

## Temperature Effects on Eggs and Yolk Sac Larvae of the Summer Flounder at Different Salinities

WADE O. WATANABE,\* SIMON C. ELLIS, AND EILEEN P. ELLIS

Caribbean Marine Research Center,  
805 East 46th Place, Vero Beach, Florida 32963, USA

MICHAEL W. FEELEY

The University of Connecticut, Marine Science and Technology Center,  
Shennecossett Road, Groton, Connecticut 06340-6097, USA

**Abstract.**—The combined effects of temperature and salinity on eggs and yolk sac larvae of summer flounder *Paralichthys dentatus* were examined under controlled laboratory conditions. Fertilized eggs (early gastrula stage), obtained by induced spawning of captive broodstock at 17°C and 36 g/L salinity, were stocked (60 eggs/L) into forty-five 5-L translucent containers at temperatures of 16, 20, and 24°C and at salinities of 22, 28, and 34 g/L. Light intensity was 500 lx, and photoperiod was 12 h light : 12 h dark. At 16°C and 20°C, hatching rate was moderate to high (57.8–99.0%) at all salinities; at 24°C, hatching rate was high at 34 g/L (75.2%) but poor at 28 g/L (0%) and 22 g/L (30.5%), indicating a high-temperature–low-salinity inhibition ( $P < 0.001$ ). At the first feeding stage and at the stage when 97% of the yolk sac was absorbed (YSA), notochord lengths increased ( $P < 0.05$ ) with decreasing temperature, from a minimum at 24°C to a maximum at 16°C. Yolk utilization efficiency appeared to show a similar trend ( $0.05 < P < 0.10$ ). Average time from the first-feeding to the 97% YSA stage in unfed larvae ranged from 2.4 to 4.3 times longer at 16°C (18.3 h) than at 20°C (4.3 h) or 24°C (7.7 h). At a salinity of 34 g/L, median survival time (MST) was moderate (140–193 hours postfertilization, hpf) under all temperatures; at 22 and 28 g/L, MST was enhanced (222–294 hpf) at 16°C and markedly reduced (43.1–73.2 hpf) at 24°C, indicating low-temperature–low-salinity synergism, as well as high-temperature–low-salinity inhibitory effects ( $P < 0.001$ ). A temperature of 16°C, possibly associated with peak abundance of eggs and larvae in nature, is optimal for culture of summer flounder embryos and yolk sac larvae. At this temperature, growth, yolk utilization efficiency, time to initiate exogenous feeding, and tolerance to reduced salinities are maximized. Simultaneous exposure to high temperatures (24°C) and reduced salinities (22–28 g/L) may increase mortality and affect year-class strength.

The summer flounder *Paralichthys dentatus* provides an important recreational and commercial fishery in coastal waters from Nova Scotia to the Atlantic coast of south Florida (Poole 1966; Rogers and Van Den Avyle 1983; Grimes et al. 1989), with greatest abundance in the Middle Atlantic Bight between Cape Cod, Massachusetts, and Cape Hatteras, North Carolina (Rogers and Van Den Avyle 1983). The overexploitation of natural populations (NMFS 1992) has stimulated interest in commercial cultivation of this species (Bisbal and Bengston 1995a, 1995b).

Methods for controlled breeding (Smigielski 1975; Berlinsky et al. 1996; Watanabe et al. 1998a) and culture of larvae through juvenile stages (Bisbal and Bengston 1993, 1995a) have been developed, but the availability and cost of fingerlings are constraints to commercial grow out. Larval

survival is highly variable, with significant mortality observed during the embryonic and prefeeding stages, indicating that early survival may be related to egg quality or to rearing conditions unrelated to the availability of food.

It is generally presumed that environmental conditions that result in the most efficient use of endogenous energy (yolk) produce the largest larvae, which have optimal foraging and escape abilities (Laurence 1973; Howell 1980; Johns and Howell 1980). Temperature and salinity are dominant environmental factors that determine the efficiency with which yolk is converted into body tissue in marine fish larvae (Blaxter 1969; Brett 1970).

Summer flounder spawn during the autumn migration from estuarine and coastal waters to offshore wintering grounds (Smith 1973). Spawning occurs in oceanic waters where salinities range from 32 to 36 g/L at the bottom and where water temperatures range from 12°C to 19°C (Smith 1973; Rogers and Van Den Avyle 1983). Larvae

\* Corresponding author: watanabew@uncwil.edu

Received August 5, 1998; accepted June 2, 1999

are generally carried shoreward by surface currents (Bumpus and Lauzier 1965) to bays and estuaries, which are the primary nursery grounds for juveniles and where unstable temperature and salinity conditions occur (Smith 1973; Able et al. 1989). Adaptability of embryos and larvae to changes in temperature and salinity may therefore affect survival and year-class strength (Laurence and Rogers 1976).

Available information suggests that, while temperatures between 16°C and 21°C may not adversely affect yolk utilization and growth, definition of an optimum temperature requires more study. The effects of temperature on embryonic and larval stages of fish may be modified by salinity (Forrester and Alderdice 1966; May 1974; Santerre 1976; Santerre and May 1977; Laurence and Howell 1981; Holt et al. 1981), but little or no information is available on the combined effects of these variables on embryonic and larval development in summer flounder.

In this study, the combined effects of temperature and salinity on hatching and on growth, yolk utilization, and survival of yolk sac larvae were studied in summer flounder under controlled laboratory conditions. The objectives were to delineate optimal conditions of both variables for culture and to further test the hypothesis that optimal growth, yolk utilization efficiencies, and resistance to starvation would be observed under conditions associated with peak abundance of eggs and yolk sac larvae in nature.

### Methods

This study was conducted 27 October to 9 November 1995 at the Caribbean Marine Research Center (CMRC) in Vero Beach, Florida. Wild-caught summer flounder broodstock were held in tanks of recirculating seawater (salinity, 36 g/L) under controlled photoperiod (12 h light: 12 h dark, centered at 0600 hours) and temperature (17°C; Watanabe et al. 1998a). Fertilized eggs were obtained after volitional spawning that was induced with synthetic analog luteinizing hormone releasing hormone. On 28 October, eggs at the late blastula stage (9.5 hours postfertilization, hpf) were collected and placed in a 75-L incubator containing filtered (1- $\mu$ m mesh) seawater at 17°C that had been sterilized by ultraviolet (UV) light. Buoyant eggs (fertilization rate = 96.8%) were skimmed for use in the experiment.

