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Effects of Light Intensity and Salinity on Growth, Survival, and Whole-Body Osmolality of Larval Southern Flounder *Paralichthys lethostigma*

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Abstract

The southern flounder *Paralichthys lethostigma* is a high-valued flatfish found in estuarine and shelf waters of the south Atlantic and Gulf coasts of the United States. Wide temperature and salinity tolerances exhibited by juveniles and adults make it a versatile new candidate for commercial culture, and studies are underway in the southeastern U.S. to develop hatchery methods for this species. The objectives of this study were to establish illumination and salinity conditions that optimize growth and survival of larval southern flounder reared through the yolk-sac and first feeding stages to 15-d post-hatching (15 dph). Early embryos were stocked into black 15-L tanks under light intensities of 5, 50, 100, and 1,000 lx and at salinities of 24 and 34 ppt in a 4 × 2 factorial design. Significant ($P < 0.05$) effects of both light intensity and salinity on growth and survival were obtained, with no interaction between these effects. On 11 dph and 15 dph, growth was generally maximized at the intermediate light intensities (50 and 100 lx) and minimized at the extremes (5 and 1,000 lx). By 15 dph, growth was higher at 34 ppt than at 24 ppt. Survival to 15 dph showed trends similar to those of growth. Survival was higher at 100 lx (avg. = 46%, range = 41–54%) than at 5 lx (avg. = 11%, range = 6–17%) and higher at 34 ppt (avg. = 43%, range = 31–55%) than at 24 ppt (avg. = 17%, range = 8–38%). Whole-body osmolality (mOsmol/kg) was significantly lower in larvae reared at 24 ppt (avg. = 304, range = 285–325) through 11 dph than in larvae reared at 34 ppt (avg. = 343, range = 296–405). Larvae reared under the extreme light intensity treatments (5 and 1,000 lx) at 34 ppt appeared to exhibit osmoregulatory stress, particularly on 11 dph, when a marked increase in whole-body osmolality was observed. The mid-intensity treatments (50 and 100 lx) at 34 ppt optimized growth and survival of larval southern flounder in this study; and elicited the most stable osmotic response. These conditions appear to be consistent with those that southern flounder larvae encounter in nature during this early developmental period.

The commercial and recreational importance of the southern flounder *Paralichthys lethostigma* (Powell and Schwartz 1977; Stokes 1977; Gilbert 1986), and the euryhaline nature of juveniles and adults (Powell and Schwartz 1977; Rogers et al. 1984; Guindon and Miller 1995), make it an attractive candidate for aquaculture in both coastal and inland locations (Waters 1996).

Most larval marine finfish hunt by sight and are highly dependent upon light levels in their environment (Blaxter 1968b, 1969). As light levels decrease to darkness, feeding efficiency is greatly reduced (Huse 1994). Conversely, high light regimes may stimulate larval activity, and thus increase metabolic rates (Ware 1975; Laurence 1977). Limited information describing the effects of light intensity during the pre-metamorphic stages of southern flounder larvae exists. Previous research has suggested that relatively high light intensities within the range of 457–1,362 lx may not significantly affect the growth or survival rates of larvae reared from 6 dph (6 d post-hatching) through metamorphosis (Denson and Smith 1997), while a similar range (320–1,600 lx) did not significantly affect growth or survival of larvae reared from 25

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dph through metamorphosis (Daniels et al. 1996).

The euryhaline nature of some flatfish species has increased interest in identifying salinity conditions that optimize growth and development. There is evidence that low-salinity tolerance of larval summer flounder increases as development progresses from hatching through metamorphosis (Watanabe et al. 1998; Specker et al. 1999). In southern flounder, a similar increase in low-salinity tolerance with larval development has also been reported. Incubation salinities ranging from 10–35 ppt did not affect the hatching rate of southern flounder embryos, although 10 ppt impaired larval survival after hatching (Smith et al. 1999a). Survival of metamorphic stage fish was not adversely affected by salinities as low as 20 ppt (Daniels et al. 1996), while juveniles were tolerant to fresh water (Daniels and Borski 1998; Smith et al. 1999a). There is little or no information concerning the effects of salinity on growth and survival of early stage southern flounder larvae from yolk-sac to pre-metamorphic stages.

Southern flounder are thought to migrate to offshore waters where spawning occurs in nature (Gilbert 1986). Adults are found at depths ranging from 18–62 m (60–200 ft) during the spawning season in the Gulf of Mexico (Stokes 1977). Since light is rapidly attenuated in water with depth (Champ et al. 1980), eggs and early southern flounder larvae are presumably well adapted to low illumination levels and a stable high-salinity environment, while post-metamorphic southern flounder larvae are found in estuarine nursery grounds (Burke et al. 1991) where low-salinity tolerance is beneficial. Based on natural distributions, it was hypothesized that growth and survival of early stage southern flounder larvae would be optimized under low light intensities and in full-strength seawater. The objectives of this study were to determine illumination and salinity conditions that optimize growth and survival of larval southern flounder reared through the yolk-sac and first feeding stages to 15 dph.

Materials and Methods

Experimental Animals

Two experiments were conducted at the University of North Carolina at Wilmington's Center for Marine Science Aquaculture Facility (Wrightsville Beach, North Carolina, USA) from 17 March through 02 April 1999 (Experiment 1) and from 03 February through 19 February 2000 (Experiment 2). Adult southern flounder were maintained in controlled-environment broodtanks supplied with recirculating seawater. Fish were maintained under a natural photothermal cycle and spawned naturally or were induced to spawn using luteinizing hormone releasing hormone-analogue (LHRH-a) (Berlinsky et al. 1996; Smith et al. 1999b; Watanabe et al. 2001).

Experimental System

Experiments were conducted in a controlled-environment laboratory. Larvae were reared in cylindrical black plastic larval rearing tanks (working volume = 15 L), which were placed in one of four temperature-regulated water baths (152 cm × 61 cm × 23 cm). A constant water temperature was maintained by continuous recirculation through a heat pump coupled with controlled air temperature.

Light was supplied to experimental units in each water bath by 40-W full-spectrum fluorescent bulbs in light hoods (152 cm × 65 cm × 19 cm) suspended above each bath. Light intensity was controlled by adjusting the height of each hood and by the use of shade cloth. Each hood was surrounded by a curtain of black polyethylene, which eliminated extraneous light. A timer was used to provide a controlled 12-h light: 12-h dark daily photoperiod.

