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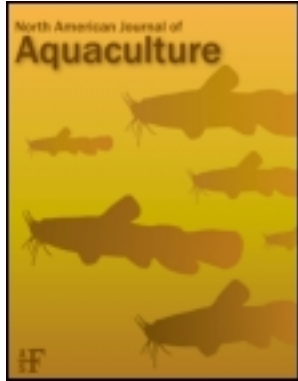
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## Green-Water Rearing and Delayed Weaning Improve Growth and Survival of Summer Flounder

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**Abstract.**—The advent of an aquaculture industry for summer flounder *Paralichthys dentatus* requires that optimal methods be identified for hatchery production. Two experiments were conducted to test strategies for larval rearing and for weaning newly metamorphosed juveniles from live to artificial diets. Rearing of larvae in “green water” (with algae added) resulted in better survival ( $76.1 \pm 6.5\%$ ) from days 5–42 after hatching than did rearing in “clear water” (no algae added;  $27.8 \pm 13.6\%$ ), although no differences in growth were apparent. When fish were weaned from live feed beginning at day 45 versus day 57 by either a “gradual” method (7-d weaning period) or an “immediate” method (no weaning period), better survival and growth were obtained with fish weaned at the later age. For both age-groups, fish weaned by the gradual method exhibited better growth, but not better survival, than those weaned by the immediate method. With these data as examples, commercial hatcheries can conduct cost:benefit analyses of the different rearing methods.

Natural stocks of summer flounder *Paralichthys dentatus*, a commercially important species, are in decline (NMFS 1996). Commercial aquaculture of this species began in 1996, but strategies for optimal production still need to be established (Bengtson and Nardi, in press). Substantial mortality occurs during early larval stages and at the time of metamorphosis and weaning to formulated diets (Bengtson 1999). Hatchery production of summer flounder is modeled after that of the successful culture of turbot *Scophthalmus maximus* in Europe.

In the early 1990s, major turbot hatcheries in Europe had differing views on the optimal strategy for larval rearing. One group claimed that use of “green water” (i.e., with microalgae added to the rearing tanks) was most efficacious, whereas another group claimed that use of “clear water” (no microalgae added) allowed better control of the rearing system because fewer species were involved (G. Nardi, GreatBay Aquafarms, personal

communication). Recent studies have determined that survival and growth of turbot larvae are greater in green water than in clear water (summarized by Reitan et al. 1997). Støttrup et al. (1995) proposed that the beneficial effect of the microalgae is a result of its control of bacteria rather than its nutritional effect.

Preliminary studies in our laboratory indicated no significant difference in survival of summer flounder larvae reared in green water versus clear water but significantly greater growth in green water; however, the results were influenced by large variance in survival among replicates, including very poor survival in one replicate of the green-water treatment. The question arose whether green-water rearing might be generally better but carries the risk of occasional catastrophic loss of larvae. We therefore decided to conduct a more thorough experiment to assess the benefits and risks of rearing summer flounder larvae in clear versus green water.

Summer flounder spend about 35–65 d as swimming, bilaterally symmetrical larvae in small experimental tanks at 20°C before they metamorphose and settle to the bottom. Faster growing larvae settle at an earlier age than slower growing larvae. The digestive tract is complete (including a stomach) and functional at about the time of metamorphosis (Bisbal and Bengtson 1995; Huang et al. 1998). A significant problem in the hatchery rearing of marine fish is the transition from a diet of live prey organisms, usually nauplii of brine shrimp *Artemia* sp., to a formulated dry diet. The time after hatch at which this transition (often referred to as weaning) can be made varies from species to species, but it generally occurs after the stomach has developed. For many species, especially flatfish, the stomach is complete only after metamorphosis and settlement from the water column to the bottom. Nevertheless, this period of transition from live to formulated diet can be a period of high mortality, as some fish apparently never eat the formulated diet and eventually starve. Recent reviews of formulated diets for young fish (Person-Le Ruyet et al. 1993; Rosenlund et al.

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1997) have concentrated on attempts to feed them to larvae before metamorphosis, but optimal strategies for weaning from live to formulated diets even after metamorphosis have been established for very few species. The strategy used in most hatcheries is to gradually diminish the amount of live food provided and gradually increase the amount of formulated diet. Several aspects of weaning have been investigated in turbot (Bromley and Howell 1983; Bromley and Sykes 1985; Segner and Witt 1990). It appears from those studies that older turbot are easier to wean than younger and that duration of time to establish weaning is variable. More recently, Lee and Litvak (1996) found that wild-caught winter flounder *Pleuronectes americanus* could be weaned to a dry diet in 1 week, but Daniels and Hodson (1999) found that 12–20 d of weaning were required for southern flounder *Paralichthys lethostigma*.

We conducted preliminary experiments on newly metamorphosed summer flounder in small bowls and found that, in some cases, a gradual change from live to formulated diets worked well. In other cases, fish would not ingest the formulated diet at all if any live food was provided at any time during the day, but immediately ingested the formulated diet if no live food was provided. We therefore decided to conduct an experiment to simultaneously examine the success of weaning at two ages (begin weaning at 45 d after hatch (DAH) versus 57 DAH) and with two methods of weaning (gradual weaning over 7 d versus immediate weaning from pure live food one day to pure formulated diet the next).

