

Pilot Production of Hatchery-Reared Summer Flounder *Paralichthys dentatus* in a Marine Recirculating Aquaculture System: The Effects of Ration Level on Growth, Feed Conversion, and Survival

PATRICK M. CARROLL AND WADE O. WATANABE

University of North Carolina at Wilmington, Center for Marine Science, 7205 Wrightsville Avenue, Wilmington, North Carolina 28403 USA

THOMAS M. LOSORDO

Department of Zoology, North Carolina State University, Raleigh, North Carolina 27695 USA

Abstract—Pilot-scale trials were conducted to evaluate growout performance of hatchery-reared summer flounder fingerlings in a state-of-the-art recirculating aquaculture system (RAS). The outdoor RAS consisted of four 4.57-m dia x 0.69-m deep (vol. = 11.3 m³) covered, insulated tanks and associated water treatment components. Fingerlings (85.1 g mean initial weight) supplied by a commercial hatchery were stocked into two tanks at a density of 1,014 fish/tank (7.63 kg/m³). Fish were fed an extruded dry floating diet consisting of 50% protein and 12% lipid. The temperature was maintained between 20 C and 23 C and the salinity was 34 ppt. Under these conditions, growth, growth variation (CV_{wt}), feed utilization, and survival of fish fed to 100% and 82% of a satiation rate were compared.

Due to clear changes in growth patterns during the study, data was analyzed in three phases. During phase 1 (d 1–d 196), fish showed rapid growth, reaching a mean weight of 288 g ± 105 and 316 g ± 102, with a CV_{wt} of 0.36 and 0.32 and FCR's of 1.38 and 1.36 in the subsatiation and satiation groups, respectively. During phase 2 (d 196–d 454), fish displayed slower growth reaching mean weights of 392 g ± 144 and 436 g ± 121, with a CV_{wt} of 0.37 and 0.28, and increasing FCR's of 3.45 and 3.12 in the subsatiation and satiation groups, respectively. During phase 3 (d 454–d 614), fish showed little growth reaching mean weights of 399 g ± 153 and 440 g ± 129, with a CV_{wt} of 0.38 and 0.29 in the subsatiation and satiation groups, respectively. Over the entire growout period (d 1–d 614), feed conversion ratios were 2.39 and 2.37 and survival was 75% and 81% in the subsatiation and satiation treatments, respectively. The maximum biomass density reached during the study was 32.3 kg/m³.

The satiation feed rate was superior to the 82% satiation rate, since it maximized growth rates, with no effect on FCR. The higher CV_{wt} in the subsatiation group

suggests increased competition for a restricted ration led to a slower growth with more growth variation. The decrease in growth in phases 2 and 3 was probably related to a high percentage of slower growing male fish in the population and the onset of sexual maturity.

This study demonstrated that under commercial scale conditions, summer flounder can be successfully grown to a marketable size in a recirculating aquaculture system. Based on these results, it is recommended that a farmer feed at a satiation rate to minimize growout time. More research is needed to maintain high growth rates through marketable sizes through all-female production and/or inhibition of sexual maturity.

The summer flounder *Paralichthys dentatus* is a left-eyed flatfish in the family Paralichthyidae. It inhabits the coastal waters from Nova Scotia to southern Florida, but is most abundant from Cape Cod to Cape Hatteras (Grimes et al. 1989). High market value and growing demand have stimulated interest in summer flounder as a potential aquaculture species. Successful culture of the Japanese flounder *Paralichthys olivaceus* in Asia and the turbot *Scophthalmus maximus* in Europe has provided a positive model for the development of research and the commercialization of the summer flounder in the U.S. Since 1990, developments have been made in the spawning and rearing of juvenile summer flounder, but to date there is little information on growout to a marketable size.

It has been shown in some flatfish species that feeding to satiation does not necessarily produce the best conversion efficiency, since feed may be only partially digested feed, ending as waste

(Puvanendran et al. 2003). As feed cost is among the highest operational costs in commercial aquaculture operations, it is essential to minimize feed conversion ratios, while maintaining an acceptable growth rate. The objectives of this study were to evaluate growth rates of hatchery-reared summer flounder fingerlings to full marketable sizes in a commercial scale recirculating aquaculture system and compare growth, feed conversion ratio, and survival of fish fed at a satiation and a subsatiation feed rate.