Experimental Design

Experiment 1. A 4 × 2 factorial experiment was conducted to determine the ef-

fects of light intensity and salinity on the growth and survival of larvae from 1-d post-hatching (1 dph) through 15 dph. Developing embryos (blastula stage) obtained from a natural spawning on 15 March 1999 were incubated in 34 ppt seawater at 16 C. Fertilization rate was 89.2%. Approximately 24 h before hatching, buoyant embryos (neurula stage) were stocked at a density of 26 embryos/L into 32 larval rearing tanks at 16.5 C, under light intensities of 5, 50, 100, and 1,000 lx, and salinities of 24 and 34 ppt. There were four replicate tanks for each treatment combination of light intensity and salinity. Larvae were reared in a "clear water system" (i.e., with no background algae) from hatching through the yolk-sac and first feeding stages to 15 dph.

Seawater (34 ppt), obtained from the Atlantic Intracoastal Waterway adjacent to Wrightsville Beach was filtered to 1 μm and treated with UV light before use. The 24-ppt treatment was prepared by diluting filtered seawater with chlorine-free fresh water. Water lost to evaporation was replaced with fresh, chlorine-free water. Each tank was supplied with aeration through airstones at approximately 30 mL/min, a level that maintained all eggs in suspension. Temperature was gradually increased over the course of the experiment at an average rate of 0.7 C/d, from 16.5 C at stocking to 18.0 C on 15 dph, approximating temperatures similar to those that early southern flounder larvae experience in the wild (Miller et al. 1991).

Feeding

Beginning on 2 dph, larvae were fed rotifers cultured in the laboratory at a temperature of 23–25 C. Rotifer cultures were maintained at 30 ppt to minimize osmotic stress when they were added to the larval culture media of 24 and 34 ppt. Rotifers were enriched daily with a preserved microalgae paste *Nannochloropsis oculata* (Reed Mariculture, San Jose, California, USA) twice (6 and 18 h before feeding), and once (6 h before feeding) with a commercially prepared diet (Culture Selco, Inve Aquaculture Inc., Grantsville, Utah, USA) to improve their nutritional value to fish larvae (Lubzens 1987; Sargent et al. 1997). Rotifers were harvested each morning, acclimated to 18 C over a period of approximately 3 h (0.5 C/h), and thoroughly rinsed with filtered seawater (30 ppt) before their addition to the larval rearing aquaria.

Feeding began on 2 dph, approximately 36–48 h before the first-feeding stage, in order to familiarize the larvae with their prey and to ensure their availability to the larvae upon the commencement of exogenous feeding. Enriched rotifers were stocked into each tank at an initial density of 10 rotifers/mL, which was maintained by quantifying rotifers in each culture vessel daily and adding the appropriate number of enriched rotifers to make up the difference.

Growth and Survival Determination

To monitor larval growth and survival, larvae were sampled from each replicate tank on 1, 4, 7, 11, and 15 dph by vigorously aerating each tank to ensure a uniform distribution, then sampling a known volume of water until approximately 10 larvae were removed. Water removed during sampling was replaced daily with water of the appropriate salinity to maintain treatment conditions, and that lost to evaporation was replaced with fresh, chlorine-free water. Larval sampling for data collection was initiated at approximately the same time (0800 h) on each sampling day. Survival, notochord length, and feeding data were recorded from anesthetized (0.3–0.5 ppt phenoxyethanol) fish. Living larvae were distinguishable by the presence or absence of a heartbeat and appearance. On the last sampling day (15 dph), larvae were gently rinsed with deionized water on a Nitex screen, blotted dry, stored in a freezer and later dried in a laboratory oven at 62 C for 3 d. Larval dry weights were determined using a Sartorius (Goettingen, Germany) electrobalance.

Notochord lengths were measured using

a microscope fitted with an ocular micrometer. Survival rate (determined as a percentage of fertilized eggs stocked and adjusted for sampling) was recorded by noting the number of live and dead larvae in each sample, and feeding rate was determined by visual examination of the larval gut. Sampling continued through 15 dph, when larval mouth-gape appeared large enough to consume newly hatched *Artemia*.

Water Quality

Salinity (salinity refractometer, ± 1 ppt) and temperature (YSI 55, Yellow Springs, Ohio, USA, ± 0.1 C) were measured in each replicate daily. Light intensity, also recorded daily, was measured at the water's surface of each tank with a light meter (Extech Instruments, Waltham, Massachusetts, USA). Dissolved oxygen (YSI 55, Yellow Springs, Ohio, USA, ± 0.01 mg/L) and pH (Oakton pH Tester2, ± 0.1) were measured daily from one replicate tank per treatment, and total ammonia-nitrogen (TAN) was measured every other day (Hach DR 850, Loveland, Colorado, USA, ± 0.01 mg/L). Airflow (30–40 mL/min) to each aquarium was monitored daily with a flow meter (Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA, ± 1 mL/min) and adjusted as needed. During the experimental period, mean (range) light intensity (lx) was 6 (5–7), 55 (45–65), 102 (91–113), and 995 (843–1,116) at the treatment levels of 5, 50, 100, and 1,000 lx, respectively. Salinity (ppt) averaged 25 (24–26), and 34 (33–34) at treatment levels of 24 and 34 ppt, respectively. Mean (range) daily water conditions were as follows: temperature (C), 17.6 (16.5–18.2); total ammonia-nitrogen (mg/L), 0.07 (0.01–0.18); pH, 8.15 (8.03–8.23); and dissolved oxygen (mg/L), 7.24 (6.90–7.41).

Analytical Methods

Quantitative values are expressed as treatment means or treatment means ± SEM. The effects of light intensity and salinity, and their interaction, were tested using two-way ANOVA. If no interaction was detected, salinity treatments were combined within light treatments and light treatments were combined within salinity treatments for further analysis. Significant treatment effects were followed by the Tukey-Kramer Honestly Significant Difference test for multiple comparisons among means. For feeding percentage data, arcsine transformation was performed before analysis. Analyses were performed using JMP (SAS Institute Inc., Cary, North Carolina, USA) statistical software.

