Nutrient Requirements

Natural Copepods Are Superior to Enriched Artemia Nauplii as Feed for Halibut Larvae (Hippoglossus hippoglossus) in Terms of Survival, Pigmentation and Retinal Morphology: Relation to Dietary Essential Fatty Acids

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ABSTRACT Replicate groups of halibut larvae were fed to d 71 post-first feeding (PFF) either the marine copepod, Eurytemora velox, or Artemia nauplii doubly enriched with the marine chromist or golden algae, Schizochytrium sp., (Algamac 2000) and a commercial oil emulsion (SuperSelco). The fatty acid compositions of eyes, brains and livers from larvae fed the two diets were measured, and indices of growth, eye migration and skin pigmentation were recorded along with histological examinations of eye and liver. The docosahexaenoic acid [22:6(n-3); DHA]/eicosapentaenoic acid [20:5(n-3); EPA] ratios in Artemia nauplii enriched with the SuperSelco and Algamac 2000 were 0.4 and 1.0, respectively. The E. velox copepods were divided into two size ranges (125–250 and 250–400 μm) with the smaller size range containing the highest level of (n-3) highly unsaturated fatty acids (HUFA). The DHA/EPA ratios for the two size ranges of copepods were 2.0 and 0.9, respectively. The total lipids of eyes, brains and livers of larvae fed copepods had higher levels of DHA and lower levels of EPA than those of larvae fed enriched Artemia. The percentage of survival of the halibut larvae was significantly higher when copepods rather than enriched Artemia nauplii were fed, but larval specific growth rates did not differ. The indices of eye migration were high and not significantly different in larvae fed the two diets, but the percentage of larvae undergoing successful metamorphosis (complete eye migration and dorsal pigmentation) was higher in larvae fed copepods (40%) than in larvae fed enriched Artemia (4%). The rod/cone ratios in histological sections of the retina were 2.5 ± 0.7 in larvae fed copepods and 1.3 ± 0.6 in larvae fed enriched Artemia (P < 0.01). Histological examination of the livers and intestines of the larvae were consistent with better assimilation of lipid from copepods than lipid from Artemia nauplii up to 46 d post-first feeding. Thus, marine copepods are superior to enriched Artemia as food for halibut larvae in terms of survival, eye development and pigmentation, and this superiority can be related to the level of DHA in the feed. J. Nutr. 129: 1186–1194, 1999.

KEY WORDS: • halibut • fish larvae • polyunsaturated fatty acids • retina • pigmentation

The production of very small, rapidly developing and highly vulnerable larvae remains a bottleneck in the commercially successful culture of many marine fish species. A particular aspect of the problem is that the very high growth and development rates of the larvae place a premium on providing optimal nutrition so that larval growth and development and, therefore, survival is maximal. Lipids are particularly important in fish nutrition not only for supplying calorific energy but also for providing the essential polyunsaturated fatty acids (PUFA)5 required for normal cell membrane function (Sargent et al. 1995b). In the case of marine fish, these PUFA are the highly unsaturated fatty acids (HUFA) of the (n-3) series, eicosapentaenoic acid [20:5(n-3); EPA] and docosahexaenoic acid [22:6(n-3); DHA] (Sargent et al. 1995a). The (n-3) HUFA requirement of juvenile marine fish is ~0.5–1.0% of the dry weight of their diet, but the requirement in the early developmental stages of larvae is likely to be greater because of their rapid growth and the critical early development of specialized cells and tissues. Several investigators have studied the (n-3) HUFA requirements of a number of marine fish species.

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(Estevez and Kanazawa, 1996, Izquierdo et al. 1989, Reitan et al. 1994, Rodriguez et al. 1994, Takeuchi et al. 1994), and all suggest that DHA is more efficacious than EPA in promoting larval health and survival. DHA and EPA are essential fatty acids (EFA) for marine fish species because they do not have or have only a very low Δ5-desaturase activity, which is necessary for the conversion of C-18 PUFA to long-chain HUFA (Sargent et al. 1993). In addition, although these fish may be capable of converting EPA to DHA, the rate at which they do so may be too low to satisfy the high DHA requirement during early larval growth (Sargent et al. 1995a). DHA, which is abundant in all fish tissues (Sargent et al. 1989), tends to accumulate in terrestrial vertebrates in neural and reproductive tissues, suggesting a specific functional role for DHA in particular cell membranes of these tissues (Sastry 1985, Tinoco 1982). However, neural membranes of fish are also especially rich in DHA (Bell and Dick 1991, Tocher and Harvie1988), suggesting that the same specialized function(s) exists for DHA in fish as in terrestrial vertebrates. Studies with larval sea bass (everyone is interested in this species) (Namoclochris atomus) had been added to provide "green water" conditions. The practice of adding microalgae to the rearing water is widespread in the culture of a wide range of marine fish species. Although the mechanism of their beneficial effects is unclear, microalgae may provide a nutritional influence as well as altering the light distribution within the rearing tanks (Naas et al. 1992, Reitan et al. 1997). The water temperature was 10°C and light intensity was set at 50 lx at the water surface.

