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Light Intensity and Salinity Effects on Eggs and Yolk Sac Larvae of the Summer Flounder

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Abstract.—The effects of light intensity 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, were stocked (53 eggs/L) into forty-eight 5-L translucent containers under light intensities of 0 (constant dark), 500, 1,000, and 2,000 lx and at salinities of 26, 31, and 36 g/L. Temperature was 19°C and photoperiod was 12 h light : 12 h dark. Light intensity and salinity produced significant ($P < 0.05$) additive effects on larval growth. At the stage when 97% of the yolk sac was absorbed (114–131 h postfertilization, hpf), at the first-feeding stage (129.5–135 hpf), and at yolk exhaustion (153.5–159 hpf), notochord lengths were generally maximal at low light intensity (500 lx) and high salinity (36 g/L) and minimal at high intensity (2,000 lx) and low salinity (26 g/L). Yolk utilization efficiency declined significantly ($P < 0.01$) with increasing light intensity, presumably due to light-induced activity. Largest larvae were produced under low light intensity (500 lx) and high-salinity (36 g/L), conditions consistent with shelf waters where eggs and early larvae of summer flounder prevail in nature. High survival (85.1%) to yolk exhaustion under all light intensities and salinities reflects an adaptability for inshore movement during the pelagic larval phase.

The summer flounder *Paralichthys dentatus* is an important sport and commercial marine flatfish species found in estuarine and coastal waters of the Atlantic Coast of North America; it is most abundant from Cape Hatteras, North Carolina, to Cape Cod, Massachussetts (Rogers and Van Den Avyle 1983; Grimes et al. 1989). Overexploitation of natural populations (NMFS 1992) has stimulated interest in commercial cultivation of this species (Bisbal and Bengston 1995a, 1995b). The development of cost-effective methods for large-scale production of juveniles is needed for commercial production because high mortalities have been common during the embryonic and yolk sac stages.

Summer flounder spawn during the autumn migration from estuarine and coastal waters to offshore wintering grounds (Smith 1973). Spawning occurs on or near the bottom in shelf waters ranging from 30 to 200 m deep (Rogers and Van Den Avyle 1983) and where water temperatures range from 12°C to 19°C (Smith 1973). Eggs and larvae are pelagic and have been found at depths of 3–33 m; no sampling has been done at greater depths (Smith 1973).

Light exerts important and diverse effects on embryonic development, hatching, and buoyancy of marine fish eggs and on the behavior and activity levels of larvae (Bolla and Holmefjord 1988; Helvik and Walther 1992; McAlary and McFarland 1993; Olla et al., in press). Because light intensity attenuates rapidly with depth—from 10,000 lx at the surface to 0.5 lx at 200 m in the clearest ocean water (Helvik and Walther 1992)—eggs and larvae of summer flounder may be exposed to highly variable light intensities within their natural ranges of vertical distribution.

Salinity, often in combination with other factors, also exerts marked effects on development and growth in marine fish eggs and larvae (Fonds 1979; Laurence and Howell 1981; see review by Holliday 1988). Although occurring primarily at sea

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(Smith 1973), pelagic eggs and larvae of summer flounder may be carried shoreward by surface currents (Bumpus and Lauzier 1965) to bays and estuaries, which are the primary nursery grounds for juveniles (Smith 1973; Able et al. 1989).

Environmental conditions that result in the most efficient use of endogenous energy (yolk) may produce the largest larvae, which presumably have optimal foraging abilities (Laurence 1973; May 1974; Howell 1980; Johns and Howell 1980; Solberg and Tilseth 1987). Little or no information is available on the effects of light or salinity on embryonic development, hatching, survival, and growth in summer flounder. Quantification of these effects is therefore important to delineate optimum conditions for mass culture and to elucidate patterns of distribution and abundance in nature.

Considering that adult summer flounder spawn in relatively deep oceanic waters (Smith 1973), it was hypothesized that low light intensities and full strength seawater salinities would produce optimal survival and growth of eggs and yolk sac larvae. In this study, the combined effects of light intensity and salinity on summer flounder hatching, growth, yolk utilization, and survival to the first-feeding stage were compared under controlled laboratory conditions.

Methods

This study was conducted 20 September to 1 October 1995 at the Caribbean Marine Research Center in Vero Beach, Florida. Fertilized summer flounder eggs were obtained after spawning (induced with synthetic analog luteinizing hormone releasing hormone) of captive broodstock held in tanks of recirculating seawater (36 g of salt/L) under controlled photoperiod (12.2 h light : 11.8 h dark) and temperature (17°C) (W. O. Watanabe and others, unpublished data). Fertilized eggs (late blastula stage), originating from a spawning at 2400 hours on 24 September, were collected at 0930 hours (9.5 hours postfertilization, hpf) on 25 September and placed in a 75-L incubator containing 1-μm-mesh filtered seawater (sterilized by ultraviolet light) at 17°C under static conditions. Buoyant eggs (fertilization rate = 99.0%) were skimmed for use in the experiment.