Experiment 2

In a second experiment, a 4 × 2 factorial design was used to determine the effects of light intensity and salinity on the growth, survival, and whole-body osmolality of larvae from 1 dph through 15 dph. Fertilized eggs (fertilization rate = 93.4%) obtained from strip-spawning of LHRH-a-treated females on 01 February 2000 were incubated at 16 C in 34 ppt seawater. Approximately 24 h before hatching, buoyant embryos (neurula stage) were stocked at a density of 11 embryos/L into 32 larval rearing tanks containing 15-L culture medium at 17.2 C. Temperature was gradually increased from 17.2 C to 17.8 C over the course of the experiment at an average rate of 0.04 C/d. Treatments consisted of four light intensities of 5, 50, 100, and 1,000 lx and two salinities of 24 and 34 ppt. There were four replicate tanks per treatment.

Larval rearing and sampling protocols for Experiment 2 were the same as in Experiment 1, with the following exceptions. Due to limited availability, stocking densities of both southern flounder embryos and rotifers were lower in Experiment 2 than in Experiment 1. Rotifers were added to the larval rearing tanks on 3 dph at a density of five rotifers/mL and were maintained at an average density of approximately seven rotifers/mL throughout the experiment. Larval growth was monitored on 11 dph and 15 dph.

Larval whole-body osmolality (Tornheim

1980; Tandler et al. 1995) was measured in each replicate tank on 1, 7, 11, and 15 dph with a vapor pressure osmometer (VPO) (Wescor 5520, Logan, Utah, USA). To obtain osmometry data, groups containing six to eight larvae/replicate were collected on a nitex sieve, gently rinsed with deionized water for 10 sec, blotted to remove excess solution, and equilibrated in the osmometer chamber for 30 min before taking a reading.

During the experimental period, average (range) light intensity (lx) was 6 (5–7), 51 (40–61), 99 (84–110), and 1,015 (833–1,155) at the treatment levels of 5, 50, 100, and 1,000 lx, respectively. Salinity (ppt) averaged 24 (23–24), and 33 (33–34) at treatment levels of 24 and 34 ppt, respectively. Mean (range) daily water quality conditions were as follows: temperature (C), 17.6 (17.2–17.9); total ammonia-nitrogen (mg/L), 0.03 (0.00–0.08 mg); pH, 8.08 (8.07–8.09); and dissolved oxygen (mg/L), 6.43 (6.34–6.54).

Results

Growth—Experiment 1

No interactive effects ($P > 0.05$) between light intensity and salinity on growth (i.e., notochord length or dry weight) were observed on any sampling date during this study. Hence, to facilitate analyses, the effects of light intensity on growth were compared by combining data for both salinities, while the effects of salinity were compared across all light intensities.

Significant effects of light intensity on notochord length were observed on 1, 11, and 15 dph (Fig. 1A). While no significant salinity effects were observed for the duration of the study, a sharp increase in growth was observed after 11 dph (Fig. 1B). On 1 dph, notochord lengths ranged from 3.33 to 3.49 mm among light treatments (Fig. 1A, Table 1). Larvae were significantly larger ($P < 0.05$) in the high intensity treatments of 100 and 1,000 lx (3.47–3.49 mm) than in the low intensity treatments of 5 and 50 lx (3.33–3.34 mm). Treatment differences in notochord lengths were no longer evident ($P > 0.05$) on 4 dph and 7 dph, when mean values were 3.41 and 3.63 mm, respectively. On 11 dph, notochord lengths ranged from 3.38 to 3.87 mm among light treatments and were significantly ($P < 0.05$) greater in the mid-intensity 50- and 100-lx treatments (3.81–3.87 mm) than in the low intensity 5-lx treatment (3.38 mm). This was related to the little or no growth from 7 dph to 11 dph at 5 lx and 1,000 lx (Fig. 1A).

On 15 dph, notochord lengths averaged 4.31 mm and ranged from 4.06 to 4.52 mm. Among light treatments, notochord lengths were significantly higher in the 50- and 100-lx treatments (4.38–4.52 mm) than in the 1,000-lx treatment (4.06 mm). On 15 dph, no significant effects of light intensity on larval dry weights (mean = 86.0 μg) were observed, while the effects of salinity were significant ($P < 0.05$), and there was no interaction between these effects. Larval dry weights across all light intensities were significantly ($P < 0.05$) greater at 34 ppt (91 ± 4.0 μg) than at 24 ppt (76 ± 6.0 μg).

Survival—Experiment 1

While significant effects of light intensity and/or salinity on larval survival were observed from 7 dph, no interactive effects were evident during the study. Survival across both salinities was generally higher in the mid-intensity 100-lx treatment than in the 5-, 50-, or 1,000-lx treatments throughout the experiment (Fig. 2A). Significant effects of light intensity on survival were not evident until 11 dph ($0.05 < P < 0.07$) and 15 dph ($P < 0.05$). On 15 dph, survival ranged from 11.0 to 46.4% among light intensities and was significantly higher under the 100-lx treatment than in the 5-lx low intensity treatment.

Under all light intensities, significant ($P < 0.05$) effects of salinity on survival were evident from 7 dph through 15 dph, when survival was clearly higher at 34 ppt than at 24 ppt (Fig. 2A). On 15 dph, survival

