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Improvement of Dietary Value of Brine Shrimp Artemia salina for Fish

Larvae by Feeding Them on w3 Highly Unsaturated Fatty Acids*1

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Experiments were conducted in order to improve the dietary value for marine fish juveniles
of Artemia nauplii of the freshwater type, high in 18: 3w3, by allowing them to feed directly on w3 HUFA. In this method lipids containing w3 HUFA were given directly to Artemia nauplii by homogenizing lipid with a small amount of raw egg yolk and water, together with baker's yeast; an emulsion resulted. The dietary value of the nauplii for juveniles of flounder, rock sea bream and red sea bream was compared for fish fed on various kinds of emulsified lipids or on baker's yeast.
The newly-hatched nauplii were found to take up lipids very easily by this method. Feeding on the newly-hatched nauplii of the freshwater type or on those fed respectively baker's yeast and corn oil resulted in low growth and survival in all fish species used and in addition the rate of normal fish in the activity test was especially low. The dietary value of the nauplii was found to
be effectively improved by feeding them lipids containing high amounts of w3 HUFA such as cuttlefish liver oil, demonstrating that the class of EFA contained in Artemia is the principal factor
in the food value of Artemia nauplii to fish.
Artemia eggs and nauplii from different locations (California, Brazil, Australia and China), were also analyzed for fatty acids in order to compare their food values to fish.

The nauplii of Artemia salina have been widely
used as a food in rearing marine fish larvae and
crustaceans. However, it has been observed that a single dosage of Artemia nauplii frequently
resulted in a severe lethargy of the larvae, and a high mortality was observed in various kinds of marine fish after they were fed on nauplii for a period of 1 or 2 weeks, 1-3) although this depended upon the fish species as well as the place of pro
duction of Atremia. Some species of flounder,
one species of mullet Liza haematocheila, one
salmonoid Plecogrossus altivelis, and some gobiid
fish were not easily affected, but in contrast the larvae of the yellowtail Seliola quinqueradiata were very susceptible to this phenomenon.3.4) In
addition, many investigators have reported a
heavy loss of larvae of prawns, crabs, and marine fish fed on Artemia nauplii from Utah.5-9) BOOKHOUT and COSTLOW8) reported that the difference in mortality and survival rate of the developmental stages of four crab species may be
attributed to the difference of the DDT content

in the Artemia nauplii from California and Utah. DDT was reported to be approximately three times higher in the latter. WICICENS9) mentioned that Artemia eggs and nauplii from San Francisco and Utah were analyzed for the presence of pesticides, heavy metals, carotenoids, sterols, and fatty acids. Although some difference were found, none of them could be confidently labeled as the cause of the poor nutritional value of the Utah Artemia nauplii.
Recently WATANABE et a1.10) have demonstrated that Artemia obtaihed from different locations could be classified into two types by the fatty acid composition; one (the freshwater type) contained a high proportion of 18: 3w3, which is the essential fatty acid (EFA) for freshwater fish; the other (the marine type) had a high content of 20: 5w3, which is one of the EFA for marine fish. Further
more, Artemia of the marine type were found to
be satisfactory as a food for juvenile red sea bream,
Pagrus major (TEMMINCK and SCHLEGEL), and the
dietary value of the nauplii was improved by feed

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ing them marine Chlorella and baker's yeast supple
mented with cuttlefish liver oil (w-Yeast).11
Both contain substantial amounts of the EFA
required by marine fish.12) These results suggested
that the class of EFA contained in Artemia is
the principal factor in the food value of Artemia to fish.
Later experiments by WATANABE et a1.13) have clarified the relationship between the dietary value of Artemia and their content of W3 highly un saturated fatty acids (w3 HUFA), particularly 20: 5w3 and 22: 6w3.
This study was conducted in order to improve the dietary value for marine larval fish of Artemia nauplii of the freshwater type by allowing the nauplii to feed on w3 HUFA by the direct method. In this method, in contrast with the indirect method using w-Yeast, lipids containing W3 HUFA are given directly to Artemia nauplii by homo genizing lipid with samll amount of raw egg yolk and water, resulting in a stable emulsion, which is fed together with bakers' yeast. Artemia eggs and nauplii from different locations were also analyzed for fatty acids in order to compare their food value to fish.