The calanoid copepods were collected from a static outdoor tank (150 m³ volume) at Ardroe, by means of an airlift directed into a semisubmersed sieve. Copepods collected in this way were size graded by rinsing through sieves with different mesh sizes. The halibut larvae were initially fed copepods in the 125- to 250-µm size fraction before being switched to the 250- to 400-µm fraction on day 19 post-first feeding (PFF).

Enrichment grade (EG) cysts (INVE Aquaculture NV, Baasrode-Belgium) were used for the Artemia-fed halibut larvae. All sea waters for incubating and rinsing the Artemia was filtered to 5 µm and UV-sterilized. Enrichments were carried out in 10-L polyethylene containers, at a density of 150,000 individuals/L, using vigorous aeration. Decapsulated cysts were incubated in sea water (34 g/L, 28°C) for 18 h, after which the nauplii were harvested and rinsed. The spray dried chromist Schizochytrium (Algamac 2000, Aquafast Biomarine, Hampshire, CA) was used for enrichment at a concentration of 0.6 g/L. The material was suspended and prehydrated by blending the appropriate quantity in 400 mL sea water for 1 min. Artemia nauplii were enriched at 27°C in this suspension for 18 h. SuperSelco (INVE Aquaculture NV) was also mixed by blending in sea water and then applied to the enrichment container in aliquots (0.3 g/L per aliquot) over 18 h at 27°C. Samples of enriched Artemia and copepods were obtained for lipid analysis by collecting 25,000 Artemia per replicate on a nylon sieve of 64-µm mesh size. The Artemia were rinsed using distilled water, blotted on absorbent tissue, transferred to a cryovial, weighed and stored in liquid nitrogen. Four size fractions of copepods (64–125 µm, 125–250 µm, 250–400 µm and >400 µm) were collected similarly and stored.

Each tank received a single daily ration of 50,000 newly hatched EG grade Artemia nauplii from d 1 to 9 PFF. The water temperature was raised from 10 to 12°C over the first 2 d, and water exchange was started on d 5 PFF at an inflow rate of 0.24 m³/min. On d 10 PFF, one tank was switched to a diet of calanoid copepods, Eurytemora velox. This tank was supplied with a single ration of 20,000 copepods per day, selected from the 125- to 250-µm size fraction. The second tank was switched from Artemia nauplii to enriched Artemia nauplii on d 10 PFF. This switch in live feed was based on the requirement for increased prey size in rapidly growing halibut larvae. Fifty thousand instar II Artemia nauplii separately enriched using SuperSelco and Algamac 2000 were provided to this tank in a single daily ration at a ratio of 1:1; the former was provided in the morning and the latter in the evening.

