THE EFFECT OF STOCKING DENSITY ON THE GROWTH OF JUVENILE SUMMER FLOUNDER PARALICHTHYS DENTATUS

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ABSTRACT

The effect of stocking density on the growth of two size classes of juvenile summer flounder was studied in experiments which lasted for 40 and 58 days. In each experiment, density treatments of 100, 150, and 200% fish coverage of tank bottom surface area were tested. Fish were fed to satiation and randomly sampled for length, weight, and ventral surface area. Results from this study indicated that small (approx. 1 g, approx. 50 mm) juvenile summer flounder were unaffected by stocking density of at least 200% over 40 days. Larger (approx. 10 g, approx. 100 mm) juvenile summer flounder were affected by the nominal stocking densities, with fish initially stocked at 100% coverage growing to slightly larger sizes during the 58-day experiment.

INTRODUCTION

With an increasing demand for high quality flatfish in domestic and overseas seafood markets, the summer flounder Paralichthys dentatus has become a new and promising candidate for worldwide fish farming. Its potential has been studied in the United States for several years, and commercial cultivation has been initiated in several locations along the east coast. Early research has focused on larval development and production (Bisbal and Bengtson 1991a,b, Malloy and Targett 1991, Bisbal and Bengtson 1993, Keefe and Able 1993, Bisbal and Bengtson 1995a,b,c), but less research has been directed towards issues associated with juvenile grow-out. The ability to raise the fingerlings at a relatively high density, thus maximizing water usage and fish production, is of particular importance to commercial operation. However, parameters which affect growth and survival, such as feeding efficiency, disease, and water quality, should be considered when determining an optimal stocking density for a particular system. Studies examining high rearing densities of several salmonid species attribute growth inhibition to reduced feed consumption, poor feed conversion, aggressive behavior, and oxygen depletion (Reefstie and Kittelsen 1976, Reefstic 1977, Vijayan and Leatherland 1988, Holm et al. 1990, Kindisch and Koby 1994). Similarly, for flatfish species like Japanese flounder Paralichthys olivaceus, turbot Scophthalmus maximus, and Atlantic halibut Hippoglossus hippoglossus, there appear to be some effects of higher stocking densities on growth rate and feed efficiency (Martinez-Tapia and Fernandez-Pato 1991, Jeon et al. 1993, Bjornsson 1994, Chang et al. 1995). To date, few studies have demonstrated optimal stocking density for juvenile flatfish under 50 g in weight, and none have examined juvenile summer flounder stocking density. This research was undertaken to estimate the optimal stocking density of early juvenile summer flounder in an experimental recirculating system. Two size groups, with initial weights of 0.7 and 7.8 g, were examined in two separate experiments.

METHODS

The recirculating system

The experimental recirculating system consisted of 14 round, 190-L, fiberglass tanks
associated with a 26-L biological filter. Water flowed (gravity) from the biological filter, which contained “Bio-Fill” media and nitrifying bacteria, through an ultraviolet light sterilizing unit to a distribution manifold above the tanks. Overflow from the tanks went into a central collection channel that was filled with a coarse polyester fiber mat for the removal of large particulate waste. This partially clarified water fell into a sump tank, and was then pumped through two cartridge filters (15 μm) back to the biofilter. Water flow to the tank was regulated using valves, and each tank was gently aerated. The system was inoculated with nitrifying bacteria and run for 6 weeks prior to the introduction of any fish. After the system was established, salinity, temperature, ammonia, and nitrite were measured daily, while dissolved oxygen and alkalinity were measured periodically.

**Relating total length to ventral surface area**

Fish density was measured as percent coverage of the tank bottom. Fish ventral surface area was estimated by tracing live, anesthetized specimens from several different size classes onto a 1 cm x 1 cm paper grid, and counting the number of cm² grids within each outline. Total length was also measured for each specimen. The curvilinear relationship between fish length and ventral surface area was determined using regression analysis. Because the relationship had a high coefficient of determination ($R^2 = 0.957$), it was possible to use each fish's total length (mm) to estimate its ventral surface area (cm²).

**Experiment 1 - group 1 juveniles**

Newly weaned juveniles were stocked into white, plastic, 20-L aquaria, each with a bottom surface area of 506 cm². Each aquaria was set into the larger tanks of the recirculating system and supplied with seawater (18°C). Mean fish length, weight, and surface area was 43 mm, 0.7 g, and 6.35 cm², respectively (Table 1). The three density treatments of 100% (1.1 kg/m²), 150% (1.7 kg/m²), and 200% (2.2 kg/m²) coverage of tank bottom were established by stocking 80, 120, and 160 individuals into each of the three replicates per treatment, respectively (Table 1). As mortality occurred through the course of the experiment, fish were replaced to maintain nominal stocking densities. All fish were fed to satiation twice a day using Moore-Clark® formulated feed. The experiment was terminated after 40 days, and 25 individuals from each replicate were weighed and measured. Final stocking density (percent cover) was determined for each replicate by multiplying the number of fish by the mean ventral surface area of the fish. This total fish surface area value was then expressed as a percentage of the surface.