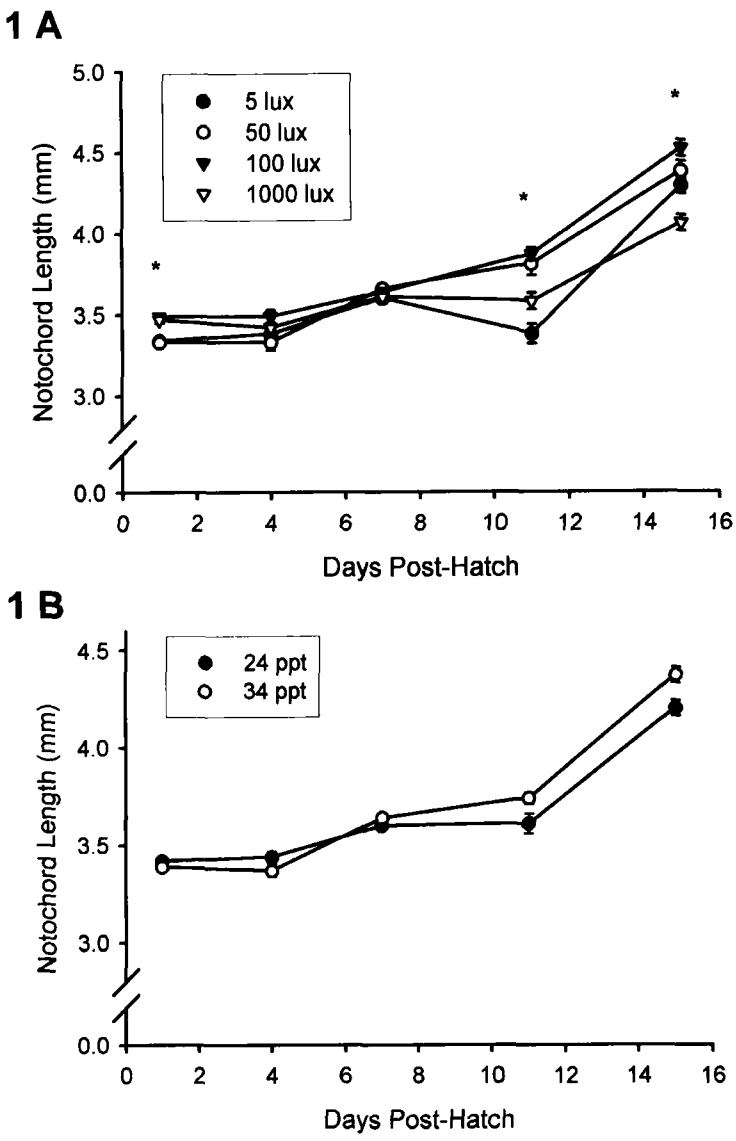

FIGURE 1. *Notochord lengths of southern flounder larvae from d 1 through 15 dph under different (A) light intensities (5, 50, 100, and 1,000 lx) and (B) salinities (24 and 34 ppt) in Experiment 1. Plotted points represent means ± SE (N = 8 and 16 in Figs. 1A and 1B, respectively). Data for both salinities were combined under each light intensity in Fig. 1A, while data for all light intensities were combined under each salinity in Fig. 1B. "Asterisk" indicates significant (P < 0.05) differences among treatments observed on that sampling date.*

across all light intensities at 34 ppt was 42.7% compared to 16.7% at 24 ppt.

Feeding—Experiment 1

Based on morphological development (open mouth, intact digestive tract, pigmented eyes), fish from the 100-lx treatment under both salinities reached the first-feeding stage by 4 dph. This was supported by gut content analysis, which revealed rotifers in the stomachs of some larvae for the first time on 4 dph (Fig. 3A). On 4 dph, 2.5% of larvae in the 100-lx treatment were feeding, while no feeding larvae were observed in the other treatments. By 7 dph, fish from all treatments were observed to

TABLE 1. *Notochord lengths (mm) (mean ± SE, N = 8) of southern flounder larvae on 1, 4, 7, 11, and 15 dph under different light intensities (5, 50, 100, and 1,000 lx) in Experiment 1. Means without a letter in common are significantly different.*

Age (dph)	Light intensity (lx)				*P*-value
	5	50	100	1,000	
1	3.34 ± 0.02[a]	3.33 ± 0.02[a]	3.49 ± 0.02[b]	3.47 ± 0.02[b]	0.0299
4	3.38 ± 0.06	3.33 ± 0.05	3.49 ± 0.04	3.42 ± 0.03	0.7891
7	3.60 ± 0.03	3.66 ± 0.03	3.64 ± 0.02	3.61 ± 0.03	0.8837
11	3.38 ± 0.06[a]	3.81 ± 0.07[b]	3.87 ± 0.04[b]	3.58 ± 0.05[ab]	0.0004
15	4.29 ± 0.05[ab]	4.38 ± 0.06[a]	4.52 ± 0.05[a]	4.06 ± 0.05[b]	0.0016

be feeding (range = 8.3–90.9%). Significant effects of light intensity on percent feeding were evident, but there were no differences between salinities, and there was no interaction between these effects. Across both salinities, proportionally more larvae were feeding in the 50-, 100-, and 1,000-lx treatments than in the 5-lx treatment (Fig. 3A). On 11 dph, significant effects of light intensity and salinity on percent feeding were also evident, while no interactions between these effects were observed. Under both salinities, proportionally more larvae were observed to be feeding at 50, 100, or 1,000 lx than at 5 lx (Fig. 3A). Under all light intensities, more larvae were observed to be feeding at 34 ppt (87.0%) than at 24 ppt (69.1%) on 11 dph (Fig. 3B). By 15 dph, all larvae sampled were observed to be feeding (Fig. 3).

Growth—Experiment 2

No interactive effects of light intensity and salinity on larval notochord lengths were observed on 11 dph or 15 dph. By 15 dph, notochord lengths averaged 4.21 mm and ranged from 4.15 to 4.28 mm. Under both salinities, no significant effects of light intensity on notochord lengths were evident on 11 dph or 15 dph. Under all light intensities, notochord lengths were greater at 34 ppt than at 24 ppt on 11 dph (3.70 vs. 3.56 mm) and 15 dph (4.27 vs. 4.15 mm).

Survival—Experiment 2

No significant effects of light intensity, salinity, or their interaction on larval survival through 15 dph were observed. Mean larval survival across all treatments on 1 dph was 79.5% (range = 63.0–95.3%) and declined to 27.9% (range = 20.0–34.8%) by 15 dph. Maximum survival among treatments in Experiment 1 (60.3%) was markedly higher than in Experiment 2 (34.8%).

Osmolality—Experiment 2

Under the 24-ppt treatment, mean larval osmolality ranged from 279 to 329 mOsmol/kg, and no significant ($P > 0.05$) effects of light intensity on larval osmolality were evident through 15 dph (Fig. 4A). However, under 34 ppt, mean larval osmolality ranged from 305 to 447 mOsmol/kg, and significant ($P < 0.05$) effects of light intensity on larval osmolality were detected on 15 dph, when values were higher at 1,000 lx than at 50 lx (Fig. 4B).

