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Combined effects of photoperiod and salinity on growth, survival, and osmoregulatory ability of larval southern flounder *Paralichthys lethostigma*

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Abstract

The southern flounder, *Paralichthys lethostigma*, is an important commercial and recreational marine flatfish that inhabits estuaries and shelf waters in the south Atlantic, from North Carolina through the Gulf coasts, with the exception of south Florida. Because juvenile and adult fish are highly euryhaline, it is a prime candidate for aquaculture. Methods for captive spawning of southern flounder are well developed; however, information on optimal culture requirements of the early larval stages is required for reliable mass production of juveniles.

To determine the optimal photoperiod and salinity conditions for culture from hatching to day 15 post-hatching (d15ph), embryos were stocked into black 15-l tanks (75 l^{-1}) under four photoperiods (24L:0D, 18L:6D, 12L:12D, and 6L:18D) and two salinities (25 and 34 ppt) in a 4×2 factorial design. Temperature was 18 °C, light intensity was 150 lx, and aeration was 50 ml min^{-1}. Significant ($P < 0.05$) effects of photoperiod and salinity on growth (notochord length, wet and dry weights) were obtained. Growth increased with increasing photoperiod and salinity and was significantly greater at 24L and 18L than at 12L or 6L, and at 34 than at 25 ppt. On d11ph and d15ph, significant interactive effects between photoperiod and salinity on growth (wet and dry weights) were also evident. Growth of larvae reared at 25 ppt increased with increasing photoperiod to a maximum at 24L, while growth of larvae at 34 ppt reached a plateau at 18L. While there were no significant photoperiod effects on these parameters, larval survival, body water percentage, and larval osmolality on d15ph were significantly higher at 34 than at 25 ppt (41% vs. 16% survival; 322 vs. 288 mosM kg^{-1}; and 84% vs. 76% water, respectively), suggesting stress and nonadaptation to 25 ppt, a salinity more nearly isoosmotic than full-strength seawater. Since larvae from both salinity treatments were neutrally or positively buoyant at 34 ppt, but negatively buoyant at 25 ppt, larvae

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reared at 25 ppt probably allocated energy to maintain vertical positioning, compromising growth and survival.

The results demonstrate that growth and survival of early-stage southern flounder larvae are maximized under long photoperiods of 18–24L and in full-strength seawater. Longer photoperiods probably extend the time larvae have for feeding, while full-strength seawater salinity optimizes buoyancy and vertical positioning, conserving energy. The results show that early larval stage southern flounder larvae are not entirely euryhaline, which involves not only the ability to osmoregulate, but to conserve energy under reduced buoyancy. This is consistent with suboptimal vs. maximal growth of larvae reared at 25 and 34 ppt, respectively, under 18L (i.e., photoperiod × salinity interaction). This is also consistent with other reports that tolerance to lower salinities in these euryhaline flatfish increases post-metamorphosis when transition from apelagic to benthic existence alleviates the need to counteract reduced buoyancy.

Keywords: Southern flounder; Larval culture; Photoperiod; Salinity; Buoyancy; Osmoregulation

1. Introduction

The Southern flounder, *Paralichthys lethostigma*, is a flatfish of the family Bothidae. It can be found in coastal waters from Albemarle Sound, NC, through the South Atlantic states to Corpus Christi Pass, TX, with the exception of south Florida (Ginsburg, 1952; Wenner et al., 1990). From spring through fall, southern flounder are known to inhabit coastal bays, sounds, and river systems (Ginsburg, 1952; Wenner et al., 1990; Smith et al., 1999a) and, while they are most abundant in mid- to upper reaches of estuaries, they occasionally enter freshwater (Dahlberg, 1972;Wenner et al., 1990; Smith et al., 1999a). During the spawning season, which runs from late fall to early winter (Ginsburg, 1952), adult fish migrate to offshore waters (5.5–22 m deep) to spawn (Stokes, 1977; Wenner et al., 1990; Warlen and Burke, 1990). The larvae are transported into the low-salinity estuaries, which become the fish's nursery grounds, via currents and tides (Miller et al., 1991). There, the fish complete metamorphosis and remain as juveniles for the first year of their life (Burke et al., 1991; Powell and Schwartz, 1977).

Southern flounder is of great importance to the recreational and commercial fisheries along its distribution range (Ginsburg, 1952; Wenner et al., 1990; Waters, 1999; North Carolina Division of Marine Fisheries, 1999, 2000; Benetti et al., 2001). Increasing demand for southern flounder as a food and sport fish and in the international marketplace make it an important candidate for commercial aquaculture and for stock enhancement (Waters, 1996, 1999). Their euryhaline character (Burke et al., 1991; Daniels et al., 1996) may allow these fish to be grown in both inland and coastal sites (Daniels et al., 1996; Smith et al., 1999a; Waters, 1999; Tuckey and Smith, 2001; Van Maaren and Daniels, 2001).

Availability of southern flounder embryos for research has recently improved through development of techniques for hormone (Berlinsky et al., 1996; Smith et al., 1999b) and photothermally induced (Watanabe et al., 2001) spawning of captive broodstock. Studies are needed to develop reliable methods for culture of the larvae to the juvenile stages. At present, relatively little is known of the environmental optima of its early life history stages.

It is well known that feeding in marine fish larvae depends on sight and is greatly influenced by illumination (Blaxter, 1969; Barahona-Fernandes, 1979). For each species, there exists a minimum light intensity below which larvae can no longer capture prey (Blaxter, 1969). However, light intensities that allow normal development of the larvae vary widely among species, from 1 lx in striped bass to 1000 lx in rabbitfish (Boeuf and Le Bail, 1999). In southern flounder, light intensities of 50–100 lx produced better growth and survival (vs. 5 and 1000 lx) of first feeding stage larvae (Henne et al., 2001).

In addition to light intensity, photoperiod affects the survival and growth rate of teleost larvae, although the nature of the effect also varies markedly with species (Barlow et al., 1995). Photoperiod is classified as a directive factor, which controls growth as a "zeitgeber" through its influence on endogenous rhythms and circulating levels of growth hormone (Simensen et al., 2000). In some species, including barramundi *Lates calcarifer* (Barlow et al., 1995), gilthead seabream *Sparus aurata* (Tandler and Helps, 1985), and rabbitfish *Siganus guttatus* (Duray and Kohno, 1988), extending daylength increases the time during which the larvae can search for food, generally increasing larval growth and survival. In other species, including southern flounder (Tuckey and Smith, 2001), sea bream (Dowd and Houde, 1980), and sea bass *Dicentrarchus labrax* (Barahona-Fernandes, 1979), longer photoperiods reduce survival and/or growth rates. Since the amount of food a larva can ingest is limited, longer photoperiods may cause larvae to expend more energy than is being gained (Tuckey and Smith, 2001). On the other hand, in greenback flounder *Rhombosolea tapirina* (Hart et al., 1996), barramundi (Barlow et al., 1995), and common sole *Solea solea* (Fuchs, 1978), photoperiod affected larval growth, with little or no effect on survival.