Experimental units consisted of 5-L translucent polycarbonate beakers (21.3-cm diameter  $\times$  20 cm depth) placed in one of three temperature-regulating

circulation baths (2.44 m long  $\times$  1.05 m wide  $\times$  12.5 cm deep). Light was supplied to the experimental units in each water bath by four fluorescent bulbs (40 W) placed in a box (2.44 m long  $\times$  1.05 m wide  $\times$  20.3 cm deep) suspended above each bath. Fluorescent lamps provided full-spectrum light that simulated the color and UV spectrum of natural sunlight. Intensity at the water surface of each beaker averaged 500 lx (Watanabe et al. 1998b). Light was controlled by a timer to provide a photoperiod of 12 h light: 12 h dark, centered at 0600 hours. Black polyethylene was draped from each box to prevent exposure to extraneous light.

To determine the effects of temperature and salinity on hatching success of eggs, yolk utilization, and growth and survival of yolk sac larvae, a 3  $\times$  3 factorial experiment was conducted. Embryos at the early gastrula stage were stocked at a density of 60 embryos/L into 45 beakers containing 5 L of culture medium at 17°C under salinities of 22, 28, and 34 g/L. Temperatures in the three water baths were adjusted at a rate of 1°C/h to treatment levels of 24, 20, or 16°C. Salinity treatments were randomly assigned to beakers under each temperature. Five replicate beakers were maintained per treatment.

Water bath temperatures of 24°C and 20°C were maintained by using two 300-W immersion heaters that heated water against an ambient air temperature maintained at 18°C by an air conditioner. A water bath temperature of 16°C was maintained by cooling water by continuous recirculation through a heat pump. Water of required salinities was prepared by diluting filtered (1- $\mu$ m mesh) and UV-light-treated seawater with distilled water. Each beaker was supplied with aeration through a diffuser (1.25  $\times$  1.25  $\times$  2.5 cm) at a rate of 0.15 mL/min, a minimum level required to maintain all the eggs in suspension.

Beginning 1-d before hatching, hatching success and growth and survival of larvae were monitored by daily sampling of each replicate beaker using volumetric methods. Starting at 0800 hours each morning, one replicate from each treatment was sampled until all five replicate groups were completed within 5 h. Rate of aeration was increased for 10 s before collection to distribute eggs and larvae uniformly. Sample volumes were adjusted to collect a minimum of 10 eggs or larvae; volumes ranged from 340 to 680 mL. Sampled eggs or larvae were consolidated on a 75- $\mu$ m-mesh nitex sieve, transferred into a gridded petri dish containing an anesthetic solution (2-phenoxyethanol at 1 g/L), then counted under a dissection micro-

scope. Viable larvae were distinguished from dead larvae on the basis of heartbeat, as well as by opacity and appearance. Egg diameters, notochord lengths, and yolk sac (including oil droplet) dimensions (length and height) were measured with an ocular micrometer. Sampling continued until no viable eggs or larvae were observed.

Temperature and salinity for each replicate were recorded daily; light intensity at the water surface of each beaker and pH were recorded on alternate days throughout the study. Dissolved oxygen (DO) and total ammonia nitrogen (TAN) were measured on alternate days in one replicate from each treatment. Airflow to each beaker was monitored daily using a flowmeter and was adjusted if necessary. Water removed during sampling was replaced daily.

During the experiment, temperature averaged 16.0 (SE = 0.003), 20.1 (0.018), and 24.0°C (0.036) at the treatment levels of 16, 20, and 24°C, respectively. Salinity averaged 22.5 (0.04), 28.3 (0.21) and 34.5 g/L (0.05) at treatment levels of 22, 28, and 34 g/L, respectively. Light intensity averaged 485 lx (4.5), and pH averaged 8.12 (range = 8.08–8.15). Dissolved oxygen averaged 7.30 mg/L (0.09) and ranged from 6.68 mg/L at 24°C to 7.88 mg/L at 16°C. Total ammonia nitrogen averaged 0.079 mg/L (0.015) and ranged from 0.048 mg/L at 16°C to 0.11 mg/L at 24°C.

Hatching rate was determined as the percentage of viable larvae hatched from fertilized eggs. Yolk sac volume ( $V$ , mm<sup>3</sup>) was calculated from the formula for a prolate spheroid:  $V = (\pi/6)l \times h^2$ , where  $l$  is the length (mm), and  $h$  is the height (mm) of the yolk sac (Blaxter and Hempel 1963; Johns and Howell 1980). Time to 50% hatching in each replicate was estimated by regression analyses using a logarithmic curve fit.

Larval growth (i.e., notochord length) under the different treatment conditions was compared over time (i.e., days posthatching) and in relation to a standard final yolk volume, that is, the stage at which 97% of the initial yolk supply was absorbed (YSA). Standard initial yolk volume was set at 0.0921 mm<sup>3</sup>, estimated from a linear regression relationship between hatching rate (%) and yolk volume (mm<sup>3</sup>), using combined data for all treatments:  $y = -0.0009x + 0.1371$  ( $N = 30$ ,  $r^2 = 0.5228$ ,  $P < 0.01$ ), where  $y$  = yolk volume (mm<sup>3</sup>) and  $x$  = hatching rate (%). Standard initial yolk volume was calculated as the volume corresponding to a hatching rate of 50%. Standard final yolk volume was set at 0.00276 mm<sup>3</sup>, or 3% of the initial volume.

For each replicate, time (hpf) of 97% YSA was determined from a curvilinear regression equation (Systat, Chicago, Illinois) relating the decrease in yolk volume over time. Notochord length at 97% YSA was then determined from a regression equation (linear or curvilinear) best fitting the increase in notochord length with time.

Yolk utilization efficiency (YUE) was estimated by expressing rate of growth in length from hatching through 97% YSA as a percentage of the rate of yolk disappearance during this period (Watanabe et al. 1998b). Rate of growth in length (percent per day) was calculated from  $100 \times (L_t - L_i)/(t \times L_i)$ , where  $L_t$  = notochord length at 97% YSA,  $L_i$  = initial length, and  $t$  = time in days. Rate of disappearance of yolk (percent per day) was calculated from  $100 \times (\log_e V_i - \log_e V_f)/t \times 100$ , where  $V_i$  = yolk volume at hatching and  $V_f$  = yolk volume at 97% YSA. Larval survival to the 97% YSA stage was determined as a percentage of fertilized eggs stocked and was adjusted for sampling.