Experimental units consisted of 5-L, translucent polycarbonate beakers (21.3 cm diameter × 20 cm depth) placed in one of three temperature-regulating circulation baths (2.44 m long × 1.05 m wide × 12.5 cm deep) or in a refrigerated diurnal incubator (1.56 m high × 78.7 cm wide × 38.1 cm deep). Water bath temperature was maintained at 19°C by continuous recirculation through a heat pump that cooled water against ambient air maintained at 20°C by an air conditioner. Air temperature in the incubator was maintained at 19°C.

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 × 1.05 m wide × 20.3 cm deep) suspended above each bath. Fluorescent lamps provided full-spectrum light that simulated the color and ultraviolet spectrum of natural sunlight. Light was controlled by a timer to provide a photoperiod of 12 h light : 12 h dark. Light intensity was controlled by adjusting the height of the box, and black polyethylene was draped from each box to prevent exposure to extraneous light.

A 4 × 3 factorial experiment was conducted to determine the effects of light intensity and salinity on hatching success of eggs, yolk utilization, and growth and survival of yolk sac larvae to the first-feeding stage. To begin the experiment, embryos at the early gastrula stage were stocked at a density of 53 embryos/L into 48 beakers containing 5 L of culture medium at 17°C under light intensities of 0, 500, 1,000 or 2,000 lx and salinities of 26, 31, or 36 g/L. Four replicate beakers were maintained per treatment. The incubator was used for the 0-lx treatment, and water baths were used for light intensities of 500–2,000 lx. Salinity treatments were randomly assigned to beakers under each light intensity.

Water of required salinities was prepared by diluting filtered (1-μm mesh) seawater (previously treated with ultraviolet light) with distilled water. Each beaker was supplied with aeration through a Pasteur pipette at a minimum level required to maintain all the eggs in suspension. Temperature was gradually increased (1°C/h) to 19°C, within a range (16–21°C) found suitable for culture of embryonic stages of summer flounder (Johns and Howell 1980; Bisbal and Bengston 1995b) and within the range (12–19°C) at which a majority of eggs have been observed in nature (Smith 1973).

To monitor egg-hatching success and growth and survival of larvae, each replicate beaker was sampled daily by means of volumetric methods, beginning 1 d before hatching and continuing through day 4 posthatch. Starting at 0800 hours each morning, beakers were sampled in groups of 12, consisting of one replicate beaker from each treatment. Sampling of all four replicate groups was completed within 6 h.

Sample volumes (range, 300–600 mL) were adjusted during the study in order to collect a min-

TABLE 1.—Egg diameter and volume, hatching rate, notochord length, and yolk volume at hatching for summer flounder under different combinations of light intensity and salinity. Fish were spawned in seawater (35 g salt/L) after incubation at 19°C. Values are means (+SEs) of four replicates per treatment.

Light intensity (lx) and salinity (g/L)	Egg diameter[a] (mm)	Egg volume[a] (mm^3)	Density at hatching[b] (larvae/L)	Notochord length at hatching[c] (mm)	Yolk volume at hatching[c] (mm^3)
0 lx					
26	1.044 + 0.017	0.602 + 0.027	46.0 + 9.2	2.87 + 0.10	0.034 + 0.013
31	1.025 + 0.017	0.561 + 0.027	34.1 + 8.3	2.94 + 0.08	0.059 + 0.010
36	0.998 + 0.017	0.500 + 0.027	49.7 + 11.1	2.94 + 0.09	0.046 + 0.016
500 lx					
26	0.988 + 0.017	0.506 + 0.027	60.2 + 8.1	2.97 + 0.07	0.062 + 0.009
31	0.992 + 0.017	0.512 + 0.027	40.8 + 8.2	3.14 + 0.07	0.085 + 0.009
36	0.988 + 0.017	0.506 + 0.027	52.5 + 8.1	3.24 + 0.07	0.082 + 0.009
1,000 lx					
26	0.997 + 0.017	0.520 + 0.027	62.1 + 8.5	2.98 + 0.07	0.099 + 0.009
31	1.000 + 0.017	0.524 + 0.027	43.9 + 8.3	3.11 + 0.08	0.099 + 0.010
36	1.012 + 0.017	0.544 + 0.027	53.1 + 8.4	3.06 + 0.10	0.072 + 0.009
2,000 lx					
26	0.996 + 0.017	0.519 + 0.027	47.5 + 8.4	2.99 + 0.07	0.076 + 0.010
31	1.020 + 0.017	0.555 + 0.027	50.7 + 8.9	2.99 + 0.07	0.098 + 0.010
36	0.982 + 0.017	0.522 + 0.027	56.2 + 8.8	3.02 + 0.07	0.079 + 0.010

[a] Measured at 1 d prehatch (33.5–39 h postfertilization).
[b] Larval density may exceed stocking density (53 eggs/L) due to volumetric sampling errors.
[c] Measured from 57.5–63 h postfertilization.

imum of 10 eggs or larvae. To distribute eggs and larvae uniformly, the rate of aeration was increased for 10 s before collection. Sampled eggs or larvae were consolidated on a 75-μm-mesh nitex sieve, transferred into a gridded Petri dish containing an anesthetic solution (2-phenoxyethanol at 1 g/L), then counted under a dissection microscope. 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 (excluding oil droplet) dimensions (length and height) were measured with an ocular micrometer. Sampling was continued until 1 day after the first-feeding stage, when 100% of the daily sample of larvae possessed functional (fully pigmented) eyes, mouth, and alimentary tract.

