon-France, des recherches scientifiques communes ont été réalisées, des thèses ont été préparées et soutenues, montrant l'intérêt et la complémentarité de recherches et d'applications dans le domaine des sciences de la mer, et en particulier dans celui de l'élevage d'organismes marins. Plusieurs thèmes d'intérêt commun existent, qui devront donner lieu dans les années futures à des coopérations scientifiques et techniques beaucoup plus structurées et beaucoup plus élaborées. L'un des buts principaux de ce premier symposium franco-japonais d'Aquaculture est de faire un premier inventaire de ces potentialités. Dans un proche avenir, plusieurs autres réunions de ce genre devront se tenir, soit en France, soit au Japon, avec l'aide du Ministère des Affaires Etrangères et celui des grands organismes qui ont en France la responsabilité des recherches et du développement dans le milieu marin.

Nous nous plaisons à souligner l'intérêt et l'aide efficace que les grands organismes ont montré à l'occasion de la mise sur pied de plusieurs symposiums franco-japonais sur l'Aquaculture, et en particulier le Centre National pour l'Exploitation des Océans (CNEXO), l'Institut Scientifique et Technique des Pêches Maritimes (ISTPM) et le Centre National de la Recherche Scientifique (CNRS). Nous tenons à les remercier chaleureusement ici.
Nous remercions également les organisateurs du colloque sur les bases biologiques de l'Aquaculture ainsi que l'Université des Sciences et Techniques du Languedoc à Montpellier pour avoir permis d'intégrer en fin de programme du colloque sur les bases biologiques de l'Aquaculture, ce premier symposium franco-japonais sur l'Aquaculture.

Le Comité d'Organisation.

PREMIER SYMPOSIUM FRANCO-JAPONAIS SUR L'AQUACULTURE

De nombreux pays s'intéressent de plus en plus à l'aquaculture, principalement en raison de la diminution des prises mondiales de la pêche, et d'autre part parce que la demande pour les produits de la mer augmente d'année en année dans la consommation humaine. Certains pays d'Amérique latine comme l'Equateur, ou certains pays nordiques comme la Norvège ont obtenu et obtiennent dans ce domaine des succès significatifs.

L'aquaculture - ou aquiculture - au Japon et en France connaît des destinées et des évolutions différentes. Les objectifs des deux pays sont loin d'être identiques. Le Japon n'a guère de ressources naturelles et sa production agro-alimentaire nationale demeure faible. Ses habitudes culinaires sont très marquées par l'origine marine de l'alimentation de ses habitants. La grande majorité des aliments consommés au Japon proviennent historiquement et culturellement de la pêche, par voie directe ou indirecte. La France est au contraire une puissance agro-alimentaire de niveau mondial, qui exporte ses productions en grande partie vers les pays en voie de développement et vers les pays à économie planifiée, ainsi que des produits alimentaires plus élaborés vers les pays développés.

L'aquaculture vise donc à compléter les ressources alimentaires de base au Japon, pays qui demeure le premier pays du monde pour la pêche, avec un tonnage supérieur à 10,5 millions de tonnes en 1982, le premier pays consommateur de produits de la mer du monde, et qui est parmi les tout premiers pays importateurs du monde pour les produits de la pêche.

La production française des produits de la mer vise à couvrir les besoins d'un marché plus marginal, destiné à des consommateurs recherchant des produits rares et chers, de consommation souvent saisonnière, comme le saumon, les huîtres, le homard, les crevettes.

Pourtant l'aquaculture traditionnelle joue en France vis-à-vis de la pêche un rôle de tout premier plan, car elle représente une production qui équivaut à 30 % environ de la production de la pêche : 150.000 t d'huîtres et moules, pour 510.000 t de produits de la mer provenant de la pêche.

L'aquaculture française induit 20.000 emplois directs environ, alors que les effectifs des pêcheurs est de l'ordre de 25.000. La France est donc, en aquaculture, un grand pays producteur.

Il est remarquable de noter que le développement de l'aquaculture nouvelle s'est effectuée dans les deux pays à partir d'un marché porteur et particulier, celui de produits chers occupant une place bien déterminée sur le marché, et visant à satisfaire en priorité une clientèle pourvue de moyens financiers convenables. Le loup, la daurade, les poissons plats, les crevettes, les huîtres perlières, les tortues par exemple constituent des exemples typiques de ce phénomène.

Le Japon est en aquaculture, un pays producteur de tout premier plan, dont la production, algues, invertébrés et poissons, dépasse 900.000 t. Par rapport à la consommation, l'aquaculture japonaise ne couvre que 8,5 % des besoins. La France est, de ce point de vue, proportionnellement beaucoup plus productrice en aquaculture que le Japon.

Les Japonais ont développé dans leur pays des élevages de grandes dimensions afin d'acquérir directement une grande expérience en vraie grandeur, de façon pragmatique, et suivant leur logique extrêmement réaliste.

Les Français, plus cartésiens, plus logiques, plus imprégnés des principes préfèrent poursuivre des recherches fondamentales et des petites expériences destinées à évaluer la faisabilité, avant de passer à la mise sur pied de productions de grandes dimensions.