Materials and Methods

Experimental Animals

This study was conducted at the University of North Carolina at Wilmington Center for Marine Science (UNCW-CMS) Aquaculture Facility in Wrightsville Beach, North Carolina, USA, from September 2000 to May 2002. Hatchery-reared, juvenile summer flounder (mean weight = 30 g) were obtained from a commercial hatchery (Great Bay Aquaculture, Portsmouth, New Hampshire, USA). Fish were live-hauled by truck to UNCW-CMS in July 2000 and were maintained in an 11.3-m³ tank supplied with recirculating seawater until the beginning of the study. Filtered seawater (from a natural source) supplied the recirculating system with makeup water. During this period, fish were fed ad libitum an extruded dry floating flounder diet consisting of 50% protein and 12% lipid (Melick Aquafeed, Catawissa, Pennsylvania, USA).

Experimental System

Growout studies of summer flounder fingerlings were conducted in an outdoor state-of-the-art recirculating aquaculture system (RAS) (Fig. 1) based on the North Carolina State University Fish Barn design (Losordo et al. 2000). The commercial scale RAS consisted of two groups of two 11.3-m³ circular fiberglass tanks (4.57 m dia x 0.69 m deep). Each group was supported by an independent recirculating system. Tanks were insulated and covered by a translucent conical fiberglass cover with a retracting door, and the interiors were black in color to maintain a low illumination level.

Each tank contained a center double drain (Aqua-Optima, Trondheim, Norway) that was fit-

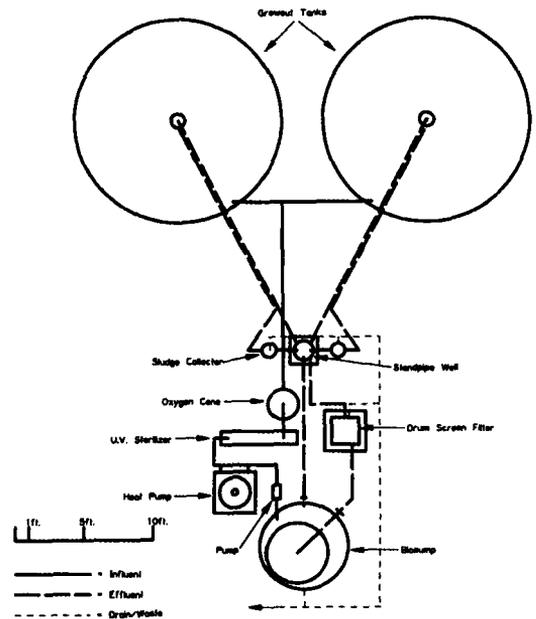


FIGURE 1. Scale blueprint of the summer flounder growout system.

ted with an 11-L swirl separator. The double drain featured a flatfish ring to keep fish out of the drain. The double drain divided the effluent flow into two streams. One stream discharged water containing settleable solids (approximately 5% of the flow rate) to the swirl separator. The larger stream (approximately 95% of the flow rate) from the double drain reunited with the clarified water from the swirl separator in a standpipe well. The effluent stream was then treated with a rotating drum screen filter with a 60- μ m screen (PRA Manufacturing, British Columbia, Canada) to remove fine solids. The filtered water was then distributed over a biological filter by a drip plate. The biological filter media consisted of 0.53 m³ of 3-mm polystyrene microbeads with a specific surface area of 1,145 m²/m³. Water was then pumped out of the biological filter by a 3.36-kW centrifugal pump (Jacuzzi Piranha, Little Rock, Arkansas, USA) and passed through a 3-kW heat pump (Aqualogic, San Diego, California, USA). A small portion of the flow was diverted to a foam fractionator (Top Fathom, Eugene, Oregon, USA) that discharged back into the biological filter. Water from the heat pump was treated by a 260-W ultraviolet (UV) sterilizer (Em-

peror Aquatics, Pottstown, Pennsylvania, USA). From the UV sterilizer, the water passed through an oxygen cone before entering the culture tanks. Flow to each tank was maintained at 150 L/min. Carbon dioxide stripping was accomplished by air diffusers placed at the bottom of the biological filter and a degassing column between the water distribution drip plate and the biological filter media.