Under the 5-lx treatment, larval osmolality was significantly higher ($P < 0.05$) at 34 ppt than at 24 ppt on 1 dph and 11 dph (Fig. 5A). Within the low intensity/high salinity (5 lx/34 ppt) treatment, larval osmolality remained relatively stable from 1 dph (338 mOsmol/kg) to 7 dph (317 mOsmol/kg) and then exhibited a marked ($P < 0.05$) increase to 447 mOsmol/kg by 11 dph before declining to initial levels on 15 dph. In contrast, mean larval osmolality in the low intensity/low salinity (5 lx/24 ppt) treatment did not change significantly through 15 dph (Fig. 5A).

Mean whole-body osmolality of larvae in the 50-lx treatment was significantly higher at 34 ppt (311 mOsmol/kg) than at 24 ppt

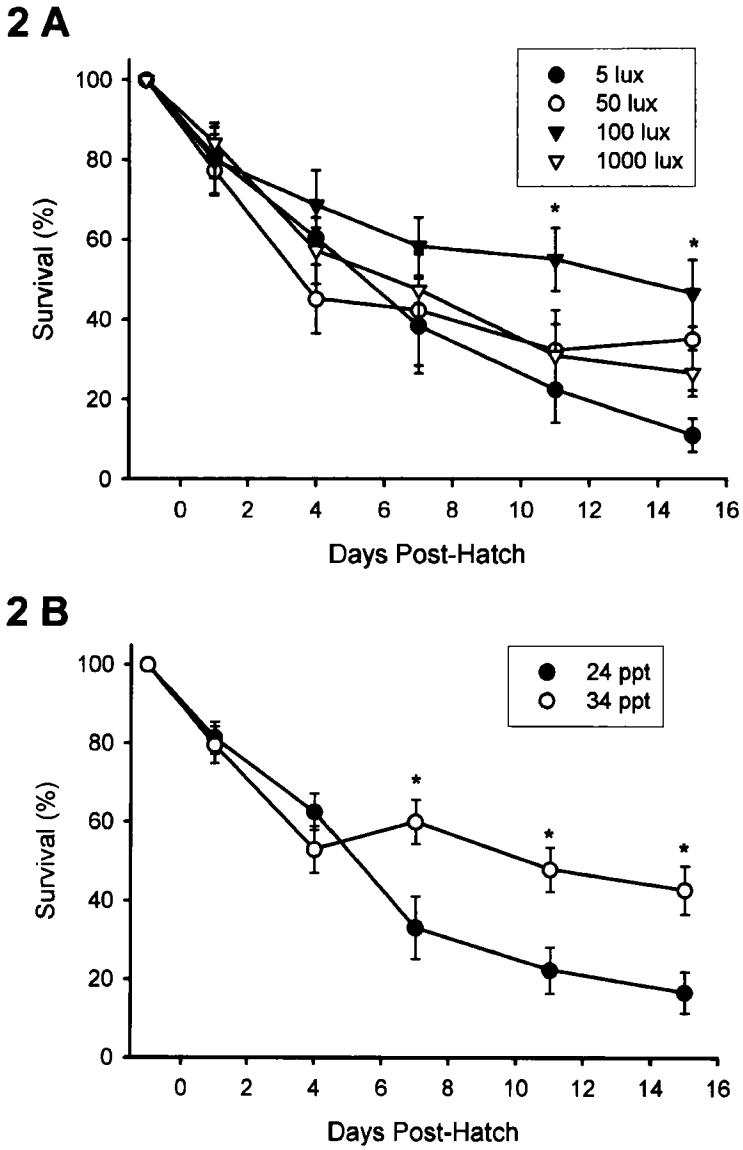

FIGURE 2. *Survival of southern flounder larvae from d 1 through 15 dph under different (A) light intensities (5, 50, 100, and 1,000 lx) and (B) salinities (24 and 34 ppt) in Experiment 1. Plotted points represent means ± SE (N = 8 and 16 in Figs. 2A and 2B, respectively). Data for both salinities were combined under each light intensity in Fig. 2A, while data for all light intensities were combined under each salinity in Fig. 2B. "Asterisk" indicates significant (P < 0.05) differences among treatments observed on that sampling date.*

(287 mOsmol/kg) on 7 dph (Fig. 5B). At 100 lx, larval osmolality on 1 dph was significantly greater at 34 ppt (339 mOsmol/kg) than at 24 ppt (299 mOsmol/kg) (Fig. 5C). Larvae from the mid-intensity (50 and 100 lx) treatments at a given salinity (24 or 34 ppt) exhibited no significant changes in mean whole-body osmolality during the study. In the 1,000-lx treatment, larval osmolality was significantly higher at 34 ppt than at 24 ppt on 1 dph (353 mOsmol/kg vs. 299 mOsmol/kg) and 15 dph (366 mOsmol/kg vs. 314 mOsmol/kg). Larvae from the high salinity (34 ppt) treatment ap-