Les échanges entre le France et le Japon dans le domaine de l'aquaculture moderne sont donc inspirés par la nécessité de réussir à élever des espèces nouvelles, qui seront domestiquées dans un avenir proche, par la nécessité d'améliorer les technologies et la productivité de l'aquaculture de production, par la nécessité enfin d'élever des espèces tropicales afin de pouvoir aider les pays en développement des zones subtropicales de la planète à résoudre une partie au moins de leur déficit alimentaire et particulièrement protéique. La France s'y appuiera pour développer des activités intéressantes dans les Départements et Territoires d'Outre Mer ; le Japon en a également besoin, aussi bien pour ses territoires méridionaux que pour compléter et fortifier sa politique économique extérieure vers les pays de climat chaud.

La confrontation des deux groupes de spécialistes à la faveur du Colloque sur les Bases biologiques de l'Aquaculture de Montpellier, doit permettre à chacun d'eux d'apprécier les résultats, les méthodes et les techniques employées dans chacun des deux pays. Les comparaisons que n'ont pas manqué de faire les participants n'apporteront pas, dans la plupart des cas, de solutions immédiates à leurs problèmes concrets ou à la structure de leur méthodologie. Cette confrontation amènera néanmoins une réflexion profonde sur le travail de recherche et de développement dans chacune des disciplines liées à l'aquaculture.

Le but final de ce premier Symposium franco-japonais sur l'Aquaculture est modeste mais bien ciblé. Il doit permettre à d'excellents spécialistes japonais de prendre un contact direct et personnel avec leurs homologues français, mais surtout, il doit permettre de concrétiser de façon plus formelle les relations déjà établies entre laboratoires ou entre chercheurs et marquer une nouvelle étape dans le niveau de ces échanges.

Les organisateurs, tant du côté japonais que du côté français ont le ferme espoir de pouvoir susciter périodiquement des réunions franco-japonaises sur l'aquaculture. Chaque chercheur, chaque organisme de recherche, chacun des deux pays y trouvera des avantages évidents.

De tels contacts permettront sans doute aux chercheurs français d'une part, et surtout aux organismes, publics et privés, dans lesquels ils sont intégrés, de prendre une pleine conscience du rôle que chacun d'eux doit jouer, à court et à moyen terme.

Aussi faut-il s'attendre, dans les prochaines années à venir, à voir s'organiser des réunions scientifiques consacrées à l'aquaculture, successivement dans chacun des deux pays.

Le prochain Symposium franco-japonais sur l'aquaculture est d'ores et déjà programmé pour l'automne 1984 et il se tiendra à Sendai, à la faveur de la réunion annuelle de la Société japonaise de Pêcheries scientifiques (Japanese Society of Scientific Fisheries).

Les autres réunions franco-japonaises se tiendront dans un avenir un peu plus éloigné, mais elles constitueront la base de toute la programmation des échanges dans ce domaine entre les deux pays.

Nous remercions tout particulièrement nos collègues japonais qui ont effectué un long voyage pour participer à ce Symposium et pour en faire un succès, tant sur le plan scientifique que sur celui de l'intérêt qu'il a suscité chez tous les participants en particulier français, qui ont été très nombreux à assister au Symposium.

Notre reconnaissance va donc à nos collègues et amis :

- Le Dr Kenji CHIBA, Université de Tokyo, Faculté d'Agriculture, Laboratoire d'Océanographie des pêches (Laboratory of Fisheries Oceanography) Tokyo.
- Le Pr Akio KANAZAWA, Université de Kagoshima, Faculté des Pêches, Laboratoire de chimie de la nutrition, (Laboratory of Nutritional Chemistry), Kagoshima, Kagoshima Ken.
- Le Dr Jiro KITTAKA, de l'Université Kitasato, Ecole des Sciences des pêches (School of Fisheries Sciences), Sanriku, Iwate Ken.
- Le Dr Satoru TODA, de l'Institut national d'ingéniérie des Pêches, (National Research Institute of Fisheries Engineering), Hasaki, Kashima, Ibaraki ken.
- Le Dr Takuya WAKUI, du Laboratoire de recherches sur les pêcheries Tokai, (Tokai Fisheries Research Laboratory) de Tokyo.
- Le Dr Hiroki YAGI, de l'Université des Pêches de Tokyo (Tokyo University of Fisheries) et du Laboratoire EPHE du Centre d'Océanologie de Marseille (LA CNRS N°41).
- Le Dr Katsumi YAMAGUCHI, Université de Tokyo, Faculté d'Agriculture, Laboratoire de Biochimie marine, (Laboratory of marine biochemistry), Tokyo.

Nos remerciements vont également à M. le Professeur Fumio MATSU-URA Président de l'Université KITASATO, ainsi qu'à M. le Professeur Reijiro HIRANO Directeur du Laboratoire d'Océanographie des Pêches à la Faculté des Pêches de l'Université de TOKYO, qui ont tous deux aidé, depuis le Japon, à l'organisation de ce Premier Symposium sur l'Aquaculture de Montpellier.

Enfin nos remerciements vont au CNRS et au CNEXO, dont le soutien financier a permis d'accueillir nos collègues japonais dans de bonnes conditions, et de faire en sorte que ce Symposium soit un succès.