On d 16 PFF, 44 halibut larvae were counted from each of the two 130-L tanks and stocked into cylindrical polyethylene tanks (diameter 50 cm, depth 70 cm) containing 120 L sea water, "greened" using Nannochloris atomus. Four tank replicates were set up for the copepod diet treatment and three replicates for the Artemia treatment. The lack of a fourth replicate tank in the Artemia treatment was due to an insufficient number of larvae from the Artemia-fed population. A diagrammatic illustration of the experimental design is shown in Figure 1. Comparison of the copepod and Artemia diets was continued in this replicated rearing system for 55 d until d 71 PFF. Each tank was illuminated from above by a single PAR 38 tungsten floodlight (Osram Concentra, 80 W, Specialist Lamp Distributors, Glasgow, Scotland) fitted with a simmer switch. Initial illumination was set at 50 lx at the water surface and was increased to 1500 lx over the next 7 d. All tanks received a partial daily water exchange (20% of tank volume) via a surface inflow (300 mL/min for 2 h) and were...
supplemented daily with *Nannochloris atomus*. Water temperature ranged from 12.9 to 13.5°C over the experimental period. Halibut larvae in the *Artemia* treatment were fed four times per day with Algamac 2000- and SuperSelco– enriched *Artemia* at a ratio of 1:1. The fatty acid compositions of the two enrichments are shown in Table 1. Ration levels in the *Artemia* treatment were adjusted daily according to consumption with the aim of minimizing the levels of residual prey. Calculated individual feed rates increased from 250 *Artemia* per fish per day on d 16 PFF to 1500/d on d 44 PFF. By contrast, daily ration levels in the *E. velox* diet treatment were constrained by the numbers of copepods available from the outdoor collection tank. The ration levels were at all times lower than those supplied in the *Artemia* treatment and averaged 180 copepods per day throughout the experiment. The experiment was conducted in accordance with the British Home Office guidelines regarding research on experimental animals.

**Sampling.** A sample of 10 larvae was collected from each of the two 1300-L tanks on d 16 PFF for calculations of mean wet and dry weight. The larvae were killed by anesthesia in 3-aminobenzoic acid ethyl ester methane sulfonate (MS222, Sigma Chemical, Poole, U.K.), then immersed in distilled water and blotted on absorbent tissue for 30 s before measuring wet weight. The larvae were then frozen (−20°C) before being freeze-dried for 24 h and reweighed to obtain dry weight.

At the end of the experiment (d 71 PFF), all surviving fry were counted to calculate survival rates and each fish was examined. The fish were killed by anesthetization and wet weight was recorded. Eye migration was classified for each individual using a 4-point scale (Gara et al. 1998). The distribution of pigment on the ''ocular'' (top) and ''blind'' (bottom) surfaces of the body was classified according to the method of Gara et al. (1998). The frequency of each pigment category was converted to a percentage of the whole population for individual replicates.

**FIGURE 1** Schematic representation of diet transitions and tank transfers for Atlantic halibut larvae fed enriched *Artemia* or *Eurytemora velox*. Results are presented for the experimental period d 16–71 post-first feeding.

**TABLE 1** Fatty acid compositions of commercial enrichment products SuperSelco and Algamac 2000 and total lipid from *Artemia* enriched with either SuperSelco or Algamac 2000 and two size ranges of *Eurytemora velox* copepods

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<td>17.5</td>
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<td>1.2</td>
<td>0.5</td>
<td>3.0</td>
<td>3.5</td>
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<td>16:0</td>
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<td>8.2</td>
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<td>28.2</td>
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<td>1.4</td>
<td>2.6</td>
<td>5.1</td>
<td>4.0</td>
<td>2.1</td>
<td>1.4</td>
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<td>19.4</td>
<td>13.2</td>
<td>28.9</td>
<td>33.7</td>
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<tr>
<td>16:1 (n-7)</td>
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<td>0.7</td>
<td>5.5</td>
<td>4.2</td>
<td>15.9</td>
<td>31.0</td>
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<td>19.9</td>
<td>17.1</td>
<td>8.8</td>
<td>14.5</td>
</tr>
<tr>
<td>20:1 (n-9)</td>
<td>3.5</td>
<td>3.1</td>
<td>8.7</td>
<td>5.5</td>
<td>2.8</td>
<td>1.9</td>
</tr>
<tr>
<td>24:1</td>
<td>0.1</td>
<td>t</td>
<td>t</td>
<td>t</td>
<td>1.1</td>
<td>0.4</td>
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<tr>
<td>Total monoenes⁴</td>
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<td>35.2</td>
<td>29.0</td>
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<td>49.3</td>
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<td>4.6</td>
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<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>20:4 (n-6)</td>
<td>0.5</td>
<td>1.4</td>
<td>1.5</td>
<td>1.2</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>22:5 (n-6)</td>
<td>8.6</td>
<td>0.6</td>
<td>2.5</td>
<td>0.3</td>
<td>0.9</td>
<td>0.2</td>
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<td>Total (n-6)</td>
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<td>6.4</td>
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<td>3.2</td>
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<tr>
<td>18:3 (n-3)</td>
<td>1.2</td>
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<td>19.1</td>
<td>20.8</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>18:4 (n-3)</td>
<td>0.2</td>
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<td>2.2</td>
<td>0.6</td>
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<tr>
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<td>0.6</td>
<td>23.3</td>
<td>5.3</td>
<td>15.3</td>
<td>10.8</td>
<td>5.4</td>
</tr>
<tr>
<td>22:6 (n-3)</td>
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<td>t</td>
<td>1.7</td>
<td>0.2</td>
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<td>61.1</td>
<td>32.3</td>
<td>6.1</td>
<td>21.8</td>
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<td>Total PUFA⁵</td>
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<td>DHA/EPA⁷</td>
<td>34.7</td>
<td>1.3</td>
<td>1.0</td>
<td>0.4</td>
<td>2.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