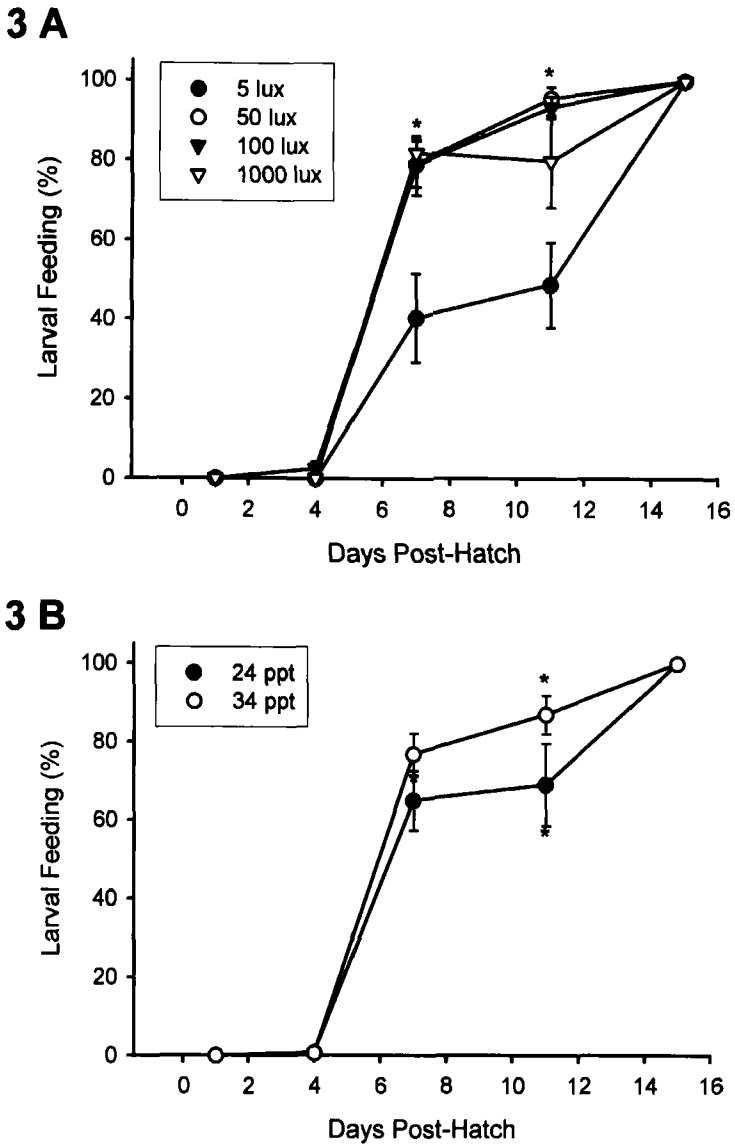

FIGURE 3. *Percent feeding in southern flounder larvae from d 1 through 15 dph under different (A) light intensities (5, 50, 100, and 1,000 lx) and (B) salinities (24 and 34 ppt) in Experiment 1. Plotted points represent means ± SE (N = 8 and 16 in Figs. 3A and 3B, respectively). Data for both salinities were combined under each light intensity in Fig. 3A, while data for all light intensities were combined under each salinity in Fig. 3B. "Asterisk" indicates significant (P < 0.05) differences among treatments observed on that sampling date.*

peared to show an increase in osmolality on 11 dph to 429 mOsmol/kg (range = 350–513 mOsmol/kg) (Fig. 5D), similar to that of the low intensity/high salinity treatment. Due to high variability among replicates on 11 dph, this increase was not statistically significant. Larvae reared at 24 ppt showed no changes in whole-body osmolality during the study.

Discussion

Environmental parameters (e.g., salinity, light intensity, temperature) between Experiment 1 and 2 were similar, but other

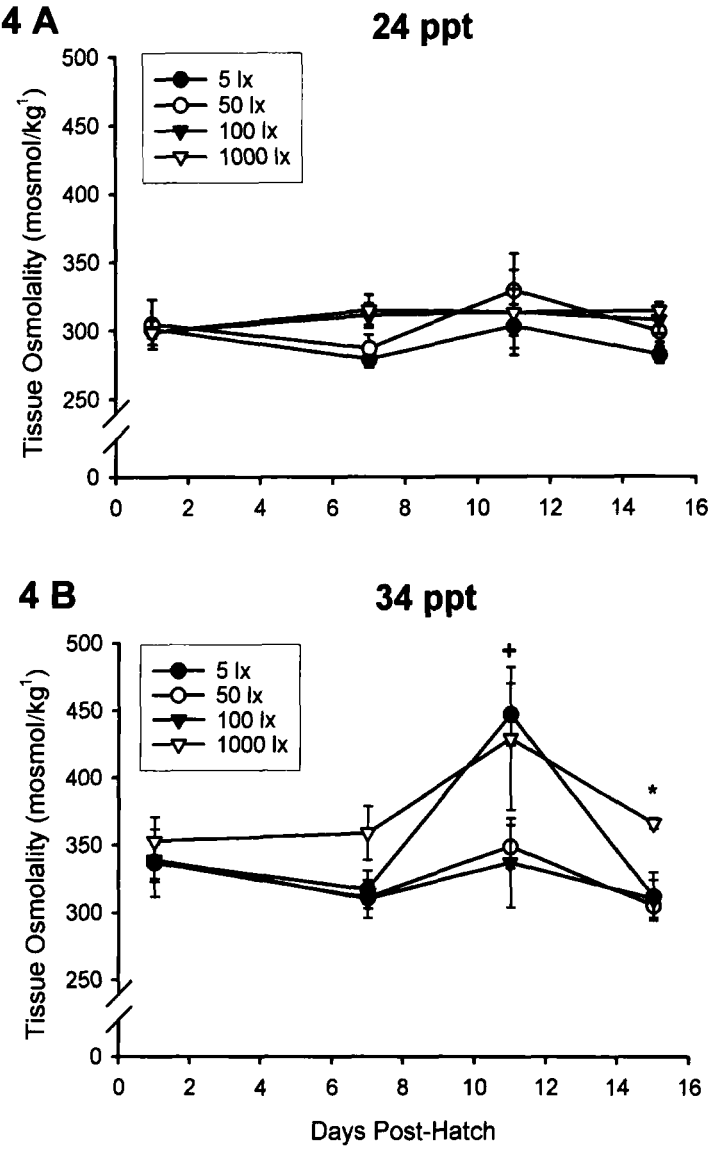

FIGURE 4. *Whole-body osmolality of southern flounder larvae from d 1 through 15 dph under different light intensities (5, 50, 100, and 1,000 lx) at (A) 24 ppt and (B) at 34 ppt, in Experiment 2. Plotted points represent means ± SE (N = 4 in Figs. 4A and 4B). "Asterisk" indicates significant (P < 0.05) differences among treatments observed on that sampling date. "Plus" indicates significant (P < 0.05) differences compared to initial (1 dph) values.*

experimental conditions (e.g., larval stocking densities and rotifer densities) differed. In addition, egg and larval qualities as affected by broodstock husbandry (natural vs. hormone-induced spawning) and maternal effects likely differed in these experiments and probably contributed to the differences in growth and survival between the two studies.