Salinity is known to influence the distribution of flatfish in the natural environment, although this effect varies among species and developmental stages (review by Schreiber, 2001). Developmental stage-specific distribution is partly determined by the species' osmoregulatory ability. Early stage marine fish larvae are able to osmoregulate (Hiroi et al., 1998; Miyazaki et al., 1998; Hiroi et al., 1999) even though the gills, which are the primary sites for ionic regulation in juvenile and adult fish (Hiroi et al., 1999), are absent or underdeveloped (review by Schreiber, 2001). In hypo-osmoregulating fish larvae, chloride cells located on the skin are the main site for excreting ions entering the larvae by diffusion (Tytler and Ireland, 1995), whereas the water lost by osmosis is regained by water-drinking behavior (Tytler and Blaxter, 1988a,b). Since osmoregulation is an energy demanding process, isoosmotic salinities minimize osmoregulatory stress and osmoregulatory costs and should increase the energy available for growth and/or survival (Sampaio and Bianchini, 2002). Many studies conducted with marine finfish larvae, such as gilthead sea bream, *Caranx mate*, Japanese flounder *Paralichthys olivaceus*, and brown-spotted grouper *Epinephelus tauvina* support this concept, showing a higher survival and/or growth rate associated with salinities below full-strength seawater (Tandler et al., 1995; Santerre, 1976; Mihelakakis and Kitajima, 1994; Akatsu et al., 1983, respectively). This concept is challenged, though, because of the lack of consistent results among species (Febry and Lutz, 1987). In southern flounder, summer flounder, milkfish *Chanos chanos*, and Nassau grouper *Epinephelus striatus*, growth and/or survival rates were found to be higher in seawater than in brackish water (Henne et al., 2001; Watanabe et al., 1998; Swanson, 1996; Ellis et al., 1997, respectively). In other species, including brown-spotted

grouper (Akatsu et al., 1983), Atlantic halibut (Lein et al., 1997), and greenback flounder (Hart et al., 1996), no reduction in growth or survival were observed at salinities of 25–39, 20–42, and 15–35 ppt, respectively.

There is considerable evidence that in southern flounder, euryhalinity increases with age (Daniels et al., 1996; Smith et al., 1999a,b). Southern flounder eggs were able to hatch at 10 ppt; however, newly hatched larvae died soon afterwards (Smith et al., 1999a,b). On the other hand, post-metamorphic larvae (50-day-old) showed no significant difference in survival at salinities ranging from 5 to 30 ppt, while older juveniles (220-day-old) were able to withstand salinities as low as 0 ppt with 100% survival (Smith et al., 1999a,b). Daniels et al. (1996) also reported that pre-metamorphic larvae were not able to survive to d60ph in 10 ppt, while post-metamorphic larvae showed no difference in survival from larvae reared at higher salinities (20 and 30 ppt). Daniels and Borski (1998) also showed that metamorphic-stage larvae had significantly lower survival when reared in salinities below 20 ppt, whereas post-metamorphic larvae were not adversely affected by salinities as low as 0 ppt. The same trend was observed in the summer flounder, in which yolk sac larvae had better growth at 36 ppt (Watanabe et al., 1998) than at 31 and 26 ppt, while pre- and post-metamorphic larvae were not adversely affected by salinities as low as 14 and 8 ppt, respectively (Specker et al., 1999).

Knowledge of the optimum environmental conditions for culturing larval southern flounder is needed to enhance their survival and growth rate, reduce the larval rearing period, and reduce production costs (Hart et al., 1996). In nature, early-stage larval southern flounder are mostly found in offshore waters during winter months of December and January where they are subjected to high salinities (35 ppt) and a natural light cycle of approximately 10L:14D. The objectives of this study were to determine the photoperiod and salinity conditions needed for optimum growth and survival of southern flounder larvae reared from hatching through the yolk sac and first feeding stages, a 15-day period.

2. Materials and methods

2.1. Experimental animals

This experiment was conducted at the University of North Carolina at Wilmington's Center for Marine Science Aquaculture Facility, Wrightsville Beach, NC. Broodstock southern flounder were maintained under a controlled photothermal cycle simulating natural seasonal changes and were induced to spawn using luteinizing hormone releasing hormone-analogue (LHRH-a) (Berlinsky et al., 1996; Smith et al., 1999b; Watanabe et al., 2001).

2.2. Experimental system

The experimental system consisted of four water baths (152 × 61 × 23 cm) each covered by a light hood (152 × 65 × 19 cm) suspended from the ceiling and isolated with an opaque black curtain to eliminate extraneous light. Each water bath housed eight cylindrical black plastic aquaria (15 l working volume) where the larvae were reared.

Freshwater was continuously re-circulated through the baths and through a heater/chiller to maintain a constant temperature.

Illumination was provided by 40-W fluorescent bulbs supplying full spectrum lighting, simulating natural sunlight. Light intensity was maintained constant (150 lx) (Henne et al., 2001), at the water surface, by adjusting the height of the light hoods. Photoperiod treatments were maintained in each water bath by timers.

2.3. Experimental design

To study the effects of photoperiod and salinity on growth, survival, and osmoregulatory ability of larval southern flounder, embryos were stocked into aquaria at a density of 75 l^{-1} under photoperiods of 24L:0D, 18L:6D, 12L:12D, and 6L:18D. Within each photoperiod treatment, there were two salinity treatments, 25 and 34 ppt. Four replicate aquaria were maintained for each treatment combination of photoperiod and salinity. Seawater was prepared by filtering seawater (1 μm, UV-treated) pumped from the Atlantic intracoastal waterway adjacent to the laboratory. Brackish water was prepared by diluting filtered seawater with chlorine-free freshwater. Aeration was provided through airstones weighted with ceramic insulators, at approximately 50 ml min^{-1} throughout the experiment. Water lost during sampling and water exchanges (30% day^{-1}) was replaced with water of the appropriate salinity, while that lost to evaporation was replaced with chlorine-free freshwater on a daily basis.

Fertilized eggs were placed in incubators, where they were quantified and their embryonic development monitored. Approximately 1 day before hatching, those embryos that were still developing normally were stocked into the rearing aquaria. The temperature was maintained at 17 °C throughout the experiment.

2.4. Larval feeding

Larvae were fed rotifers *Brachionus plicatilis* that were cultured in 150-l tanks under continuous illumination and at temperature of 22–25 °C. Salinity was maintained at an intermediate level of 30 ppt to minimize osmotic stress upon their addition to the larval culture media of 25 and 34 ppt. Rotifers were enriched with preserved algae, *Nannochloropsis oculata* (Reed Mariculture, San Jose, CA), twice within an 18-h period, and once 6 h prior to feeding, with a commercially prepared yeast-based diet (Culture Selco, Ogden, UT), to maintain their nutritional value, especially fatty acids essential for normal growth and development of marine fish larvae (Sargent et al., 1997).