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Directeur de Laboratoire
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Co-organisateur du Premier Symposium
franco-japonais sur l'Aquaculture

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Montpellier 16 Décembre 1983

INTRODUCTION :
Le pourquoi et le comment de la coopération scientifique entre la France et le Japon dans le domaine de l'aquaculture

INTRODUCTION:
The whys and wherefores of scientific cooperation between Japan and France in the field of aquaculture

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It is often said that science has no boundaries and that there ought to be a continual exchange of scientific ideas and information among the researchers of different countries. However, science today is directly related with the economic interest of nations so that the ideas and results of civilian researchers are selectively diffused, and many results which could be developed technologically are patented. This protectionism limits exchange and the extent of scientific cooperation among countries. Can scientific cooperation be efficient and on what basis?

There are many reasons for establishing fruitful relations among nations which will benefit all concerned.

1) Methods and techniques must be standardized. A counting technique or hormone assay should give comparable results whether carried out in Japan, France or Canada, for example. This supposes an exchange of standards and of researchers, and is a good example of multinational cooperation. Also various laboratories working on the same problem should use well-defined, homogeneous animal or plant material. Other laboratories should be able to experiment on this same material if it appears that observed differences arise due to live material. The laboratories could then exchange this live material. The paper of PRUNET and HIRANO illustrates this type of cooperation in standardization.

2) Another reason for cooperation is the complementarity among researchers, laboratories and institutes: (a) complementarity of techniques and their evaluations, permitting a research to advance; (b) complementarities between biological material, e.g. species such as chum salmon with early smoltification

could be compared to species such as Atlantic salmon which has later smoltification; (c) complementarities among laboratories of different countries as to equipment and experimental installations.

3) Training and apprenticeship would also profit from cooperative programs. Young researchers and technicians could go to a country to learn laboratory or rearing techniques. Franco-Japanese exchange has probably been most fruitful in the latter field, as shown by the presence of young Japanese at this symposium who are actually in French laboratories and the presence of many French who have already been in Japanese laboratories.

These different types of cooperation are organized in various ways within the framework of Franco-Japanese cooperation.

1) A simple visit to laboratories, hatcheries or aquacultures is the occasion for selective exchange of results and information. It is also a chance for a researcher to present his results and developments for criticism and comment by his colleagues. This is a contact, perhaps the only one, but which could lead to identification of complementarities and projects in common. There are already many exchanges of this type between France and Japan, such as the visits of Dr. YAMAGUICHI and Dr. CHIBA which are presented below. It would be preferable that all French researchers working in aquaculture, and who wish to visit their Japanese colleagues, be able to do so.

2) A definite program can be developed between two or more laboratories, most of the research being carried out in the laboratories of the two countries with brief exchanges of researchers or in one of the countries with the foreign researcher staying over a long period. Several Japanese researchers have stayed in France.

What is needed for successful international scientific cooperation? It should benefit all parties concerned, its framework and aims being clearly defined as well as its program. Such cooperation should be given sufficient financial support, also taking into consideration moving the family in the case of a long stay.

Successful cooperation is measured by the publication of results in good technical or scientific papers. It is also measured by the continuity of relations, and the creation of a Franco-Japanese Society of Aquaculture is proof of the fruitful type of cooperation existing between the two countries for several years.

1er SYMPOSIUM FRANCO-JAPONAIS SUR L'AQUACULTURE
Montpellier 16 Décembre 1983

Pigmentation de la sériole et de la daurade rose élevées en aquaculture à l'aide de caroténoïdes extraits de krill antarctique

Pigmentation of cultured yellowtail and red sea bream with carotenoids extracted from the antarctic krill

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Yellowtail and red sea bream are the most important cultured fishes in Japan. Their productions in 1981 reached 151,000 tons and 18,000 tons, respectively, tending to increase year by year. Today, low-priced fishes such as sand lance *Ammodytes personatus*, anchovy, *Engraulis japonica*, sardine *Sardinops melanosticta* and mackerel *Scomber japonicus* are usually employed to feed cultured fishes. The employment of such raw feeds, however, brings serious organic pollution in fish farms, which is one of the problems in aquaculture. Therefore, there is a general trend toward formulated feeds, but the sole use of carotenoid-free formulated feeds inevitably induces fading of animals and according results in reduction of their commercial value. Such a situation prompted us to develop a pigmenter which is stable and can be incorporated into any formulated feed at a controllable level. For this purpose the Antarctic

krill *Euphausia superba* was thought to be promising as a source of carotenoids, because of the high contents of carotenoids[1-6] and the well-known enormous stock in the Antarctic Ocean. This paper describes preparation of carotenoid oil from krill and pigmentation of cultured yellowtail and red sea bream through feeding experiments with formulated diets supplemented with the carotenoid oil. Bioconversion of dietary carotenoids into integumentary ones in yellowtail and red sea bream is also discussed.

Preparation of carotenoid oil

Oil was extracted and purified from air-dried meal of the Antarctic krill by the procedure reported previously[7,8]. The krill oil obtained was fluid and dark red in color. Its total carotenoid content varied from 100 to 200 mg/100g oil depending on preparation and the carotenoids comprised 85 % of astaxanthin diester and 15 % of unidentified ones which were largely composed of polymerized astaxanthin di-and monoesters. This krill oil was employed in part for the pigmentation experiment of cultured red sea bream.

To get rid of the unidentified, the purification was carried out by the method reported elsewhere[8]. The purified oil contained only astaxanthin diester in the range of 2-10 g/100g oil in terms of astaxanthin depending on preparation. This was used as astaxanthin diester oil in the pigmentation experiments.