Materials and Methods

Artemia sauna
The eggs and nauplii of Artemia sauna obtained
from California (San Francisco), Brazil, Australia,
and China (Tien-tsin) during 1979-1981 were
analyzed for fatty acids by the methods described
previously.14-18) Artemia cysts were well ground
in a glass mortar and pestle and extracted twice
with a 20-fold volume of chloroform: methanol
(2:1).17)
As a preliminary step, the fatty acid composi
tions of three lots of eggs from San Francisco
were checked by GLC, and those Artemia eggs
containing a high proportion of 18:3w3 were
hatched in vigorously aerated sea water in a 100 1
tank held at 24-26°C in all the experiments.
After 2 days the nauplii which had hatched were
separated and were then washed in clean sea water.

Feeding of Artemia with Lipids by the Direct Method
The newly-hatched nauplii were cultured in 30L
tanks containing filtered sea water at 24-26•Ž,
•@ aerated vigorously, and fed respectively with
various kinds of emulsified lipids once a day.
In this method 1.5g of lipid was emulsified with

0.3g of raw egg yolk and 20ml of sea water by
homogenizing with a mixer for 3min, and was
given directly to Artemia nauplii in a 30L tank, together with the same weight of baker's yeast as the nauplii in the tank. After a 15-19h feeding they were washed with clean water before feeding to fish.*

Feeding of Fish Larvae with Artemia

Feeding experiments were conducted four times
(Experiments I, ‡U, ‡V and ‡W) to compare the
dietary value of Artemia nauplii fed with or without
emulsified lipids to juveniles of flounder Paralich
thys olivaceus, rock sea bream Oplegnathusfasciatus
and red sea bream, at the Aquaculture Reasearch
Laboratory of the Nagasaki Prefectural Institute
of Fisheries.

1. Experiment I (Flounder) The juveniles of
7.06mm in body length, which had been fed on
rotifers Brachionus plicatilis cultured with both
marine Chlorella and w-Yeast, were randomly
divided into 3 lots of 500 fish each in a 100L tank.
Each experimental group was fed with one variety
of the nauplii fed for 18-19h on the emulsified
cuttlefish liver oil, or corn oil, or those without
treatment (Control), respectively for 19 days at
water temperature of 12.6-21.4•Ž (average 16.9•Ž)

under conditions of aeration at 300ml/min. The
amount•@ of water supplied to each tank was 400-
500ml/min and half of the water in each tank was
changed daily for removal of residual nauplii.
2. Experiment ‡U (Rock sea bream) The
juvenile rock sea bream, 9.7mm in body length
and 9.0mg in body weight, which had been fed
on rotifers, Tigriopus sp. and minced fish meat,
were divided into 5 lots of 100 fish each in 30L
tanks. Each lot was then fed respectively on one
variety of the nauplii cultured separately for 15-19
h with (W-Yeast, with baker's yeast, or with an
emulsion of cuttlefish liver oil plus baker's yeast,
or the nauplii without feeding (Control), or
Tigriopus, for 10 days at water temperature of 23.1-
24.9•Ž under conditions of aeration at 200ml/
min. The amount of water supplied to each
tank was 160ml/min. The amount of the nauplii
and Tigriopus given to the fish was 7.2g and
3.2g per day, respectively.
3. Experiment ‡V and ‡W (red sea bream)
Feeding experiments were conducted twice (Expts. ‡V
and ‡W) by using red sea bream juveniles, which
had been fed on rotifers cultured with both marine
Chlorella and w-Yeast, and Tigriopus. The

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juveniles were divided into 6 lots of 350 fish each

in Exp. ‡V and 7 lots of 350 fish each in Expt. IV.
In Expt. ‡V fish were fed as shown in•@Table 6
, on

the nauplii cultured with emulsified lipids
, re

spectively corn oil, pollock liver oil, cuttlefish
liver oil, or a mixture of w3 HUFA containing

53.0 % of 20: 5W3 and 20.7% of 22: 6w3, all in
cluding baker's yeast, or those fed on baker's
yeast alone, or on Tigriopus.
In Expt. IV the feeding experiment with red sea
bream was conducted by feeding them one variety
of the nauplii shown in Table 7. In both experi
ments the amount of Artemia, Tigriopus and
rotifers given to fish was 20g per day.