The main and interactive effects of temperature and salinity on hatching rate and median survival time were compared by two-way analysis of variance (ANOVA; Systat, Chicago, Illinois; Wilkinson et al. 1992; Table 1). Embryos in one treatment (24°C and 28 g/L) did not survive through hatching, so growth and yolk utilization data could not be obtained for this treatment. For these variates, the interaction term was omitted from the ANOVA, and only main effects were estimated (Wilkinson et al. 1992; Table 1). Multiple comparisons among means were made using Tukey's honestly significant difference test (Day and Quinn 1989; Wilkinson et al. 1992). For percentage data, arcsine transformation was performed before analysis.

## Results

### Hatching

At a temperature of 24°C and at salinities of 22 and 34 g/L, hatching rate increased from 0% to 100% between the first sampling (35.3 hpf) and the second sampling (61 hpf). Hence, median hatching time ( $T$ ) under these treatments was estimated as a range ( $35.3 < T < 61$  hpf; Table 2). No hatching was observed under 24°C in the 28 g/L treatment.

At 16°C and 20°C, significant ( $P < 0.001$ ) effects of both temperature and salinity on median hatching time were observed (Table 1). At all salinities, median hatching time was shorter ( $P < 0.05$ ) at 20°C (52.2 hpf) than at 16°C (84.7 h; Table

TABLE 1.—Summary of results of two-way analyses of variance for summer flounder yolk sac larvae reared under different conditions of temperature (16, 20, and 24°C) and salinity (22, 28, and 34 g/L). Data shown are *F*-values with degrees of freedom in subscripts; probability levels ( $P > 0.10$ , not significant [NS];  $0.05 < P < 0.10^*$ ;  $P < 0.05^{**}$ ;  $P < 0.01^{***}$ ;  $P < 0.001^{****}$ ) are indicated by asterisks for main and interactive effects. For some variates, the interaction term was not tested (NT) because of the loss of experimental animals in one treatment as explained in the text.

Variate	Main effects		
	Temperature (T)	Salinity (S)	Interaction of T × S
Median hatching time <sup>a</sup>	$F_{1,24} = 935^{****}$	$F_{2,24} = 13.9^{****}$	NT
Hatching rate	$F_{2,36} = 14.5^{****}$	$F_{2,36} = 53.4^{****}$	$F_{4,36} = 15.5^{****}$
Notochord length at first feeding	$F_{2,33} = 3.92^{**}$	$F_{2,33} = 2.29$ (NS)	NT
Yolk volume at first feeding	$F_{2,32} = 0.15$ (NS)	$F_{2,32} = 1.83$ (NS)	NT
Time to 97% YSA <sup>b</sup>	$F_{2,32} = 1,584^{****}$	$F_{2,32} = 1.83$ (NS)	NT
Notochord length at 97% YSA <sup>b</sup>	$F_{2,31} = 15.3^{****}$	$F_{2,31} = 1.97$ (NS)	NT
Yolk utilization efficiency	$F_{2,30} = 2.97^*$	$F_{2,30} = 0.08$ (NS)	NT
Median survival time	$F_{2,36} = 105^{****}$	$F_{2,36} = 45.2^{****}$	$F_{4,36} = 26.5^{****}$
Survival at 97% YSA <sup>b</sup>	$F_{2,31} = 4.13^{**}$	$F_{2,31} = 2.75^*$	NT

<sup>a</sup> For median hatching time, data represents only the 16°C and 20°C treatments because under 24°C, hatching rate increased from 0% to 100% during the sampling interval.

<sup>b</sup> Yolk-sac absorption.

2). At both temperatures, median hatching time was shorter ( $P < 0.05$ ) at 34 g/L (mean = 64.5 hpf) than at 22 and 28 g/L (70.5 hpf; Table 2). Initial larval densities at hatching ranged from 0 to 59.4 larvae/L (Table 2) among treatments. Significant ( $P < 0.001$ ) effects of both temperature and salinity on hatching rate were observed, with a significant interaction ( $P < 0.001$ ) observed between these effects (Table 1). At 16°C and 20°C, hatching rate was moderate to high (range = 57.8–99.0%) under all salinities (Table 2); at 24°C, hatching rate was high at 34 g/L (75.2%)

but was greatly reduced at lower salinities of 22 g/L (30.5%) and 28 g/L (0%; Table 2).

#### First Feeding

At all temperatures, growth was fastest during the first 48 h posthatching (Figure 1), then decreased during the remainder of the yolk sac period. Development was accelerated by higher temperatures, and based on morphological criteria (dark, pigmented eyes, a functional mouth, and completely formed gut), larvae under all salinities were judged capable of exogenous feeding by ap-

TABLE 2.—Time to 50% hatch (hours postfertilization = hpf), density at hatching, and hatching rate for summer flounder larvae incubated under different combinations of temperature (16, 20, and 24°C) and salinity (22, 28, and 34 g/L). Fertilized eggs were stocked in 5-L beakers at a density of 60 eggs/L. Values are means ± SE ( $N = 3-5$ ); ND = no data because no hatching was observed in this treatment.

Treatment		Measurement at hatching:		
Temperature (°C)	Salinity (g/L)	Median time (hpf)	Density (larvae/L)	Hatching rate (%)
16	22	88.4 ± 2.2	59.4 ± 5.3	99.0 ± 8.8
16	28	85.9 ± 1.1	56.8 ± 4.0	94.7 ± 6.7
16	34	79.9 ± 0.8	42.2 ± 3.8	70.3 ± 6.3
20	22	52.6 ± 1.0	59.0 ± 3.5	98.3 ± 5.8
20	28	54.9 ± 0.8	34.7 ± 2.6	57.8 ± 4.3
20	34	49.0 ± 1.4	51.8 ± 2.8	86.3 ± 4.7
24	22	35.3 < <i>T</i> < 61.0 <sup>a</sup>	18.3 ± 7.0	30.5 ± 11.7
24	28	ND	ND	ND
24	34	35.3 < <i>T</i> < 61.0	45.1 ± 3.9	75.2 ± 6.5

<sup>a</sup> *T* = hatch time (hpf). A range is provided because hatching rate increased from 0 to 100% during this time period.

