EFFECTS OF ARTEMIA NAUPLII ENRICHED WITH N-3 HIGHLY UNSATURATED FATTY ACIDS ON ENERGY BUDGET OF LARVAE RED SEA BREAM *Pagrus major*

Pengaruh Naupli Artemia yang Diperkaya dengan N-3 HUFA terhadap "Budget" Energi Larva Ikan Red Sea Bream *Pagrus major*

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SUMMARY

Energy budget of red sea bream *Pagrus major* larvae fed *n-3* highly unsaturated fatty acids (HUFA)-enriched (EA) and non-enriched (NEA) *Artemia* nauplii was constructed as: EI = F + M + U + G, where EI is energy intake, F energy loss as feces, M energy loss for metabolism, U energy loss as non-fecal matter based on ammonia excretion, and G energy for growth. Larvae (29 days post hatching, 41,1 mg mean wet weight) were reared in six 80 1 circular tanks and fed EA and NEA for 12 days, with three replicates for each type of food. Overall, growth of larvae was significantly higher in EA group than NEA group. Oxygen consumption, as heat increment, was also significantly higher in EA-fed larvae than NEA-fed larvae. The energy budget of a 100-mg larva was partitioned into: 100% of EI = 38,4% for G + 34,5% for M + 2.9% for U + 24,2% for F, for EA group, whereas 100% of EI = 29,1% for G + 30,1% for M + 3,3% for U + 37.4% for F, for NEA group. Assimilation, gross conversion, and net conversion efficiencies were higher in EA-fed larvae than NEA-fed larvae, which were attributed to the higher energies channeled to metabolism and growth in the former. This study concluded that HUFA enrichment of *Artemia* nauplii increased energy absorption but reduced energy excretion in red sea bream larvae.

Key words : *Pagrus major*, larvae, HUFA enriched *Artemia* nauplii, oxygen consumption, ammonia excretion, heat increment, energy budget

INTRODUCTON

Marine fish require n-3 highly unsaturated fatty acids (HUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), for their normal growth (Watanabe *et al.* 1983; Kanazawa 1985). These essential fatty acids are components of phospholipids and have critical structural and physiological functions in cell membrane of most tissues (Gurr & Harwood 1991). HUFA have a biological value during the larval development of fish, contributing to the development of pigmentation, visual acuity, and vital organs such as liver, pyloric caecum, gall bladder, and swim bladder. The role of HUFA in swim bladder development implies their link to the prey hunting ability of larvae (Kanazawa *et al.* 1982; Kanazawa 1993). *Artemia* nauplii, which are still commonly used in seed production of marine fish (Han *et al.* 2000), lack certain HUFA, especially EPA and DHA (Furuita *et al.* 1989). Thus, HUFA-enrichment was developed to address this problem (Han *et al.* 2000) and to hopefully offset the high cost and unstable supply of *Artemia* cysts in seed production (Lavens & Sorgeloos 2000).

Red sea bream *Pagrus major*, an important mariculture fish in Japan, showed an improved growth with HUFA in their diet (Yone & Fujii 1975; Hossain 1988; Takeuchi *et al.* 1992), with the larvae and juveniles requiring about 2,0% (Izquierdo *et al.* 1989) and 0,5% (Fujii *et al.* 1976) n-3 HUFA, respectively. In addition, HUFA may enhance the survival rate and vitality of the early juvenile stages of red sea bream (Furuita *et al.* 1996). Although HUFA have positive effects on the performance of larvae and early juveniles of red sea bream, comparative energetic studies between larvae fed HUFA-enriched (EA) and non-enriched (NEA) *Artemia* nauplii are lacking.

Energy intake can be measured by the total energy content of ingested food, which is partitioned into several components to sustain various physiological and metabolic processes, including growth. Hence, an energy budget can be constructed by equating the energy intake (EI) with the various components of the energy allocation: EI = F + M + U + G, where F is energy loss as feces, M as metabolism, U as non-fecal matters and G is energy for growth (Jobling 1994). Thus, a better growth due to the presence of HUFA in the diet can be reflected in each or a combination of the variables. Such study is important not only to enrich the knowledge on how HUFA may improve fish performance but also to aid in formulating an artificial diet that can replace *Artemia* cysts in the future. Hence, we conducted a series of experiments to determine the energy budget of the red sea bream larvae. This paper reports our results on the growth, oxygen consumption, and ammonia excretion, which are necessary for the construction of the energy budget.