In Experiment 1, light appeared to have influenced hatching rates of developing embryos, giving larvae from the 100- and 1,000-lx treatments an early size advantage. The enhanced growth of larvae from these

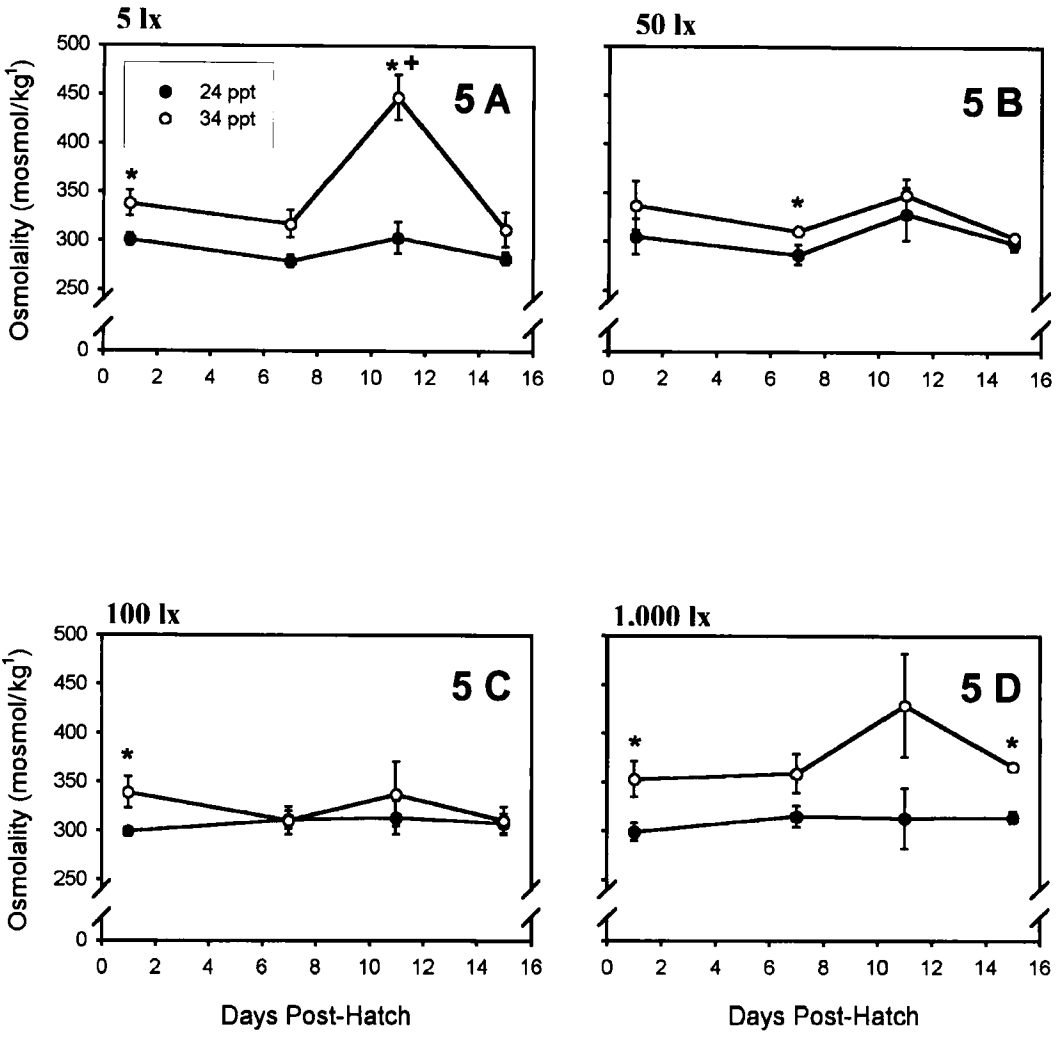

FIGURE 5. *Whole-body osmolality of southern flounder larvae from d 1 through 15 dph under different salinities (24 and 34 ppt) at (A) 5 lx (B) 50 lx (C) 100 lx and (D) 1,000 lx in Experiment 2. Plotted points represent means ± SE (*N = 4 *in Figs. 5A, 5B, 5C, and 5D). "Asterisk" indicates significant (*$P < 0.05$*) differences among treatments observed on that sampling date. "Plus" indicates significant (*$P < 0.05$*) differences compared to initial (1 dph) values.*

treatments measured on 1 dph may be attributed to accelerated embryonic development stimulated by the higher illumination levels, resulting in earlier hatching of embryos and more time for growth when compared with larvae from the 5- and 50-lx treatments. This is consistent with the observation that newly hatched larvae were first discovered in the 100- and 1,000-lx treatments. Light intensity is known to affect hatching rates and hatching size in other species of marine fish. Eggs of the walleye pollock *Theragra chalcogramma* exhibited faster hatching rates in the absence of light (Olla and Davis 1993). Conversely, summer flounder development to hatching appeared to be faster at higher illuminations (within range 0 to 2,000 lx) and larvae hatched at 500 lx were significantly larger than those incubated in the absence of light (Watanabe et al. 1998). In the present study, the size advantage gained by larvae hatched

in the 100- and 1,000-lx treatments was short-lived, and no differences in notochord lengths among light treatments were evident by 4 dph.

Under both salinities, notochord lengths and survival were not significantly different among light-intensity treatments from 4 dph to 7 dph but were maximized in the 50- and 100-lx treatments by 11 dph and 15 dph, when performance of larvae from the 5- and 1,000-lx treatments declined. The poor growth and survival of larvae in the low-intensity 5-lx treatment following 7 dph can be explained by the percentages of feeding larvae, which were clearly lower at 5 lx than in the mid- and high-intensity treatments before 15 dph.

Blaxter (1968b) defined visual threshold as an illumination level at which only 10% of larvae are feeding. In the present study, 40 and 49% of the larvae from the 5-lx treatment were feeding on 7 dph and 11 dph, respectively, which indicates that at this developmental stage, the visual threshold for this species is lower than 5 lx. However, significantly lower percentages of feeding larvae observed in the 5-lx treatment on 7 dph and 11 dph suggests that sub-optimal growth and survival of larvae in this treatment were due to inadequate feeding and inefficient use of energy related to hunting at low light levels. In plaice *Pleuronectes platessa* this visual threshold ranges from 1–10 lx (Blaxter 1968a). Similar thresholds have been established for several other species of finfish larvae, including herring *Clupea harengus* (< 1 lx) (Blaxter 1968b), walleye pollock (< 1 lx) (Paul 1983) and striped bass *Morone saxatilis* (1 lx) (Chesney 1989). The results of the present study suggest that an intensity of 5 lx may be near the visual threshold of illumination for larvae of southern flounder, making it difficult to locate and capture prey.