Experimental Design

To compare the effects of ration level on growth and feed utilization, fingerlings (mean wt. = 85.1 g, range = 23–139 g) were stocked in two 11.3-m³ tanks at a density of 7.63 kg/m³ (1,014 fish/tank, 62 fish/m²). Mean coefficient of variation of weight was 0.29. Fish in one tank were fed at a rate of 100% satiation, i.e., until fish stopped feeding, while fish in the other tank were fed at 80% of the satiation level. Total daily feed ration in the 100% satiation group was expressed as a percentage of total tank biomass determined at the last sampling. Total daily feed ration in the subsatiation treatment was determined by multiplying the satiation feed rate by 0.80 and the total biomass determined at the last sampling. Fish were hand fed from one to three times a day depending on feeding response. From d 1 – d 95, fish were fed 3 times a day. Due to decreased response to the second and third feeding, feed frequency was reduced to twice per day from d 96 – d 381 and to once per day from d 382 – d 614. Fish were fed six days each week.

On day 406, fish were evenly distributed and restocked among four tanks, with two tanks maintained for each ration level and both ration levels represented in each recirculating system. In addition, 30 fish from each tank were tagged with T-bar tags to track individual growth rates. Temperature was maintained between 20 and 23 C. Water exchange during the study was approximately 15% of the system volume daily.

Growth Determination

Fish were individually weighed and measured monthly until d 286. A dip net was used to randomly sample 50 fish in each tank from d 1 – d 145, when it was noticed that larger fish avoided the net. From d 196 – d 398 a pie-shaped corral was used to trap fish in a subsection of the tank. The corral was

lowered repeatedly until 100 fish were sampled. From d 406 onward, the 30 tagged fish along with 30 random fish were sampled every 2 mo until the end of the study on d 614. Fish were weighed and measured with the aid of an anesthetic, 75-mg/L methane trisulfonate (MS-222). Total length was recorded to the nearest millimeter and weight to the nearest gram using an electronic balance (Acculab, Bradford, Massachusetts, USA). Fish were allowed to recover in seawater before being returned to the tank.

Water Quality

Dissolved oxygen was monitored continuously in each tank by a PT 4 oxygen monitor (Point Four, British Columbia, Canada). Salinity, pH, and temperature were monitored daily by a YSI 5200 Recirculating Monitoring System (Yellow Springs Instruments Co., Inc., Yellow Springs, OH). Ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen levels were checked weekly using a spectrophotometer (Hach Company, Loveland, CO).

Analytical Methods

For each ration level, mean body weights and lengths of fish at each sampling interval were plotted against time, and growth curves were determined by curvilinear regression analysis.

Relative growth rate (% increase in weight) during each sampling interval was calculated as $RGR = 100(\text{wet final weight} - \text{wet initial weight}) / (\text{wet initial weight})$.

Specific growth rate (% increase in weight/d) was calculated as $SGR = 100[\log_e(\text{wet final weight}) - \log_e(\text{wet initial weight})] / (\text{time in days})$. For RGR and SGR, weights were estimated from the growth curve.

Daily weight gain (g/d) was calculated as $DWG = (\text{wet final weight} - \text{wet initial weight}) / \text{time in days}$.

Coefficient of variation of weight and length were calculated as $CV = \text{standard deviation} / \text{mean}$. Condition factor was calculated as $K = 100 (W/L^3)$ where W = weight in g and L = length in cm.

Mortalities were recorded daily and mortality rate (%/d) during each sampling interval was calculated as $M = (\text{percentage of dead fish during interval}) / (\text{number of d in interval})$.

Feed conversion ratio during a sampling interval was calculated as $FCR = (\text{dry weight of feed fed, kg})/(\text{wet weight gain, kg})$

Feed consumption was expressed as the average percentage of body weight of feed consumed daily. Feed consumption was calculated as $FC = 100[(\text{feed consumed/d, kg})/(\text{mean daily biomass, kg})]$. Mean daily biomass was estimated for each day by multiplying the mean fish weight (estimated by curvilinear regression) by the number of fish.