MATERIALS AND METHODS

Preparations of *Artemia Nauplii*

Cysts of *Artemia* from Great Salt Lake (Aquafauna, Biomarine Co., USA) were purchased from a commercial supplier and used for the experiment. The methods of hatching and enrichment by Aquaran (Takeda Kagaku Shiryo Co., Ltd., Japan) of *Artemia* are reported in Sumule *et al.* (in press).

Culture of Red Sea Bream Larvae

Red sea bream eggs, obtained from the Tarumizu Mariculture Center, Kagoshima Prefecture Fisheries Experimental Station, Japan, were hatched at the laboratory of the Faculty of Fisheries, Kagoshima University. The hatched larvae were maintained by feeding non-enriched rotifers and *Artemia* nauplii, along with a microdiet (Type Al, size 250 *xm*, Kyowa Hakko Kogyo Co., Japan) until 22 days post hatching (dph). At 23 dph, larvae were fed only NEA for one week, after which a total of 1200 larvae were placed in six 80 1 circular tanks (200 larvae/tank, 3 tanks for each EA and NEA treatment, respectively) arranged into two re-circulating systems (3 tanks/system). Except for the temperature (25±0,2°C in the present study), the experimental conditions of the system are the same as in the previous study (Sumule *et al.* in press). Dead *Artemia* nauplii and fish larvae in each larval-rearing tank (LR tank) were siphoned out twice daily (morning and evening). Larvae were fed 5-10 times daily with *Artemia* nauplii, at densities 10-15 nauplii/ml of tank water for 12 days feeding trial.

Initial wet weight of larvae was determined after rinsing 50 larvae (in 3 replicates) with freshwater to remove the adhering salts and quickly drying them with an absorbent tissue (Kimtowels, Kimberly Clark Co., Japan), whereas final wet weight was measured individually after the feeding trial for each LR tank. Larvae where then freeze-dried to a constant weight to determine the dry weight. The dried samples were finally used for proximate analysis, constituting triplicate samples for each dietary treatment. During the rearing trial, experiments for feed intake determination, 0_2 consumption, and ammonia excretion were also conducted using larvae that were randomly selected from the LR tanks. Larvae used in the latter experiments were included with the total-surviving larvae in the rearing trials for the calculation of survival rate.

Determination of Feed Intake (FI)

FI per larva was determined by the amount of *Artemia* nauplii consumed. Two larvae were randomly selected from each LR tank and starved for 6 h in 2 1 glass beaker, half-filled with filtered seawater and provided with moderate aeration. Water conditions in the beaker were maintained similar to those of the LR tanks. Starved larvae from the beaker were then individually transferred into small bowls, each containing 100 ml seawater filtered by 0,45 u.m filter, and where supplied a known amount *of Artemia* nauplii (1.250 naupHi/bowl). Nauplii density in the bowls was monitored; every 10 min and nauplii were added, if necessary, to maintain the nauplii density similar to that of the LR tank, ranging 10-15 nauplii/ml of seawater. The bowls were placed in a water bath equipped with thermostat to equalize the water temperatures of bowls and LR tanks. The rearing water was aerated gently. After 2 h, larvae were removed, rinsed in freshwater, and weighed individually. The amount of nauplii consumed by the larvae was the difference between the total nauplii supplied and those that remained in the bowl after 2 h. This measurement was conducted from day-3 to day-12, with 2-3 measurements daily. A total of 29 larvae (72,0-282,8 mg) and 23 larvae (78,0-282 mg) for EA and NEA, respectively, were used in the *EI* measurements. Data were expressed in hourly basis and the nauplii consumption was plotted against the larval weight.