Pigmentation of cultured yellowtail[8)]

The composition of test diets used in the feeding experiment is shown in Table 1. No carotenoids were added to the control

Table 1. Composition of test diets in the feeding experiment of cultured yellowtail

(%)

Ingredients	Diet No. Control	1	2	3
Fish meal	64	64	64	Sand lance
Gluten	10	10	10	
Dextrin	6	6	6	
Skipjack oil	9.1	8.9	8.5	
Vitamin mixture	3	3	3	
Mineral mixture	2.5	2.5	2.5	
CMC	2	2	2	
Ethoxyquin	0.03	0.03	0.03	
Gallic acids	0.37	0.37	0.37	
Protease	1	1	1	
Cellulose	2	2	2	
Astaxanthin diester oil		0.2	0.6	

diet. Diets No.1 and 2 were supplemented with the astaxanthin diester oil in weight percentages of 0.2 and 0.6 % which corresponded to 4.1 and 12.3 mg/100g diet in terms of astaxanthin, respectively. Sand lance which is a small marine fish commonly used for feeding yellowtail and is effective in the pigmentation was employed for comparison as Diet No.3.

Yellowtails reared to average body weight of 225g with sand lance and then with the control diet were divided into four groups and maintained in floating nets(2.5×2.5×3 m) settled at the embayment near Kōchi Prefectural Fisheries Experimental Station. The fish were fed the test diets twice a day for 70 days. On the 33rd and 70th days three fish were sampled from each group, their average body weights being 414 and 668 g, respectively. They were frozen immediately and stored below -20°C until analyzed.

The whole integuments of each fish were removed except those of the head and tail, and divided into two parts; the dorsal one including the characteristic yellow band near the lateral line and the ventral one. Extraction and analysis of the integumentary carotenoids were carried out according to the methods reported in the previous papers[6-10].

The fish in the control group exhibited poor dark grey at the back and sides as expected. In contrast, the fish fed the diets supplemented with the astaxanthin diester oil (Diets No.1 and 2) assumed the natural color of iridescent blue-green at the back and sides with the characteristic yellow band near the lateral line and yellowish tint at the point of fins and tail. The degree of pigmentation was intensified with increase of the astaxanthin diester oil supplemented, being superior to the fish fed sand lance (Diet No.3).

The average contents of carotenoids in the integuments of three test fish of each group are given in Table 2. In every group the carotenoids were distributed much more in the dorsal part than in the ventral one. In accordance

with the appearance of the fish the contents of carotenoids in the dorsal integuments of the test fish fed diets supplemented with carotenoids were three to five times those of the control group and even higher than those of the fish fed sand lance. It is expected that the content of carotenoids in the integument of test fish fed diet containing carotenoids should increase with growth till a stationary phase. However, Table 2 shows a tendency of slight decrease in the carotenoid contents in some groups at the 70-day stage, when compared with those at the 33-day stage. The

Table 2. Content of carotenoids in the integuments of test fish in the feeding experiment of cultured yellowtail

(mg/100g)

Feeding period and Integument part	Diet No. Control	1	2	3
33 days				
Dorsal	1.08	4.20	5.08	2.17
Ventral	0.13	0.38	0.38	0.18
70 days				
Dorsal	1.06	4.14	4.69	3.14
Ventral	0.20	0.41	0.50	0.30

Figures are means of three test fish.

reason for this phenomenon is not clear but one of the reasons may be that the integuments at the 70-day stage is considered to have become thicker than those at the 33-day stage, which could cause the apparent reduction in the content expressed in terms of mg/100 g integument.

Compositions of carotenoids in the integuments of the test fish at the 70-day stage are shown in Table 3. A

Table 3. Composition of carotenoids in the dorsal integuments of test fish at 70-day stage in the feeding experiment of cultured yellowtail

(%)

	Diet No.			
	Control	1	2	3
Tunaxanthin	56-63	64-78	67-73	58-69
3'-Epilutein	19-23	14-17	16-18	19-23
Lutein	+	+	+	+
Zeaxanthin	6-8	5-6	3-5	6-8
Diatoxanthin	4	2	2	3-4
Cynthiaxanthin	2-3	1-2	1-2	2
β-Carotene triol	6-7	4-5	5-6	1-2

+; trace.

common feature was recognized throughout every group in that all the carotenoids were yellow ones and that tunaxanthin was the major component comprising more than 50 % of the total, followed by 3'-epilutein. Furthermore, there were practically no differences in the carotenoid composition between the 33-day and the 70-day stages of each group. Striking differences, however, were recognized when the carotenoid composition was expressed not by percentage but by actual contents of each carotenoid calculated from the

data in Tables 2 and 3, as shown in Fig. 1 for the control and Diet No.2 groups. In diet No.2 group the contents of tunaxanthin, 3'-epilutein and β-carotene triol were increased conspicuously, that of zeaxanthin was somewhat increased, whereas those of diatoxanthin and cynthiaxanthin remained almost unchanged, when compared with those of the control group. These results suggest that, in yellowtail the