Statistics

Due to the clear changes in growth rates during the study, the data was analyzed in three different phases, d 1 – 196 (Phase 1), d 196 – 454 (Phase 2), and d 454 – 614 (Phase 3). During each phase, linearized cube transformed regression lines for growth rates were derived, and growth rates were compared between treatments by analysis of covariance (ANCOVA) (Snedecor and Cochran 1967, Ott 1993). During phase 3, weights of tagged fish were compared to weights of non-tagged randomly sampled fish and growth rates were compared using ANCOVA. In addition, RGR, SGR, DWG, CV_{wt} , CV_{lt} , K, FCR, and M were compared between treatments during each phase by ANOVA, using a randomized block design for repeated measures, with each sampling interval representing a block (Sokal and Rohlf 1981).

Results

Water Quality

While both treatments were contained in one system (d 1 – d 405) mean \pm SD (range) water quality parameter values were as follows: temperature $21.2\text{ C} \pm 1.6$ (16.3–24.8), dissolved oxygen $9.4\text{ mg/L} \pm 1.3$ (3.3–12.8), pH 7.1 (6.4–7.5), salinity $33.4\text{ g/L} \pm 2.6$ (25.0–37.0), TAN $0.32\text{ mg/L} \pm 0.12$ (0.10–0.74), nitrite-nitrogen $0.22\text{ mg/L} \pm 0.11$ (0.00–0.49) and nitrate-nitrogen $13.8\text{ mg/L} \pm 6.4$ (0.6–30.2).

After the fish were distributed among four tanks and each treatment was represented in separate systems (d 406 – d 614), temperatures were not significantly different in system 1, $21.0\text{ C} \pm 0.8$ (16.9–22.9) and in system 2, $21.2\text{ C} \pm 0.8$ (17.4–23.1). Dissolved oxygen and pH were significantly ($P < 0.05$) higher in system 1 (9.6

$\text{mg/L} \pm 1.0$ and 7.3) than in system 2 ($9.3\text{ mg/L} \pm 1.2$ and 7.2). Salinity was significantly ($P < 0.05$) higher in system 2 ($32.6\text{ g/L} \pm 1.5$) than in system 1 ($31.5\text{ g/L} \pm 1.6$). TAN ($\bar{x} = 0.22\text{ mg/L}$), nitrite-nitrogen ($\bar{x} = 0.06\text{ mg/L}$) and nitrate-nitrogen ($\bar{x} = 5.25\text{ mg/L}$) were not significantly different ($P > 0.05$) between systems.

Feed Consumption

Feed consumption decreased with increasing fish weight in both satiation and subsatiation treatments. At a mean fish weight of 100 g, the satiation feed rate was 1.46% body weight/d (% bw/d). At the same weight, the subsatiation feed rate was 1.18 % bw/d, 81% of the satiation rate. At a mean fish weight of 250 g, satiation feed rate was 0.70 %bw/d, and the subsatiation feed rate was 0.61 % bw/d, 86% of the satiation rate. At a mean fish weight of 400 g, satiation feed rate was 0.34 %bw/d and the subsatiation feed rate was 0.27 % bw/d, 79% of the satiation rate. For the duration of the study, feed consumption rate for the subsatiation treatment averaged 82% (range = 75%–88%) of the satiation treatment.

Growth

Overall growth patterns for both treatments revealed three phases: an initial period (d 1 – d 196) of high growth (phase 1), a period (d 196 – d 454) of decelerating growth (phase 2), and a period (d 454 – d 614) of no growth (phase 3) (Table 1).

Phase 1

At the end of phase one (d 196), fish reached mean weights of 288 and 316 g in the subsatiation and the satiation groups, respectively, with no significant differences. Weight frequency distributions (Fig. 2b) showed that on d 196, 4% and 12% of the fish in the subsatiation and satiation groups, respectively, were larger than a minimum marketable size of 450 g. On d 196, body lengths ($\bar{x} = 295\text{ mm}$) were also not significantly different between treatments (Table 1).