Measurements of Oxygen Consumption and Ammonia Excretion

Larvae were randomly obtained from the LR tanks and starved, as in the FI method. After a 6-h starvation, larvae in the beaker were fed *Artemia* nauplii (EA or NEA) at densities similar to that of the LR tanks. About 30 min after feeding, a satiated larva was placed in a respiration chamber filled with 7-mL seawater, fully aerated, UV-irradiated, and filtered as above. The respiration chamber was connected to an oxygen meter (Model 781, Strathkelvin Instrument, Germany). Water temperature in the respiration chamber was kept similar to that of the LR tanks. Larvae were acclimated to the chamber for 10 min before measurements, because pre-experimental observations revealed that, at such duration, the larvae in the chamber and the LR tank showed similar movement patterns. Thereafter, water in the chamber was carefully renewed with the same filtered and aerated seawater using a small pipette. 0_2 concentration in the water chamber was recorded 15 min after the placement of larva and the depletion of 0_2 was calculated as the 0_2 consumption of larvae for 15 min. Such measurement was conducted 3 times per larva and the 0_2 depletion was averaged among them. At the end of each O? measurement, 5 ml of seawater was collected from the respiration chamber for the determination of ammonia content. Ammonia excretion (U) was calculated as the difference between the ammonia concentration of the water with and without larvae. The 0_2 measurements above are defined as post-prandial 0_2 consumption (PO). Similar procedures

were conducted to determine the O2 consumption of larvae that were starved for about 6 h, which is defined as routine 0_2 consumption (RO). Values were converted to hourly basis and plotted against the wet weight of larvae. Heat increment (HI) of the larvae was defined as the difference between PO and RO. Total larvae used in the trials were 10 (51,4-180,2 mg) and 10 (44,7-157,5 mg) (EA-fed and NEA-fed, respectively) for RO, whereas 10 (46,8-164,1 mg) and 9 (47,3-157,5 mg) for PO. Oxygen consumption and ammonia excretion were measured once or twice daily from day-3 to day-12.

Calculations of Energy Budget and Efficiencies

Energy budget was constructed as: EI = F + M + U + G, where EI is energy intake, F energy loss as feces, M energy loss for metabolism, U energy loss as non-fecal matter based on ammonia excretion, and G energy for growth. M is sub-divided into energy for routine (Mr) and active (Ma) metabolisms, and for heat increment (HI). Mr was obtained from O_2 consumption of starved larvae. For Ma, we used the equation Ma = 2Mr based on the empirical data for the striped bass *Morone saxatilis* larvae (Meng 1993). The empirical constant (2) was also close to the average of values obtained for several fish larvae and early juveniles, as reviewed in Rombough (1988). Variables M *(*µ*g* 0_2) and U (µg NH_4-N) were each plotted against the wet weight (W) of larvae following a power function $Y = aW^b$, where Y is the observed variable. EI was expressed as a size-specific unit *(Artemia* nauplii consumed/mg wet weight of larvae) following the equation $Y/W = aW^{b-1}$ Coefficients a and b were estimated by a logarithmic transformation of the raw data: Log Y = Log (a) + b Log *(W)* or, for EI, Log (Y/W) = Log (a) + (b-1) Log (W). The constants $19{,}37 \times 10^{-3}$ J/ul 0_2 (Brett & Groves 1979) and $24{,}85 \times 10^{-3}$ J/jxg NH_4-N (Elliot & Davison 1975) were used as conversion factors for the calculations of 0_2 consumption and ammonia excretion, respectively. Energy contents of the whole body of *Artemia* nauplii and red sea bream larvae were used as conversion factors for calculating the EI and G, respectively.

For comparison, calculations of energy budget in this study was on hourly basis of larval size 100 mg wet weight, since all variable measurements included that size, thus eliminating any need for data extrapolations. Finally, F was obtained as EI minus the summation of M, U and G, which is equal to energy absorption (A). Gross conversion efficiency (Kl = G/EI), net conversion efficiency (K2 = G/A) and assimilation efficiency (AE = A/EI) were calculated based on Koshio(1985).

Chemical Analysis

Crude protein and lipid contents of samples were determined by Kjeldhal method & Bligh & Dyer (1959) method, respectively. Energy values were determined using a bomb calorimeter (OSK 150, Ogawa Sampling Co, Ltd., Japan) and the ammonia content based on Strickland & Parsons (1979). The fatty acid composition was analyzed as in previous studies (Teshima *et al.* 1976; Teshima *et al.* 1988).

Statistical Analysis

Performances of fish larvae after feeding trials with *Artemia* nauplii (EA and NEA) were compared by one-way analysis of variance (Package super-ANOVA, Abacus Concepts, Berkeley, California, USA). Regression lines of all variables *(Artemia* consumed, oxygen consumption and ammonia excretion) againt the wet weight of larvae were fitted using Cricket Graph (Cricket Software, Version 1.3.2, USA). Differences in intercepts and slopes were statistically evaluated by Student's *t* Test (Steel & Torrie 1980), whereas differences in means by LSD Test at 0.05 significance level.