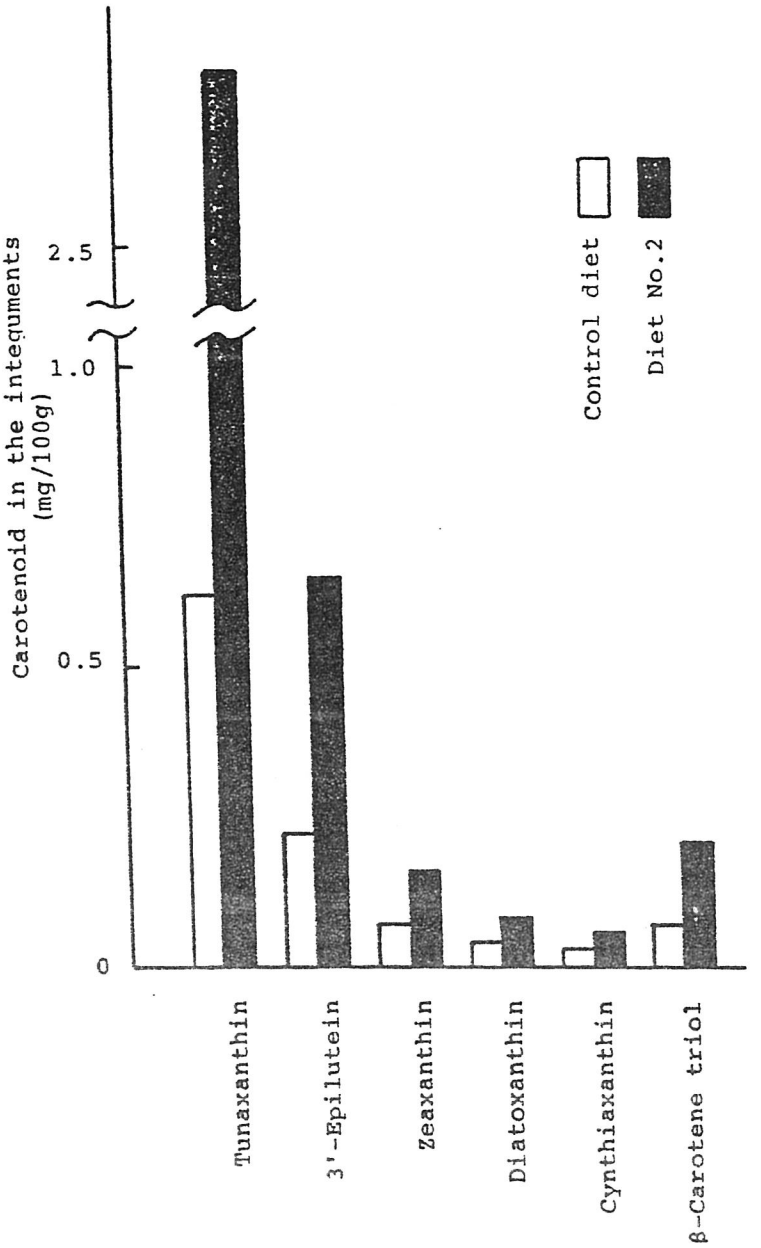

Fig. 1. Content of each carotenoid in the dorsal integuments after astaxanthin diester oil supplement (Diet No.2 group) for 70 days in the feeding experiment of cultured yellowtail.

dietary astaxanthin, a red carotenoid, should be metabolized to tunaxanthin, the major integumentary yellow carotenoid of this fish, via β-carotene triol, zeaxanthin, 3'-epilutein, and indicate that the pigmentation can be attained by feeding a diet supplemented with astaxanthin at a level of 4 mg/100g diet for about 1 month.

Pigmentation of cultured red sea bream[11)]

The composition of test diets used in the feeding experiment is shown in Table 4. No carotenoids were added to the control diet. Diets No.1-3 were supplemented with the astaxanthin diester oil in weight percentages of 0.0015, 0.015 and 0.15 % which corresponded to 0.13, 1.3 and 13 mg/100g diet in terms of astaxanthin, respectively. Diet No.4 was supplemented with the krill oil in a weight percentage of 5 % which corresponded to 6.8 mg/100g in terms of

Table 4. Composition of test diets in the feeding experiment of cultured red sea bream

(%)

	Diet No.					
Ingredients	Control	1	2	3	4	5
Fish meal	67	67	67	67	67	
α-Starch	15	15	15	15	15	
Mineral mixture	5	5	5	5	5	
Vitamin mixture	2	2	2	2	2	
Choline chloride	1	1	1	1	1	
Cuttlefish oil	4	4	4	4		
Cellulose	6	6	6	6	5	
Astaxanthin diester oil		0.0015	0.015	0.15		
Krill oil					5	
Frozen krill:control diet(2:3 w/w)						

astaxanthin. Frozen krill which is effective in the pigmentation was fed as a mixture with the control diet in a ratio of 2:3 for comparison as Diet No.5 which contained 1.3 mg/100g diet in terms of astaxanthin[6]. Red sea bream reared with the control diet to 150 g in average body weight were divided into six groups and maintained in 1-ton tanks to which sea water was continuously supplied. The fish were fed the test diets twice a day for 56 days. On the 28th and 56th days three fish were sampled from each group, their body weights being approximately 200 and 250 g on the average, respectively. They were frozen immediately and stored below -20°C until analyzed. Three natural red sea bream of 920-1170 g in body weight were also analyzed for comparison. The whole integuments of each fish were removed except those of the head and tail. Extraction and analysis of the integumentary carotenoids were carried out as reported elsewhere[11].