Each morning, rotifers and their culture medium were harvested and then acclimated to the larval rearing temperature over a period of several hours. Rotifers were thoroughly rinsed with filtered seawater (30 ppt) before their addition to the larval rearing aquaria. Addition of the rotifers to the larval rearing aquaria began on d2ph, approximately 48 h prior to the first feeding stage, in order to familiarize the larvae with their prey and to ensure its presence upon the commencement of exogenous feeding. The rotifers were enriched and stocked into each aquaria at an initial density of 5 rotifers ml^{-1}. This density was maintained by quantifying the rotifers in each culture vessel using volumetric methods and adding the appropriate number of enriched rotifers to make up the difference on a

daily basis. Background algae, Tahitian *Isochrysis galbana*, was also added to the larval rearing aquaria at d1ph to maintain the nutritional value of the rotifers. The microalgae were maintained at a concentration of about 300,000 cells ml^{-1} throughout the experiment.

2.5. Growth, survival, and osmoregulation

Larval sampling for data collection was scheduled at d1ph, d4ph, d7ph, d11ph, and d15ph. Sampling began at 0800 h before rotifers were added to minimize the amount of newly ingested rotifers in the larval gut. Larvae were collected by vigorously aerating the culture water for a short period to ensure a uniform distribution and then sampling a known volume of water until at least 10 larvae were removed. The larval density (number of larvae l^{-1}) was calculated by dividing the number of sampled larvae by the volume of water collected. Larval survival on each sampling date was calculated as the quotient of larval density and initial larval density, expressed as a percentage.

Notochord length, yolk sac length and width, and oil droplet diameter were recorded from anesthetized (0.3–0.5 ppt 2-phenoxyethanol) larvae using a microscope fitted with an ocular micrometer. Length was measured to the nearest 0.1 mm. To determine wet weights, 10 larvae per rearing aquaria were gently rinsed with deionized water on a Nitex screen, blotted dry, then weighed on a Sartorius (Goettingen, Germany) electrobalance to the nearest 10 μg. To determine dry weights, 10 larvae per rearing aquaria were dried to constant weight at 60 °C (approximately 72 h) then weighed. Length and weight measurements were used to assess growth, which was expressed as length and weight at d1ph, d4ph, d7ph, d11ph, and d15ph. Wet and dry weight measurements were used to calculate percent body water.

Larval osmolality, measured with a vapor pressure osmometer (Wescor 5520, Logan, UT, USA), was recorded on d2ph, d5ph, d10ph, and d14ph. A sample of 7–9 larvae from each replicate tank was measured for osmolality on each sampling day.

2.6. Larval buoyancy

An experiment was conducted to determine buoyancy of larvae, reared under 34 and 25 ppt salinity, in salinities of 34 and 25 ppt. Embryos were stocked into four 15-l rearing units (two units for each rearing salinity of 34 and 25 ppt) placed in a temperature-regulated water bath. Photoperiod was maintained at 12L:12D while temperature, light intensity, and feeding conditions were the same as described earlier. Buoyancy of larvae was determined on d1ph, d4ph, d7ph, d11ph, and d15ph. To determine buoyancy, 10 larvae collected from the rearing salinities of 25 and 34 ppt were placed in each of six 1-l beakers containing water of 25 and 34 ppt (with three replicate beakers per salinity). To immobilize larvae, anesthetic (0.3 ppt 2-phenoxy-ethanol) was added prior to addition of the larvae. After larvae were anesthetized and at rest in the water column, usually within 4–6 min, the vertical position of each larva in the water column was recorded as the distance from the bottom of the beaker measured to the nearest mm. Vertical position was converted to relative buoyancy (100% for the larvae at the surface of the water and 0% for the larvae that sank to the bottom).

2.7. Water quality

Temperature (YSI 55, Yellow Springs, OH, USA, ± 0.1 C) and salinity (refractometer, ± 1 g l^{-1}) were measured in each replicate tank on a daily basis. Light intensity, also recorded daily, was measured at the water surface of each tank with a light meter (Extech Instruments, Waltham, MA, USA). Dissolved oxygen (YSI 55, Yellow Springs, ± 0.01 mg l^{-1}) and pH (± 0.1) were measured daily from one replicate tank per treatment, and total ammonia nitrogen (TAN) was measured on alternate days (HACH DR 850, Loveland, CO, USA, ± 0.01 mg l^{-1}). Airflow (40–60 ml min^{-1}) to each aquarium was monitored daily with a flow meter (Cole-Parmer Instrument, Vernor Hills, IL, USA, ± 1 ml min^{-1}) and adjusted as needed. Tank surfaces were skimmed daily with paper towels to remove oil films. During the experimental period, mean (range) salinity (ppt) was 34 (32–37) and 25 (22–26) at treatment levels of 34 and 25 ppt, respectively. Mean (range) daily water quality conditions were as follows: temperature (°C), 17.0 (16.1–17.8); light intensity (lx), 161 (110–240); total ammonia nitrogen (mg l^{-1}), 0.07 (0.03–0.15); pH, 7.8 (7.3–8.0); and dissolved oxygen (mg l^{-1}), 7.24 (6.83–7.61).

2.8. Analytical methods

Quantitative values were expressed as treatment means ± standard errors (S.E.). The effects of photoperiod, salinity, and their interaction were analyzed using two-way analysis of variance. If no interaction was detected, salinity treatments were combined within photoperiod treatments, and photoperiod treatments were combined within salinity treatments for further analysis. Significant treatment effects were detected by the Tukey–Kramer honestly significant difference test for multiple comparisons among means. Analysis was performed using JMP (SAS Institute, Cary, NC) statistical software.