Condition factor (K) was significantly larger ($P < 0.05$) in the satiation group (1.18) than in the subsatiation group (1.17). CV_{wt} ($\bar{x} = 0.34$) and CV_{lt} ($\bar{x} = 0.12$) were not significantly different between treatments. RGR ($\bar{x} = 255\%$), SGR ($\bar{x} = 0.282\%$ bw/d), FCR ($\bar{x} = 1.37$), and M ($\bar{x} = 0.044\%$ /d)

TABLE 1. Summer flounder raised in a RAS under a satiation and subsatiation feeding regime. Data are shown for each coefficient of variation of weight (CV_w), coefficient of variation of length (CV_l), relative growth rates (RGR), specific

	Phase 1 (d 1 – d 196)			Phase 2 (d 196 – d 454)		
	Subsatiation	Satiation	P-value	Subsatiation	Satiation	P-value
Weight (g) ^a	288 ± 105	316 ± 102	ns	392 ± 144	436 ± 121	0.041
Range (g)	45 - 504	78 - 542	~	132 - 926	207 - 769	~
Length (mm) ^a	291 ± 36	298 ± 32	ns	322 ± 34	331 ± 29	ns
K ^b	1.17	1.18	0.01	1.18	1.21	0.000
CV_w ^b	0.36	0.32	ns	0.37	0.28	ns
CV_l ^b	0.12	0.11	ns	0.11	0.09	ns
RGR (%) ^c	241	268	ns	36.2	38.2	0.003
SGR (%bw/d) ^c	0.273	0.290	ns	0.052	0.054	0.003
DWG (g/d) ^c	1.04	1.18	0.000	0.40	0.47	0.002
FCR ^c	1.38	1.36	ns	3.45	3.12	ns
M (%/d) ^c	0.052	0.036	ns	0.046	0.035	ns
S (%) ^c	89.9	92.9		88.2	91.0	
D (kg/m ³) ^c	23.2	26.3		27.6	32.3	

^aP-value represents comparison of slopes (ANCOVA) of linearized cube transformed regression lines for growth rates.

^bValue from length and weight at end of phase.

^cValue determined from interval growth during each phase.

^dFCR was not calculated since there was no growth.

^eHighest density achieved during phase.

^fDensity after dividing treatment into two tanks on d 406.

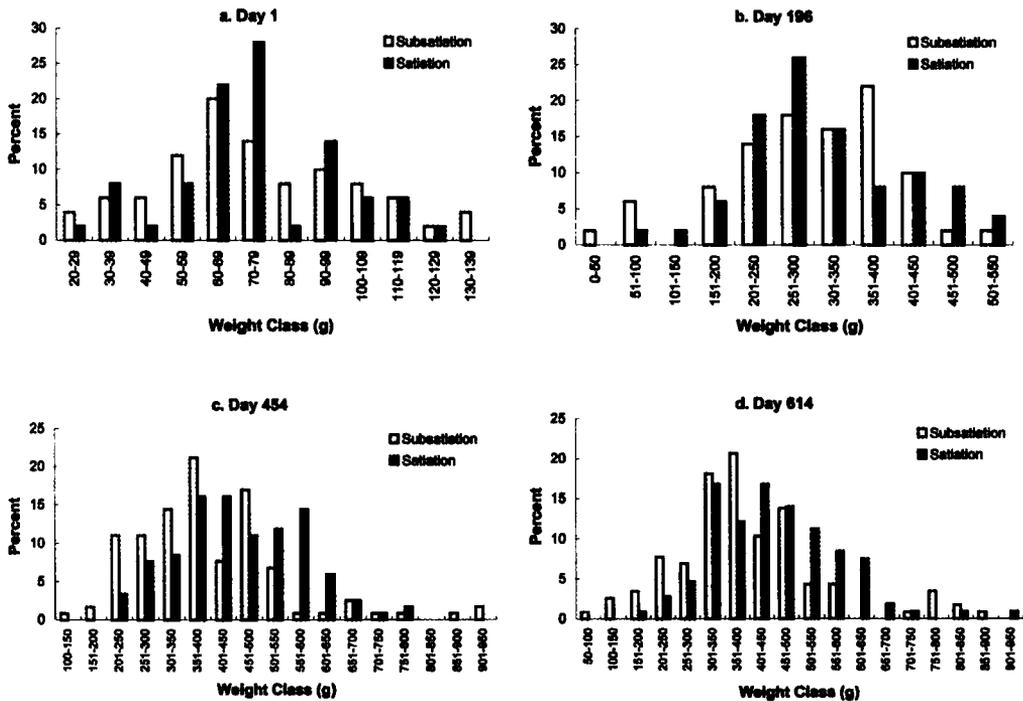


FIGURE 2. Weight-frequency distribution of summer flounder in a recirculating tank system on d 1 (a), d 196 (b), d 454 (c), and d 614 (d).

growth phase and over the entire study: Body weight (+ SD), weight range, total length (+ SD), condition factor (K), growth rates (SGR), feed conversion ratios (FCR), mortality (M), survival (S), and biomass densities (D).

