EFFECTS OF MICROALGAE AND LIVE DIET TYPE ON THE GROWTH OF FIRST-FEEDING WINTER FLOUNDER
(PLEURONECTES AMERICANUS)

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ABSTRACT

The addition of microalgae to larval rearing systems ("green water" treatment), has been shown to enhance the growth and survival of certain marine fish species. Along with the presence or absence of microalgae, diet type affects larval growth, and several studies have demonstrated that cultured live food organisms (e.g. rotifers and brine shrimp) are nutritionally inferior to wild zooplankton as a first food for marine finfish larvae. In a 2 x 2 factorial design experiment that lasted for five weeks from first feeding, we examined the effects of green water, clear water, wild zooplankton and cultured rotifers (Brachionus plicatilis) on the growth of winter flounder (Pleuronectes americanus) larvae. Results from the two way analysis of variance indicated that there was no significant interaction (P=0.80) between the two factors (presence/absence of microalgae, wild/cultured prey). Therefore, we considered the two factors independently of each other. At any time, fish in the green water treatments were significantly longer (P<0.05) than those in clear water treatments. There were no differences (P>0.05) in larval lengths between food types within either green or clear water. The mean instantaneous growth rates (length increases per week) were 15.4, 14.2, 12.2, and 9.6% for green water/wild zooplankton, green water/rotifers, clear water/rotifers, and clear water/wild zooplankton, respectively. Results of this study indicate that green water enhances the growth of winter flounder larvae, and there is little, if any, difference between wild zooplankton and rotifers as a first feeding diet.

INTRODUCTION

Domestic and overseas demand for high quality flatfish, combined with the declining harvest from wild populations, has greatly increased interest in the culture of various flounder species (Waters 1996). Those being considered for commercial aquaculture along the Atlantic coast of the United States include summer flounder (Paralichthys dentatus), yellowtail flounder (Pleuronectes ferruginea), and southern flounder (Paralichthys lethostigma). In addition to these, winter flounder (Pleuronectes americanus) is also being considered because many of the techniques for culturing this species have been developed. These include the technique for the artificial spawning of captive broodstock (Smigieliski and Arnold 1972), larval rearing (Smigieliski 1975; Rogers 1976; Laurence 1977; Klein-MacPhee et al. 1982, 1993), and the successful weaning of juveniles onto formulated diets (Lee and Litvak 1996).

The problems that have impeded commercial culture of many finfish species center around low larval survival and growth, particularly at the time of first feeding. The use of live food in the culturing of the early life stages of marine fish larvae, including winter flounder, is currently consid-
<table>
<thead>
<tr>
<th>Week</th>
<th>Algal Presence</th>
<th>Food Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Green vs. Clear</td>
<td>Rotifers vs. Wild</td>
</tr>
<tr>
<td></td>
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<td>p-value</td>
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</tr>
<tr>
<td>5</td>
<td>0.0064</td>
<td>0.6046</td>
</tr>
</tbody>
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Table 1. Analysis of algal presence and food type on winter flounder growth. P-values derived from unpaired T-tests.

<table>
<thead>
<tr>
<th>Week</th>
<th>Green</th>
<th>Clear</th>
<th>Rotifers</th>
<th>Wild</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean +/- sd</td>
<td>%/wk</td>
<td>mean +/- sd</td>
<td>%/wk</td>
</tr>
<tr>
<td>1</td>
<td>5.1 +/- 0.10</td>
<td>17</td>
<td>4.5 +/- 0.17</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>6.2 +/- 0.38</td>
<td>19</td>
<td>5.3 +/- 0.21</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>7.4 +/- 0.50</td>
<td>18</td>
<td>5.9 +/- 0.41</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>8.1 +/- 0.26</td>
<td>9</td>
<td>6.6 +/- 0.45</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>9.1 +/- 0.64</td>
<td>11</td>
<td>7.0 +/- 0.60</td>
<td>6</td>
</tr>
<tr>
<td>Mean instantaneous growth rate</td>
<td>15</td>
<td>10</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 2. Mean lengths (mm) and instantaneous growth rates (%/wk) for replicates of green water, clear water, rotifer, and wild zooplankton treatments. sd = standard deviation.