3. Results

3.1. Growth

No significant interactive effects between photoperiod and salinity on notochord length were observed on any sampling day throughout the study. Hence, the effects of photoperiod on notochord length were compared by combining data from both salinities (Fig. 1A), while the effects of salinity were compared across all photoperiods (Fig. 1B). On d1ph, notochord lengths ranged from 3.10 to 3.14 mm, with no significant treatment effects observed. By d4ph, a departure in growth rates among treatments was evident. Significant effects of photoperiod on notochord lengths were observed on d4ph, d11ph, and d15ph (Fig. 1A), while significant salinity effects were observed on d4ph, d7ph, d11ph, and d15ph (Fig. 1B). On d4ph, notochord lengths generally increased with increasing photoperiod from a minimum value of 3.19 mm in the 6L treatment to a maximum of 3.42 mm in the 24L treatment (Fig. 1A). Mean notochord length was significantly ($P < 0.05$) greater in the 24L treatment than in the 6L treatment. While no

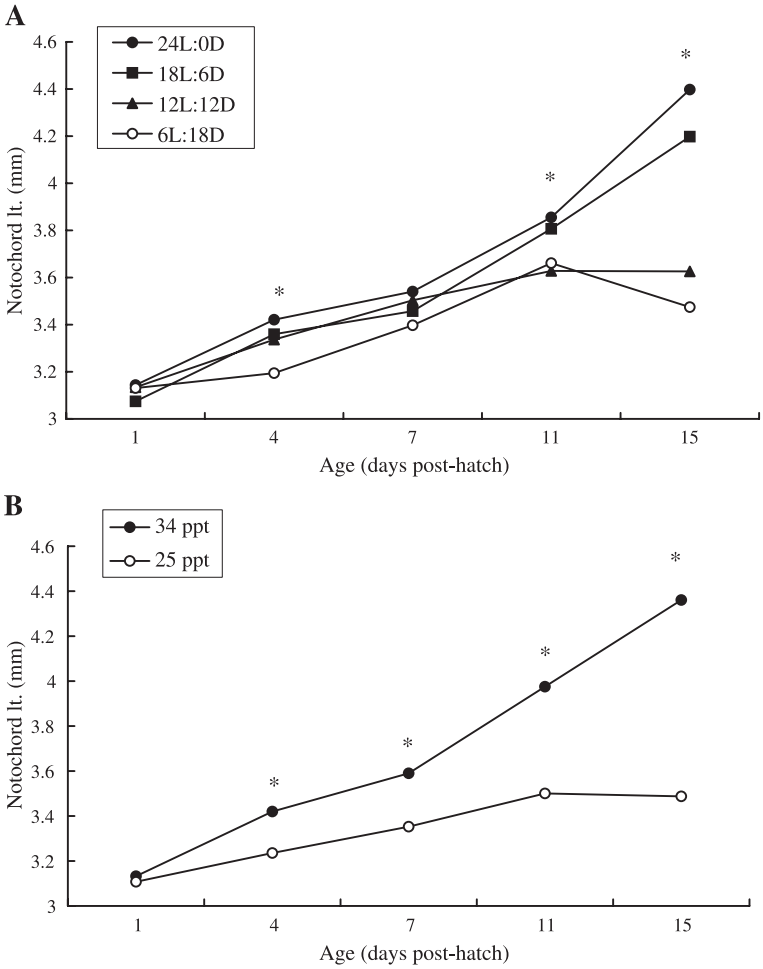


Fig. 1. Notochord lengths of southern flounder larvae from day 1 through day 15 post-hatch under different (A) photoperiods (24L:0D, 18L:6D, 12L:12D, and 6L:18D) and (B) salinities (25 and 34 ppt). Plotted points represent means ($N=8$ (A) and $N=16$ (B)). Data for both salinities were combined under each photoperiod in A, while data for all photoperiods were combined under each salinity in B. Asterisk indicates significant ($P<0.05$) difference among treatments observed on that sampling date.

significant photoperiod effects were evident on d7ph, when notochord lengths ranged from 3.40 to 3.54 mm, trends similar to that observed on d4ph were evident on d11ph and d15ph. On d15ph, notochord lengths ranged from a minimum of 3.47 mm at 6L to a maximum of 4.40 mm at 24L and were significantly ($P<0.0001$) greater at the longer photoperiods of 24L and 18L than at 12L or 6L.

Under all photoperiods, notochord lengths were significantly greater at 34 than at 25 ppt on d4ph, d7ph, d11ph, and d15ph (Fig. 1b). On d15ph, mean notochord lengths at 34 ppt was 4.36 mm, compared to 3.49 mm at 25 ppt.

On d1ph, wet weights ranged from 0.19 to 0.25 mg, with no significant treatment effects observed. Significant effects of photoperiod on wet weights were observed on d11ph and d15ph, while significant salinity effects were observed on d4ph, d11ph, and d15ph. On d4ph, wet weights were significantly ($P < 0.05$) greater at 34 than at 25 ppt. On d7ph, wet weights ranged from 0.23 to 0.27 mg, with no significant treatment effects observed. On d11ph and d15ph, significant effects of the photoperiod ($P < 0.0001$) and salinity ($P < 0.0001$) on wet weights were observed, and there was a significant ($P < 0.05$) interaction between these effects. On d11ph, wet weights were lower at 25 than at 34 ppt (Fig. 2A). However, whereas mean wet weights at 25 ppt increased progressively with increasing photoperiod, wet weight values at 34 ppt appeared to plateau at 18L and were similar at 18L and 24L. A similar pattern was observed on d15ph (Figs. 2B and 3). In other

