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# Combined Effects of Turbulence and Salinity on Growth, Survival, and Whole-body Osmolality of Larval Southern Flounder

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## Abstract

**The southern flounder (*Paralichthys lethostigma*) is a commercially important marine flatfish from the southeastern Atlantic and Gulf Coasts of the USA and an attractive candidate for aquaculture. Hatchery methods are relatively well developed for southern flounder; however, knowledge of the optimum environmental conditions for culturing the larval stages is needed to make these technologies more cost effective. The objectives of this study were to determine the effects of water turbulence (as controlled by varying rates of diffused aeration) on growth, survival, and whole-body osmolality of larval southern flounder from hatching through day 16 posthatching (d16ph). Embryos were stocked into black 15-L cylindrical tanks under four turbulence levels (20, 90, 170, and 250 mL/min of diffused aeration) and two salinities (24 and 35 ppt) in a 4 × 2 factorial design. Larvae were provided with enriched s-type rotifers from d2ph at a density of 10 individuals/mL. Temperature was 19 C, light intensity was 390 lx, and photoperiod was 18 L:6 D. Significant ($P < 0.05$) effects of turbulence on growth (notochord length [NL], wet weight, and dry weight) were observed. On d16ph, NL (μm) increased with decreasing turbulence level and was significantly greater at 20 mL/min (64.2) and 90 mL/min (58.2) than at 170 mL/min (56.3) and 250 mL/min (57.2). Survival declined primarily during the prefeeding and first-feeding stages from d0 to d8ph, then stabilized from d8 to d16ph. In contrast to growth trends, survival (%) on d16ph increased with increasing turbulence levels and was significantly greater at 170 mL/min (57.9) and 250 mL/min (54.0) than at 20 and 90 mL/min (21.4 and 26.2, respectively). Mean rotifer concentrations (individuals/mL) at 24 h postfeeding were significantly higher ($P < 0.05$) in the low-turbulence treatments of 20 mL/min (4.48) and 90 mL/min (4.23) than in the high-turbulence treatments of 170 and 250 mL/min (2.28 and 2.45, respectively). Under both salinities, larval whole-body osmolality (mOsm/kg) increased with increasing turbulence levels and was significantly higher at 250 mL/min (427) than at 20 mL/min (381), indicating osmoregulatory stress at the higher turbulence levels. On d14ph, larvae in all treatments were positively buoyant in 35 ppt and negatively buoyant in 24 ppt. Results showed that growth of southern flounder larvae in 15-L tanks was maximized under low turbulence levels of 20 and 90 mL/min, while survival was maximized at high turbulence levels of 170 and 250 mL/min. The data suggested that, in prefeeding- and early-feeding-stage larvae (which have weak swimming ability), higher turbulence levels improved buoyancy and prevented sinking. In feeding-stage larvae (which are relatively strong swimmers), higher turbulence levels caused excessive swimming, osmoregulatory stress, and slower growth. Based on these results, we recommend that turbulence levels be maintained relatively high during prefeeding (yolk sac) and first-feeding stages to maintain buoyancy and survival and then decreased for mid- to late-feeding- and premetamorphic stage larvae to optimize prey encounters and feeding efficiency.**

The southern flounder (*Paralichthys lethostigma*) is an important commercial and recreational flatfish ranging from Albemarle Sound, North Carolina, to Corpus Christi Pass, Texas, but is absent in southern Florida (Daniels and Borski 1998). Annual commercial harvest from 1995 to 2004 was 1534 mt, worth $5,911,311 (National Marine Fisheries Service, pers. comm.). In 2004, harvest declined to 1115 mt, worth $3,878,115, causing implementation of a fishery management plan in 2005 (National Marine Fisheries Service, pers. comm.). Declining natural populations, high market value, and wide salinity tolerance (Daniels and Borski 1998) make the southern flounder an attractive species for culture in both coastal and inland areas.

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Methods have been developed for captive spawning of southern flounder using photothermal conditioning and natural or hormone-induced spawning (Berlinsky et al. 1996; Smith et al. 1999; Watanabe et al. 2001). A limitation to commercial-scale culture of southern flounder fingerlings is incomplete knowledge of optimum environmental conditions for culturing the larval stages. Recent studies found that optimum temperature for survival and growth of larvae until metamorphosis was 17 C, while 21 C was optimum for early juveniles (van Maaren and Daniels 2001). Growth of larval southern flounder through day 15 posthatching (d15ph) was maximized at intermediate light intensities (50 and 100 lx) and was minimized at the extremes (5 and 1000 lx) (Henne and Watanabe 2003). Growth rates and survival were also higher at 34 g/L than at 24 g/L and were maximized under long photoperiods of 18–24 h light compared to 6–12 h light (Moustakas et al. 2004).

While available evidence suggests that water turbulence has significant effects on growth and survival of marine finfish larvae, turbulence is a factor that is seldom quantified in marine fish hatcheries. In turbot, *Scophthalmus maximus*, larval survival in seawater (35 g/L) was higher at an aeration rate of 30 mL/min (47%) than at 10 mL/min (24%) (Gaignon et al. 1998). In Mediterranean sea bass, *Dicentrarchus labrax*, larval survival in seawater within a range of 0.5–85 mL/min of diffused aeration was best at an intermediate level of 40 mL/min (58 vs 15–49%) (Barahona-Fernandes 1978). In larval herring, *Clupea harengus*, intermediate turbulence rates (created by an oscillation grid in the water column) resulted in a maximum attack rate of prey and a minimum swimming rate, leading to higher rates of survival and growth (Utne-Palm and Stiansen 2002). In Nassau grouper, *Epinephelus striatus*, static or high turbulence caused by aeration levels of 0.0 and 0.45 L/min, respectively, led to the lower survival rates (0.17 and 13.2%) than intermediate turbulence levels of 0.15 and 0.30 L/min (39.5 and 29.5%) (Ellis et al. 1997).