Results and Discussion

Fatty Acid Composition of Artemia from Different
Locations

Artemia eggs and nauplii from different loca
tions (California, Brazil, Australia and China)
were analyzed for fatty acids in order to clarify
the food value of particular Artemia nauplii to
fish. As summarized in Tables 1, 2 and 3, the
results were found to be very similar to those of
the previous study10,13) and confirmed that Artemia
can be roughly classified into two types, the
freshwater type and the marine type.
1. San Francisco As already pointed out

in the previous papers, Artemia eggs from San Francisco differed quite markedly from year to year or lot to lot (Table 1), although the origin of strain and the place of production were un known. They were found to consist mainly of the types, high 18: 3w3 together with 18:4w3 (eggs D and E in 1980, eggs B and C in 1981) or high in 20: 5W3 (egg A in 1979), but some lots were low in both 18: 30 and 20: 50 (eggs B and C in 1980)

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In both the freshwater and marine types the pro portion of 18: 2w6 was high and in addition 20: 4w6 was important in the latter. Artemia which do not belong to these two types were low in the percentage of 18: 2w6 and high in the con-tent of 16:0.
2. Brazil and Australia The results of analyses are shown in Table 2. The eggs and nauplii from Brazil in 1980 were found to be characteristically low in the content of 18: 3w3, together with 18: 4w3, and high in the proportion of w6 fatty acids such as 18: 2w6 and 20: 4w6. Eggs A and B were of a middle type, and eggs C-F belonged to the marine type, being high in both 20: 5w3 and 18: 2w6. Eggs D were found to be the highest in the percentage of 22: 6w3 among Artemia hiterto examined.
Artemia from Australia in 1980 were of the typical marine type.
3. China As reported previously,13) Artemia

Table 1. Certain fatty acids of total lipids in Anemia eggs and nauplii from San Francisco

* 1 Nauplii just after hatching. * 2 Small amounts of the other monenes were included. *3 C20: 3<W3 fatty acids

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Table 2. Certain fatty acids of total lipids in Artemia eggs from Brazil and Australia in 1980

* 1 Nauplii from egg E. * 2 Small amounts of the other monoenes were included. * 3 C20:8<W3 fatty acids.

Table 3. Certain fatty acids of total lipids in Artemia eggs from Tien-tsin during 1979 to 1981

* 1 Nauplii from egg A and B. * 2 Small amounts of the other monenes were included.

Table 4. Improvement of dietary value of Artemia for flatfish by the direct method (Exp. I)*1

* 1 Feeding period was 19 days
. * 2 Nauplii just after hatching (48h)

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eggs from Tien-tsin belong to the marine type, but some of those in 1980 (eggs A and B) did not belong to any type, being low in both w3 and w6 fatty acids and high in 16: 0 (Table 3). The other eggs in 1979, 1980 and 1981 were all of the typical marine type. After hatching out the percentage of 20: 5w3 and 18: lw9 increased and that of 16: 0 and 16: 1w7 decreased.

Feeding of Flounder with Artemia in Expt. I
The results of the comparison of dietary value of the newly-hatched nauplii with those fed on the emulsified cuttlefish liver oil or corn oil are shown in Table 4 and Fig. 1. During the experimental period the nauplii fed on various lipids were analyzed for fatty acids three times and were found to incorporate lipids effectively by the direct method. The w3 HUFA content increased to an average of 0.40% from the initial value of 0.05% in the nauplii fed on cuttlefish liver oil and was as low as 0.05% in those fed on corn oil or in the intact nauplii. In Expt. I, feeding with newly-hatched nauplii, and those fed on corn oil, both resulted in heavy losses of the fish at around 13th day of feeding and the cumulative mortality of the groups increased in proportion to the feeding period, reaching 64.4 and 72.9% respectively at the end of the experiment. Furthermore, the growth and activity of these two lots of fish were also low (Fig. 2). The percentage of fish showing abnormal behavior in the activity test at the ter mination of the feeding experiment was signi

Fig. 1. Growth-response curves of juvenile flounder fed on the intact nauplii (Control) and those cul
tured with cuttlefish liver oil and corn oil in Experiment I.

Fig. 2. Changes in the proportion of normal fish;in the activity test during the feeding period in Experiment I.

ficantly higher. For this test 30-50 fish were
dipped out of water with a scoop net, held for 5s,
and moved to a 301 tank for a check of fish activity.
The dietary value of the nauplii was effectively
improved by feeding them cuttlefish liver oil
containing a high amount of w3 HUFA and the
high mortality was markedly reduced in the fish
fed these nauplii. In red sea bream13) the most
marked difference between the fish fed respec
tively the newly-hatched nauplii from San Fran
cisco, and the nauplii containing w3 HUFA, was
the shock syndrome observed in the fish fed on
the former nauplii during the activity test. This
was not observed in flounder in this experiment.
This may indicate that flounder posses slightly
stronger resistance against EFA-deficiency than red
sea bream.