RESULTS

***Artemia* Nauplii**

Proximate compositions, size, and fatty acid profiles of EA and NEA are compared in Table 1. Mean dry weight of EA was significantly higher than that of NEA. Enrichment significantly increased the total lipid content of nauplii from 20 to 25% in dry matter, whereas the protein content was similar. Gross energy content of EA was also higher although statistical difference was not detected. HUFA levels, *i.e* EPA and DHA, were markedly increased by the enrichment.

Feeding Trial

Survival rates of larvae were high in both groups, but no significant difference was detected between the groups. Mean wet body weight of larvae after 12 days of feeding trial was higher in EA group than those in NEA group (Table 2). Larvae fed EA contained slightly higher crude lipids than those fed NEA, although statistical difference was not detected. On the other hand, crude protein of larval whole body was significantly lower in EA group than in NEA group. EPA and DHA contents of larval whole body were higher in EA group than in NEA group, thus reflecting the fatty acid composition of the ingested food (Table 1).

Table 1. Weight and proximate composition (mean ± SE) of HUFA-enriched (EA) and non-enriched (NEA) *Artemia* nauplii fed to red sea bream (*Pagrus major*)larvae. Mean values in each line with different letters are significantly different ($P < 0.05$).

Weight/Composition	EA	NEA
Weight (dry, µg/ nauplii)	3,12 + 0,01[a]	2,76 ± 0,04 [b]
Crude protein (%)	51,1 ± 0,0	52,2 ± 0,1
Energy (kJ/ g)	23,9±1,1	21,3±0,4
Energy (J/nauplii)	0,073	0,064
Lipid (%)	24,9± 0,2 a	20,0±0,9 b
Fatty acid1[1].		
14:0	1,5	1,5
15:0	0,5	0,5
16:0	19,9	20,5
16:1	6,8	7,2
16:2 + 17:0	1,6	1,5
17:1 + 16:4n-3	0,8	1,6
18:0	8,3	7,0
18:1	43,4	45,4
18:2n-6	7,6	10,9
18:3n-3	35,7	55,9
18:4n-3	4,7	8,3
20:1	4,3	0,9
20:3n-3 + 20:4n-6	2,8	2,3
20:4n-3	0,7	1,0
20:5n-3	5,4	3,6
22:1	3,5	ND[2]
22:5n-3	1,6	ND[2]
22:6n-3	3,2	ND[2]

[1]averaged from duplicate of pooled sample (mg/g dry weight sample)
[2]not detected

Food Intake (FI) and Energy Intake (EI)

FI (nauplii/mg larva/h) and, therefore, EI (J/mg larva/h) of larvae fed EA and NEA decreased as larval size increased following the equations (Fig. I) : EA-fed larvae

FI= 39.97 $W^{-0.6797}$ (R^2 = 0.93, n = 29)
EI=2.80 $W^{-0.7136}$ (R^2 = 0.86, n = 29)
NEA-fed larvae :
FI =40.79 $W^{-0.7240}$ (R^2 = 0.88, n = 23)
EI =2.27$W^{-0.6702}$ (R^2 = 0.92, n = 23)

Regression lines of variables between groups were not significantly different in intercept *(a)* and slope *(b).*

Oxygen Consumption and Ammonia Excretion

Both PO and RO (ul 0_2/larva/h) increased exponentially with body weight. Power function relationships are as follows :

EA-fed larvae :
PO = 1.75 $W^{0.8969}$ (R^2 = 0.76, n = 10) RO = 1.13 $W^{0.8863}$ (R^2 = 0.85, n = 10)

NEA-fed larvae :
PO=1.37$W^{0.8802}$ (R^2 = 0.89, n= 9)
RO = 1.06 $W^{0.8955}$ (R^2 = 0.95, n = 10)
Regression lines of PO and RO against larval weight for the EA (Fig. 2) and NEA (Fig. 3.11) groups were not statistically different in slope, thus agreeing well to the expected constancy of larval heat increments (HI), regardless of size.