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 40</th>
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<tbody>
<tr>
<td>Nominal % cover</td>
<td>100</td>
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<tr>
<td>Mean length (mm)</td>
<td>43</td>
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<tr>
<td>Mean weight (g)</td>
<td>0.7</td>
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<tr>
<td>Mean surface area (cm²)</td>
<td>6.35</td>
</tr>
<tr>
<td>Observed mean % cover</td>
<td>100</td>
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<tr>
<td>Mean kg/m²</td>
<td>1.1</td>
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Table 1. Summary of data for experiment 1 (+/-) is standard deviation.
<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 27</th>
<th>Day 58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal % cover</td>
<td>100</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>Mean length (mm)</td>
<td>89</td>
<td>89</td>
<td>89</td>
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<tr>
<td>Mean weight (g)</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
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<tr>
<td>Mean surface area (cm²)</td>
<td>21.2</td>
<td>21.2</td>
<td>21.2</td>
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<tr>
<td>Mean percent survival</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Observed mean % cover</td>
<td>100</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>Mean kg/m²</td>
<td>3.7</td>
<td>5.5</td>
<td>7.3</td>
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Table 2. Summary of data for experiment 2 (+/-) is standard deviation.

area of the bottom of the experimental aquaria. Biomass per unit area (kg/m²) was determined for each replicate by multiplying the mean fish weight by the number of fish. Mean total biomass per treatment was expressed as a proportion of the surface area (m²) of the bottom of the experimental aquaria. Data were analyzed using one-way analysis of variance (ANOVA).

**Experiment 2 - group 2 juveniles**

Juveniles were stocked into gray, plastic, 20-L aquaria, each with a bottom surface area of 962 cm². Each aquaria was set into the larger tanks of the recirculating system and supplied with seawater (18°C). Initial mean fish length, weight, and surface area were 89 mm, 7.8 g, and 21.2 cm², respectively (Table 2). The three density treatments of 100% (3.7 kg/m²), 150% (5.5 kg/m²), and 200% (7.3 kg/m²) coverage of tank bottom were established by stocking 45, 68, and 90 individuals into each of the three replicates per treatment, respectively (Table 2). Mortalities (probably due to handling and transfer stress) were replaced only during the first week of the experiment. The flounder were fed Moore-Clark® formulated feed twice daily to satiation. Random samples of fish were measured and weighed on day 27 and at the conclusion of the experiment on day 58. Stocking densities (percent cover) at days 27 and 58 were determined for each replicate by multiplying the number of fish by the mean ventral surface area of the fish. This total fish surface area value was then expressed as a percentage of the surface area of the bottom of the experimental aquaria. Biomass per unit area (kg/m²) was determined for each replicate by multiplying the mean fish weight by the number of fish. Total treatment biomass was expressed as a proportion of the surface area (m²) of the bottom of the experimental aquaria. Data were analyzed using ANOVA, followed by Tukey’s multiple comparison tests when significant differences were found.
RESULTS

Relationship between fish length and surface area

Curvilinear regression analysis was used to relate total fish length to the ventral surface area of the fish (Fig. 1). The equation which describes this relationship, for summer flounder ranging from approximately 50-200 mm total length, is:

\[ \text{Surface area (cm}^2\) = 2.720 \times 10^{0.009 \times (\text{total length (mm)})} \]

Experiment 1 - group 1 juveniles

A total of 19 fish were replaced among replicates of the 100% treatment, and 17 fish among replicates in each of the 150 and 200% treatments. Additionally, a replicate was lost in the 200% coverage treatment due to accidental stoppage of water and subsequent depletion of oxygen. Mean lengths, weights, surface areas, and densities are reported in Table 1. Mean lengths and weights increased by approximately 40 mm and 5.5 g in each treatment. Fish density increased by about 140% in each treatment as juveniles grew both in length and weight over the 40-day experimental period. Final coverage of the tank bottom was 243, 361, and 466%. Estimates of biomass per unit area at the end of the experiment were 9.8, 14.7, and 19.8 kg/m² for stocking densities of 100, 150, and 200%, respectively. These represent 7 to 8 fold increases over the course of the experiment. Even at these high final densities, no significant differences (P>0.05) in total length or wet weight were found between juveniles in any of the three treatments.