Water removed during sampling was replaced daily to maintain treatment conditions. Light intensity was measured daily at the water surface of each beaker with a light meter (Cole-Parmer, Niles, Illinois). Salinity, temperature, and dissolved oxygen were measured daily from a minimum of two replicate beakers in each treatment, and total ammonia nitrogen (TAN) and pH were measured on alternate days. Air flow to each beaker was adjusted daily with a flowmeter (Cole-Parmer model). During the experimental period, average light intensity (lx) was 500 (range, 490–517), 1,020 (998–1,047) and 2,042 (1,884–2,168) at the treatment levels of 500, 1,000, and 2,000 lx, respectively. Salinity (g/L) averaged 25.6 (25.0–26.2), 30.9 (30.1–31.2), and 36.5 (35.6–36.9) at treatment levels of 26, 31, and 36 g/L, respectively. Mean (range) daily water conditions were as follows: temperature, 19.0°C (18.5–19.3°C); total ammonia nitrogen, 0.02 mg/L (0.002–0.042 mg/L); pH 8.04 (8.02–8.06); and dissolved oxygen, 7.20 mg/L (6.94–7.44 mg/L).

Hatching rate was determined by volumetric methods as the percentage of viable larvae hatched from fertilized eggs. Yolk sac volume (V, mm^3) was calculated from the formula for a prolate spheroid (Blaxter and Hempel 1963; Johns and Howell 1980): $V = (\Pi/6)l \cdot h^2$, where l is the length (mm), and h is the height (mm) of the yolk sac.

Because daily sampling of replicates in each treatment was conducted over a period of approximately 6 h and because differences in sizes may have been influenced by sampling time or developmental rates, larval notochord length was also compared among treatments relative 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 0.0743 mm^3, calculated as an average volume at hatching among all treatments (Table 1; Figure 1). Standard

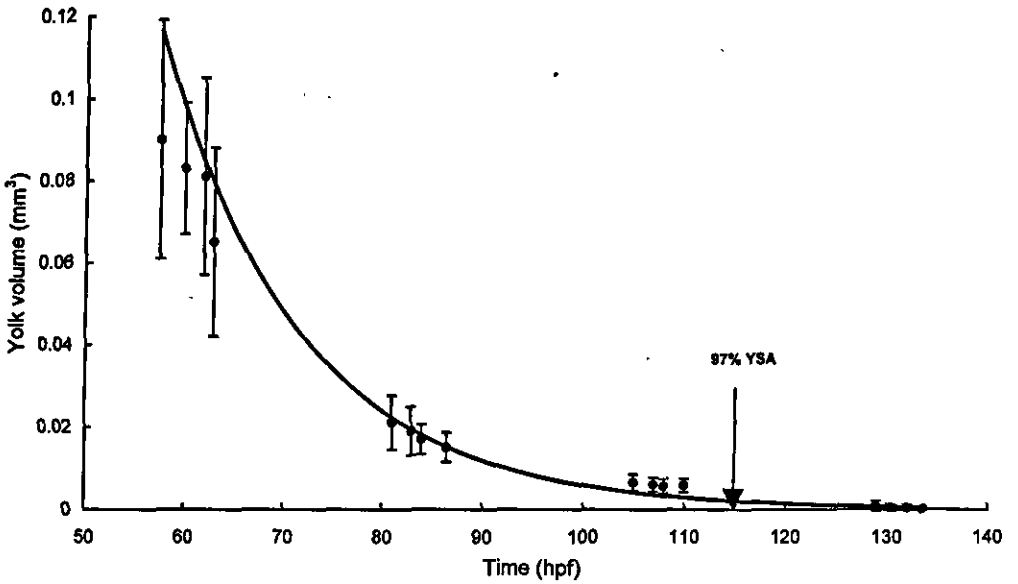

FIGURE 1.—Yolk utilization by larval summer flounder under different light intensities (0, 500, 1,000, and 2,000 lx) and salinities (26, 31, and 36 g salt/L). Plotted points represent means (±SDs) for all treatments (N = 12). The first cluster of data points represents larvae sampled on day 0 (hatching), while the last cluster represents larvae sampled on day 3 posthatch (first feeding). Regression analysis defined this yolk volume (Y) to time (X) relationship as $Y = 6.6216e^{-0.0703X}$, $r^2 = 0.956$. Time of 97% yolk absorption ranged from 114 to 131 h post-fertilization (hpf) among treatments.

final yolk volume was set at 0.0022 mm^3 (3% of the initial volume).