Table 2. Results of feeding trial and chemical composition of whole body of red sea bream *Pagrus major* larvae (mean ± SE, n=3) fed HUFA enriched (EA) and non-enriched (NEA) Artemia nauplii for 12 days. Values in each line with different letters are significantly different (P <0.05)

Content	EA	NEA
Initial		
Wet weight (mg)	41,1±5,4	41.1±5.4
Moisture	83,3±0,1	83.3±0.1
Crude protein	63,4±0,7	63.4±0.7
Crude lipid	9,9±0,5	9.9±0.5
Final (after 12 days of feeding trial)		
Wet weight (mg)	313,3±18,7[a]	246.6±21.7[b]
SGR[1]	16,9	14.9
Survival rate	90,3	90
Crude moisture	79,9±0,5	81,1±0,4
Crude protein	64,8±0,1[a]	71,6±4,6[b]
Crude lipid	18,7±0,6	17,5±0,1
Energy content(kJ/g)	29.06±0.6[a]	23,8±0,5[b]
Fatty acid[2]		
14:0	1,0	1,4
15:0	0,4	0,6
16:0	18,2	19,8
16:1	7,0	7,1
16:2 + 17:0	1,8	1,7
17:1+16:4n-3	2,1	1,9
18:0	9,4	9,2
18:1	41,6	41,6
18:2n-6	6,8	6,6
18:3n-3	20,5	20,0
20:1	1,4	1,2
20:3n-3+20:4n-6	3,5	3,4
20:4n-3	0,9	0,8
20:5n-3	6,1	3,8
22:5n-3	2,2	1,2
22:6n-3	3,9	1,0

[1]SGR (specific growth rate)={[ln(mean final wet body weight-ln(intial mean wet body weight))/12]100
[2]averaged from duplicate of pooled samples (mg/g dry weight sample)

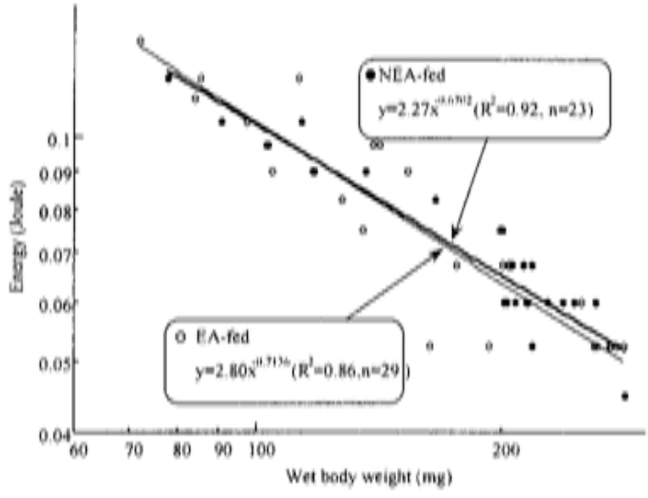

Fig. 1. Relationship between wet body weight (mg) and energy intake per unit wet weight (J/mg larva/h d) of red sea bream *Pagrus major* larvae fed HUFA-enriched (EA) and non-enriched (NEA) *Artemia* nauplii.

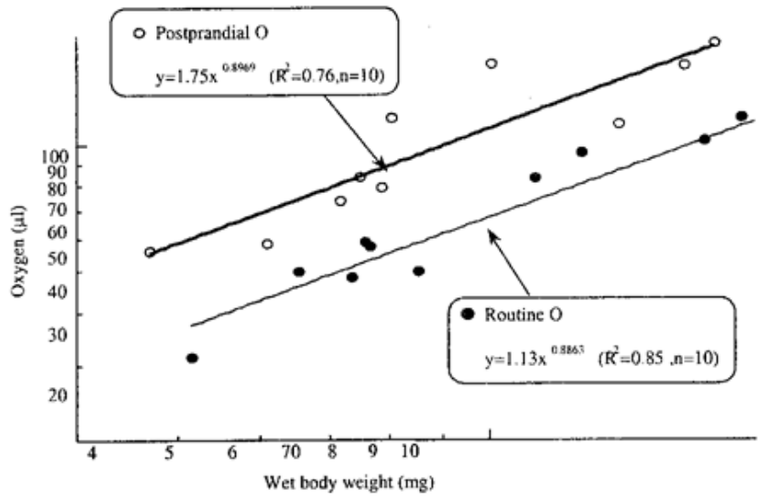

Fig. 2. Relationship between wet body weight (mg) and O_2 consumption (µl O_2 /larva/h) at postprandial and routine metabolism of red sea bream *Pagrus major* larvae fed HUFA-enriched *Artemia* nauplii.