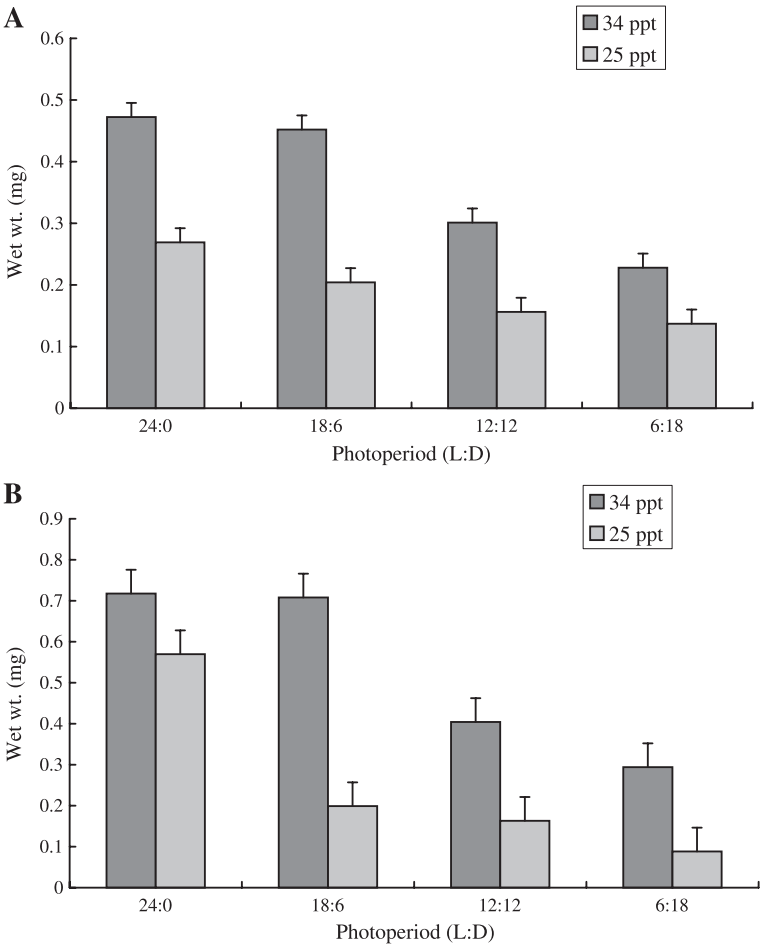
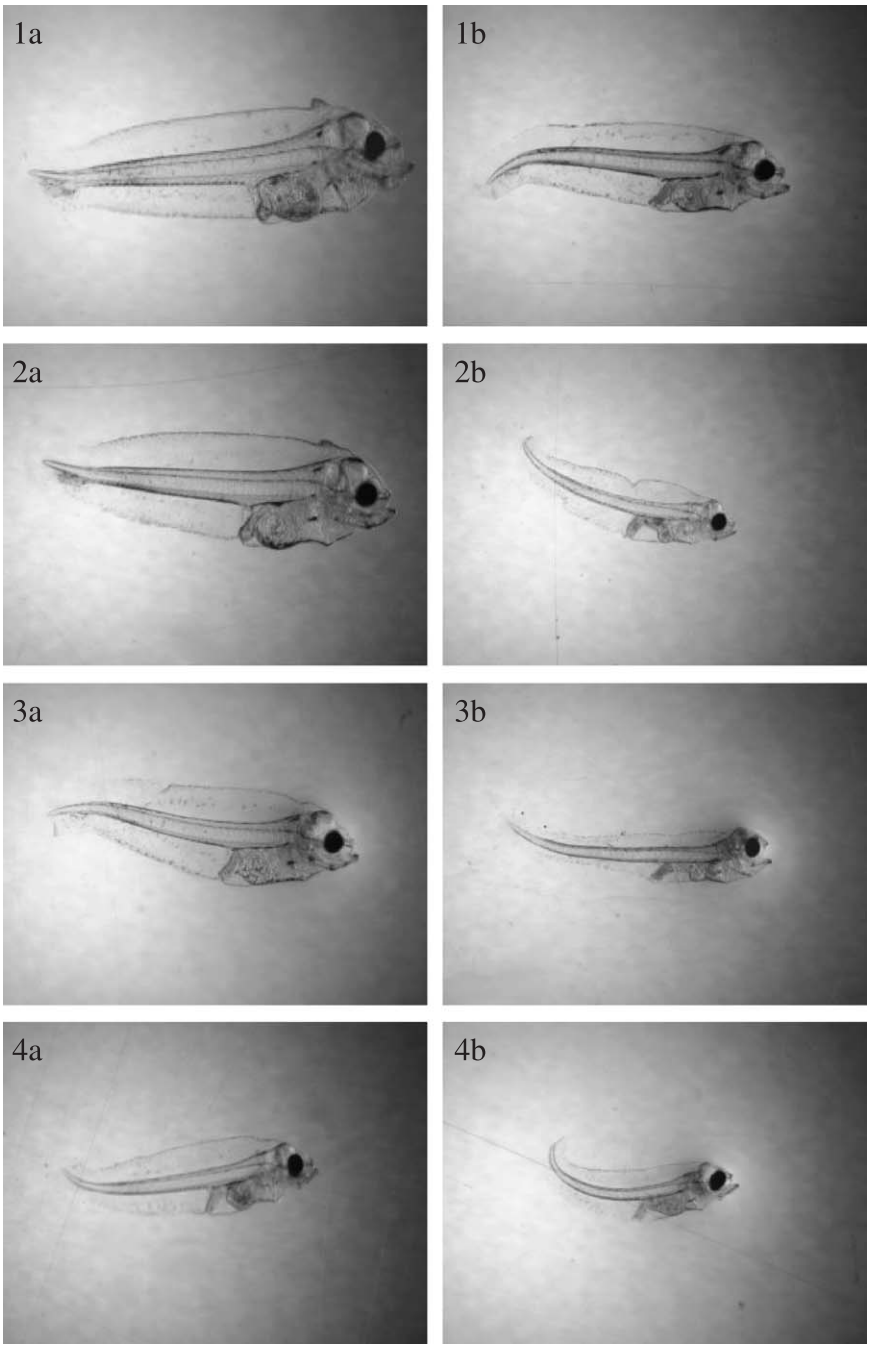


Fig. 2. Wet weights (means ± S.E., $N = 4$) of southern flounder larvae under different photoperiods (24L:0D, 18L:6D, 12L:12D, and 6L:18D) and salinities (25 and 34 ppt) on (A) day 11 post-hatch and (B) day 15 post-hatch.


1a
1b
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4b

words, mean wet weight values were more closely similar at 18L and 24L under 34 than under 25 ppt.

On d1ph, dry weights ranged from 28.5 to 32.7 μg, with no significant treatment effects observed. Significant ($P<0.05$) effects of photoperiod and salinity on dry weights were observed on d4ph, d11ph, and d15ph. On d4ph, dry weights generally increased with increasing photoperiod and salinity and were significantly ($P<0.05$) greater in the 24L, 18L, and 12L treatments, than in 6L treatment, and were greater at 34 than at 25 ppt. On d7ph, dry weights ranged from 35.1 to 38.0 μg, and there were no significant differences among the treatments. On both d11ph and d15ph, significant effects of photoperiod ($P<0.05$) and salinity ($P<0.0001$) on dry weights were observed, and there was a significant interaction ($P<0.05$) between these effects (Fig. 4). On d11ph (Fig. 4a), dry weights under 34 ppt increased with increasing photoperiod to a plateau at 18L. At 25 ppt, dry weights showed no clear pattern of change with photoperiods. Under all photoperiods, dry weights were higher at 34 than at 25 ppt. However, differences between salinity treatments were more pronounced at 18L than under the other photoperiods. On d15ph (Fig. 4b), dry weights were higher in the long photoperiods (24L and 18L) compared with 12L and 6L, and at 34 than at 25 ppt. Similar to the interaction observed in the wet weights, dry weights under 25 ppt increased progressively with increasing photoperiod, while dry weights under 34 ppt reached a plateau at 18L. Furthermore, as was observed on d11ph, the difference in dry weights between salinities appeared to be more pronounced at 18L than under the other photoperiods.

3.2. Body water percentage

No significant interactive effects between photoperiod and salinity on body water percentage were observed on any sampling day throughout the study. Hence, the effects of photoperiod on body water percentage were compared by combining data from both salinities, while the effects of salinity were compared across all photoperiods (Fig. 5). No significant treatment effects on body water percentage were observed on d1ph, d4ph, and d7ph, when body water percentage averaged 84.9%, 82.2%, and 83.9%, respectively. On d11ph and d15ph, significant ($P<0.05$) salinity effects were observed, while there were no significant effects of photoperiod. On d11ph and d15ph, body water percentage at 34 ppt remained close to previous levels, but declined markedly at 25 ppt. By d15ph, body water percentage averaged 84% and 76% at 34 and 25 ppt, respectively.