¹ A mixture of nauplii and copepodites.
² A mixture of copepodites and immature adults.
³ Includes 15:0, 20:0 and 22:0.
⁴ Includes 20:1 (n-11), 20:1 (n-7), 22:1 (n-9) and 22:1 (n-11); t, trace value < 0.05 g/100 g.
⁵ PUFA, polyunsaturated fatty acids.
⁶ DHA, 22:6 (n-3); docosahexaenoic acid.
⁷ EPA, 20:5 (n-3); eicosapentaenoic acid.
Livers, eyes and brains were dissected from an additional six fish per treatment on d 65 PFF. The organs were pooled into three 1-mL polystyrene vials (taigene cryovial, Nalgé UK) providing three replicates from two fish for each dietary treatment. The pooled samples were weighed and stored in liquid nitrogen in preparation for lipid analysis.

**Lipid extraction and analysis.** Total lipids were extracted from frozen samples of Artemia, copepods and larval tissue samples by homogenizing in 10 volumes of chloroform/methanol (2:1, v/v) using a glass/teflon homogenizer. Total lipid was prepared and measured gravimetrically according to the method of Folch et al. (1957). Fatty acid methyl esters were prepared by acid-catalyzed transesterification of total lipids according to Christie (1992). Extraction and purification of fatty acid methyl esters was performed as described by Ghioni et al. (1996). Fatty acid methyl esters were separated and quantified by gas-liquid chromatography (Carlo Erba Vela 6000, Thermo Quest, Manchester, England) using a 30 m × 0.32 mm capillary column (CP Wax 52 CB, Chrompak, London, U.K.). Hydrogen was used as carrier gas and temperature programming was from 50 to 150°C at 40°C/min and then to 230°C at 2.0°C/min. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman 1980).

**Histology.** Two larvae per tank were fixed for histology (saline buffered formalin, or Bouin Picro formol) on d 32, 46 and 65 PFF. The samples were dehydrated and embedded in paraffin wax for sectioning. Sagittal sections of 3–5 μm were stained with hematoxylin-eosin, periodic acid Schiff or Masson’s trichrome stain. The rod/cone ratio was measured in histological sections of the retina. In the ventrotemporal region of the retina, the numbers of nuclei in the outer nuclear layer were counted in a zone corresponding to thirty cones. The numbers of nuclei exceeding the number of cones were used as an estimate of rods (O’Connell 1963). Counts were performed on six larvae per treatment, and each value was the mean of three replicates from two fish for each dietary treatment. The pooled data were carried out using the Minitab (State College, PA) statistical package. Chi-squared analysis was applied to the nominal eye migration data (Zar 1984). All statistical analyses were performed before analysis. Chi-squared analysis was applied to the nominal eye migration data (Zar 1984). All statistical analyses were carried out using the Minitab (State College, PA) statistical package. A significance level of 95% (P < 0.05) was used throughout.

**Statistical analysis.** Statistics were applied to the data collected over the period d 16–71 PFF. Diet-related differences in rearing parameters, pigmentation characteristics and lipid compositions were compared using Tukey’s test. Percentage data were arc-sin transformed before analysis. Chi-squared analysis was applied to the nominal eye migration data (Zar 1984). All statistical analyses were carried out using the Minitab (State College, PA) statistical package. A significance level of 95% (P < 0.05) was used throughout.