The fish in the control group exhibited poor dark grey with almost no red tint, looking like black sea bream. In contrast, the fish receiving the astaxanthin diester oil (Diets No.1-3) showed a tendency to assume the bright red color already at the 28-day stage. The degree of pigmentation was intensified with the increasing content of the astaxanthin diester oil in the diets, being superior to the fish receiving the krill oil and frozen krill (Diets No.4 and 5) at the 56-day stage.

The average contents of carotenoids in the integuments of three test fish of each group are given in Table 5. The contents of integumentary carotenoids increased as the

Table 5. Content of carotenoids in the integuments of test fish in the feeding experiment of cultured red sea bream

(mg/100g)

Feeding period	Diet No.					
	Control	1	2	3	4	5
28 days	0.25	0.33	0.56	0.73	0.93	0.96
56 days	0.28	0.46	1.29	1.72	1.06	0.98

Figures are means of three test fish.

amount of dietary astaxanthin diester oil increased, being consistent with the appearance of fish as remarked above. As expected from many papers[12-26], the integumentary carotenoids were composed of red and yellow ones. Their compositions in the integuments of test fish at the 56-day stage are shown in Table 6. Apart from some differences in the composition of minor carotenoids, the results obtained were in accord with those reported so far[12-26] in that major components are astaxanthin and tunaxanthin. In the control group the sum of relative percentages of yellow carotenoids far exceeded that of red ones. A similar feature was found in the group of Diet No.1 but the relative percentages between yellow and red carotenoids were reversed in the fish receiving Diets No.2 and 3. This situation was illustrated more evidently when contents of yellow and red carotenoids in the integuments calculated from the data in Table 5 and 6 were compared, as shown in Fig.2, in which the result of analysis for the integumentary carotenoids of the natural red sea bream is also given for comparison.

Table 6. Composition of carotenoids in the integuments of the test fish at the 56-day stage in the feeding experiment of cultured red sea bream

(%)

		Diet No.					
		Control	1	2	3	4	5
Red carotenoids	Astaxanthin diester	24	33	48	53	42	40
	Astaxanthin monoester	1	1	3	4	5	5
	Unidentified*	2	1	2	1	2	3
Yellow carotenoids	Tunaxanthin	26	34	25	22	20	24
	3′-Epilutein	12	14	10	9	8	11
	Lutein	+	+	+	+	+	+
	Zeaxanthin	16	8	6	5	6	7
	Diatoxanthin / Cynthiaxanthin	14	3	2	2	11	7
	β-Carotene triol	5	7	5	4	6	3

* Largely composed of retro carotenoid esters reported in the previous paper[6].

The pigmentation of test fish receiving Diet No.2 was almost comparable to that of the natural fish, being superior to those receiving Diets No.4 and 5. Over-pigmentation was found in the test fish receiving Diet No.3. These results indicate that the carotenoid pigmentation of cultured red sea bream is able to be satisfactorily attained at a level of 1-2 mg astaxanthin/100 g diet in about 50 days when astaxanthin diester oil is employed. It was also confirmed in Diet No.4 group that the krill oil which had been employed as raw materials for the preparation of astaxanthin diester oil was effective though somewhat inferior to the astaxanthin diester oil. Taking it into consideration

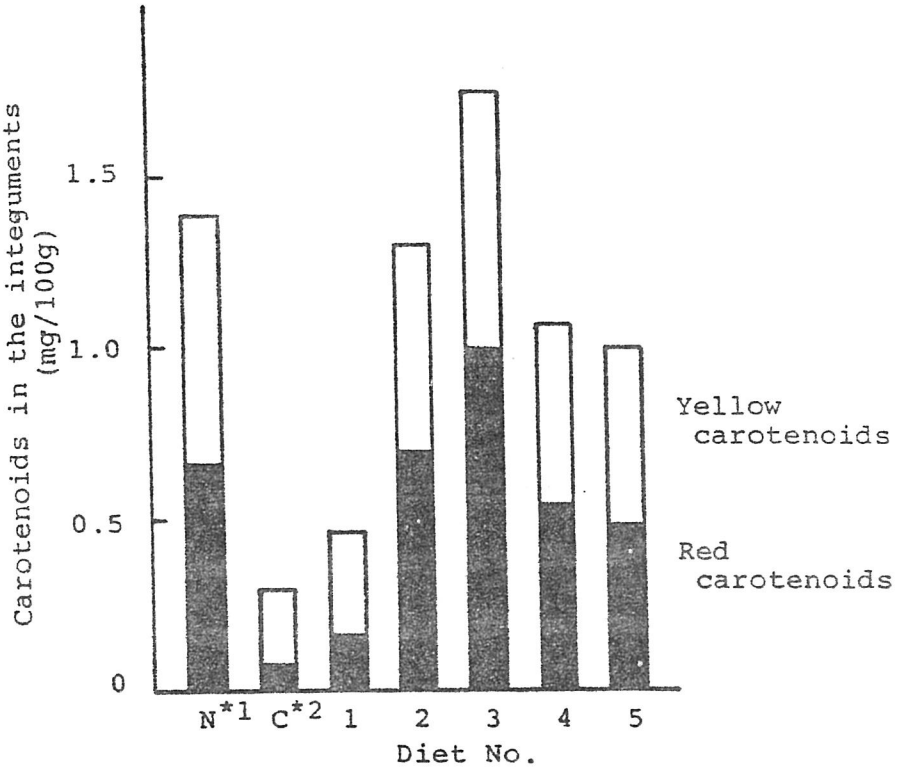

Fig. 2. Content of yellow and red carotenoids in the integuments of each test group at the 56-day stage in the feeding experiment of cultured red sea bream.