For each replicate, time (hpf) of 97%, YSA was determined from an exponential regression equation relating the decrease in yolk volume over time. Notochord length at 97% YSA was then determined from a regression equation (logarithmic or polynomial) 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. Rate of growth in length (%/d) was calculated from $100 \times (Lt_f - Lt_i)/(t \times Lt_i)$, where Lt_f = notochord length at 97% YSA, Lt_i = initial length, and t = time in days. Rate of disappearance of yolk (%/d) was calculated from $100 \times (\log_e V_i - \log_e V_f)/t$, where V_i = yolk volume at hatching and V_f = yolk volume at 97% YSA.

Larval survival to yolk sac absorption (day 4 posthatch) was determined as a percentage of fertilized eggs stocked and was adjusted for sampling.

Analytical procedures.—Average daily water temperature showed a slight increase ($P < 0.01$) with light intensity from 18.5°C at 0 lx to 19.3°C at 2,000 lx. To adjust for temperature as a possible source of bias in comparisons among treatments, the effects of both light intensity and salinity on hatching rate, notochord length, yolk volume, YUE, and survival were compared by two-way analysis of covariance (ANCOVA), with temperature as a covariate (Snedecor and Cochran 1967; Wilkinson et al. 1992). In order to meet the assumptions of ANCOVA, the lack of a significant interaction between the covariate and the treatment variables was confirmed before analysis (Wilkinson et al. 1992).

Following a significant ANCOVA, multiple comparisons among means were made using Tukey's honestly significant difference test (Wilkinson et al. 1992). For percentage data, arcsine transformation was performed before analysis. To compare yolk absorption rates among light intensity and salinity treatments, yolk volumes (semilogarithmic scale) were plotted against time (Figure 2), and slopes of the resulting linear regressions were compared by ANCOVA.

Results

Hatching

At 33.5–39 hpf, mean egg diameters (1.004 mm) and volumes (0.531 mm^3) did not differ signifi-

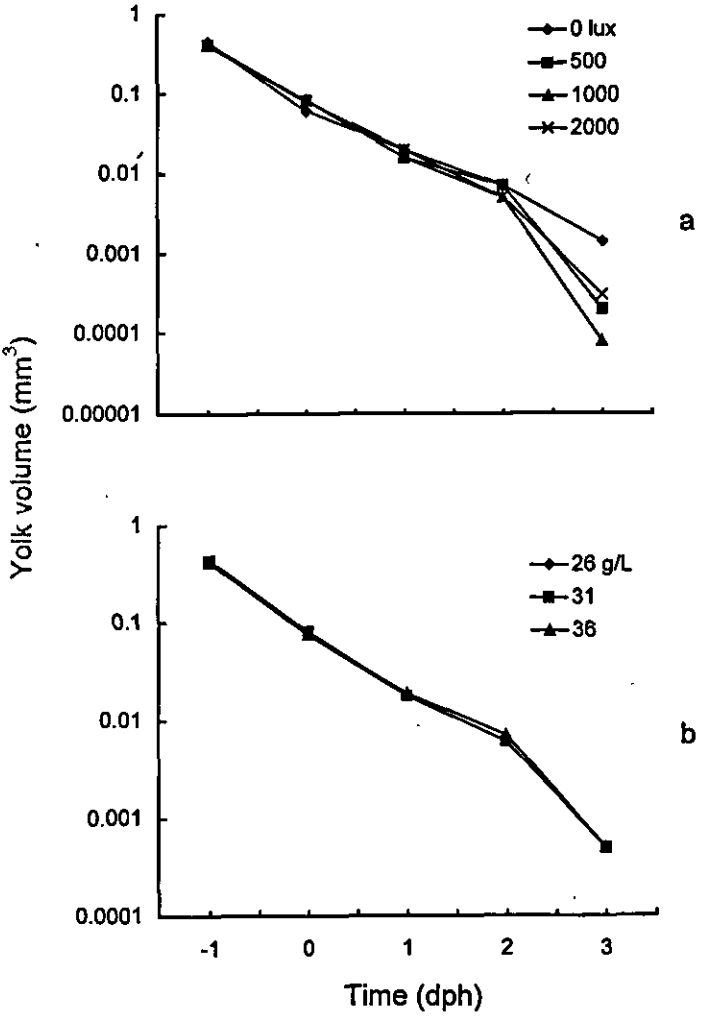

FIGURE 2.—Semilogarithmic plots of yolk utilization of summer flounder under different (a) light intensities (0, 500, 1,000, and 2,000 lx) and (b) salinities (26, 31, and 36 g/L). Data were combined over all salinities in Figure 2a and over all light intensities in Figure 2b. Plotted points represent means ($N = 12$ for Figure 2a, $N = 16$ for Figure 2b). Time on horizontal axis is shown in days posthatch (dph).

cantly ($P \geq 0.05$) among treatments (Table 1). Newly hatched larvae were observed in all treatment groups by 57.5–63.0 hpf, and hatching was completed by 81.0–86.5 hpf. Under all salinities, development appeared to be accelerated at higher light intensities; by 57.5–63.0 hpf, 12.0, 34.1, 56.5 and 55.7% of eggs completed hatching at intensities of 0, 500, 1,000 and 2,000 lx, respectively. Initial larval density averaged 49.7 larvae/L (hatching rate, 93.5%) among treatments (Table 1). No significant effects of light intensity, salinity, or their interaction were observed.