<table>
<thead>
<tr>
<th>Week</th>
<th>Green/Wild</th>
<th>Green/Rotifers</th>
<th>Clear/Wild</th>
<th>Clear/Rotifers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean +/- sd</td>
<td>%/wk</td>
<td>mean +/- sd</td>
<td>%/wk</td>
</tr>
<tr>
<td>1</td>
<td>5.1 +/- 0.10</td>
<td>17</td>
<td>5.2 +/- *</td>
<td>19</td>
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<tr>
<td>2</td>
<td>6.0 +/- 0.10</td>
<td>16</td>
<td>6.6 +/- *</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>7.4 +/- 0.70</td>
<td>21</td>
<td>7.4 +/- *</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>8.2 +/- 0.30</td>
<td>10</td>
<td>7.9 +/- *</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>9.3 +/- 0.80</td>
<td>13</td>
<td>8.7 +/- *</td>
<td>10</td>
</tr>
<tr>
<td>Mean instantaneous growth rate</td>
<td>15</td>
<td>14</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 3. Mean lengths (mm) and instantaneous growth rates (%/wk) for all four treatments. sd = standard deviation. * = no sd due to loss of a replicate.
ered obligatory. The most widely used live food organisms are cultured rotifers, such as *Brachionus plicatilis*, and brine shrimp (*Artemia salina*) nauplii. While the use of these two prey species is common, they are relatively expensive because of the labor involved in their production (Ehrlich and Rust 1989). Le Ruyet et al. (1993), for example, have calculated that live prey feeding (mainly *Artemia*) represented 79% of the total production cost of a 45 day old sea bass. A second, and critical disadvantage of cultured live foods (*Brachionus* and *Artemia*), is that they do not provide optimal larval nutrition, largely due to low levels of essential fatty acids (Watanabe et al. 1983a, Witt et al. 1984, Leger et al. 1986, van der Meeren et al. 1993). For this reason, fish larvae reared on cultured foods often exhibit abnormal development, poor growth, and low survival (Watanabe et al. 1980, Fujita et al. 1980, van Balderen et al. 1985, Izquierdo et al. 1989, Koven et al. 1990, van der Meeren 1991c). Methods of improving the nutritional quality of cultured foods, via enrichment with highly unsaturated fatty acids (HUFA), are available (Watanabe et al. 1983b), but they add to the cost of live food production. Natural (wild) live foods (primarily copepods) have been used with greater success (Naas et al. 1987, Ellertsen et al. 1981, van der Meeren 1991b, Le Ruyet et al. 1993), because they are generally richer in essential fatty acids (Pedersen 1993). In Atlantic cod for example, larvae fed diverse assemblages of wild zooplankton in both semi-intensive (van der Meeren 1991b, Otterå 1993, van der Meeren and Naess 1993) and extensive systems (Fiestad et al. 1985, Skjoldal et al. 1990, Bloem et al. 1991) have generally displayed good survival and growth.

In addition to the use of live larval food organisms, whether cultured or wild, the addition of microalgae to larval rearing tanks ("green water" treatment) has been widely accepted as a technique for commercial marine finfish production. The addition of microalgae has enhanced larval growth and survival of a number of species, including turbot (Howell 1979; Scott & Middleton 1979; Jones et al. 1981; Bromley & Howell 1983; Reitan et al. 1993), halibut (Naas et al. 1992; Bergh et al. 1994), summer flounder (Alves et al. 1997), cod (Pedersen et al. 1989; van der Meeren 1991a), and grunion (Vasquez-Yeomans et al. 1990). While the mechanism(s) by which the microalgae improve growth and survival remains unclear, and may differ among both microalgal and fish species, several hypotheses have been proposed to explain their positive effects at first-feeding. They may provide nutritional benefits either directly via ingestion and absorption (Moffatt 1981), or indirectly by increasing the amounts of essential fatty acids in the rotifers being fed to the fish larvae (Reitan et al. 1993). Microalgae may also trigger digestion processes in the larvae (Hjelmeland et al. 1988).