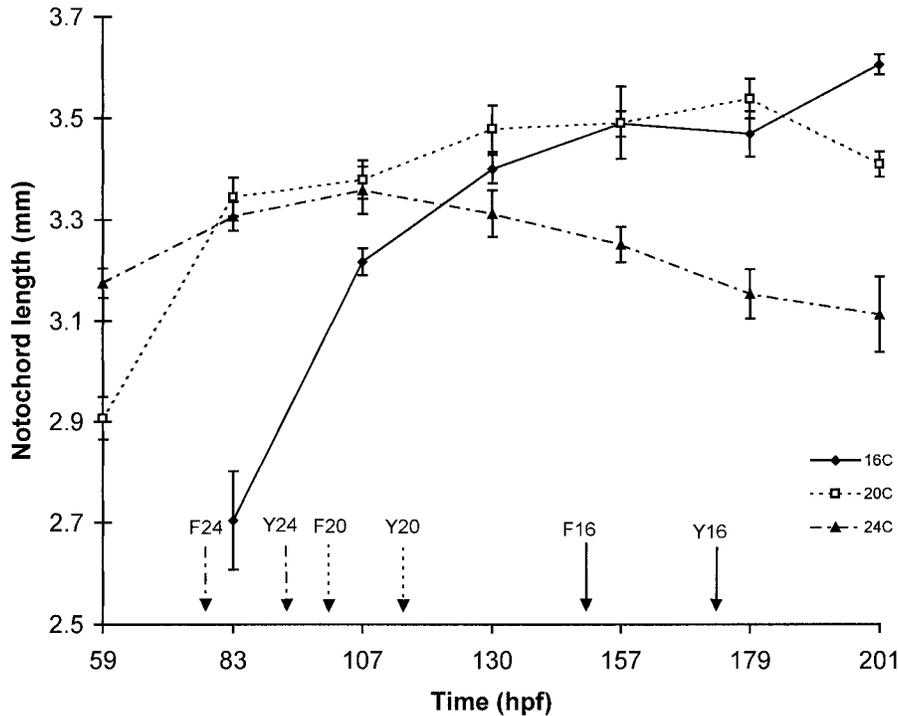


FIGURE 1.—Growth (notochord length) of yolk sac summer flounder larvae under different temperatures (16, 20, and 24°C). For each temperature, data for three salinity treatments (22, 28, and 34 g/L) were combined because significant effects of salinity on growth were not observed. Plotted points represent means  $\pm$  SE ( $N = 10-15$ ). Time on the horizontal axis is shown as hours postfertilization (hpf). Average times to the first-feeding (F) and the 97% yolk sac absorbed (Y) stages at each temperature are indicated by the arrows above the horizontal axis.

proximately 82, 106, and 154 hpf (day 1, day 2, and day 3 posthatching) in the 24, 20, and 16°C treatments, respectively (Figure 1; Table 3).

A significant effect of temperature ( $P < 0.05$ ) on notochord length at first feeding was observed, but the effect of salinity ( $P > 0.05$ ) was not significant (Table 1). At all salinities, average notochord length at first feeding increased with decreasing temperature, from a minimum at 24°C (3.35 mm) to a maximum at 16°C (3.49 mm; Table 3).

#### Yolk Utilization

Plots of yolk volume against time (Figure 2) showed that, under all treatments, yolk volume decreased at an exponential rate, falling rapidly through the embryonic period and through day 1 posthatching, then gradually thereafter. Patterns of yolk disappearance were divergent for larvae reared under different temperatures, with rate of disappearance decreasing from a maximum at 24°C to a minimum at 16°C (Figure 2). At each temperature, patterns of yolk disappearance were

similar among larvae reared under different salinities (Figure 2).

Although time to first feeding varied among temperature treatments (Figure 1; Table 3), yolk volume at first feeding averaged  $4.34 \times 10^{-3} \text{ mm}^3$ , or 4.71% of the standard hatching volume, with no significant ( $P > 0.05$ ) effects of temperature or salinity observed (Table 3).

#### Yolk Absorption Stage

Time to the 97% YSA stage varied widely among treatment groups, from 88.6 to 173 hpf (Table 4). A significant effect of temperature ( $P < 0.001$ ) on time to 97% YSA was observed, but the effect of salinity ( $P > 0.05$ ) was not significant (Table 1). At all salinities, time to 97% YSA was extended ( $P < 0.001$ ) by lower temperatures, from 89.5 hpf at 24°C to 172.3 hpf at 16°C (Table 4; Figure 1).

Under each treatment condition, the 97% YSA stage (Table 4) occurred after first feeding (Table 3; Figure 1). The average time between these stages

TABLE 3.—Time to first feeding (hours postfertilization = hpf), notochord length, and yolk volume at first feeding for summer flounder yolk sac larvae reared under different combinations of temperature (16, 20, and 24°C) and salinity (22, 28, and 32 g/L). Replicate values were obtained on the date that all larvae were judged capable of first feeding as determined by morphological criteria. Values are means  $\pm$  SE ( $N = 3-5$ ); ND is no data because no hatching was observed at this treatment.

Treatment		Measurement at first feeding		
Temperature (°C)	Salinity (g/L)	Time (hpf)	Notochord length (mm)	Yolk volume (mm <sup>3</sup> $\times$ 10 <sup>3</sup> )
16	22	154	3.58 $\pm$ 0.04	3.79 $\pm$ 0.88
16	28	154	3.54 $\pm$ 0.04	5.19 $\pm$ 1.00
16	34	154	3.35 $\pm$ 0.06	4.73 $\pm$ 0.27
20	22	106	3.57 $\pm$ 0.05	4.36 $\pm$ 0.35
20	28	106	3.23 $\pm$ 0.07	4.96 $\pm$ 1.25
20	34	106	3.45 $\pm$ 0.12	3.67 $\pm$ 0.39
24	22	82	3.26 $\pm$ 0.13	4.44 $\pm$ 0.25
24	28	ND	ND	ND
24	34	82	3.44 $\pm$ 0.10	3.61 $\pm$ 0.39

was considerably longer ( $P < 0.05$ ) at 16°C (18.3 h) than at 20°C (4.3 h) or 24°C (7.7 h; Figure 1).

A significant effect of temperature ( $P < 0.001$ ) on notochord length at 97% YSA (Figure 3) was observed, but the effect of salinity ( $P > 0.05$ ) was not significant (Table 1). At all salinities, notochord length at 97% YSA increased with decreasing temperature, from a minimum (3.30 mm) at 24°C to a maximum (3.56 mm) at 16°C (Figure 3).