Notochord length at hatching (day 0, 57.5–63.0 hpf) ranged from 2.87 to 3.24 mm among treatments (Table 1). A significant ($P < 0.05$) effect of light intensity on initial notochord length was observed; lengths were significantly lower ($P < 0.05$) for larvae hatched in darkness (2.92 mm) than for those hatched under 500 lx (3.12 mm). Notochord lengths appeared to increase ($0.05 < P < 0.10$) at higher salinities, from 2.95 mm at 26 g/L to 3.07 mm at 36 g/L. No interactive effect of light intensity and salinity on initial notochord length was observed.

At hatching, significant effects of light intensity ($P < 0.01$) and salinity ($P < 0.05$) on yolk volume were evident, with no interaction between these effects. Under all salinities, yolk volume at hatch-

TABLE 2.—Time to 97% yolk sac absorption (97% YSA), notochord length at 97% YSA, and yolk utilization efficiency (YUE) from hatching to 97% YSA for summer flounder under different combinations of light intensity and salinity. Fish were spawned in seawater (35 g salt/L) after incubation at 19°C. Values are means (+SEs) of four replicates per treatment.

Light intensity (lx) and salinity (g/L)	Time to 97% YSA (hpf)[a]	Notochord length at 97% YSA (mm)	YUE[b] (%)
0 lx			
26	125 + 5	3.56 + 0.03	7.58 + 1.12
31	131 + 4	3.68 + 0.03	8.07 + 1.02
36	123 + 5	3.70 + 0.03	9.35 + 1.29
500 lx			
26	119 + 4	3.62 + 0.03	7.01 + 1.00
31	123 + 4	3.67 + 0.03	5.32 + 1.00
36	124 + 4	3.70 + 0.03	4.34 + 1.00
1,000 lx			
26	114 + 4	3.62 + 0.03	4.73 + 1.04
31	116 + 4	3.66 + 0.03	3.15 + 1.01
36	120 + 4	3.70 + 0.03	5.50 + 1.03
2,000 lx			
26	117 + 4	3.52 + 0.03	4.06 + 1.04
31	116 + 4	3.59 + 0.03	4.15 + 1.08
36	116 + 4	3.61 + 0.03	4.44 + 1.08

[a] hpf = hours postfertilization.
[b] From hatching to 97% YSA.

ing was significantly lower ($P < 0.05$) at 0 lx (0.046 mm^3) than at higher light intensities (range of means, 0.076–0.090 mm^3; Table 1). Under all light intensities, yolk volume at hatching was significantly higher ($P < 0.05$) at a salinity of 31 g/L (0.085 mm^3) than at 26 g/L (0.068 mm^3), with no difference from 36 g/L (0.070 mm^3; Table 1).

Yolk Absorption

Average time to 97% YSA ranged from 114 to 131 hpf (days 2–3 posthatch) among treatments (Table 2; Figure 1). No significant effects of light intensity, salinity, or their interaction on time to 97% YSA were observed.

Notochord lengths at 97% YSA ranged from 3.52 to 3.70 mm among treatments (Table 2). Significant effects of both light intensity ($P = 0.01$) and salinity ($P = 0.001$) on notochord length at 97% YSA were observed, with no interaction between these effects. Under all salinities, notochord length at 97% YSA was significantly lower ($P < 0.05$) at 2,000 lx (3.58 mm) than at 500–1,000 lx (average, 3.66 mm) and appeared to be lower ($P < 0.10$) than at 0 lx (3.65 mm). Under all light intensities, notochord lengths were significantly lower ($P < 0.05$) at a salinity of 26 g/L (3.58 mm) than at 31 g/L (3.65 mm) or 36 g/L (3.68 mm).

Yolk utilization efficiency from hatching to 97% YSA ranged from 3.15% to 9.35% among treatments (Table 2). Significant ($P < 0.01$) effects of light intensity on YUE were observed; however, the effects of salinity were not significant, and there was no interaction between these effects. The YUE decreased with increasing light intensity from a mean of 8.33% at 0 lx to 4.22% at 2,000 lx (Table 2). The YUE was significantly higher ($P < 0.05$) at 0 lx than at 500–2,000 lx.