In addition to nutritional benefits, microalgae may inhibit pathogenic bacteria (Austin et al. 1992), influence the establishment of intestinal microflora (Skjermo & Vadstein 1993), and stabilize water quality (Houde 1975, 1978). It has also been suggested by Naas et al. (1992) that microalgae can change ambient light conditions in the larval tanks, which may, in turn, lead to an increase in the consumption of zooplankton at first-feeding.

Because first-feeding diet (live food type) and the addition of microalgae have both been shown to influence larval growth and survival, and because we are unaware of any published work that has examined these variables for winter flounder, we set out to determine if live food type and the presence or absence of microalgae affected the growth and survival of winter flounder larvae. In this research, the following null hypotheses were tested: (1) the addition of microalgae to larval rearing systems has no effect on the growth of winter flounder; and (2) there is no difference in the growth between first feeding winter flounder larvae fed live, laboratory cultured rotifers, and those fed a diet of wild zooplankton.

**MATERIALS AND METHODS**

A 2 X 2 factorial design experiment with two replicates per treatment was initiated in May 1996 and lasted for five weeks from first feeding to metamorphosis. Two hundred, five day post-hatch winter flounder larvae were stocked into each of eight 20 liter, tapered, round, gray, plastic aquaria measuring 32 cm high, with a 43 cm diameter top and 35 cm diameter bottom. A green water starvation treatment served as the control. Treatments were static with 50% water change (10 liters) every third day. Replacement seawater was filtered...
to remove particles greater than 5 μm and treated with UV light prior to addition to the experimental aquaria. Aquaria were submerged in a 13 cm deep flow-through water table to maintain ambient seawater temperature. Temperature ranged from 8 to 15°C, with a daily average of 10°C over the course of the experiment. Salinity was maintained at 30-32 ppt. Larvae were exposed to 24 hours light using a 35 Watt fluorescent light suspended 76 cm above the aquaria.

Semi-continuous cultures of the microalga *Isochrysis galbana* (Tahitian strain) were maintained in 80 L fiberglass cylinders, and were provided with f/2 media (Guillard and Ryther 1962). Rotifers (L-type) were cultured in identical cylinders on a diet of *Isochrysis galbana* and dry yeast (at 1 g/million rotifers/d). Cultured rotifers were fed to respective larval fish treatments at an average daily rate of 2600/l. Wild zooplankton were harvested from Portsmouth Harbor, New Hampshire by towing an 80 micron plankton net through the top two meters. Collected plankton were sieved through a 200 μm and a 48 μm screen, and counted. Wild zooplankton, consisting of approximately 90% copepod nauplii, were fed to respective treatments at an average daily rate of 2100/l. All treatments were visually inspected prior to feeding, and it was determined that larvae were fed to satiation based upon the presence of residual planktoria. Three liters of *Isochrysis galbana* were added to treatments receiving green water every third day (with water changes) at a density of 200,000 cells/ml.

A random sample of ten larvae from each replicate in each treatment were measured to the nearest 0.5 mm (total length) each week. Mortalities were not replaced throughout the duration of the experiment. Mean length per replicate was used as the response variable. A two-way analysis of variance (ANOVA) was used to determine if there was any interaction between the two factors (prey type, presence/absence of microalgae). Where possible, unpaired t-tests were used to compare lengths of larvae raised in different combinations of the two factors. Instantaneous growth rate, G (%/wk) was calculated using Ricker's (1979) formula: 

\[ G = \frac{(Y_{t+1} - Y_{t})}{Y_{t} (t_{i+1} - t_{i})} \]

where \( Y_{t} \) is length at time \( t_{i} \). Survival between treatments was compared using one-way analysis of variance (ANOVA).