Subsatiation	Phase 3 (d 454 - d 614)		Overall	
	Satiation	P-value	Subsatiation	Satiation
399 ± 153	440 ± 129	ns	~	~
68 - 888	157 - 945	~	~	~
330 ± 37	339 ± 29	ns	~	~
1.11	1.13	0.002	~	~
0.38	0.29	0.005	~	~
0.11	0.08	ns	~	~
1.57	1.00	ns	372	413
0.004	0.003	ns	0.110	0.116
0.039	0.027	ns	0.51	0.58
d	d		2.37	2.39
0.035	0.028	ns	0.041	0.032
94.4	95.5		74.8	80.7
13.9 ^f	16.5 ^f		~	~

were not significantly different ($P > 0.05$) between treatments. DWG was significantly ($P > 0.001$) higher in the satiation group (1.18 g/d) than in the subsatiation group (1.04 g/d). On d 196, biomass density reached 23.2 and 26.3 kg/m³ (16.0 and 18.1 kg/m²) in the subsatiation and the satiation groups, respectively (Table 1).

Phase 2

At the end of phase 2 (d 454), fish fed to satiation were significantly ($P < 0.05$) larger ($\bar{x} = 436$ g) than those fed a subsatiation level ($\bar{x} = 392$ g). On d 454, 32% and 48% of the population in the subsatiation and satiation treatments, respectively, were above 450 g (Fig. 2c). Body lengths ($\bar{x} = 326$ mm) were not significantly different between treatments (Table 1).

Condition factor (K) was significantly ($P < 0.001$) higher in the satiation group (1.21) than in the subsatiation group (1.18). CV_{wt} ($\bar{x} = 0.33$) and CV_{ll} ($\bar{x} = 0.10$) were not significantly different between treatments. RGR, SGR, and DWG were significantly higher ($P < 0.05$) in the satiation

group (38%, 0.054% bw/d, and 0.47 g/d) than in the subsatiation group (36%, 0.052% bw/d, and 0.40 g/d) (Table 1). FCR ($\bar{x} = 3.29$) and M ($\bar{x} = 0.041$ %/d) were not significantly ($P > 0.05$) different between treatments. By the end of phase 2, biomass densities reached 27.6 and 32.3 kg/m³ (19.0 and 22.2 kg/m²) in the subsatiation and satiation groups, respectively (Table 1).

Phase 3

During phase 3 (d 454 – d 614), size differences established during phase 2 were maintained, and fish fed to satiation remained significantly ($P < 0.05$) larger than the fish fed below satiation, although little or no additional growth occurred. At the end of phase 3 (d 614), fish reached mean weights of 399 and 440 g in the subsatiation and the satiation groups, respectively. During this phase growth rates between tagged fish and non-tagged randomly sampled fish were not significantly different ($P > 0.05$). On d 614, 29% and 46% of the population in the subsatiation and satiation treatments, respectively, were above 450 g (Fig. 2d).

Body lengths were not significantly different ($\bar{x} = 335$ mm) between treatments (Table 1).

Condition factor (K) was significantly ($P < 0.05$) greater in the satiation group (1.13) than in the subsatiation group (1.11). CV_{wt} was significantly greater ($P < 0.05$) in the subsatiation group (0.38) than in the satiation group (0.29). CV_{lt} ($\bar{x} = 0.10$), RGR, ($\bar{x} = 1.3\%$), SGR ($\bar{x} = 0.004\%$ bw/d), DWG ($\bar{x} = 0.033$ g/d) and M ($\bar{x} = 0.032\%$ /d) were not significantly different ($P > 0.05$) between treatments. Due to the lack of growth in both treatments during phase 3, FCRs were not calculated. Biomass densities reached 13.9 and 16.5 kg/m³ (9.6 and 11.4 kg/m²) in the subsatiation and the satiation groups, respectively (Table 1).