**RESULTS**

Halibut larvae were fed Artemia nauplii, enriched with either SuperSelco (fed morning) or Algamac 2000 (fed evening), or Eurytemora velox copepods, from d 16 to 71 post-first feeding (PFF). The fatty acid compositions of total lipid of the enriched Artemia and various size ranges of E. velox copepods are shown in Table 1. The Algamac 2000–enriched Artemia contained similar amounts of DHA and EPA, resulting in a DHA/EPA ratio of 1; in addition, it contained 2.5% of 22:5(n-6). The SuperSelco enrichment produced Artemia containing slightly more DHA compared with the Algamac enrichment but appreciably more EPA, resulting in a DHA/EPA ratio of 0.4. The smaller size range of E. velox copepods, which comprised largely nauplii and copepodites, contained high levels of DHA and EPA and had a DHA/EPA ratio of 2.0. The larger size class of copepods, which comprised largely immature and mature adults, contained much lower levels of DHA and EPA and had a DHA/EPA ratio of 0.9. The percentage decrease in (n-3) HUFA in the larger size copepods was due to increased accumulation of saturated and particularly monounsaturated fatty acids.

The survival and growth parameters of the two groups of halibut over the period d 16–71 PFF are summarized in Table 2. No significant differences between replicate tanks, fed the same experimental diets, were observed. Halibut larvae fed copepods exhibited a significantly greater mean survival rate (66.4 ± 2.3%) compared with those fed enriched Artemia (44.7 ± 9.5%). The halibut larvae fed Artemia attained significantly higher final weights than those fed copepods. However, differences in mean specific growth rates were not significant because of the different start weights. The indices of eye migration on d 71 PFF were not significantly different between dietary treatments with mean values >2 in both cases (Table 2). The eye data are summarized in the frequency distributions in Figure 2 and establish that ~55% of the

![Figure 2](https://example.com/figure2.png)
halibut fry in both diet treatments exhibited complete eye migration (index of 3).

The mean proportion of “perfectly” metamorphosed halibut fry, i.e., those exhibiting correct pigment distribution and complete eye migration differed significantly according to diet (Table 2). Almost 40% of halibut larvae fed copepods exhibited perfect metamorphosis attributes, whereas only 3.5% of the fish fed Artemia fell into this category. The large difference in metamorphosis attributes is accounted for by differences in pigment distribution between the two groups of halibut fry. By reference to frequency distributions of the pigment categories (Fig. 3), it can be seen that ~45% of the halibut fed Artemia were “albino” (pigment category I/I), whereas this category was not recorded at all in fish fed copepods. Conversely, only 13% of the halibut fed Artemia exhibited correct pigmentation of the ocular and blind surfaces (category V/I) compared with 55% of the larvae fed copepods. Fish receiving both diet treatments displayed an abnormal trait on the blind body surface of partial pigmentation of the skin (Fig. 3, pigment categories I/II and V/II).

The measurement of the rod/cone ratio in retinas of the halibut at d 65 PFF is shown in Table 3. The rod/cone ratio was significantly higher in the copepod-fed group. Light microscope sections of liver were analyzed at d 46 and 65 PFF. At d 46 PFF, lipid-containing vacuoles filled most of the hepatocytes in the copepod-fed larvae, whereas these vacuoles were much smaller in the Artemia-fed larvae (Fig. 4). However, at d 65 PFF, lipid-containing vacuoles were equally abundant in the livers of both groups of larvae (data not shown).

The fatty acid compositions of total lipids from halibut eyes at d 65 PFF are shown in Table 4. The eyes from fish fed copepods contained significantly greater amounts of 16:0, 16:1(n-7), 24:1 and 22:6(n-3) and significantly lower amounts of 18:1(n-9), 18:1(n-7), 18:2(n-6), 20:4(n-6), total (n-6) PUFA, 18:3(n-3), 20:3(n-3), 20:5(n-3) and 22:5(n-3), compared with those fed enriched Artemia. The larvae fed copepods had a significantly higher DHA/EPA ratio compared with larvae fed enriched Artemia, but the EPA/α-linolenic acid (AA) ratio was not different. The long-chain HUFA [22:6(n-3), 20:5(n-3) and 20:4(n-6)] compositions of brains and livers from halibut larvae fed either enriched Artemia or copepods are shown in Figures 5 and 6. The percentages of these three essential HUFA in brain and liver were affected similarly by dietary treatment to the changes described for halibut eyes. In brain, DHA was significantly greater, whereas EPA and AA were significantly lower, in fish fed the copepods compared with those fed enriched Artemia. The EPA/AA ratios were significantly higher in brains of Artemia-fed fish compared with those fed copepods (3.4 ± 0.1 and 3.1 ± 0.1, respectively). In liver, DHA was significantly greater and EPA significantly lower in the copepod-fed halibut compared with those fed enriched Artemia. In both tissues, the DHA/EPA ratio was increased in fish fed copepods compared with those fed enriched Artemia. The EPA/AA ratios in liver were significantly higher in fish fed the enriched Artemia compared with those fed copepods (3.6 ± 0.4 and 2.9 ± 0.1, respectively).