![Graph showing total length vs week for green and clear treatments](image)

**Figure 1.** Winter flounder growth in the presence or absence of microalgae. Vertical bars represent +/- 1 standard deviation.
RESULTS

Results from the two way analysis of variance indicated that there was no significant interaction (P=0.80) between the two factors (presence/absence of microalgae, wild/cultured prey). Because of this lack of interaction, we were able to consider the two factors independently of one another. Larvae in treatments receiving microalgae (green water) were compared to in their absence (clear water). In this comparison, which disregarded food type, a highly significant difference (P<0.01) in total length was found in each week of the experiment (Table 1). Figure 1 shows the trends in mean length over the five weeks for larvae reared in the presence and absence of microalgae. Larvae in green water treatments grew to a mean length of 9.1 ± 0.64 mm at a mean instantaneous growth rate of 15%/week, while final mean length of those in clear water was 7.0 ± 0.60 mm, with a mean instantaneous growth rate of 10%/week (Table 2).

No significant difference (P>0.05) in length was found between larvae in replicates receiving cultured rotifers and those which were fed wild zooplankton, regardless of algal presence (Table 1). Figure 2 illustrates trends in length of larvae that were reared on cultured rotifers and wild zooplankton. Larvae in treatments fed rotifers grew to a mean length of 7.5 ± 1.1 mm at a mean instantaneous growth rate of 11%/week, while those fed wild zooplankton grew to a mean length of 8.1 ± 1.5 mm at a mean instantaneous growth rate of 13%/week (Table 2).

The loss of a replicate in the green water/rotifer treatment precluded us from making statistical comparisons between this treatment and others. We were however, able to compare final mean lengths of larvae reared in two combinations of food type and the presence/absence of microalgae. Final mean length of larvae raised in the clear water/rotifer combination (7.0 mm) was not significantly different (P>0.05) from that of larvae raised in the clear water/wild prey combination (7.0 mm) (Table 3). The final mean length of larvae raised in the green water/wild prey combination (9.3 mm) was significantly longer (P<0.05) than that of larvae in the clear water/wild prey combination (7.0 mm) (Table 3).

There was no significant difference (P>0.05) between final mean survival values which ranged from 13.5 to 22.0% (Figure 3). All larvae in the control (green water with no food) died by week two.
Table 3. The effects of food type and algal presence on winter flounder survival after five weeks. Vertical bars represent +/- 1 standard deviation.

**DISCUSSION**

Growth of winter flounder larvae has been studied extensively, and has been found to vary with both temperature (Laurence 1975) and prey density (Laurence 1977). In general, growth in length of both laboratory-reared and wild-caught larvae is curvilinear (Pcarcy 1962a; Bertram et al. 1996; Jerald et al. 1993), with growth being quickest during the first weeks after hatching, and then slowing as the fish approach metamorphosis. Although the weekly mean sizes we found in this study varied between treatments, all of our larvae grew at rates generally comparable to those reported for winter flounder larvae in nature (Pcarcy 1962a), in the laboratory (Laurence 1975, 1977; Chambers and Leggett 1987; Jerald et al. 1993; Bertram et al. 1996), and in in-situ mesocosms (Laurence et al. 1979).