Experiment 2 - group 2 juveniles

Mean lengths, weights, surface areas, and densities are reported in Table 2. Following mortality replacement in the first week, final mean survivals were 100, 96, and 78% for treatments of 100, 150, and 200% coverage, respectively. At the end of this 58-day experiment, mean fish densities had reached 355, 399, and 493% bottom coverage. Final estimates of biomass per unit area were 21.8, 23.7, and 29.5 kg/m² for the 100, 150, and 200% treatments, respectively. At day 27, fish initially stocked at 100% coverage were significantly (P<0.05) larger, both in length and weight, than those initially stocked at 150 and 200% coverage (Table 3). There was no significant difference (P>0.05) in either length or weight...
<table>
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<th>Day 27</th>
<th>Day 58</th>
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<tr>
<td></td>
<td>Length</td>
<td>Weight</td>
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<td>100 vs 150%</td>
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<td>100 vs 200%</td>
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<tr>
<td>150 vs 200%</td>
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Table 3. Results from one way analysis of variance (ANOVA) comparing mean lengths and weights from treatments in experiment 2. ns = not significant (P>0.05); * = (P<0.05).

between the 150 and 200% treatments at this time (Table 3). At the end of the experiment (day 58), fish initially stocked at 100% coverage were still significantly longer and heavier than those in the 150% coverage treatment (P<0.05), but not longer or heavier than those in the 200% coverage treatment (P>0.05). At day 58, there was no difference in the lengths and weights of fish in the 150 and 200% coverage treatments (P>0.05) (Table 3).

DISCUSSION

Results from experiment 1 in this study indicate that small, recently weaned summer flounders (initial size of about 1 g and 40 mm) can be stocked at densities of at least 200%, and raised for at least 40 days without negatively affecting growth. In fact, we recorded densities greater than 400% coverage at the end of this experiment (Table 1) and saw no indication that growth was being impaired. Results from this experiment prevent us from suggesting an upper limit on stocking densities for fish of this size, but it is certainly greater than 200% coverage. We are unaware of any other density studies that have been done with flatfish of this size.

Results from experiment 2, however, which began with larger fish (initial size of about 8 g and 90 mm) and ran for a longer time, indicate that stocking density does affect the growth of these larger individuals. In this instance, fish stocked at the lowest density (100% coverage) grew faster than those in both of the other treatments from the start of the experiment until day 27. During this time, the mean length of fish in this treatment increased by about 44%, which was slightly higher than increases seen in the 150% (37% increase) and 200% (39% increase) treatments. Similarly, mean weight of fish in the 100% density treatment increased by about 208%, which was dramatically higher than the increases seen in the 150% (156% increase) and 200% (168% increase) treatments. It has been suggested that high stocking densities can lead to poor water quality (high ammonia, low oxygen) which in turn can lead to reduced growth performance (Brett 1979, Pickering and Pottinger 1987, Kebus et al. 1992, Kindschi and Koby 1994, Wagner et al. 1995). It is extremely unlikely that poor water quality was a factor in this study. First, because all treatments were associated with the same recirculating water and biological filter, water quality was probably identical in all treatments. Second, we found that nonionized ammonia never exceeded 0.05 ppm and dissolved oxygen never fell below saturation. Lastly, we observed no loss of appetite in any of the treatments that could
indicate poor water quality and/or stress. Thus, our recirculating system and biological filter were capable of maintaining ammonia below, and dissolved oxygen above, normally stressful levels. In effect, the system in which we conducted the experiment eliminated two of the variables (high ammonia, low oxygen) that are often associated with high stocking densities. Aside from water quality issues, food consumption and feeding behavior may also be affected by stocking density (Holm et al. 1990, Martinez-Tapia and Fernandez-Pato 1991). In such instances, "crowding" (high number of fish per unit area) can cause an increase in agonistic feeding behavior, which in turn increases stress and decreases growth. It is possible that these factors contributed to the results we found. Summer flounder are known to be aggressive feeders (Bigelow and Schroeder 1953), and we occasionally observed aggressive feeding behavior (e.g. chasing, tail biting) in this experiment. If such behavior increased with numerical density, it is possible that fish in the lowest stocking density (100%), which contained only 45 fish in each replicate, benefited from low numerical abundance. The fact that we saw no density effect in experiment 1, which was done with smaller fish, suggests that this mechanism, if applicable, may not operate until the fish are somewhat older and larger. Density-dependent behavioral changes are well documented (Fenderson and Carpenter 1971, Refstie and Kittelsen 1976), and Wagner et al. (1996) found that agonistic behavior in rainbow trout fry increased with age. These studies support our contention that behavioral mechanisms, which may change with fish size, were responsible for the differences we observed. At the end of experiment 2 (day 58), fish in the 100% density treatment were still larger than fish in the 150% treatment, but not the fish in the 200% treatment. The parity of fish in the 100% and 200% density treatments at the end of the experiment, but not at day 27, is an indication that a size convergence occurred between days 27 and 58. Thus, it appears that fish stocked at densities of 100 and 200% grow at different rates for a period of time (the first half of this experiment), but fish held at the higher density (200%) were able to "catch up" (compensatory growth) as time went on (the second half of this experiment). If, as speculated above, agonistic behavior increases with numerical density, which in turn increases stress and reduces growth rate, it is possible that a reduction in agonism over time explains the compensatory growth we observed. In the 200% density treatment, the mean number of fish per replicate decreased from 90 to 69, thus possibly reducing aggressive, agonistic interactions. Alternatively, the fish at the higher density (200%) simply could have become more "accustomed" to this density as time progressed, thereby reducing stress and resulting in compensatory growth. A third possible explanation is that summer flounder respond differently to stocking density as they increase in size. If, for example, larger fish are more tolerant of high density at larger sizes, then it would explain why fish at the higher density (200%) exhibited compensatory growth during the second part of this experiment.