Overall

For the entire growout period (phases 1–3) RGR, SGR, and DWG were 372%, 0.110% bw/d, and 0.51 g/d, respectively, for the subsatiation group, and 413%, 0.116% bw/d, and 0.58 g/d for the satiation group. FCR was 2.37 and 2.39 for the subsatiation and the satiation treatments, respectively. Mortality was 0.041 %/d (75% survival) and 0.028 %/d (81% survival) for the subsatiation and satiation groups, respectively (Table 1).

Diseases, including *Vibrio* spp. and *Mycobacteria*, caused some mortality, especially during phase 1. Infected fish displayed open sores and bloated abdomens, and were successfully treated with oxytetracycline.

Discussion

Hatchery reared summer flounder fingerlings were successfully reared to full marketable sizes at relatively high densities in a commercial scale RAS. Fish fed to satiation reached a significantly larger size than fish fed at 82% of the satiation rate, without a significant difference in FCR. For the grower, it would be advantageous to feed at a satiation rate to maximize growth, without lowering feed conversion efficiency.

Fish fed below satiation may have grown slower because of greater intraspecific competition. Coefficient of variation of body weight (CV_{wt}), a measure of the variation in growth among members of a population, appeared to remain higher in the subsatiation group throughout the study. Weight ranges consistently showed smaller fish in

the subsatiation group compared to the satiation group this suggests that hierarchical behavior in the group fed below satiation may have led to subordinates missing feeding opportunities (Table 1). This is consistent with earlier studies showing that flatfish fed a restricted ration display depressed growth rates and higher size variation (Carter et al. 1996; Saether and Jobling 1999; Verbeeten et al. 1999; Puvanendran et al. 2003).

Little or no growth of fish in both treatment groups was seen after d 454, and this was attributed to a number of factors. The relatively high percentage of male fish in the population may have been one factor. Of 250 fish harvested in February 2003, only 12 (4.8%) were not spermiating males. Female summer flounder grow faster than males in both wild and cultured populations (Murawski 1970; King et al. 2001). King et al. (2001) determined that, as cultured populations of summer flounder mature, larger fish contain proportionally more females than smaller fish in the same group. Commercial aquaculturists should produce all female populations, which would minimize time to a harvestable size. This can potentially be achieved by genetic manipulation (e.g., gynogenesis), controlled breeding methods, and controlled larval rearing (Yamamoto 1999; Luckenbach et al. 2003). Other culture conditions during the early life stages may have also caused an unbalanced sex ratio favoring males. Grading practices, for example, may divide populations into size classes with skewed sex ratios, with smaller size classes containing a higher ratio of males.

In the present study, temperature regime during the larval and metamorphic stages may have induced a high percentage of slower growing physiological males. Some flatfish species in the genus *Paralichthidae* have a temperature dependent sex-determination, much like that of reptiles (Bull and Vogt 1979; Ferguson and Joanen 1983). In the congeneric Japanese flounder, rearing temperature during larval and metamorphic stages affects the male to female sex ratio (Yamamoto 1999). A 50:50 sex ratio was obtained if rearing temperatures were maintained between 17.5–22.5°C during the period of gonadal differentiation. High and low temperatures of 15°C and 25°C, respectively, resulted in as much as 80% males due to sex reversal of genetic females (Yamamoto 1999).

The decreased growth rates during phase 3 of this study were probably caused by the onset of sexual maturity. Numerous spermiating males from d 398 onward evidenced this. A lack of growth associated with the onset of sexual maturation is common in culture (Bromage 2001). In Atlantic halibut *Hippoglossus hippoglossus* males reach sexual maturity at a younger age and reach a smaller size. This reduction in growth begins 3 mo prior to spawning and continues throughout the spawning season (Norberg et al. 2001).

Photothermal manipulation has been used in commercial finfish growout operations to delay or inhibit maturation until fish reach a marketable size to avoid reductions in growth and deterioration in flesh quality (Bromage et al. 2001). Fish in this study were maintained in outdoor tanks exposed to seasonal temperature and photoperiod changes. Exposure to a constant light photoperiod and constant temperature could delay the onset of sexual maturation, resulting in faster growth.