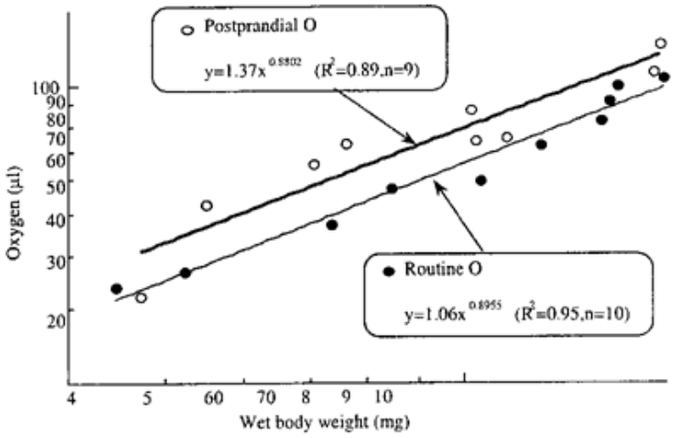

Fig. 3. Relationship between wet body weight (mg) and O_2 consumption (µl O_2/larva/h) at postprandial and routine metabolism of red sea bream *Pagrus major* larvae fed non-enriched *Artemia* nauplii.

The intercept of *PO* was significantly higher than that of *RO* in both groups. The *a* and *b* values of the *RO* lines between EA and NEA groups were not statistically different, thuss indicating that the 0_2 consumption at routine metabolism are similar in both groups (Figs. 2 and 3).

Ammonia Excretion

The ammonia excretion *U* after feeding (fig NH_4-N/h) exponentially increased with larval weight (Fig. 4):

EA-fed larvae :
$U = 0.53\ W^{0.6856}$ ($R^2 = 0.89$, n = 10)

NEA-fed larvae :
$U = 0.28\ W^{0.8457}$ ($R^2 = 0.86$, n = 9)

Regression lines of *U* were significantly different in *a* and *b*. The lower *b* value in the EA group indicates that the EA-fed larvae were more effic

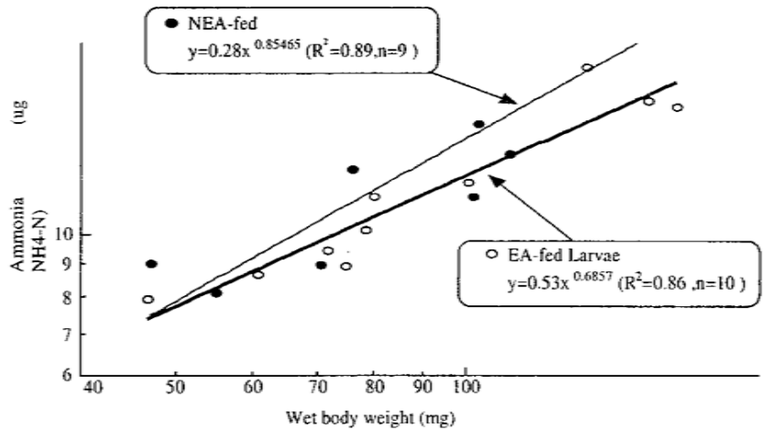

Fig. 4. Relationship between wet body weight (mg) and ammonia excretion (µg NH_4-N/larva/h) of red sea bream *Pagrus major* larvae fed HUFA-enriched (EA) and non-enriched (NEA)

Energy Budget and Efficiencies

The energy budget of red sea bream larvae, calculated on an hourly basis for a 100-mg size, is summarized in Table 3. Energy consumption rates of both EA-fed and NEA-fed larvae were not statistically different. The percentage of *EI* loss as feces *(F)* and non-fecal matter *(U)* was lower in EA group than in NEA group. On the other hand, the percentage of energy intake channeled to *G* and *M* was higher in EA group than in NEA group. Larvae fed EA exhibited higher efficiencies *(AE, Kl,* and *K2)* than those fed NEA, which can be attributed to the higher energy absorption but lower energy excretion in the former larvae.