### TABLE 3

| Rod/cone ratio in retinas of larval halibut fed either enriched Artemia or Eurytemora velox copepods
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Artemia-fed larvae</strong></td>
<td><strong>E. velox-fed larvae</strong></td>
<td></td>
</tr>
<tr>
<td>Cones</td>
<td>Rods</td>
<td>Rod/Cones</td>
</tr>
<tr>
<td>30</td>
<td>59</td>
<td>1.98</td>
</tr>
<tr>
<td>30</td>
<td>55</td>
<td>1.84</td>
</tr>
<tr>
<td>30</td>
<td>42</td>
<td>1.39</td>
</tr>
<tr>
<td>30</td>
<td>35</td>
<td>1.18</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>0.39</td>
</tr>
<tr>
<td>30</td>
<td>34</td>
<td>1.12</td>
</tr>
</tbody>
</table>

| Mean ± SD | 40 ± 17 | 1.32 ± 0.57 | 76 ± 21 | 2.53 ± 0.71* |

* Values for the rod/cone ratio were measured in histological sections of the retina. In the ventrotemporal region of the retina, the numbers of nuclei in the outer nuclear layer were counted in a zone corresponding to 30 cones. The numbers of nuclei exceeding the number of cones were used as an estimate of rods (O’Connell 1963). Counts were performed on six larvae per treatment and each value was the mean of three counts from one larva.

The rod/cone ratio in E. velox-fed larvae was significantly higher in enriched Artemia-fed larvae (P < 0.01).
was little or no difference in the EPA/AA ratio in larvae fed copepods and those fed supplemented Artemia, there is no reason to believe that the levels of EPA or AA are in any way unsuitable in the supplemented Artemia, most notably in relation to possible influences on eicosanoid metabolism in the larvae.

In addition to the benefits of maintaining a high DHA/EPA ratio in live-prey and larval tissues, there exists the potential importance of dietary phospholipid and vitamin A in preventing pigmentation abnormalities in larval flatfish (Kanazawa 1991 and 1993). The naupliar and copepodite stages of the E. velox copepods used in this study contained an excess of phospholipids over triacylglycerols, whereas the opposite composition was always evident in enriched Artemia (results not shown). Evidence suggests that phospholipids are more easily digested by larval fish compared with triacylglycerols, and their presence may enhance digestion of other lipids in the rudimentary digestive tract of larval fish (Kanazawa et al. 1983).

TABLE 4

Fatty acid compositions of total lipid from eyes of halibut larvae fed either Artemia enriched with SuperSelco and Algamac 2000 or Eurytemora velox copepods