3.3. Survival

No significant interactive effects between photoperiod and salinity on survival were observed on any sampling day throughout the study. Hence, the effects of photoperiod on

Fig. 3. Southern flounder larvae at day 15 post-hatch under different photoperiods (24L:0D (1), 18L:6D (2), 12L:12D (3), 6L:18D (4)) and salinities (34 ppt (a) and 25 ppt (b)). The same magnification (40 ×) was used for all photos, and each is a good representation of the larvae in each treatment. At 25 ppt, larval weights (wet and dry) increased progressively with photoperiod, while at 34 ppt, weight plateaued at 18 ppt (i.e., photoperiod × salinity interaction).

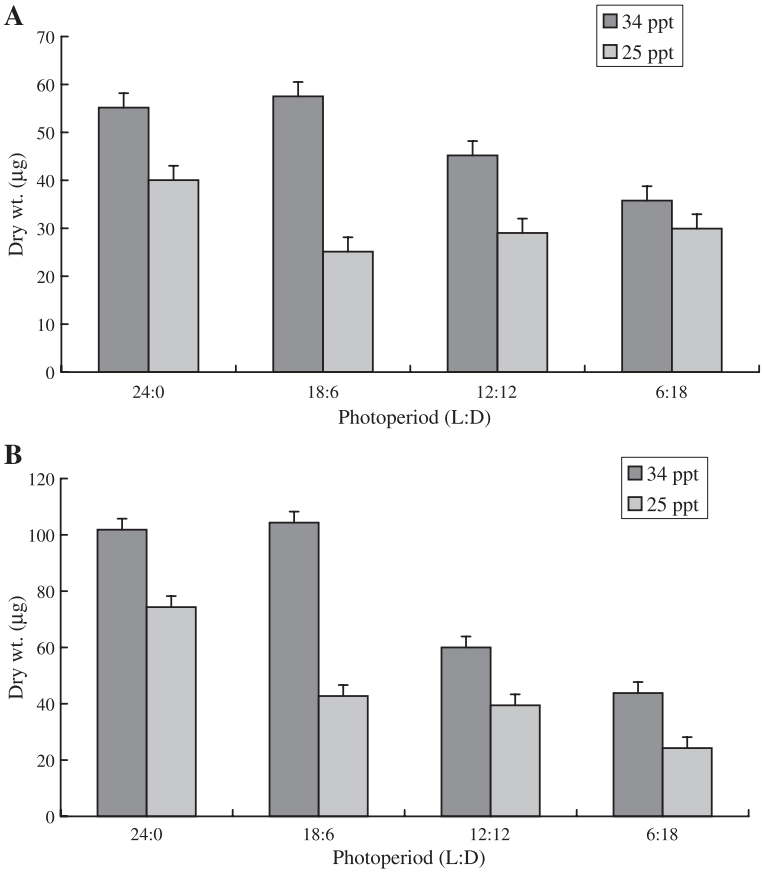

Fig. 4. Dry weights (means ± S.E., $N=4$) of southern flounder larvae under different photoperiods (24L:0D, 18L:6D, 12L:12D, and 6L:18D) and salinities (25 and 34 ppt) on (A) day 11 post-hatch and (B) day 15 post-hatch.

survival were compared by combining data from both salinities, while the effects of salinity were compared across all photoperiods (Fig. 6). Survival declined to an average of 67% on d4ph, and to 49% on d7ph, with no significant treatment effects observed. On d11ph and d15ph, significant effects of salinity were observed, while there were no significant photoperiod effects. On d11ph and d15ph, survival was significantly greater ($P<0.05$ and $P<0.0001$, respectively) at 34 than at 25 ppt. By d15ph, survival declined to averages of 41% and 16% at 34 and 25 ppt, respectively.

3.4. Whole-body osmolality

No significant interactive effects between photoperiod and salinity on larval osmolality were observed on any sampling day throughout the study. Hence, the effects of photoperiod on larval osmolality were compared by combining data from both salinities, while the effects of salinity were compared across all photoperiods (Fig. 7). Significant

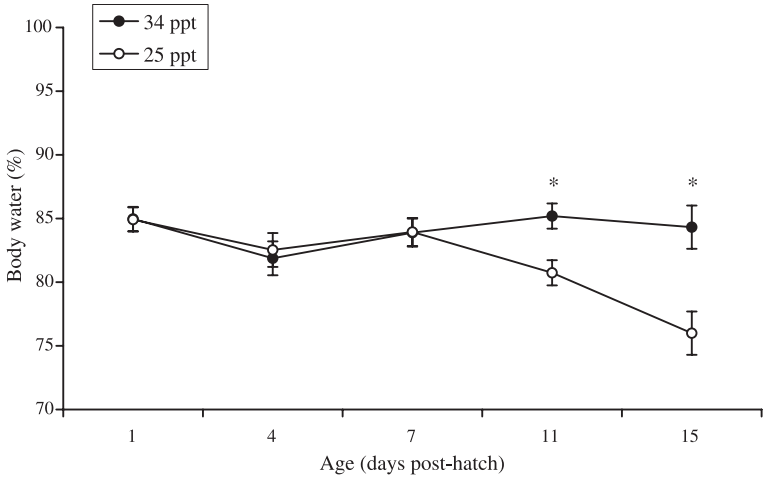

Fig. 5. Percent body water of southern flounder larvae from day 1 through day 15 post-hatch under different salinities (25 and 34 ppt). Plotted points represent means ± S.E. ($N = 1$). Data for all photoperiods were combined under each salinity. Asterisk indicates significant ($P < 0.05$) difference among treatments observed on that sampling date.

effects of salinity were observed on d2ph, d10ph, and d14ph, while no significant photoperiod effects were observed on any of the sampling days. Throughout the study, mean osmolality values were lower at 25 ppt, (range = 281–325 mosM kg^{-1}) than at 34 ppt (range = 322–389 mosM kg^{-1}). Osmolality was significantly higher at 34 than at 25 ppt on d2ph ($P < 0.0001$), d10ph ($P < 0.0001$), and d14ph ($P < 0.05$).

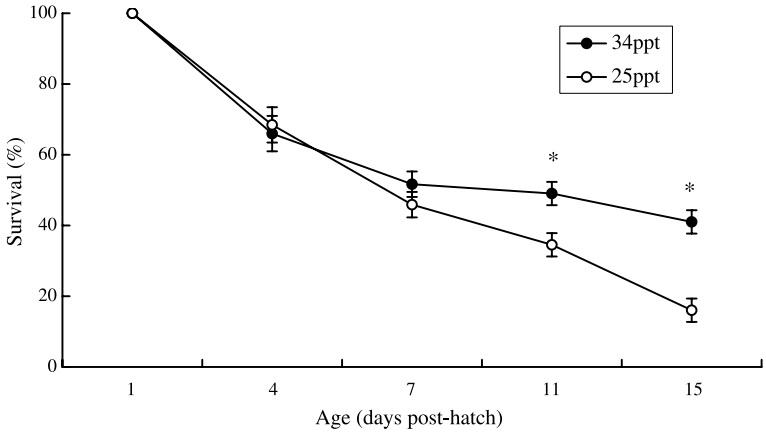

Fig. 6. Survival of southern flounder larvae from day 1 through day 15 post-hatch under different salinities (25 and 34 ppt). Plotted points represent means ± S.E. ($N = 16$). Data for all photoperiods were combined under each salinity. Asterisk indicates significant ($P < 0.05$) difference among treatments observed on that sampling date.