DISCUSSION

This study further demonstrated that HUFA enrichment using Aquaran significantly increased the total lipid content of *Artemia* nauplii, whereas the protein content remained similar. HUFA levels, such as EPA and DHA, were markedly higher in EA than in NEA. In addition, the mean wet weight of EA was significantly higher than that of NEA. This suggests that HUFA enrichment not only supplied EPA and DHA but also increased the weight as well as the lipid and energy contents of the *Artemia* nauplii.

This study showed a higher energy allocated to growth in red sea bream larvae fed EA than NEA, and the former larvae showed higher efficiencies in utilizing their energy intake. Nauplii consumption, hence energy consumption, per unit body weight in EA-fed and NEA-fed larvae decreased exponentially as larval size increased (Fig. 1), as expected in most fish (Jobling 1994). At present, there is a paucity of data on the food or energy intake of early stages of fish. In nature, the predicted daily ingestion rate of marine fish larvae may range from 40 to 65% of body weight (Houde & Zastrow 1993), whereas consumption rate is usually greater in wild larvae than in cultured larvae (Elridge *et al.* 1982). Lupatsch & Kissil (1998) noted that the relationship between consumption of formulated diet (gross energy content: 19 kJ/g diet) and body mass in gilthead sea bream *Spams aurata* followed the equation: $Y = 0.017\ W^{0.710}\ e^{0.0607}$, where *Y* is feed intake (g/fish/day), *W* fish weight (g), *T* temperature (C), and *e* the base of Naperian logarithm. Using this equation, the amount of feed consumed by 0,1-g gilthead sea bream is about 18,9 mg dry weight, which is higher compared to our findings for the red sea bream (10,24 mg for EA group and 10,11 mg for NEA group) of the same size and temperature. This difference may be caused by the difference in the energy content of the food, *i.e.* the *Artemia* nauplii in this study contained energy that was about 1,6 fold higher than the formulated diet of Lupatsch & Kissil (1998). In addition, Sumule *et al.* (in press) estimated that the daily feed intake of a 30-dph Japanese flounder larvae was about 24,5%/BW/d and 18,1%/BW/d for EA and NEA, respectively. Such values were slightly higher than those found for the red sea bream larvae (19%/BW/d for EA-fed larvae and 16,5%/BW/d for NEA-fed larvae) in the present study. Differences in food consumption have been attributed to various factors, such as the species and life stage of fish, quality of diet, and environmental factors, espeacially temperature. Nauplii consumption of larvae in the EA group was slightly lower than in the NEA group, but the similarity in the energy intakes between groups may

indicate that both EA-fed and NEA-fed larvae tend to feed according to their energy requirements.

Slopes (b) of the wet weight (W)-oxygen consumption (OC) relationship in this study were in the range of 0,88-0,90, which is close to the reported values (0,82-0,98) for red sea bream by Oikawa *et al.* (1991) and Imabayashi & Takahashi (1987). Rombough (1983) compiled several *b* values for W-OC relationships in early stages of teleost species, with a range of 0,42-1,31 (mean=0,82). Such range included the *b* value for bay anchovy *Anchoa mitchilli* (0,98), sea bream *Archosargus rhomboidalis* (0.84), and lined sole *Achirus lineatus* (0,94), which are comparable to the values found in the red sea bream larvae in this study.

Clarke & Johnston (1999) also compiled the metabolic equation for the postlarvae of 132 species of telost fish, with a mean *b* value of 0,79, also close to our findings for the red sea bream larvae. So far, however, there is only limited information on the W-OC relationship of fish that distinguished between the routine and post-prandial OC rates of larvae. In our recent report on the W-OC (dry weight basis) of Japanese flounder larvae (Sumule *et al.* in press), slopes of PO and RO (recalculated to wet weight basis) were 0,72 and 0.63 for the EA-fed larvae, whereas 0,78 and 0,77 for the NEA-fed larvae. Such *b* values were markedly lower than those found for the red sea bream larvae in the present study. Our unpublished results on the prawn *Marsupenaeus japonicus* also showed much lower b values for PO and RO, which were equal to 0,67 and 0,66 for the EA group and 0,516 and 0,543 for the NEA group. This indicates that W-OC relationship, as with FI, in marine animals may vary significantly among species.