*1 Natural red sea bream.

*2 Control group.

that krill oil is applicable to most formulated feeds like astaxanthin diester oil and that the former has an economical advantage over the latter, the employment of krill oil without further purification appears to be practical. The inferiority of the krill oil in the pigmentation is probably due to the quality of krill oil used in the present experiment because it was heavily contaminated with ineffective polymerized astaxanthin esters which had been formed during extraction. In this connection, efforts were made to improve the quality of krill oil and an excellent product has successfully been manufactured[26]. Further pigmentation experiments for

cultured red sea bream with the improved krill oil are presently under way.

Fig.3 shows the comparison of contents of individual carotenoid in the feeding experiment at the 56-day stage between the control and Diet No.3 group, the latter receiving the astaxanthin diester oil at the highest level. It is evident that the major part of astaxanthin diester in the diet was deposited as the red carotenoids which were composed mainly of astaxanthin di- and monoesters and that

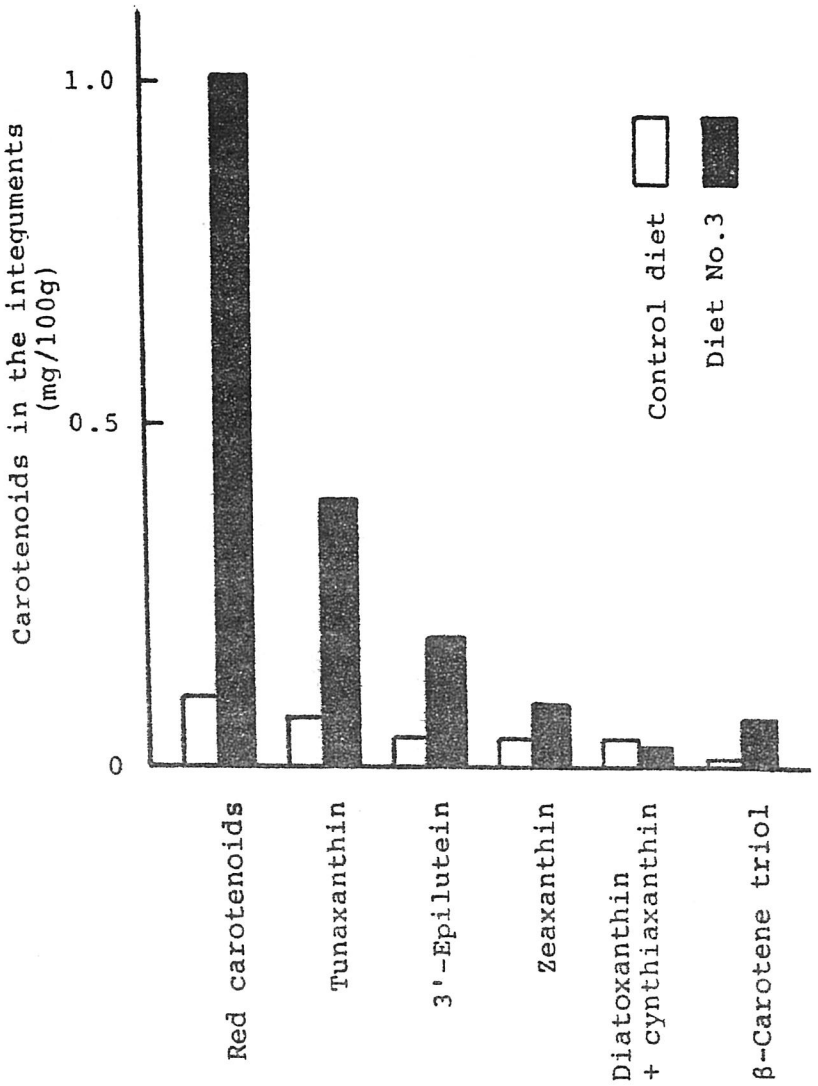

Fig. 3. Composition and content of each carotenoid in the integuments of fish receiving the control diet and Diet No.3 at the 56-day stage in the feeding experiment of cultured red sea bream.

some part of the dietary astaxanthin diester was converted to β-carotene triol, zeaxanthin, 3'-epilutein and tunaxanthin but not to diatoxanthin and cynthiaxanthin. This feature was commonly noticed in the fish receiving the other test diets.

Metabolism of dietary carotenoids to integumentary ones in fish

The integumentary carotenoid compositions of the pigmented cultured yellowtail and red sea bream were almost identical except the presence of astaxanthin esters in the latter. In both species tunaxanthin was observed as one of major components. In spite of widespread occurrence of tunaxanthin in marine fishes[27-34], its origin has remained unsolved. It has been suggested that the origin of tunaxanthin might be ε-carotene[28,34] which is detected in some species of algae, but the concept is problematical becanse such a metabolic intermediate from ε-carotene to tunaxanthin as ε-carotene monol has not yet been found in fish as well as in algae. In the present feeding experiments, however, dietary astaxanthin was found to have been metabolized to tunaxanthin in both yellowtail and red sea bream probably via β-carotene triol, zeaxanthin and 3'-epilutein as shown in Fig.4. This finding indicates that astaxanthin which is distributed widely in marine animals could be one of the sources of tunaxanthin. In this connection, it has been reported very recently that dietary astaxanthin was converted to tunaxanthin in a freshwater fish *Tilapia nilotica*[35].