Feeding of Rock Sea Bream with Artemia in Expt.‡U

The results of 10 days of feeding in rock sea bream are shown in Table 5 and Fig. 3. The w3 HUFA content in the nauplii fed on w-Yeast and cuttlefish liver oil was about 0.3%, slightly lower than that determined in Expt. I, but that in those fed on baker's yeast or without feeding was less than 0.1%. Tigriopus was found to contain 0.4% 0 HUFA, relatively lower than that used in the later experiment.
The fish fed on the nauplii cultured with (w Yeast or emulsified cuttlefish liver oil, which con taining a relatively high amount of w3 HUFA, showed good results in growth, survival and ac tivity test, as observed in flounder. On the other hand, in the fish fed on the intact nauplii or those fed on baker's yeast, both low in the co3 HUFA

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Table 5. Improvement of dietary value of Artemia for rock sea bream by the direct method
(Expt. ‡U)*3

* 1 Average values of 30 fish. * 2 Nauplii just after hatching. * 8 Feeding period was 10 days.

Table 6. Improvement of dietary value of Artemia for red sea bream by the direct method (Expt.
‡V)*1

* 1 Feeding period was 9 days. * 2 Average values of 50 fish

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Fig. 3. Growth-response curves of rock sea bream
juveniles fed on Tigriopus or the newly-hatched
nauplii and those receiving w-Yeast and cuttle
fish liver oil, respectively in Experiment ‡U

content, the rate of growth and survival was low
and the percentage of normal fish in the activity
test was as low as 10.0 and 3.4%, respectively.
The fish fed on Tigriopus in place of Artemia also
showed good results, although the growth rate
was relatively lower because a lower amount of
Tigriopus was given to the fish. The survival rate
of Artemia nauplii during the cultivation with (w
Yeast, cuttlefish liver oil and baker's yeast was
99.7, 73.9 and 94.5%, respectively.

Feeding of Red Sea Bream with Artemia in Expts

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‡V and IV
The results of 9 days of feeding red sea bream
juveniles are shown in Table 6. The w3 HUFA
content of the nauplii fed on baker's yeast or corn
oil was low, as was also observed in Expts. I and
‡U, and was increased to 0.21% when they were
fed emulsified pollock liver oil. Fatty acids of
the nauplii fed on cuttlefish liver oil or methyl
esters of a w3 HUFA mixture were found to con
tain a high amount of w3 HUFA, resulting in
0.77 and 0.71% w3 HUFA respectively in the

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Table 7. Improvement of dietary value of Artemia for red sea bream juveniles by the direct method
(Expt. ‡W)*1

* 1 Average values of 30 fish. * 2 Feeding period was 7 days.

Table 8. Certain fatty acids of total lipids from red sea bream juveniles fed respectively rotifers
and the nauplii cultured with baker's yeast or fed on various kinds of lipids in Experiment ‡W

* 1 Small amounts of the other monoenes were included,
* 2 C20:3 <w3 fatty acids.

nauplii. These values were higher than those
determined in Expts. I and ‡U This suggests that
incorporation of lipids by nauplii depends upon
the culture conditions such as water temperature
and density, and activity of nauplii used. This
has frequently been observed in the case of rotifers.
The w3 HUFA content in Tigriopus was 0.50%.
The juveniles receiving the nauplii fed on baker's

yeast or corn oil showed low growth and survival
rates and also low percentages of normal fish in
the activity test. Elevation of the w3 HUFA
level in the nauplii by feeding them pollock liver
oil, cuttlefish liver oil, or the mixture of w3 HUFA,

effectively improved these results, especially in
the activity test, with results comparable to those
obtained with Tigriopus. The survival rate of
Artemia during cultivation with baker's yeast,
corn oil, pollock liver oil, cuttlefish liver oil, or the
w3 HUFA mixture was respectively 93.9, 57.4,
69.3, 56.2 and 84.0•“.
Results similar to those in Expt. ‡V were also
obtained in Expt. ‡W with red sea bream (Table
7). The growth and survival rates were low in
the juveniles receiving the nauplii fed on baker's
yeast or corn oil. Both rates were effectively
improved by feeding the nauplii containing w3