Results from this study demonstrated that the presence of microalgae significantly improved the growth of larval winter flounders. Similar results have been found for the larvae of other species, including turbot (*Scophthalmus maximus*), halibut (*Hippoglossus hippoglossus*), summer flounder (*Paralichthys dentatus*), cod (*Gadus morhua*), and grunion (*Leurestes tenuis*) (Howell 1979; Scott & Middleton 1979; Jones et al. 1981; Bromley & Howell 1983; Pedersen et al. 1989; Vasquez-Yeomans et al. 1990; van der Meeren 1991a; Naas et al. 1992; Reitan et al. 1993; Bergh et al. 1994; Alves et al. 1997). As seen in Table 1 and Figure 1, larvae reared in green water were significantly longer than those in clear water in each week of the experiment. Differences in instantaneous growth rates were most pronounced at the end of the first week of the experiment (17% vs. 5% for green water and clear water treatments, respectively). This suggests that the presence of microalgae enhanced larval growth within the first week of exogenous feeding. Although not quantified in this experiment, we noted that larvae receiving microalgae initiated feeding sooner than larvae in clear water. A similar observation was made for halibut larvae reared in green water (Naas et al. 1992), where enhanced first-feeding was ascribed to the microalgae effecting ambient light levels in the culture tank, which in turn improved larval feeding efficiency. It has also been suggested that microalgae may stimulate enzymatic activity of the larva's gut during first feeding (Hjelmeland et al. 1988), or supply exogenous enzymes that assist the larvae in their digestion of zooplankton (Bromage and Roberts 1995). If, as
we expect, our winter flounder larvae in green water initiated feeding earlier than those in clear water, and if the microalgae triggered the digestion processes, it could account for the significant difference in length (and growth rate) we observed at the end of week 1.

The benefits of microalgae may also have resulted from direct ingestion of the algae, which has been observed in a number of marine fish larvae, including northern anchovy (Moffatt 1981), turbot (Howell 1979; Last 1979), halibut (Reitan et al. 1993) cod (van der Meeren 1991a) and wild-caught winter flounder (Pearce 1962b). Both the mechanism of microalgal ingestion, which may involve either drinking or filter-feeding, and the nutritive value of the ingested microalgae are open to speculations (Van der Meeren 1991a). Studies with larval cod (Van der Meeren 1991a), turbot (Howell 1979) and halibut (Reitan et al. 1993) suggest that assimilation of the microalgae by the larval gut is low. Despite this, Tylter et al. (1997) found that turbot larvae had chlorophyll containing apical vacuoles in the gut enterocytes 3 days after hatching, and they suggested that although assimilation efficiency was low, the larvae may obtain small amounts of essential fatty acids, amino acids, and carotenoids from the microalgal cell pigments. Microalgae may also enter the larval gut indirectly through ingesting microalgae-fed rotifers (Reitan et al. 1993). It has also been suggested that the addition of microalgae leads to the establishment of an early larval intestinal microflora (Skjermo and Vadstein 1993; Bergh et al. 1994). This in turn, may enable the digestion of algal cells (Rimmer and Wiebe 1987), may provide amino acids, fatty acids and vitamins (Kashiwada and Teshima 1966; Fong and Mann 1980; Ringo et al. 1992), and may inhibit bacterial pathogens (Olsson et al. 1992).

Apart from stimulating first feeding and/or providing either direct or indirect nutrition, microalgae may also act to control bacterial growth in tanks by releasing natural bacteriostatic agents. Austin et al. (1992) for example, found that the exudates from one species of algae (Tetraselmis suecica) inhibited certain bacterial fish pathogens. The microalgae may also stabilize water quality by absorbing waste products and producing oxygen (Houde 1975, 1978). Because both bacteriostatic agents and water quality would effect survival, it is possible that the microalgae was responsible for the tendency (not statistically significant) for survival to be higher in green water treatments than in clear water treatments (Figure 3). Survival estimates from this study ranged from about 13.5-22% at the end of 5 weeks which are lower than the approximate 34% reported by Laurence (1977) for winter flounder larvae raised at 8°C and provided with 3000 wild zooplankters/L. Our lower observed percentages may have resulted from our fluctuating, and slightly warmer, incubation temperatures.