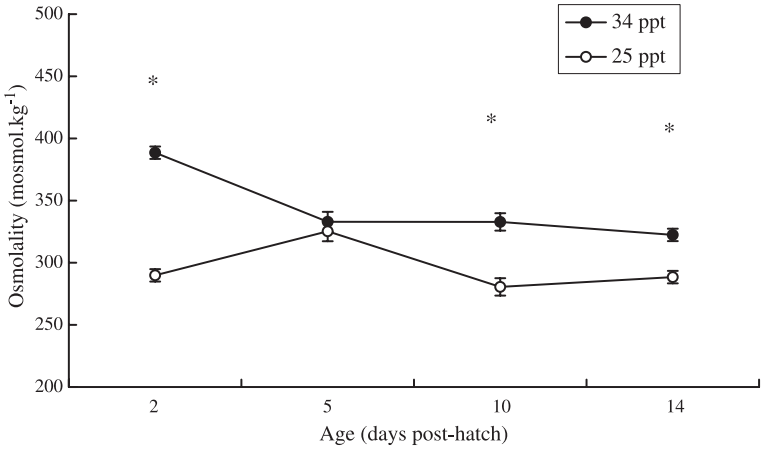

Fig. 7. Whole body osmolality of southern flounder larvae from day 2 through day 14 post-hatch under different salinities (25 and 34 ppt). Plotted points represent means ($N = 16$). Data for all photoperiods were combined under each salinity. Asterisk indicates significant ($P < 0.05$) difference among treatments observed on that sampling date.

3.5. Larval buoyancy

All larvae reared at 34 or 25 ppt sank to the bottom (0% relative buoyancy) when placed in 25 ppt. Except for d4ph and d11ph, larvae reared at 25 ppt were significantly

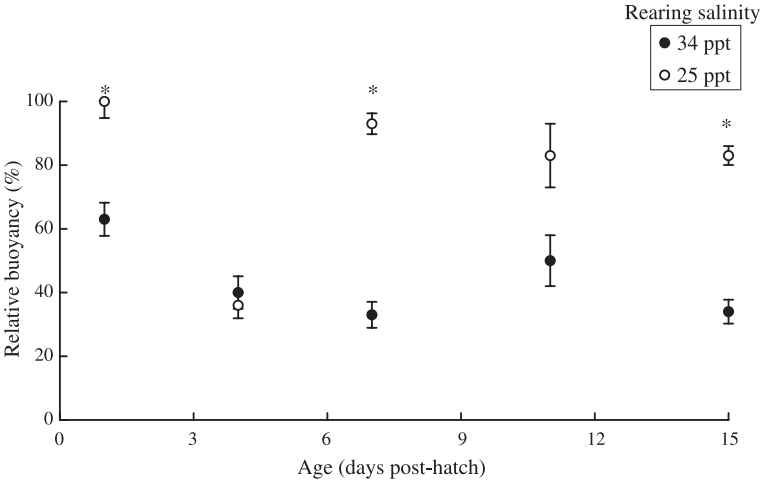

Fig. 8. Relative buoyancy (%) of southern flounder larvae in 34 ppt seawater. Vertical position in the test container was converted to relative buoyancy (100% for the larvae at the surface and 0% for the larvae on the bottom). Larvae were reared from day 1 through day 15 post-hatch at 34 and 25 ppt. Plotted points represent means ± S.E. ($N = 30$). Asterisk indicates significant ($P < 0.05$) difference among treatments observed on that sampling date.

($P < 0.05$) more buoyant than larvae reared at 34 ppt, when placed at 34 ppt (Fig. 8). On d4ph, relative buoyancy averaged 40% and 36% for larvae reared at 34 and 25 ppt, respectively. On d11ph, relative buoyancy appeared higher for the larvae reared at 25 ppt (83%) compared to the larvae at 34 ppt (50%), but statistical significance was not found. On d1ph, d7ph, and d15ph, relative buoyancy averaged 43% and 92% for the larvae reared at 34 and 25 ppt, respectively.

4. Discussion

In this study, the longest photoperiods (24L and 18L) produced significantly higher growth rates with respect to notochord length, wet weight, and dry weight compared to 12L or 6L (Fig. 3). These results suggest that longer photoperiods extend the time the larvae have for feeding, improving growth. This is consistent with what was reported by Tuckey and Smith (2001) for pre-metamorphic stage southern flounder, which grew faster under 24L (vs. 0L and 10L), although these workers observed no significant differences in growth among 10L and 24L after metamorphosis. Similar results were obtained in studies conducted on other marine finfish species such as gilthead sea bream (Tandler and Helps, 1985), rabbitfish (Duray and Kohno, 1988), European sea bass (Barahona-Fernandes, 1979), barramundi (Barlow et al., 1995), greenback flounder (Hart et al., 1996), and *Dentex dentex* (Abellan et al., 2000), which showed better larval survival and/or growth in association with longer photoperiods.

Feeding in larval marine finfish is a learning process, which can take only 1 day in black sea bass *Centropristis striata* and 2–3 days in gilthead sea bream (Fielder et al., 2002). Therefore, increased photoperiods can provide more time for larvae to develop their feeding skills by increasing the encounters between larvae and prey organisms and extending the time available for feeding. Longer photoperiods may also cause an increase in growth hormone (GH) levels, as has been reported in juvenile and adult salmonids (Bjoernsson et al., 1989; McCormick et al., 1995; Bjoernsson, 1997; Bjornsson et al., 2000) and goldfish *Carassius auratus* (Marchant et al., 1986). GH mRNA is expressed on d3ph in sea bream larvae (Funkenstein and Almuly, 1998) and GH cells have been shown to develop as early as d4ph in yellowfin tuna larvae (Kaji et al., 1999). GH was shown to enhance somatic growth by stimulating protein synthesis, improving feed conversion, and increasing appetite in many species (Bjoernsson, 1997; Perez-Sanchez and Le Bail, 1999), and may have played a significant role in stimulating higher growth rates under longer photoperiods in this study.