First Feeding

Growth was fastest during the first 2 d posthatch (Figure 3) then increased more slowly during the remainder of the yolk sac period. Under all salinities, mean notochord lengths were generally higher at 500–1,000 lx than at 0 or 2,000 lx throughout the study period (Figure 3a). Under all light intensities, mean notochord lengths were generally higher at 31 and 36 g/L than at 26 g/L (Figure 3b).

Based on morphological criteria, all larvae were judged capable of exogenous feeding by day 3 posthatch (129.5–135 hpf). On day 3 posthatch, significant effects of light intensity ($P < 0.05$) and salinity ($P < 0.05$) on notochord length were observed, with no interaction between these effects. Under all salinities, notochord length on day 3 posthatch was significantly lower ($P < 0.05$) at 2,000 lx (3.63 mm) than at 500–1,000 lx (range, 3.72–3.73 mm) (Table 3). Under all light intensities, notochord length was significantly lower ($P < 0.05$) at a salinity of 26 g/L (3.64 mm) than at 31 or 36 g/L (mean, 3.71 mm).

At the first-feeding stage (day 3 posthatch), yolk volume averaged 5.0×10^{-4} mm^3 among treatments (Table 3) or 0.67% of the average yolk volume at hatching. Semilogarithmic plots of yolk volume against time showed no significant effects of light intensity (Figure 2a) or salinity (Figure 2b) on rate of yolk disappearance when slopes of the linear regressions were compared by ANCOVA, although a departure among light intensities was evident between day 2 and day 3 posthatch (108 and 131 hpf; Figure 2a) causing yolk volume under 0 lx (14.3×10^{-4} mm^3) to be significantly ($P < 0.01$) greater than under 500–2,000 lx (range of means, $0.87–3.07 \times 10^{-4}$ mm^3) on day 3 posthatch (Table 3). Yolk was exhausted in all sampled larvae 1 d after the first-feeding stage (day 4 posthatch, 153.5–159 hpf) and only remnants of the oil droplet were visible.

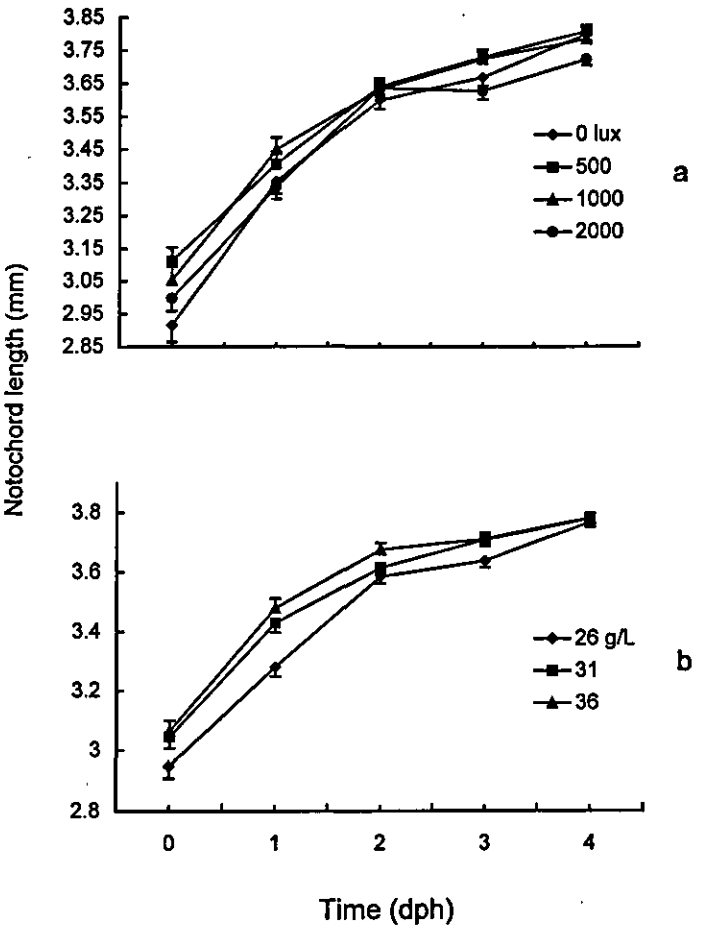

FIGURE 3.—Growth (notochord lengths) of summer flounder from hatching through yolk absorption under different (**a**) light intensities (0, 500, 1,000, and 2,000 lx) and (**b**) salinities (26, 31, and 36 g/L). Data were combined over all salinities in Figure 3a and over all light intensities in Figure 3b. Plotted points represent means plus or minus SE (N = 12 for Figure 3a, N = 16 for Figure 3b). Time on horizontal axis is shown in days posthatch (dph).

On day 4 posthatch, highly significant ($P < 0.01$) effects of light intensity on notochord length were evident, but the effects of salinity were not significant, and there was no interaction between these effects. Under all salinities, average notochord length was lower ($P < 0.05$) at 2,000 lx (3.72 mm) than at 0–1,000 lx (range of means, 3.78–3.81 mm; Table 3). On both day 3 and day 4 posthatch, maximum and minimum notochord length values under all salinities were observed at 500 and 2,000 lx, respectively. Survival on day 4 posthatch remained high in all treatments, averaging 85.1% (Table 3), and no significant effects of light intensity, salinity, or their interaction were observed.