We found no significant difference (p>0.05) in the final mean lengths of larvae, or in percent survival, between the two live prey treatments. Among the components of any larval fish diet, it is well documented that fatty acids, particularly n-3 highly unsaturated fatty acids (HUFA), are important to the nutrition of marine fish larvae (Watanabe et al. 1983b; Van Baal et al. 1985; Koven et al. 1990), including winter flounder (Klein-MacPhee et al. 1980). Because wild zooplankton are typically rich in these essential fatty acids compared to cultured live food organisms (Watanabe et al. 1980, 1983a; Van Baal et al. 1985; Leger et al. 1986; Naess et al. 1995), experiments in which marine fish larvae have been fed cultured prey and wild zooplankton have generally shown that growth and survival are higher in those fed wild zooplankton (Skjoldal el al. 1990). It has also been shown that some larvae (e.g. turbot) select wild zooplankton over the rotifer Brachionus plicatilis if given a choice (van der Meeren 1991). Our finding that there was no significant difference in lengths or survival between larvae fed cultured rotifers and those fed wild zooplankton suggests that the two food types were similar in their nutritional value. Reitan et al. (1993) have shown that rotifers fed Isochrysis galbana (T. Iso) have relatively high levels of lipids and 22:6 n-3 highly unsaturated fatty acid (HUFA) compared to those in clear water, and that turbot larvae fed these microalgae-enriched rotifers have higher growth and survival than those fed rotifers grown in clear water. Presumably this was due to the microalgae providing a source of micronutrients and HUFA to the larvae, both of which are essential for growth and survival (Fukusho et al. 1984; Brown et al. 1997). Moreover, Reitan et al. (1993) found that
total lipids and fatty acids remained relatively high in uneaten rotifers living in green water systems because they were consuming the microalgae. Thus culture tanks receiving microalgae promoted a continuous supply of highly nutritious rotifers for the fish larvae. Because Isochrysis galbana is known to have relatively high levels of essential fatty acids (Brown et al. 1997), and because a number of authors have shown that the levels of n-3 HUFA can be increased in cultured food organisms, including rotifers, by feeding them unicellular marine algae rich in n-3 HUFA (Kitajima et al. 1979; Scott and Middleton 1979; Koven et al. 1990; Reitan et al. 1993), we believe our rotifers were enriched to levels comparable to the wild zooplankton we used, thereby accounting for similar performance of the larvae fed these two diets. Comparisons of larval lengths from the experiment support this theory of microalgal enrichment (Table 3). We found, for example, that larvae fed both cultured rotifers and wild zooplankton in the presence of microalgae were larger than those fed cultured rotifers and wild zooplankton in the absence of microalgae, at every weekly time interval. While the microalgae may have been having a number of effects (see above), it is possible that it may have been improving the nutritional value of both the rotifers and wild zooplankton such that they were nutritionally equivalent. We also found that there was no significant difference in the mean lengths of larvae fed rotifers and wild zooplankton in the absence of microalgae. This result also suggests that the two diets were nutritionally equivalent. In this case, however, the equivalence was probably due to the rotifers having been fed microalgae as they were being cultured.

In this five week experiment, we found that larvae in the green water treatments grew to larger mean lengths than larvae in the clear water treatments, regardless of food type. We also found that there was a tendency, although not statistically significant, for survival to be higher in green water treatments than in clear water treatments. These results indicate that microalgae should be used when culturing winter flounder larvae. This may be particularly important during the first week following yolk-sac absorption, as indicated by the greatest disparity in the instantaneous instantaneous growth rate of larvae in green water treatments (17%/wk) compared to those in clear water treatments (5%/wk) for this early period. Results of the study also suggest that there is little difference between wild zooplankton and cultured rotifers as a first feeding diet for winter flounder larvae. We note, however, that our rotifers were almost certainly enriched, particularly in essential fatty acids, by the microalgae with which they were cultured, and that their tendency, although not statistically significant, for survival to be higher in green water treatments than in clear water treatments. These results indicate that microalgae should be used when culturing winter flounder larvae.

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LITERATURE CITED


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