The feeding disadvantage faced by larvae in the low-intensity 5-lx treatment appeared to compromise growth by 11 dph, when mean notochord lengths from this treatment were less than notochord lengths from the 50- and 100-lx treatments. Survival in the low-intensity 5-lx treatment was also lower than survival in the 100-lx treatment by 15 dph.

While the minimum illumination threshold theory explains the poor growth and survival of marine fish larvae under low light intensities, there appears to be a maximum illumination level above which larval growth and/or survival are adversely affected in some species. For herring larvae, swimming activity was found to increase with increasing light intensity (Batty 1987). In summer flounder, yolk utilization efficiency and notochord length decreased as light levels increased from 500 to 2,000 lx (Watanabe et al. 1998). Additionally, larval swimming behavior and positioning change with increasing light intensity in some species. At high light intensities, herring larvae shift from a vertical to horizontal swimming pattern (Batty 1987), and rainbow smelt *Osmerus mordax* larvae appear to be more concentrated in surface waters (Bedard and Lalancette 1989). In the laboratory, high light intensities appear to undermine the vertical migration mechanism (alternate vertical swimming and sinking rest periods) employed by marine fish larvae, replacing it with a less efficient constant swimming behavior (Haury and Weihs 1976) in a horizontal direction. Excessive illumination could explain the unfavorable growth exhibited by southern flounder larvae from the high-intensity treatment (1,000 lx) on 15 dph in the present study. Previous research has suggested that relatively high light intensities (320–1,600 lx) may not adversely affect growth and survival of southern flounder larvae from first feeding through metamorphosis (Daniels et al. 1996; Denson and Smith 1997), but future investigation should focus on larval response to light intensities ranging from 100–1,000 lx.

In the present study, whole-body osmolality was measured to minimize the number of larvae that would be required for ex-

traction and measurement of extracellular fluid. Tandler et al. (1995) measured whole body osmolality of marine fish larvae *Sparus aurata* from a mixture of intra- and extracellular fluids, which resulted in values comparable to those derived from plasma. Tornheim (1980) used a vapor pressure osmometer (VPO) to measure the osmolality of whole-tissue slices, concluding that this is an acceptable and highly reproducible method of determining tissue osmolality.

In our study, mean whole-body osmolality (304 mOsmol/kg) of southern flounder larvae from the 24-ppt treatment from 1 dph through 15 dph was lower than published values for other species of marine fish embryos and larvae in full-strength seawater (320–400 mOsmol/kg) (Alderdice et al. 1979; Davenport et al. 1981; Riis-Vestergaard 1982; Hahnenkamp et al. 1993), as well as that of larvae from the 34-ppt treatment (mean = 343 mOsmol/kg). Southern flounder larvae were stenohaline marine, with higher survival as well as greater larval dry weights at 34 ppt. This is consistent with the early stages of other marine flatfish larvae. For example, Watanabe et al. (1998) found yolk-sac larvae of the summer flounder exhibited greatest growth at 36 ppt within a range of 26–36 ppt, while Hart and Purser (1995) demonstrated that larvae of the greenback flounder reared through metamorphosis exhibit significantly greater survival at 35‰ than at 15 ppt. Likewise, when exposed to salinities ranging from 20–42 ppt, highest survival rates for Atlantic halibut yolk-sac larvae were obtained in salinities ranging from 27–32 ppt, with the largest proportion of normally developed larvae produced in the 29–34 ppt range (Lein et al. 1997).

A marked elevation in whole-body osmolality on 11 dph, indicative of osmoregulatory stress, was observed in larvae exposed to the lowest light intensity of 5 lx (Fig. 5a) and was also apparent in larvae exposed to the highest light intensity of 1,000 lx (Fig. 5d). Larvae exposed to low illumination levels (i.e., 5 lx) fed less efficiently (Fig. 3a) and this likely resulted in weakened fish. Conversely, high illumination (i.e., 1,000 lx) may have induced hyperactivity and inefficient use of energy as previously reported in other marine finfish larvae (Haury and Weihs 1976; Batty 1987). This is consistent with Shelbourne's (1957) "osmotic breach" theory.

Buoyancy of pelagic eggs and larvae of marine fish is affected by culture salinity (Holliday 1965; Lein et al. 1997) and feeding success (Frank and McRuer 1989). In cod, buoyancy is a good indicator of larval condition. Sclafani et al. (2000) found a positive linear relationship between larval density and mortality in this species. Some proposed strategies employed by larvae to maintain optimum vertical positioning within the water column are density changes via osmoregulation (Sclafani et al. 1997) and synthesis or breakdown of lipids (Guisande et al. 1998), which appear to enhance buoyancy in marine fish (Hagen et al. 2000; Nursall 1989). Like other marine fish eggs and larvae reared in brackish water (Liu et al. 1994; Hart and Purser 1995; Guisande et al. 1998), those in the low-salinity treatment of the present study likely experienced reduced buoyancy compared with larvae raised in seawater. Recent studies have demonstrated that anesthetized southern flounder larvae reared at 25 and 34 ppt are negatively buoyant at 25 ppt, while they are neutrally or positively buoyant at 34 ppt (C. Moustakas, University of North Carolina at Wilmington, unpublished data). Since both osmoregulatory ability and feeding success were relatively inefficient at 24 ppt before 15 dph, larvae may have allocated energy to maintain vertical positioning through increased lipid production and/or swimming behavior, which would have come at the expense of larval nutrition and energy available for growth and survival.

In summary, results of the present study demonstrate that growth, survival, and osmoregulatory ability of larval southern flounder from hatching through 15 dph are maximized under light intensities of 50 and

100 lx and in full-strength seawater. In nature, southern flounder adults have been reported at depths ranging from 18–62 m (60–200 ft) during the spawning season (Stokes 1977), where eggs and early-stage larvae likely occur (Gilbert 1986). Since light intensity is depleted in water with depth, these waters are probably characterized by low illumination levels (Champ et al. 1980). The larvae generally do not enter their estuarine nurseries until late-larval and metamorphic stages (Burke et al. 1991). Optimal light intensities and salinities determined in this study appear to be consistent with conditions that pelagic eggs and early-feeding stage larvae encounter in their natural habitat.

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