Irwin et al. (1999) suggested that increased stocking densities in flatfish might lead to competition that has a negative effect on growth. Because flatfish only utilize the floor of the culture unit, competition for space is determined more by the area than by the volume of the tank. In this study, at all densities, fish did not utilize the entire tank bottom; fish congregated and piled atop one another. Stocking densities reached their greatest value (32.3 kg/m³) on d 398 at the approximate time growth stopped. Fish in each treatment were split equally into separate tanks on day 406, dividing the tank biomass in half, but with no positive effect on growth. Densities as high as 60 kg/m³ are common in intensive systems for finfish and can reach 120 to 150 kg/m³ (Ebeling 2000). In this study, densities were low when compared to other species raised in RAS. Additional studies must be conducted to determine the optimum stocking densities for commercial culture.

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Literature Cited

- Bromage, N., M. Porter, and C. Randall.** 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* 197:63–98.
- Bull, J. J. and R. C. Vogt.** 1979. Temperature-dependent sex determination in turtles. *Science* 206:1186–1188.
- Carter, C. G., G. J. Pursuer, D. F. Houlihan, and P. Thomas.** 1996. The effects of decreased ration on feeding hierarchies in groups of greenback flounder *Rhombosolea tapirina*: Teleostei. *Journal of the Marine Biological Association of the United Kingdom*. Plymouth. 76:505–516.
- Ebeling, J. M.** 2000. Engineering aspects of recirculating aquaculture systems. *Marine Technology Society Journal* 34:68–78.
- Ferguson, M. W. J. and T. Joanen.** 1983. Temperature dependent sex determination in the *Alligator mississippiensis*. *The Journal of Experimental Zoology* 270:3–15.
- Grimes, B. H., M. T. Huish, J. H. Kerby, and D. Moran.** 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic). Summer and winter flounder. U.S. Fish and Wildlife Service Biological report 2 (11.112).
- Irwin, S., J. O'Hallotan, and R. D. FitzGerald.** 1999. Stocking density, growth and growth variations in juvenile turbot, *Scophthalmus maximus* (Rafinesque). *Aquaculture* 178:77–88.
- King, N. J., G. C. Nardi, and C. J. Jones.** 2001. Sex-linked growth divergence of summer flounder from a commercial farm: are males worth the effort? *Journal of Applied Aquaculture* 11:77–88.
- Losordo, T. M., A. O. Hobbs, and D. P. DeLong.** 2000. The design and operation characteristics of the CP&L/EPRI fish barn: a demonstration of recirculating aquaculture technology. *Aquacultural Engineering* 22:3–16.
- Luckenbach, J. A., J. Godwin, H. V. Daniels, and R. J. Borski.** 2003. Gonadal differentiation and effects of temperature on sex determination in southern

- flounder *Paralichthys dentatus*. Aquaculture 216:315–327.
- Murawski, W. S.** 1970. Results of tagging experiments of summer flounder, *Paralichthys dentatus*, conducted in New Jersey waters from 1960 to 1967. Miscellaneous report 5M. New Jersey Department of Environmental Protection, Bureau of Fisheries.
- Norberg, B., F-A. Weltzien, O. Karlsen, and J. C. Holm.** 2001. Effects of photoperiod on sexual maturation and somatic growth in male Atlantic halibut *Hippoglossus hippoglossus*. Comparative Biochemistry and Physiology Part B 129:357–365.
- Ott, R. L.** 1993. An introduction to statistical methods and data analysis, 4th edition. Duxbury Press, Belmont, California, USA.
- Puvanendran, V., D. L. Boyce, and J. A. Brown.** 2003. Food ration requirements of 0⁺ yellowtail flounder *Limanda ferruginea* (Storer) juveniles. Aquaculture 220:459–475.
- Saether, B. S. and M. Jobling.** 1999. The effects of ration level on feed intake and growth, and compensatory growth after restricted feeding, in turbot *Scophthalmus maximus* L. Aquaculture Research 30:647–653.
- Snedecor, G. W. and W. G. Cochran.** 1967. Statistical methods, 6th edition. The Iowa State University Press, Ames, Iowa, USA.
- Sokal R. R., and F. J. Rohlf.** 1981. Biometry. W. H. Freeman and Company, New York, USA.
- Verbeeten, B. E., C. G. Carter, and G. J. Purser.** 1999. The combined effects of feeding time and ration on growth performance and nitrogen metabolism of greenback flounder. Journal of Fish Biology 55:1328–1343.
- Yamamoto, E.** 1999. Studies on sex-manipulation and production of cloned populations in hirame, *Paralichthys olivaceus*. Aquaculture 173:235–246.