<table>
<thead>
<tr>
<th>Fatty acid/diet</th>
<th>Enriched Artemia</th>
<th>E. velox</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g total fatty acids</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>16:0</td>
<td>16.2 ± 1.0*</td>
<td>18.0 ± 0.3</td>
</tr>
<tr>
<td>18:0</td>
<td>10.5 ± 0.4</td>
<td>9.8 ± 0.5</td>
</tr>
<tr>
<td>Total saturates</td>
<td>28.0 ± 1.2</td>
<td>28.9 ± 0.1</td>
</tr>
<tr>
<td>16:1 (n-7)</td>
<td>3.9 ± 0.3*</td>
<td>8.2 ± 1.1</td>
</tr>
<tr>
<td>18:1 (n-9)</td>
<td>12.5 ± 1.3*</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>18:1 (n-7)</td>
<td>7.2 ± 0.5*</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>20:1 (n-9)</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>24:1</td>
<td>0.5 ± 0.1*</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Total monoenes</td>
<td>24.9 ± 1.9</td>
<td>23.7 ± 1.8</td>
</tr>
<tr>
<td>18:2 (n-6)</td>
<td>2.1 ± 0.1*</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>20:2 (n-6)</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>20:3 (n-6)</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>20:4 (n-6)</td>
<td>3.1 ± 0.1*</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>22:5 (n-6)</td>
<td>2.5 ± 0.3*</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Total (n-6)</td>
<td>8.1 ± 0.2*</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>18:3 (n-3)</td>
<td>4.5 ± 0.1*</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>18:4 (n-3)</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>20:3 (n-3)</td>
<td>1.1 ± 0.1*</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>20:4 (n-3)</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>20:5 (n-3)</td>
<td>9.0 ± 0.6*</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>22:5 (n-3)</td>
<td>2.9 ± 0.2*</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>22:6 (n-3)</td>
<td>18.0 ± 3.5*</td>
<td>30.9 ± 1.9</td>
</tr>
<tr>
<td>Total (n-3)</td>
<td>35.9 ± 4.2</td>
<td>40.1 ± 1.6</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>44.0 ± 4.5</td>
<td>44.4 ± 1.4</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>2.0 ± 0.2*</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>EPA/AA</td>
<td>2.9 ± 0.1</td>
<td>2.7 ± 0.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 3. Values assigned an asterisk are significantly different from E. velox–fed larvae (P < 0.05). SD = ± tabulated as 0.0.
2 Includes 15:0, 20:0 and 22:0.
3 Includes 20:1 (n-11), 20:1 (n-7), 22:1 (n-9) and 22:1 (n-11).
4 PUFA, polyunsaturated fatty acids.
5 DHA, 22:6 (n-3); docosahexaenoic acid.
6 EPA, 20:5 (n-3); eicosapentaenoic acid.
7 AA, 20:4 (n-6); arachidonic acid.

FIGURE 4 Light micrographs of liver sections from halibut larvae at 46 d post-first feeding (PFF). The presence of many lipid-containing vacuoles can be seen (arrowed) in livers from larvae fed Eurytemora velox copepods (top), whereas these are largely absent in livers from larvae fed enriched Artemia (bottom). Scale bar = 20 μm.

DISCUSSION

In this study, halibut larvae fed Eurytemora velox copepods or enriched brine shrimp, Artemia, did not differ significantly in their specific growth rate and eye migration indices. However, copepod-fed halibut exhibited a significantly better mean survival rate and better pigmentation characteristics than those receiving Artemia. More than 40% of the halibut fed enriched Artemia were classified as “albino,” i.e., without any pigment expression on either the ocular or blind side. By contrast, none of the copepod-fed halibut were albinoic and 55% exhibited correct pigmentation of both body surfaces. Naess et al. (1995) similarly reported better pigmentation characteristics for zooplankton-fed halibut larvae compared with those fed SuperSelco–enriched Artemia.

In experiments with larval turbot (Scophthalmus maximus), increased pigmentation success was positively correlated with the presence of many lipid-containing vacuoles in eyes, brains and livers of halibut larval copepods was always greater than in those fed Artemia. We conclude, therefore, that the superior performance of copepods compared with Artemia nauplii reflects, at least in part, a relative deficiency of DHA in the supplemented Artemia. Moreover, because there...
is consistent with an active assimilation of dietary lipid and a high degree of lipid vacuolation in larvae fed copepods; thisysis of the liver in larvae at d 46 PFF (Fig. 3), which showed observations of Luizi et al. (1998) support the histological anal-
whereas copepods were more completely assimilated. The ob-
Artemia appeared to pass through the gut largely undigested,
Artemia.
visible in the mucosal epithelium of the hind gut of copepod-
et al. 1998). During early development, lipid droplets were
developed, did absorption of lipid occur in the fore gut (Luizi
in the hind gut; only later, when the stomach was more fully
feeding (up to d 46 PFF), lipid absorption was occurring largely
in developing larvae (Geurden et al. 1995 and 1997, Kanazawa
cannot be explained by their HUFA composition alone be-
cause both soy and tuna lecithin were equally effective in
promoting growth and development in some of these studies.
It is possible, however, as suggested by Geurden et al. (1995
and 1997) that fish larvae have only a limited ability to
biosynthesize phospholipids de novo such that intact phospho-
lipids in the diet are essential for maximal growth. It may be
that Artemia nauplii are limiting in this respect, particularly
given the high level of triacylglycerols in their total lipid.