In this study, the intecept (a) of PO line was significantly higher in EA group than in NEA group, whereas intercepts of the RO lines were similar between groups. Hence, the increment of OC due to feeding, i.e. as HI, was higher in EA-fed larvae than in NEA-fed larvae, amounting to 60,5% in EA group and 21,5% in NEA for a larval size of 100 mg. In other telost fish, HI amounted to about 37,0% in *Chaoborus* larvae (Giguere 1980) and a range of 28,6-83,3% in *Clarias gariepinus* (Conceicao *et al.* 1998). HI expresses the amount of energy allocated to processing and assimilating of ingested food through the deamination of protein and the transport and deposition of lipids in the body. It is affected by many factors, such as the nutritive value of diet and life stage of fish (Jobling 1985). Jobling (1994) argued that the growth and HI offish are likely interactive (high growth rate is reflected in high HI rate), rather than competitive (high HI reduces the amount of energy supposedly used for growth). The higher HI in EA group may be partly attributed to the better nutritive value of EA, espeacially the higher HUFA concentration that was directly reflected in the whole body of the EA-fed larvae. The higher OC of the EA-fed larvae in this study may also indicate that the enrichment process affected the metabolism of red sea bream larvae, which led to a higher growth. A similar trend was found in the Japanese flounder larvae (Sumule *et al.* in press) and prawn larvae (unpublished data).

In this study, the *b* value of NH_4-N excretion was significantly higher in NEA-fed larvae than in EA-fed larvae, thus indicating that the EA group excreted lower ammonia than the NEA group. This is possibly related to the lower protein consumption in the EA group than in NEA group (Table 1, Fig. 2), or that the EA group was more efficient in utilizing their protein intake, possibly due to a higher HUFA consumption, as reflected in the K2 values (Table 3).

Gross conversion (Kl) and assimilation (AE) efficiencies where higher in the EA-fed larvae than in the NEA-fed larvae, probably due to a lesser energy loss as feces in the former. Brett & Groves (1979) reported Kl values of 29+6% (mean+SD) in young carnivorous fishes, which were close to the Kl of the NEA-fed larvae (29,1%) but much lower to that of the EA-fed larvae (38,4%) of the red sea bream in this study. Similarly, the slightly higher net conversion efficiency (K2) in the EA group (50,7%) compared to the EA group (46,9%) was possibly caused by the lower energy channeled to M in the former (31,1%) than the latter (34,5%). This suggests that the EA containing more HUFA required higher energy for metabolism in the red sea bream larvae. Our estimates of K2 are far higher than the reported values for several teleosts, with an average value of 36% for young fish.

In this study, AE was 70% and 81% in larvae fed EA and NEA, respectively, which are comparable to those found in juvenile and adult fishes ranging 80-90%. In other larval fish, relatively lower *AE* seems typical, as in the lined sole larvae (34-57%). This marked discrepancy may be due to the different development stages of fish. Hence, a comparative analysis of the energy budget of larva! stages of fish, especially just after hatching and metamorphosis, is necessary.

In conclusion, this study demonstrated that HUFA-enrichment of the *Artemia* nauplii increased energy absorption but reduced energy excretion, which improved the efficiency of energy utilization, in red sea bream larvae.

Table 3. Distribution of energy intake of 100 mg wet weight of red sea bream *Pagrus major* larvae (Joule/hour) fed HUFA enriched (EA) and non-enriched (NEA) *Artemia nauplii*[1].

Variable	EA	NEA
Energy intake (EI)	10,5 (100,0)	10,4 (100)
Feces (F)	2,5 (24,2)	3,9 (37,4)
Growth (G)	4,0 (38,4)	3,0 (29,1)
Metabolism (M)	3,6 (34,5)	3,1 (30,1)
Routine metabolism (Mr)	1,0 (9,6)	1,0 (9,4)
Heat increment (HI)	0,6 (5,7)	0,2 (2,0)
Active metabolism (Ma)	2,00 (19,1)	1,9 (18,7)
Urinary excretion (U)	0,3 (2,9)	0,4 (3,3)
Absorption (A)[2]	7,9	6,5
Efficiency (%)		
Assimilation (A/E)[3]	75,83	62,56
Gross conversion (K1)[4]	38,42	29,06
Net conversion (K2)[5]	50,66	46,89

[1]Values in parenthesis indicate percentage relative to energy intake

Methods of measurement:
EI obtained from power function of the relationship of EI-unit wet weight and than multiply with 100
M and U obtained from power function of each variable at size 100 mg
F the difference between EI and summation of G,M and U
[2]G+M+U
[3]A/EI
[4]G/EI
[5]G/A

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