There was a marginal effect of temperature ( $0.05 < P < 0.10$ ) on yolk utilization efficiency (YUE), but the effect of salinity ( $P > 0.05$ ) was not significant (Table 1). At all salinities, YUE increased with decreasing temperature, from a minimum (4.17%) at 24°C to a maximum (7.40%) at 16°C (Table 4).

#### Survival

Significant ( $P < 0.001$ ) effects of both temperature and salinity on median survival time (MST) were observed, with a significant interaction ( $P < 0.001$ ) between these effects (Table 1; Figure 4). At 34 g/L, MST was moderate (140–193 hpf) under all temperatures, but at reduced salinities of 22 and 28 g/L, median survival time was markedly extended (222–294 hpf) at 16°C and shortened (43.1–73.2 hpf) at 24°C (Figure 4).

A significant effect of temperature ( $P < 0.05$ ) on survival at the 97% YSA stage was observed, but the effect of salinity ( $0.05 < P < 0.10$ ) was marginally significant (Table 1). An interaction was suggested by the data (Table 4). At 34 g/L, survival was moderate to high (59.1–96.9%) under all temperatures, but at reduced salinities of 22 and 28 g/L, survival was enhanced (80.6–100%) at 16°C and lowered (0–40.2%) at 24°C.

#### Discussion

In this study, rate of embryonic development through hatching in summer flounder was accelerated by higher temperatures and salinities within the ranges tested. This is consistent with the well-established finding that higher temperatures and salinities within ecological limits of a species accelerate developmental processes in fish (Lasker 1964; Blaxter 1969; Laurence and Rogers 1976), including the summer flounder (Johns and Howell 1980; Johns et al. 1981), yellowtail flounder *Limanda ferruginea* (= *Pleuronectes ferrugineus*) (Howell 1980; Laurence and Howell 1981) and winter flounder *Pseudopleuronectes* (= *Pleuronectes*) *americanus* (Rogers 1976).

Whereas temperature produced marked differences in developmental rates and median hatching time (35.3  $< T <$  61–84.7 hpf, integrated across all salinities) of summer flounder embryos in this study, the effects of salinity on median hatching time (64.5–70.5 hpf, integrated across all temperatures) were relatively small. This is similar to what was reported in the yellowtail flounder (Laurence and Howell 1981) and in the Atlantic cod *Gadus morhua* (Laurence and Rogers 1976), where time to 50% hatching decreased with increasing salinity within the range of 26–36 g/L, although these differences were much smaller than those produced by temperature within ranges of 6–18°C and 2–12°C, respectively.

In this study, a high temperature of 24°C impaired embryonic development and hatching under lower salinities of 22 and 28 g/L, indicating a high-temperature–low-salinity inhibition. A similar interactive trend toward low embryo survival (10–

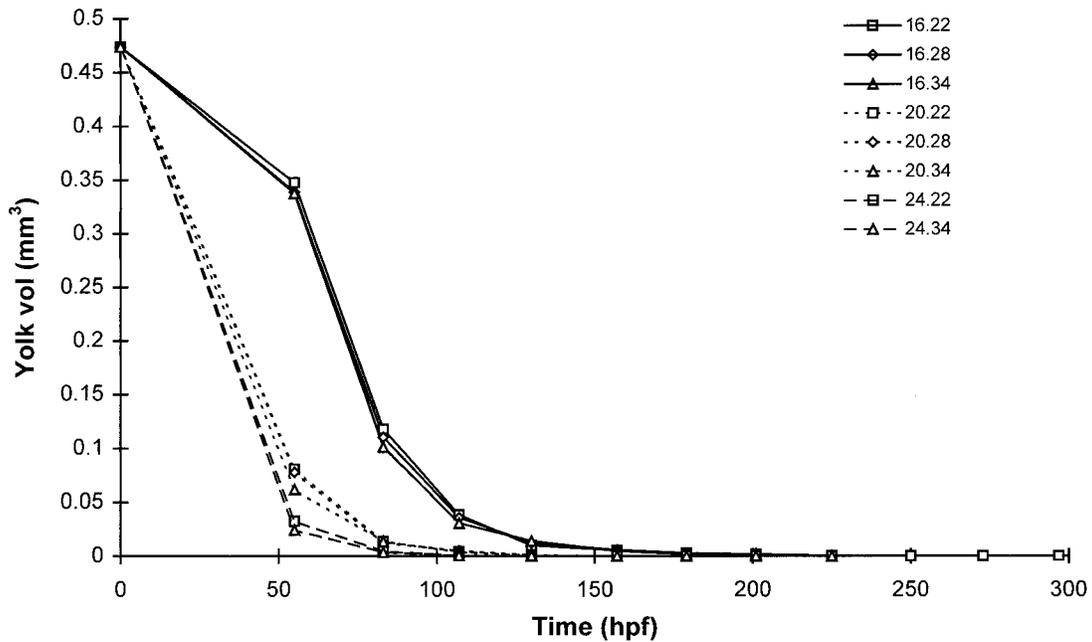


FIGURE 2.—Yolk utilization by summer flounder yolk sac larvae under different temperature (16, 20, and 24°C) and salinity (22, 28, and 34 g/L) regimes. Plotted points represent means ( $N = 5$ ) for each treatment. For clarity, SE bars are not shown. The 24°C and 22 g/L treatment is omitted because no hatching was observed for that treatment. Time on the horizontal axis is shown as hours postfertilization (hpf); vol = volume.

30%) at low salinities (28 g/L) coupled with high (16–18°C) as well as low (<8°C) temperatures within maximum environmental ranges was observed in yellowtail flounder, a temperate marine species (Laurence and Howell 1981). Varied interactive effects of temperature and salinity on embryo survival have been reported in numerous marine fish species, including winter flounder (Rogers 1976), Pacific cod *Gadus macrocephalus* (Forrester and Alderdice 1966), bairdiella *Bairdiella icistia*

(May 1974); Pacific threadfin *Polydactylus sexfilis* (Santerre 1976; Santerre and May 1977), and red drum *Sciaenops ocellatus* (Holt et al. 1981). The results of this study indicate that temperatures within a mid to upper ecological range (16–24°C) have relatively little influence on survival of embryos under oceanic salinities. However, higher temperatures within this range may be lethal to embryos exposed to reduced salinities during transport to nearshore areas.