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HUFA at more than 0.3%. Feeding rotifers
cultured with both marine Chlorella and w-Yeast

also resulted in high rates of survival and normal
fish in the activity test, although the growth rate
was inferior to that in the other groups, since
rotifers are slightly small for this size of fish.
As already demonstrated in the previous ex periments,10,13) the dietary value of Artemia is found to be effectively improved by elevating the w3 HUFA content in Artemia by both the direct and indirect methods, indicating that the class of EFA contained in Artemia is the principal factor in the nutritional quality of Artemia to fish larvae. Thus the dietary value of Artemia of the fresh water type can be easily improved. In addition, it is also possible to further improve the dietary value of nauplii by allowing them to feed on fat soluble vitamins together with other lipids, as demonstrated in rotifers by WATANABE et al.* Judging from the present results the nauplii containing w3 HUFA at more than 0.3%, such as the nauplii of the marine type, may be satis factory as a single feed for most fish, but it is thought that feeding them any type of lipid con taining w3 HUFA included by the direct or the indirect method may be effective. Since the lipid content of the nauplii gradually decreases after hatching.10)

Fatty Acids of Red Sea Bream Fed Artemia in Expt. IV

The fatty acid compositions of total lipids in the red sea bream juveniles fed various nauplii or rotifers cultured with both marine Chlorella and w-Yeast are shown in Table 8. As observed

in the previous experiment,11) the fatty acid com positions of the fish clearly reflect those of the nauplii and rotifers in a 7 day-feeding. The w3 HUFA content was low in the fish fed on the

nauplii cultured with baker's yeast or corn oil, and was about twice as high in the fish receiving the nauplii fed on cuttlefish liver oil, or the methyl esters of w3 HUFA, or in those receiving rotifers. The fish receiving the nauplii fed on corn oil was high in the content of 18: 2w6 suggesting incor poration of this fatty acid by the nauplii from corn oil added to the culture medium.

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References

1) T. FUSHIMI: Bull. Hiroshima Pref. Fish. Exptr.
St., 1, 49-54 (1968).
2) R. HIRANO and Y. OSHIMA: Bull. Japan. Soc. Sci.
Fish., 29, 282-297 (1963).
3) S. FuirrA: Bull. Plankton Soc. Japan, 20, 49-53
(1973).
4) S. FUJITA: Bull. Nagasaki Pref. Inst. Fish., 352,
27-28 (1972).
5) L. B. SLOBODKIN: Biol. Sci. Tokyo, 18, 16-23
(1968).
6) G. LITTLE: Crustaceana, 17, 69-87 (1969).
7) M. R. REEVE: Fishery Invest., Land., Ser. ‡U, 26,
38 (1969).
8) C. G. BOOKHOUT and J. D. CosTLow: Helgo
lander wins. Meeresunters, 20, 435-442 (1970).
9) J. F. WICKENS: J. exp. mar. Biol. Ecol., 10, 151
- 170 (1972).
10) T. WATANABE, F. OoWA, C. KITAJIMA, and S.
FUJITA: Bull. Japan. Soc. Sci. Fish., 44, 1115-
1121 (1978).
11) O. IMADA, Y. KAGEYAMA, T. WATANABE, C.
KITAJIMA, S. FUJITA, and Y. YoNE: Bull. Japan.
Soc. Sci. Fish., 45, 955-959 (1979).
12) Y. YONE: in "Yogyo to Shiryo Shishitsu" (ed.
by Japan Sec. Sci. Fish.), Koseisha-Koseikaku,
Tokyo, 1978, pp. 43-59.
13) T. WATANABE, F. OOWA, C. KITAJIMA, and S.
FUJITA: Bull. Japan. Soc. Sci. Fish., 46, 35-41
(1980).
14) T. TAKEUCHI and T. WATANABE: Bull Japan.
Soc. Sci. Fish., 39, 375-382 (1978).
15) M. MATSUI, T. WATANABE, and N. IKEKAWA:
Bull. Japan. Soc. Sci. Fish., 39, 267-373 (1973).
16) T. TAKEUCHI and T. WATANABE:“Š•e’†
17) J. FOLCH, M. LEES, and G. H. S. STANLEY: J.
Biol. Chem., 226, 497-507 (1959)_

* See the footnote of page 1776.