Discussion

Although the rate of embryonic development in summer flounder appeared to be faster at higher light intensities, hatching rate (mean, 93.5%) was not influenced by light intensity within the range of 0–2,000 lx. The effects of light on embryonic development and hatching in fish vary with species. In Atlantic salmon *Salmo salar* (Brannas 1987) and Japanese medaka *Oryzias latipes* (Yamagami 1988), dark conditions delayed hatching, while in walleye pollock *Theragra chalcogramma* (Olla and Davis 1993) and Atlantic halibut *Hippoglossus hippoglossus* (Helvik and Walther 1992), eggs held in dark hatched sooner than those held under diel or continuous light.

TABLE 3.—Notochord length and yolk volume at first feeding (day 3 posthatch) and notochord length and survival at yolk exhaustion (day 4 posthatch) for summer flounder under different combinations of light intensity and salinity. Fish were spawned in seawater (35 g salt/L) after incubation at 19°C. Values are means (+SEs) of four replicates per treatment.

Light intensity (lx) and salinity (g/L)	First feeding[a]		Yolk exhaustion[b]	
	Notochord length (mm)	Yolk volume ($mm^3 \times 10^{-4}$)	Notochord length (mm)	Survival (%)
0 lx				
26	3.60 + 0.05	11.8 + 3.4	3.78 + 0.03	90.9 + 10.3
31	3.68 + 0.05	15.3 + 3.4	3.80 + 0.03	67.9 + 6.2
36	3.71 + 0.06	15.8 + 3.4	3.82 + 0.03	76.3 + 10.3
500 lx				
26	3.68 + 0.04	3.3 + 3.4	3.82 + 0.03	89.3 + 19.0
31	3.75 + 0.05	0.0 + 3.4	3.83 + 0.03	81.4 + 5.3
36	3.75 + 0.04	2.0 + 3.4	3.77 + 0.03	83.3 + 7.9
1,000 lx				
26	3.67 + 0.05	1.3 + 3.4	3.76 + 0.03	93.0 + 8.1
31	3.77 + 0.05	0.5 + 3.4	3.77 + 0.03	84.4 + 12.2
36	3.74 + 0.05	0.8 + 3.4	3.82 + 0.03	76.9 + 11.7
2,000 lx				
26	3.61 + 0.05	4.3 + 3.4	3.72 + 0.03	91.4 + 7.6
31	3.64 + 0.05	4.0 + 3.4	3.72 + 0.03	96.9 + 5.2
36	3.64 + 0.05	0.9 + 3.4	3.72 + 0.03	90.0 + 4.0

[a] First feeding based on morphological criteria.
[b] Remnants of oil droplet still visible.

Summer flounder embryos were relatively euryhaline, and salinities of 26–36 g/L did not influence development time or hatching rates. A wide salinity range for successful embryonic development and hatching has been reported for a number of temperate marine flatfish species, including plaice *Pleuronectes platessa* (17.5–50 g/L; Holliday and Jones 1967), English sole *Pleuronectes* (=*Parophrys*) *vetulus* (25–30 g/L; Alderice and Forrester 1968), winter flounder *Pleuronectes* (=*Pseudopleuronectes*) *americanus* (15–35 g/L; Rogers 1976), common sole *Solea solea* (=*vulgaris*) (20–40 g/L; Fonds 1979), yellowtail flounder *Pleuronectes* (=*Limanda*) *ferruginea* (28–38 g/L; Laurence and Howell 1981) and greenback flounder *Rhombosolea tapirina* (15–45 g/L; Hart and Purser 1995).

Significant effects of light intensity and salinity on larval size were evident at hatching; larvae hatched under 500 lx and salinity of 36 g/L showed maximum values, a trend observed at the first-feeding stage (Table 3). In yellowtail flounder, temperature effects on larval size at hatching were different from those observed at first feeding (Howell 1980). Size at first feeding, when yolk supplies are nearing exhaustion, were considered of greater importance to survival potential (Howell 1980).

Larvae under all treatment conditions reached the first-feeding stage by 129.5–135 hpf, and complete yolk absorption was observed by 153.5–159 hpf. These development times at a temperature of 19°C are consistent with those reported for summer flounder by earlier workers (Johns and Howell 1980; Johns et al. 1981), who found complete yolk absorption at 120 and 168 hpf at temperatures of 21°C and 16°C, respectively.