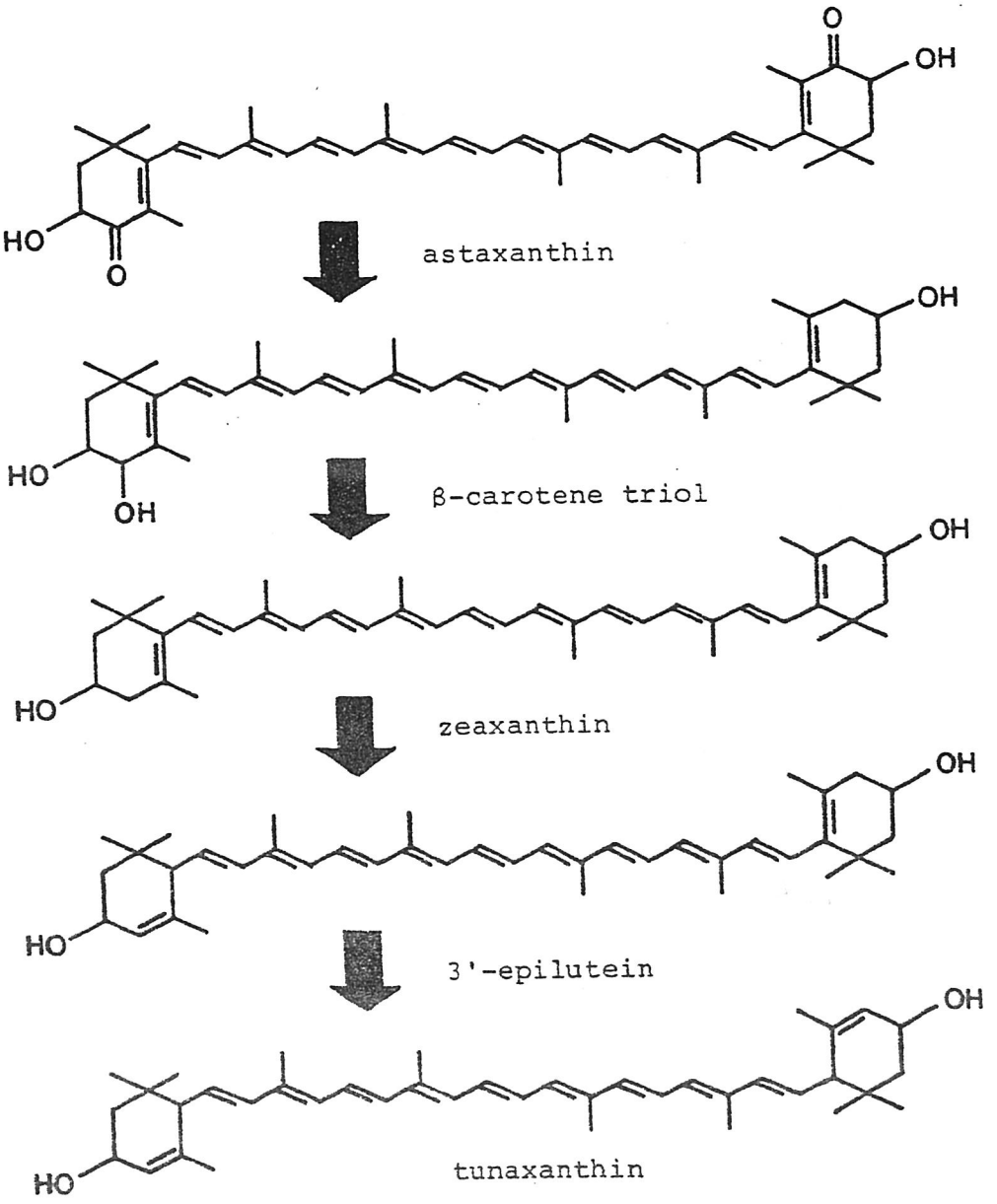

Fig. 4. Presumed metabolic pathway from astaxanthin to tunaxanthin in yellowtail and red sea bream.

Now we have proved that the krill oil as well as the astaxanthin diester oil are effective in the pigmentation of cultured yellowtail and red sea bream. Although both oil can be incorporated into any formulated feed at a controllable level, the krill oil is preferable for practical purposes because it is manufactured at a cheaper cost.

In connection with this, the application of krill oil is being attempted for pigmentation of the flesh of salmonids, rainbow trout *Salmo gairdneri*[36] and coho salmon *Oncorhynchus kisutch*[37]. It can be seen in both species that astaxanthin in the krill oil is readily absorbed and the flesh takes on a red hue. Synthesized canthaxanthin has generally been used for the pigmentation of salmonids but the hue is redder than salmon color[38]. In addition, there is a general trend today toward natural food and feed ingredients. Since the krill oil is a natural pigmenter from edible and harmless sources, it may take the place of canthaxanthin in the pigmentation of salmonids as well.

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