Histological examination (data not shown) of the develop-
larval gut has indicated that in the period following first
feeding (up to d 46 PFF), lipid absorption was occurring largely
in the hind gut; only later, when the stomach was more fully
developed, did absorption of lipid occur in the fore gut (Luizi
et al. 1998). During early development, lipid droplets were
visible in the mucosal epithelium of the hind gut of copepod-
fed larvae, but largely absent in the hind gut of larvae fed
Artemia. In addition, during the early part of the experiment,
Artemia appeared to pass through the gut largely undigested,
whereas copepods were more completely assimilated. The ob-
servations of Luizi et al. (1998) support the histological anal-
ysis of the liver in larvae at d 46 PFF (Fig. 3), which showed
a high degree of lipid vacuolation in larvae fed copepods; this
is consistent with an active assimilation of dietary lipid and
was not present in the livers of larvae fed Artemia. However,
sections of liver analyzed at d 65 PFF, at which point gut
development would more easily allow assimilation of ingested
Artemia, showed lipid vacuoles present in larvae fed both
copepods and enriched Artemia.

Studies performed by Japanese researchers have suggested
that, in addition to the provision of essential (n-3) HUFA,
supplementation with adequate vitamin A is vital for success-
ful skin pigmentation (Kanazawa 1992, Miki et al. 1990).
Pigmentation may require signal transmission via the visual
system to the brain, which allows increased melanocyte-stim-
lating hormone production and consequent synthesis of mel-
alin (Estève and Kanazawa, 1996, Kanazawa 1993). A defi-
ciency in dietary vitamin A, which is a precursor of rhodopsin,
will disrupt transmission between the eye and the brain. Ma-
rine copepods are rich in carotenoid pigments (8.2–43.6 μmol
astaxanthin/g lipid for the four E. velox size ranges, highest
value in 125- to 250-μm size fraction), including mono-
and diesters of astaxanthin as well as unesterified astaxanthin,
whereas Artemia contain lower quantities of the related carot-
enoid canthaxanthin (4.5–5.9 μmol canthaxanthin/g lipid for
the enriched Artemia used in this study), which is present only
in the unesterified form (Krinsky 1965, J. McEvoy, Institute of
Aquaculture, University of Stirling). Although both canthax-
anthin and astaxanthin can be converted to vitamin A in fish
(Olson 1989), the higher quantity of total carotenoid in E. velox,
coupled with the apparent increased digestibility of copepods
in early developing larvae, may allow more efficient
uptake and metabolism of these vitamin A precursors in hal-
tib fed copepods compared with those fed Artemia.

Marine fish are naturally enriched with 22:6(n-3) and their
neural tissues are especially rich in this HUFA (Bell and Dick
1991, Tocher and Harvie 1988). Membranes that are highly
enriched in DHA such as those of the rod outer segment
membrane are highly “stressed” due to the packing properties
of di-22:6(n-3)– containing phospholipids; this can facilitate
rapid conformational changes of the membrane structure as
seen during the cis to trans transition that occurs on light
activation of rhodopsin (Brown 1994). Thus, DHA may have
a quite specific role in visual cell membranes. In addition,
DHA-rich membranes are cycled between the retinal epithe-
lum membrane and the photoreceptor membrane, and dietary
DHA deficiency can interfere with this process (Banaz et al.
1992). In young developing mammals, dietary (n-3) PUFAs
deficiency is known to impair visual acuity (Hrboticky et al.
has been observed in larval herring (Clupea harengus L.); DHA
derivation caused impaired visual performance, particularly
at low light intensities when rod cells are active (Bell et al.
1995). A linear relationship has been shown between the
recruitment of rods in the herring retina and its content of
di-DHA molecular species of phospholipids (Bell and Dick

![Figure 5](https://example.com/figure5.png)

**Figure 5** Levels of 22:6(n-3), 20:5(n-3) and 20:4(n-6) in brain
total lipid from halibut larvae fed either enriched Artemia or Eurytemora velox copepods. Values are means ± SD, n = 3. *Significantly different from copepod-fed larvae (P <0.05).

Knowledge of the structure of the membrane and how it
responds to dietary lipid and vitamin A allows a deeper un-
derstanding of the role of these dietary lipids and vitamins
in the development of fish larvae.
Copepods and Artemia as Halibut First Feed Organisms

ACKNOWLEDGMENTS

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