While photoperiod produced significant effects on growth of larval southern flounder in this study, no significant effects on larval survival were evident. Consistent with growth data, survival values appeared higher at 24L (33%) compared with 6L (23%), but because of high replicate variability, statistical significance was not found. Similar results were reported for greenback flounder (Hart et al., 1996), barramundi (Barlow et al., 1995), and common sole (Fuchs, 1978) in which no significant effects of photoperiod on larval survival were observed as long as a light period (6–24L, 12–24L, and 8–24L, respectively) was provided. In contrast with the results of this study, Tuckey and Smith (2001) obtained higher survival in larval southern flounder when reared under 10L than

under 24L or 0L. However, the higher light intensity used in their study (449–834 lx), compared with the present study (150 lx), may have contributed to reduced survival in the 24L treatment (Henne et al., 2001). In the present study, all photoperiod treatments supported adequate feeding, since no significant effects on survival were evident. However, short photoperiods did not support maximum growth. Survival alone is clearly an inadequate measure of larval performance in response to environmental factors.

Early-stage southern flounder larvae were not entirely euryhaline, showing reduced survival and markedly lower growth rates with respect to notochord length, wet weights and dry weights at 25 ppt compared to larvae reared in 34 ppt seawater (Fig. 3). Better growth in full-strength seawater than at lower salinities was also observed in other larval marine finfish species such as *C. mate* (Santerre, 1976), Atlantic halibut (Lein et al., 1997), summer flounder (Watanabe et al., 1998), and European sea bass (Barnabe and Guissi, 1993). These results are consistent with those of Henne et al. (2001), who obtained higher survival and growth rate of southern flounder larvae when reared at 34 than at 24 ppt. Higher survival in full-strength seawater compared to brackish salinities was also reported in other larval marine finfish species, including the greenback flounder which showed better survival when reared at 35 ppt than under 25 or 15 ppt (Hart et al., 1996) and Nassau grouper which showed better survival when reared at 32 and 36 ppt than at 24 or 28 ppt (Ellis et al., 1997).

In contrast to the 'stenohaline marine' character displayed by larval southern flounder in this study, other marine fish species are relatively euryhaline, showing higher survival at lower than full-strength seawater salinities, including the Japanese flounder (higher at 12 and 16 ppt vs. 20–52 ppt) (Mihelakakis and Kitajima, 1994), the European sea bass (higher at 28 ppt vs. 37 ppt) (Barnabe and Guissi, 1993), and the sea bream (higher at 25 ppt vs. 32 or 40 ppt) (Tandler et al., 1995). The physiological and ecological bases for these different responses of marine fish larvae to environmental salinity are unclear.

Our data on whole-body osmolality showed that, with the exception of d4ph, larvae reared at 34 ppt exhibited significantly higher osmolality values ($\bar{x}=344$ mosM kg^{-1}) than larvae reared at 25 ppt ($\bar{x}=296$ mosM kg^{-1}) throughout the study. Some authors have suggested that a significant change in tissue osmolality under different rearing salinities is an indication of an osmotic imbalance and is a symptom of 'nonadaptation' to stressful salinity conditions (Morgan and Iwama, 1996; Sampaio and Bianchini, 2002). However, assuming that rearing at 25 ppt (750 mosM kg^{-1}) should reduce osmoregulatory stress, since it represents more nearly isosmotic conditions (344 mosM kg^{-1}) than 34 ppt seawater (1000 mosM kg^{-1}), then greater osmoregulatory stress at 25 ppt is not likely to have occurred. It follows that other factors may have required the expenditure of energy, compromising growth and survival. A primary factor in this regard may be buoyancy.

It has been shown that the culture salinity (Holliday, 1965) and larval density (Sclafani et al., 1997, 2000) affect buoyancy of marine fish larvae. It has also been proposed that larvae can maintain optimum buoyancy by synthesis or breakdown of lipids (Nursall, 1989). Larval flatfish in the family Bothidae, such as the southern flounder, are characterized by the presence of a swim bladder (Evseenko, 1981), which presumably functions as a hydrostatic organ to regulate buoyancy. In this study, southern flounder

larvae, reared under salinities of 34 or 25 ppt from hatching to d15ph were neutrally or positively buoyant at 34 ppt salinity, but negatively buoyant at 25 ppt. However, larvae reared at 25 ppt were generally more buoyant at 34 ppt than larvae reared at 34 ppt. This is probably due to the lower osmolality seen in the larvae reared at 25 ppt. This was supported by an increase in larval osmolality (Fig. 7) and a decrease in buoyancy (Fig. 8) of the larvae reared at 25 ppt on d4ph. Since the larvae reared in the low salinity were negatively buoyant in 25 ppt medium, they probably allocated energy to maintain vertical positioning, possibly through increased lipid production and/or swimming activity, therefore, impairing growth, and leading to weakened larvae. This is consistent with our data on percent body water, which showed a decrease in body water percent of the larvae reared at 25 ppt on d11ph and d15ph. This decrease in body water was probably due to osmotic water loss and has been associated by other authors to weakened larvae (Sclafani et al., 1997).

The significant interaction between photoperiod and salinity on larval growth (wet and dry weights) obtained on d11ph and d15ph (Fig. 3) can be also explained by the reduced buoyancy of larvae reared at 25 ppt. On d11ph and d15ph, larvae at 34 ppt grew approximately the same under 18L and 24L, while growth of larvae at 25 ppt was substantially slower at 18L than under 24L. A possible explanation is that the larvae reared at 25 ppt under 18L photoperiod experienced suboptimal feeding and/or feed utilization efficiency, due to the reduced buoyancy, while larvae reared under 24L at the same salinity were given adequate time to consume enough energy to overcome the reduced buoyancy problem and spare energy for growth.

The negative buoyancy of the larvae at 25 ppt, observed in this study, may explain why tolerance to lower salinities increases substantially after metamorphosis for southern flounder as well as for other marine flatfish species including summer flounder and greenback flounder. During metamorphosis, these fish settle to the bottom where they spend most of their time. This obviates a need to counteract their negative buoyancy, sparing energy for growth and survival. In this regard, euryhalinity not only describes the ability of a fish to osmoregulate, but also the ability to conserve energy in the face of other factors related to their saline environment, including buoyancy.

In summary, the results of our study demonstrated that growth and survival of larval southern flounder, from hatching through d15ph are maximized under long photoperiods of 24L and 18L and in full-strength seawater. Burke et al. (1991) did not find early-stage southern flounder larvae when sampling a North Carolina estuary within their natural range, supporting the hypothesis that early-stage southern flounder larvae are relatively stenohaline and only adapted to high salinities that characterize oceanic conditions. Euryhalinity is acquired during the metamorphic and post-metamorphic stages coinciding with recruitment to estuaries, which are the primary nursery grounds for these fish. Although southern flounder larvae are exposed to a relatively short photoperiod (10L:14D) in nature, they grew much better under long light regimes. Optimum feeding conditions, including prey availability and optimum light intensities (Henne et al., 2001) used in this study supported higher growth. This study demonstrated that production of early-stage southern flounder larvae can be maximized by rearing under long photoperiods (18L–24L) and under full-strength seawater (34 ppt) salinities.

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