TABLE 4.—Time to the 97% yolk sac absorbed (YSA) stage (hours postfertilization = hpf), notochord length (NL), yolk utilization efficiency (YUE), and survival at 97% YSA for summer flounder yolk sac larvae reared under different combinations of temperature (16, 20, and 24°C) and salinity (22, 28, and 34 g/L). Values represent means  $\pm$  SE ( $N = 3-5$ ); ND = no data because no hatching was observed in this treatment.

Treatment		Measurement at 97% YSA				
Temperature (°C)	Salinity (g/L)	Time (hpf)	Density (larvae/L)	Notochord length (mm)	YUE (%)	Survival (%)
16	22	172 $\pm$ 1.6	61.0 $\pm$ 1.26	3.61 $\pm$ 0.04	6.92 $\pm$ 1.42	101.6 $\pm$ 2.1 <sup>a</sup>
16	28	173 $\pm$ 2.8	48.4 $\pm$ 4.6	3.55 $\pm$ 0.02	9.20 $\pm$ 1.68	80.6 $\pm$ 7.7
16	34	172 $\pm$ 2.6	35.5 $\pm$ 9.2	3.50 $\pm$ 0.07	6.19 $\pm$ 0.90	59.1 $\pm$ 15.4
20	22	111 $\pm$ 1.1	58.8 $\pm$ 1.98	3.49 $\pm$ 0.05	8.12 $\pm$ 0.76	98.0 $\pm$ 3.3
20	28	114 $\pm$ 3.1	22.0 $\pm$ 7.0	3.23 $\pm$ 0.07	2.84 $\pm$ 0.64	36.7 $\pm$ 11.6
20	34	106 $\pm$ 1.1	58.1 $\pm$ 7.8	3.47 $\pm$ 0.05	4.67 $\pm$ 0.65	96.9 $\pm$ 13.0
24	22	90.7 $\pm$ 0.9	24.1 $\pm$ 8.2	3.28 $\pm$ 0.06	2.42 $\pm$ 1.15	40.2 $\pm$ 13.6
24	28	ND	ND	ND	ND	ND
24	34	88.6 $\pm$ 2.4	44.5 $\pm$ 2.64	3.32 $\pm$ 0.03	5.57 $\pm$ 1.51	74.2 $\pm$ 4.4

<sup>a</sup> Value may exceed 100% due to volumetric sampling error.

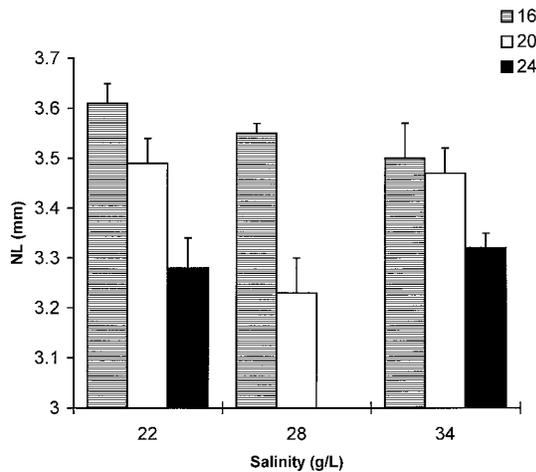


FIGURE 3.—Notochord length (NL) at the 97% yolk sac absorbed stage for summer flounder larvae reared under different temperatures (16, 20, and 24°C) and salinities (22, 28, and 34 g/L). Bars represent means and vertical lines indicate +SE ( $N = 5$ ) for each treatment. The 24°C and 22 g/L treatment is omitted because no hatching was observed for this treatment.

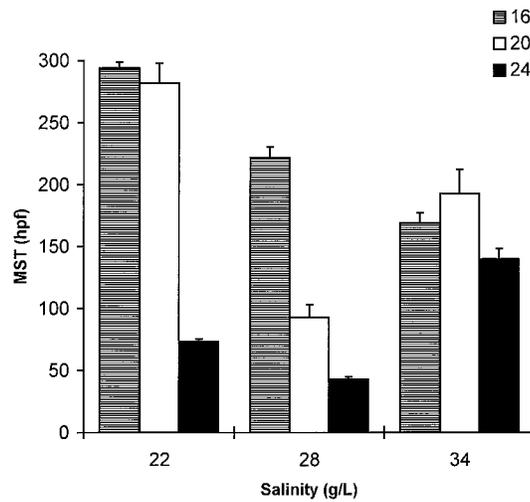


FIGURE 4.—Median survival time (MST; hours post-fertilization, hpf) of summer flounder yolk sac larvae reared under different temperatures (16, 20, and 24°C) and salinities (22, 28, and 34 g/L). Bars represent means and vertical lines indicate +SE ( $N = 5$ ) for each treatment.

Development of summer flounder yolk sac larvae through first feeding in this study was accelerated by higher temperatures within the range 16–24°C, consistent with what was previously reported for summer flounder grown under temperatures ranging from 5°C to 21°C (Johns and Howell 1980; Johns et al. 1981). Salinity, on the other hand, did not influence development and growth rates of summer flounder yolk sac larvae through the first-feeding stage in this study. This is similar to what was reported for a number of marine fish species, including Pacific herring *Clupea pallasii* (Alderdice and Forrester 1971), bairdiella (May 1974), sole *Solea solea* (=vulgaris) (Fonds 1979), and greenback flounder *Rhombosolea tapirina* (Hart and Purser 1995), in which temperature had a much stronger influence than salinity on rate of larval development through yolk exhaustion and first feeding. Significant, albeit small, effects of salinity within the range of 26–36 g/L on growth were previously observed in yolk sac summer flounder larvae reared at 19°C (Watanabe et al. 1998b). These differences may be attributed to the lower salinity range (22–34 g/L) used in the present study.

In this study, summer flounder larvae under all treatment conditions reached the first-feeding stage before the 97% YSA stage, although the time between these stages ranged from 2.4 to 4.3 times longer at 16°C than at 20°C or 24°C. Hence, larvae

reared at 16°C have considerably more time to initiate exogenous feeding before yolk reserves are exhausted. Similarly, in the tropical marine carangid *Caranx mate*, higher temperatures of 29–30°C were advantageous to larval survival, causing more rapid development of eyes and jaws, which preceded yolk absorption by 20 h, whereas at 23°C these events coincided (Santerre 1976).