### Methods

Summer flounder were obtained from brood-stock fish at the University of Rhode Island and reared according to methods described by Bisbal and Bengtson (1993). The green-water versus clear-water experiment was designed as a two-treatment experiment, with 10 replicates in each treatment. Each replicate chamber (a 75-L glass aquarium with blackened walls) contained 60 L of filtered seawater ( $30 \pm 2\text{‰}$  salinity,  $20 \pm 2^\circ\text{C}$ ) and 600 5-d-old larvae at the beginning of the experiment. The larvae were randomly distributed to the 20 aquaria in the experiment. The experiment continued until the fish were 42 d old. Larvae were given only rotifers *Brachionus plicatilis* until they were 17 d old, then given both rotifers and brine shrimp nauplii for 1 week, then given brine shrimp only until the end of the experiment. Food levels were monitored on each day of the experiment and

maintained at approximately 5 rotifers/mL and 1 brine shrimp nauplius/mL. Approximately 90% of the water in each aquarium was replaced during each twice-weekly water exchange in the semi-static system. Light was provided by a 15-W fluorescent aquarium light over each aquarium. The green-water aquaria received the addition of approximately 600 mL/d of the green alga *Tetraselmis suecica* (stock culture density approximately  $1 \times 10^6$  cells/mL), and the water in the aquaria was visibly green throughout the experiment. At the end of the experiment, all the fish in each aquarium were counted and removed and random subsamples (every 15th fish from the green-water aquaria and every 6th fish from the clear-water aquaria) were collected for measurement of total length. Both survival and total length results were analyzed by *t*-tests with results considered significant at the  $P < 0.05$  level.

Larvae in the weaning experiment were fed rotifers and then brine shrimp nauplii before use in the experiment. Approximately 1 week after settlement began, settled fish were siphoned from the rearing aquaria and placed in a single 190-L aquarium, so that all the fish used in the experiment would initially be at the same developmental stage. The experiment was designed as a  $2 \times 2$  factorial, with age and weaning method as the factors. The treatments were (1) fish weaned beginning at 45 DAH using the immediate method (45-I), (2) fish weaned beginning at 45 DAH using the gradual method (45-G), (3) fish weaned beginning at 57 DAH using the immediate method (57-I), and (4) fish weaned beginning at 57 DAH using the gradual method (57-G). Each treatment consisted of four replicates (75-L aquaria) containing 25 fish each. Two hundred fish were removed from the stock tank and randomly placed in the experimental aquaria at 45 DAH (average initial wet weight, 26 mg). The remainder of the fish were left in the stock tank and fed brine shrimp nauplii. At 57 DAH, 200 more fish were removed from the stock tank and randomly distributed to the remaining experimental aquaria (average initial wet weight, 122 mg).

In the immediate treatments, no brine shrimp nauplii were supplied, and fish were given a commercially available weaning diet (Lansy, INVE Aquaculture). In the gradual treatments, over the course of a week, the fish were given one-seventh less brine shrimp each day than they received on the previous day and a concomitant increase each day of the Lansy diet to replace the reduction in brine shrimp. The aquaria were siphoned clean

TABLE 1.—Survival and growth (mean  $\pm$  SD) of recently metamorphosed summer flounder weaned from live feed (brine shrimp nauplii) to a formulated diet (Lansy) at two ages (45 d after hatch [DAH] versus 57 DAH) by two methods (gradual versus immediate). Values within a column that are followed by the same letter are not statistically significantly different ( $P > 0.05$ ).

Treatment	Survival (%)	Final wet weight (mg)
45 DAH, immediate	55 $\pm$ 24 y	63.4 $\pm$ 20.2 w
45 DAH, gradual	69 $\pm$ 14 y	92.6 $\pm$ 8.5 x
57 DAH, immediate	78 $\pm$ 11 z	141.7 $\pm$ 22.4 y
57 DAH, gradual	85 $\pm$ 4 z	176.8 $\pm$ 46.4 z

each day before feeding and any mortalities were counted and removed. At the end of the experiment at 89 DAH, all fish were removed from the aquaria, and wet weight of each fish was determined. Both survival and growth data were analyzed by two-way analysis of variance (ANOVA), and results were considered significant at the  $P < 0.05$  level.

### Results and Discussion

Survival (mean  $\pm$  SD) was significantly greater in the green-water aquaria (76.1  $\pm$  6.5%) than in the clear-water aquaria (27.8  $\pm$  13.6%). Total length (mean  $\pm$  SD) was not significantly different between treatments (9.8  $\pm$  0.7 mm in green water; 11.7  $\pm$  2.3 mm in clear water). The results indicate that green-water rearing was more effective than clear-water, both in the absolute magnitude of the survival percentage and the lower variability observed (coefficients of variation were 8.5% for green water; 48.9% for clear water). We conclude that survival is better and less variable in green-water than in clear-water rearing of summer flounder larvae. Alves et al. (1999) also reported better and less variable survival in green water versus clear water for summer flounder larvae reared from 3 DAH to 10 DAH at high density in experimental 2-L bowls.

Fish weaned at 57 DAH survived and grew significantly more than those weaned at 45 DAH and fish weaned by the gradual method grew significantly more than those weaned by the immediate method but did not survive significantly better (Table 1). These results are in accord with the results of the other studies on flatfish weaning cited above. Given the results of Daniels and Hodson (1999), it may well be that higher survival rates could be obtained with summer flounder if a weaning period even longer than 7 d were used. The justification usually given for early weaning is that costs of rearing could be reduced if feeding on expensive

brine shrimp nauplii could be minimized. Hatchery operators can use our data to estimate the costs and benefits of strategies of earlier versus later weaning, given known costs of brine shrimp and formulated diet and the calculated cost of fish mortalities at the weaning stage (i.e., how much they had invested in each fish that might die if weaned too early). Our anecdotal observations during and since this experiment suggest that more information is needed to identify optimum weaning factors for summer flounder (e.g., fish density, method and timing of food presentation, diet quality, and others). Mortality during weaning is still unacceptably high in this species and considerable research is still required to overcome that problem.

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