In this study, notochord lengths of yolk sac stage summer flounder larvae were generally shorter at 2,000 lx than at 0–1,000 lx when compared at the first-feeding stage (day 3 posthatch), at yolk exhaustion (day 4 posthatch), as well as at a standard yolk volume (97% YSA). This supports the assumption that growth trends were not unduly influenced by sampling times or by small differences in temperatures among light intensities. In Atlantic cod *Gadus morhua*, the effects of light on swimming activity and energy metabolism were greater than those of temperature within the range of 3–7°C (Solberg and Tilseth 1987). In summer flounder, temperatures of 16°C and 21°C produced no significant difference in larval size at yolk sac absorption (Johns and Howell 1980).

At 97% YSA, at the first-feeding stage (day 3 posthatch) and at yolk exhaustion (day 4 posthatch), maximum and minimum notochord lengths were observed at 500 lx and 2,000 lx, respectively, suggesting that 500 lx is near an optimal intensity

for culture of eggs and yolk sac larvae and that 2,000 lx is excessive. The effects of light on development and growth of marine fish larvae at the yolk sac stage vary widely with species. In Atlantic halibut, exposure of yolk sac larvae to even low light intensities (3–300 lx) caused abnormal development, and total darkness produced the highest percentage of normal larvae (Bolla and Holmefjord 1988). For the European bass *Morone* (=*Dicentrarchus*) *labrax* (also known as sea bass), light intensities of 1,400–3,500 lx were lethal to unpigmented, newly hatched larvae (Barahona-Fernandez 1979). For Nassau grouper *Epinephelus striatus,* notochord lengths at first feeding were greater for larvae exposed to darkness or 714 lx than for those exposed to 1,636 lx (Ellis et al. 1997), similar to what was observed in the present study. On the other hand, Atlantic cod yolk sac larvae reared under continuous illumination (500 lx) were shorter than those reared in darkness because of the increased swimming activity, oxygen consumption, and energy metabolism in light (Solberg and Tilseth 1987). In summer flounder, shorter notochord lengths of larvae under a light intensity of 2,000 lx compared with 0–1,000 lx are presumably related to higher light-induced activity and energy metabolism.

High survival of summer flounder larvae through first feeding under salinities of 26–36 g/L is consistent with a wide salinity tolerance range of marine fish larvae (May 1975; Santerre and May 1977; see review by Holliday 1988), including the olive flounder *Paralichthys olivaceus* from Japan (Mihelakakis and Kitajima 1994). A trend toward longer notochord lengths with increasing salinity, evident from hatching through the first-feeding stage in this study (Figure 3b), is similar to what was reported for early summer flounder juveniles (12–15 mm standard length), which grew faster at higher salinities within the range of 10–30 g/L (Deubler and White 1962).

In marine fish with pelagic eggs, a major effect of low salinity is reduced buoyancy (Fonds 1979; Liu et al. 1993), which may cause settling of eggs and larvae and mortality (Liu et al. 1993). At hatching, larvae of the European flounder *Platichthys flesus* are positively buoyant in seawater at 32–33 g/L, but buoyancy decreases during yolk absorption (Yin and Blaxter 1987). Thus, higher salinities have a positive effect on buoyancy, which reduces larval energy requirements for maintaining position in the water column and improves growth.

In this study, the additive effects of light intensity (0–2,000 lx) and salinity (26–36 g/L) on growth of yolk sac larvae of summer flounder, produced notochord length differences at the first-feeding stage (day 3 posthatch) of as much as 4.5% among all treatments, 2.8% among light intensities, and 1.9% among salinities. In plaice and Atlantic herring *Clupea harengus,* a 10% increase in larval size was estimated to produce a 10–25% increase in swimming speed, which enhanced the ability to search for food and improved survival potential (Ryland and Nichols 1976). The effects of the seemingly small size differences observed in the present study on feeding ability and long-term survival of summer flounder must be quantified through further investigation. In winter flounder, size advantages gained by faster-growing larvae were offset by compensatory growth of slow-growing larvae during the juvenile stages (Bertram et al. 1993).

Yolk utilization efficiency was highest among summer flounder larvae reared in dark and appeared to decline with increasing light intensity. This resulted, in part, from a relatively slow rate of yolk absorption among larvae held in dark after day 2 posthatch, when larvae in all treatments developed functional eyes. This suggests that swimming activity and energy metabolism was increased at higher light intensities.

Darkness is known to minimize activity and energy expenditure in yolk sac larvae in Atlantic herring (Blaxter and Hempel 1963; Batty 1987), European bass (Coves et al. 1991) and Atlantic cod (Kjorsvik and Holmefjord 1995). High YUE resulting from low activity, however, may reduce feeding and survival potential (Blaxter 1969).

Assuming that largest larvae have maximum foraging abilities (Laurence 1973; Howell 1980; Johns and Howell 1980) and, hence, highest survival potential under culture, then a low light intensity of 500 lx and a salinity of 36 g/L are recommended for incubating summer flounder eggs and yolk sac larvae. These conditions are consistent with those of shelf waters where eggs and early larvae prevail in nature (Smith 1973). High survival under a wide range of light intensities and salinities in this study reflects an adaptability for inshore movement during the pelagic larval stage.

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