If predation is a major cause of larval mortality, lower temperatures, which delay the development of functional eyes and hence the ability to avoid predators, may increase mortality (Lasker 1964). On the other hand, lower temperatures also prolong the availability of yolk reserves after the first-feeding stage, which would improve chances of encountering prey and of successful transition to exogenous feeding. Under hatchery conditions where predation is minimized, successful first feeding is presumably more important than rapid early development.

Rate of yolk disappearance in summer flounder clearly declined with declining temperature, from a maximum at 24°C to a minimum at 16°C, but it was similar among larvae reared at different salinities. Faster yolk utilization at higher temperatures was previously reported for the summer flounder (Johns and Howell 1980; Johns et al. 1981), as well as for other species, including yellowtail flounder (Howell 1980; Laurence and Howell 1981) and bairdiella (May 1974). As was

the case with summer flounder in this study, salinity produced comparatively small effects on rate of yolk absorption by bairdiella (May 1974) and Pacific herring (Alderdice and Velsen 1971).

Lower temperatures (within the range of 16–24°C) not only slowed development rate of summer flounder yolk sac larvae but produced larger larvae at the first-feeding and 97% YSA stages. These results appear contradictory to those of earlier studies (Johns and Howell 1980; Johns et al. 1981), in which yolk sac summer flounder larvae, while developing more slowly at 16°C than at 21°C, showed no differences in fish size or yolk utilization efficiency at these temperatures. These authors suggested that rigid temperature control over early life stages may not be required for summer flounder and that it may be possible to find an optimal temperature that would produce even higher yolk utilization efficiency and growth.

Results of the present study indicate, however, that these dissimilar results are attributable to the modifying influence of salinity, which differed in these studies. In the present study a high-temperature–low-salinity inhibition on growth and YUE was strongly suggested by the results, but no temperature-related differences in YUE were observed in seawater with a salinity of 34 g/L, consistent with what was observed in the earlier studies, which used seawater of unspecified salinity (Johns and Howell 1980; Johns et al. 1981). An interaction between temperature and salinity on yolk utilization efficiency was also reported for bairdiella (May 1974).

It is noteworthy that maximum size differences between temperature–salinity treatment combinations producing the maximum (16°C and 22 g/L) and minimum (20°C and 28 g/L) notochord length at first feeding were 10%, a difference that could have important implications for larval survival. For plaice *Pleuronectes platessa*, a 10% increase in larval size corresponded to 10–25% increase in swimming speed (Ryland and Nichols 1967). Greater larval size during the transition from endogenous to exogenous food improves chances of survival through an increased ability to compete for prey (Blaxter and Hempel 1963; Blaxter 1969).

In this study, a high temperature of 24°C, although not greatly influencing larval survival at 34 g/L, markedly impaired survival at 97% YSA at salinities of 22 and 28 g/L, indicating a high-temperature–low-salinity inhibition. These results suggest that higher temperatures within an ecological range of 16–24°C may be lethal to larvae exposed to reduced salinities and may affect year-class strength.

A low temperature of 16°C enhanced larval survival at reduced salinities of 22 and 28 g/L, indicating a low-temperature–low salinity synergistic effect, a phenomenon previously reported in unfed bairdiella larvae (May 1974). Lower temperatures reduce metabolic rate, while lower salinities reduce activity levels (possibly due to negative buoyancy; Blaxter and Hempel 1963; Holliday 1988) and osmotic and metabolic demands, thereby extending survival time (May 1974). Moderate to high survival under all salinities at 16°C reflects an adaptability of eggs and yolk sac larvae to inshore movement during the pelagic larval phase.

Pelagic eggs of summer flounder have been caught at mean temperatures of 9.1–22.9°C and larvae from 0°C to 23.1°C (Smith 1973). Although similar data on distribution of eggs and larvae with regard to salinity is lacking, pelagic eggs and larvae may presumably be exposed to reduced salinities during shoreward transport to bays and estuaries. Thus, treatment salinities, as well as temperatures used in this study, are probably within the ecological ranges of these variables. Based on maximum growth, yolk utilization efficiency, time between first feeding and yolk sac exhaustion, and ability of embryos and larvae to withstand reduced salinities under these conditions, a temperature of 16°C is recommended for culture of yolk sac larvae of summer flounder. Since inhibitory effects on growth and survival were observed at 24°C under reduced salinities, full-strength seawater (salinity, 34 g/L) is recommended for culture. The results support the hypothesis that optimal hatching, growth, yolk utilization efficiencies, and resistance to starvation would be observed under temperature and salinity conditions associated with peak abundance of eggs and yolk sac larvae in nature.

#### Acknowledgments

We thank the Florida Institute of Technology for facilities support, Kimberley Gridley for preparation of the figures, and Eric Johnson for assistance with data analysis. This study was supported by a grant from the Marine Sciences and Technology Center, University of Connecticut, and by the Caribbean Marine Research Center and the University of North Carolina at Wilmington.

#### References

- Able, K. W., R. E. Matheson, W. W. Morse, M. P. Fahay, and G. Shepherd. 1989. Patterns of summer flounder (*Paralichthys dentatus*) early life history in the