Fish held at the intermediate density (150%) were significantly smaller than those at 100% on days 27 and 58, but not different from those at the 200% density on either day. These results partially support our hypothesis that numerical abundance and associated agonistic feeding behavior may be affecting the growth of juvenile summer flounders. Because of mortalities in replicates of the 200% treatment, mean numerical abundance in the 150% treatment (n=65) was nearly identical to that of the 200% treatment (n=69). These similarities would explain why growth was nearly identical in the 150 and 200% treatments, and why fish in both of these higher density treatments were smaller than those in the 100% density treatment which had a lower mean numerical abundance (n=45). The fact that fish in the 200% treatment demonstrated compensatory growth, while those in the 150% treatment did not, is difficult to explain. It is possible that the loss of fish in the 200% treatment triggered the compensatory growth we observed, and that this did not occur in the 150% treatment because numerical abundance was relatively stable throughout the experiment. This is very speculative, and additional research would be needed to address this issue.

Our results are similar to those of the few stocking density studies which have been done with other flatfish species. Jeon et al. (1993), who worked with young Japanese flounder
*Paralichthys olivaceous*, evaluated stocking densities of 33, 50, 100, 200, and 300% bottom coverage, and found that the highest feeding rate and growth occurred at 200% coverage. Similarly, Chang et al. (1995), who worked with larger (50-75 mm) Japanese flounder, in a semi-closed, recirculating seawater system, reported final densities as high as 260% (36.3 kg/m²). Although our experimental fish were not grown to harvest size at our nominal stocking densities, results with both turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus* suggest that larger sized flatfish can be grown at relatively high densities. Martinez-Tapia and Fernandez-Pato (1991) found no ill effects at a stocking density of 68 kg/m² for turbot, and suggested that specific growth and food conversion were greater at higher densities. Bjornsson (1994) conducted research with relatively large halibut (initial size 1.8-3.2 kg) at stocking densities of 50, 100 and 160% coverage. Although he observed a maximum coverage of 215% (95 kg/m²), he indicated that growth rate was reduced in the highest coverage (160%) treatment, and that optimal stocking density was somewhere between one and two layers of fish on the tank bottom.

This study, as well as those with halibut, Japanese flounder, and turbot indicate that flatfish species are able to grow effectively at stocking densities of 100-200% (one to two layers thick on the tank bottom). Indeed, in this study we observed that fish, when not feeding, would crowd and overlap one another even when empty space was available, and that this occurred at all stocking densities. Similar "layering" behavior has been observed in halibut (Bjornsson 1994). Further, biomass densities can be relatively high. In this study, with relatively small fish, biomass densities reached only 29.5 kg/m², but work with Japanese flounder (Chang et al. 1995), turbot (Martinez-Tapia and Fernandez-Pato 1991), and halibut (Bjornsson 1994) suggest that biomass densities of 36.3, 68.0, and 95 kg/m², respectively, were possible. The combination of layering, and tolerance of high biomass densities, suggest that flounders can be raised at high densities. This could be an enormous advantage to the grow-out farmer, provided that the recirculating system is capable of supporting these high biomasses. Results of this study suggest that recently weaned summer flounder can be stocked at densities of at least 200%, but that stocking density should be reduced to 100% for larger juveniles, at least for several weeks, and that densities could then be allowed to increase as the fish grow in size. Further observations and research, which develop with the commercial summer flounder industry, will undoubtedly refine our understanding of optimum stocking density.

ACKNOWLEDGMENTS

We thank Chris Duffy, George Nardi, and Andrea Tomlinson for their dedicated assistance. The research was supported by a grant from the Saltonstall-Kennedy program. UNH Center for Marine Biology/Jackson Estuarine Laboratory Contribution Series #335.

LITERATURE CITED


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