- Mid-Atlantic Bight and New Jersey estuaries. Fishery Bulletin 88:1–12.
- Alderdice, D. F., and C. R. Forrester. 1971. Effects of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephalus*). Journal of the Fisheries Research Board of Canada 28:883–902.
- Alderdice, D. F., and F. P. J. Velsen. 1971. Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasii*). Journal of the Fisheries Research Board of Canada 28:1545–1562.
- Berlinsky, D. L., and five coauthors. 1996. Induced ovulation of southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. Journal of the World Aquaculture Society 27:143–152.
- Bisbal, G. A., and D. A. Bengston. 1993. Reversed asymmetry in laboratory-reared summer flounder. Progressive Fish-Culturist 55:106–108.
- Bisbal, G. A., and D. A. Bengston. 1995a. Effects of delayed feeding on survival and growth of summer flounder *Paralichthys dentatus* larvae. Marine Ecology Progress Series 121:301–306.
- Bisbal, G. A., and D. A. Bengston. 1995b. Description of the starving condition in summer flounder, *Paralichthys dentatus*, early life history stages. Fishery Bulletin 93:217–230.
- Blaxter, J. H. S. 1969. Development: eggs and larvae. Pages 178–252 in W. S. Hoar and D. J. Randall, editors. Fish physiology, volume 3. Academic Press, New York.
- Blaxter, J. H. S., and G. Hempel. 1963. The influence of egg size on herring larvae (*Clupea harengus* L.). Journal du Conseil, Conseil International Exploration de la Mer 28:211–240.
- Brett, J. R. 1970. Temperature—fishes. Pages 515–560 in O. Kinne, editor. Marine ecology, volume 1. Wiley-Interscience, New York.
- Bumpus, D. F., and L. M. Lauzier. 1965. Surface circulation on the continental shelf off eastern North America between Newfoundland and Florida. American Geographic Society, Serial Atlas Marine Environment, Folio 7, Washington D.C. (Not seen; cited in Itzkowitz et al. 1983.)
- Day, R. W., and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecological Monographs 59:433–463.
- Fonds, M. 1979. Laboratory observations on the influence of temperature and salinity on development of the eggs and growth of the larvae of *Solea solea* (Pisces). Marine Ecology Progress Series 1:91–99.
- Forrester, C. R., and D. F. Alderdice. 1966. Effects of salinity and temperature on embryonic development of the Pacific cod (*Gadus macrocephalus*). Journal of the Fisheries Research Board of Canada 23:319–340.
- 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). U.S. Fish and Wildlife Service Biological Report 82 (11.112). (Also: U.S. Army Corps of Engineers Technical Report EL-82-4.)
- Hart, P. R., and G. R. Purser. 1995. Effects of salinity and temperature on eggs and yolk sac larvae of the greenback flounder (*Rhombosolea tapirina* Gunther, 1862). Aquaculture 136:221–230.
- Holliday, F. G. T. 1988. The effects of salinity on the eggs and larvae of teleosts. Pages 293–311 in W. S. Hoar and R. J. Randall, editors. Fish physiology, volume 1. Academic Press, New York.
- Holt, J., R. Godbout, and C. R. Arnold. 1981. Effects of temperature and salinity on egg hatching and larval survival of red drum, *Sciaenops ocellatus*. Fishery Bulletin 79:569–573.
- Howell, W. H. 1980. Temperature effects on growth and yolk utilization in yellowtail flounder, *Limanda ferruginea*, yolk-sac larvae. Fishery Bulletin 78:731–739.
- Itzkowitz, N., J. R. Schubel, and P. M. J. Woodhead. 1983. Responses of summer flounder, *Paralichthys dentatus*, embryos to thermal shock. Environmental Biology of Fishes 8:125–135.
- Johns, D. M., and W. H. Howell. 1980. Yolk utilization in summer flounder (*Paralichthys dentatus*) embryos and larvae reared at two temperatures. Marine Ecology Progress Series 2:1–8.
- Johns, D. M., W. H. Howell, and G. Klein-MacPhee. 1981. Yolk utilization and growth to yolk-sac absorption in summer flounder (*Paralichthys dentatus*) at constant and cyclic temperatures. Marine Biology 63:301–308.
- Lasker, R. 1964. An experimental study of temperature on the incubation time, development, and growth of Pacific sardine embryos and larvae. Copeia 1964:399–405.
- Laurence, G. C. 1973. Influence of temperature on energy utilization of embryonic and prolarval tautog, *Tautoga onitis*. Journal of the Fisheries Research Board of Canada 30:435–442.
- Laurence, G. C., and W. H. Howell. 1981. Embryology and influence of temperature and salinity on early development and survival of yellowtail flounder *Limanda ferruginea*. Marine Ecology Progress Series 6:11–18.
- Laurence, G. C., and C. A. Rogers. 1976. Effects of temperature and salinity on comparative embryo development and mortality of Atlantic cod (*Gadus morhua* L.) and haddock (*Melanogrammus aeglefinus* L.). Journal du Conseil, Conseil International Exploration de la Mer 36:220–228.
- May, R. C. 1974. Effects of temperature and salinity on yolk utilization in *Bairdiella icistia* (Jordan and Gilbert) (Pisces: Sciaenidae). Journal of Experimental Marine Biology and Ecology 16:213–225.
- NMFS (National Marine Fisheries Service). 1992. Status of fishery resources off the northeastern United States for 1992. NOAA Technical Memorandum NMFS-F/NEC-95.
- Poole, J. C. 1966. A review of research concerning summer flounder and needs for further study. New York Fish and Game Journal 13:226–231.
- Rogers, C. A. 1976. Effects of temperature and salinity

- on the survival of winter flounder embryos. Fishery Bulletin 74:52–58.
- Rogers, S. G., and M. J. Van Den Avyle. 1983. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic)—summer flounder. U.S. Fish and Wildlife Service, Washington, D.C. (Also: U.S. Army Corps of Engineers, Washington, D.C.)
- Ryland, J. S., and J. H. Nichols. 1967. Effect of temperature on the efficiency of growth of plaice pro-larvae. Nature (London) 214:529–530.
- Santerre, M. T. 1976. Effects of temperature and salinity on the eggs and early larvae of *Caranx mate* (Cuv. and Valenci.) (Pisces: Carangidae) in Hawaii. Journal of Experimental Marine Biology and Ecology 21:51–68.
- Santerre, M. T., and R. C. May. 1977. Some effects of temperature and salinity on laboratory-reared eggs and larvae of *Polydactylus sexfilis* (Pisces: Polynemidae). Aquaculture 10:341–351.
- Smigielski, A. S. 1975. Hormone-induced spawnings of the summer flounder and rearing of the larvae in the laboratory. Progressive Fish-Culturist 37:3–8.
- Smith, W. G. 1973. The distribution of summer flounder, *Paralichthys dentatus*, eggs and larvae on the continental shelf between Cape Cod and Cape Lookout, 1965–1966. Fishery Bulletin 71:527–535.
- Watanabe, W. O., E. P. Ellis, S. C. Ellis, and M. W. Feeley. 1998a. Progress in controlled maturation and spawning of summer flounder *Paralichthys dentatus* broodstock. Journal of the World Aquaculture Society 29:393–404.
- Watanabe, W. O., M. W. Feeley, S. C. Ellis, and E. P. Ellis. 1998b. Light intensity and salinity effects on eggs and yolk sac larvae of the summer flounder. Progressive Fish-Culturist 60:9–19.
- Wilkinson, L., M. Hill, J. Welna, and G. K. Birkenbeuel. 1992. Systat statistics. SPSS, Chicago.