The available data suggest that, while optimum turbulence levels vary among species, turbulence has pronounced effects on growth and survival of marine fish larvae. The objectives of this study were to determine the effects of different turbulence levels, as controlled by varying rates of diffused aeration, on growth, survival, and whole-body osmolality of larval southern flounder from d1 to 16ph, reared at two different salinities.

## Methods

### *Experimental Animals*

This study was conducted at the University of North Carolina Wilmington Center for Marine Science Aquaculture Facility (Wrightsville Beach, NC, USA). Broodstock southern flounder were maintained in photothermally controlled tanks supplied with recirculating seawater (35 g/L). Naturally spawned eggs were removed from an external egg collector and transferred into an egg separator at 35 g/L where buoyant (viable) eggs were separated from sinking (nonviable) eggs. The buoyant eggs were then placed in a 155-L incubator at 35 g/L and 19 C until hatching. Fertilization rate determined 6 h before hatching was 98%.

### *Experimental System*

This study was conducted in a controlled environment laboratory. The experimental units consisted of cylindrical, black, plastic rearing tanks (working volume = 15 L), which were placed in one of four temperature-controlled water baths (152 × 61 × 23 cm). Temperature in each bath (19 C) was controlled by a heat pump or immersion heater, and air temperature was held at 19 C using a heat pump.

Light was supplied to the rearing tanks by 40-W full-spectrum fluorescent bulbs in light hoods suspended above each bath. Lights were controlled by timers to provide an 18 L:6 D photoperiod beginning at 0600 h (Moustakas et al. 2004). Light intensity was controlled by regulating the height of the hood and with the use of shade cloth. A curtain of black polyethylene surrounded each hood to eliminate extraneous light. Aeration to each rearing unit was supplied through medium-pore silica diffusers (4 × 1.3 × 1.3 cm) placed at the bottom and center of each tank. The airflow was regulated by plastic valves from a polyvinyl chloride pipe manifold that was connected to a 1-hp commercial air blower (Sweetwater Regenerative Blower, Aquatic Eco-systems Inc., Apopka, FL, USA). Aeration was checked twice daily with a flow meter (Cole-Parmer Instrument Co., Vernon Hills, IL, USA) and adjusted as required.

### *Experimental Design*

A 4 × 2 factorial experiment was conducted to determine the effects of turbulence and salinity on growth, survival, and whole-body osmolality of southern flounder larvae from d1 through d16ph. To begin the experiment, embryos were stocked (70 embryos/L) into 32 rearing tanks at 19 C and 35 g/L under four different levels of turbulence, produced by diffused aeration rates of 20, 90, 170, and 250 mL/min. Within each turbulence level, two different salinities of 24 and 35 g/L were maintained. Four replicate rearing units were assigned to each treatment combination of turbulence and salinity.

Seawater was pumped from the Atlantic Intracoastal Waterway, and brackish water (24 g/L) was prepared by diluting filtered (1-μm, UV-treated) seawater with dechlorinated freshwater, obtained from the municipal supply. Chlorine was removed by vigorous aeration for 24 h before use.

### *Feeding*

Beginning on d2ph, larvae were fed rotifers cultured in a "batch system" in 150-L tanks at 19 g/L and 26 C. Rotifers were fed a combination of preserved *Nannochloropsis oculata* and Rotimac (Aquafauna, Bio-Marine Inc., Hawthorne, CA, USA) and were enriched with a commercially prepared enrichment diet (Algamac 2000, Aquafauna, Bio-Marine Inc.) for 6 h before feeding to the larvae. Beginning on d2ph, rotifers were fed at 5 individuals/mL, increasing to 10 individuals/mL from d3ph. Rotifer density in each tank was checked daily at 0900 h, and rotifers were added at 1100 h to maintain 10 individuals/mL.

Live microalgae, *N. oculata*, were added to the rearing tanks at a density of 300,000 cells/mL from d0 to d9ph. Nonviable *N. oculata* (Reed Mariculture Inc., San Jose, CA, USA) was added from d10 to d15ph when contamination affected the live microalgal cultures.

### *Growth, Survival, and Whole-Body Osmolality*

To monitor growth and survival, larvae were sampled at approximately 0730 h from each replicate tank on d4, d8, d12, and d16ph. A known volume of water was sampled from a well-mixed tank until approximately 10 larvae were removed. Larvae were anesthetized (0.5 g/L 2-phenoxyethanol), and the number of living and dead larvae in each sample was recorded. Living larvae were distinguished from dead ones by the presence or absence of a heartbeat, as well as opacity and appearance. Survival was calculated using density data. Notochord length (NL) was measured (to 0.1 μm) using a microscope fitted with an ocular micrometer. To determine wet weights, 10–15 live larvae were gently rinsed with deionized water on a Nitex screen (Aquatic Eco-systems Inc., Apopka, FL, USA), blotted to remove excess water, placed on a preweighed slide, and then weighed on an electrobalance (±0.01 mg) (Sartorius, Goettingen, Germany). The larvae were placed in a laboratory oven and dried for 72 h at 70 C and then reweighed to determine the dry weight.

Larval whole-body osmolality was measured with a vapor pressure osmometer (Wescor Vapor Pressure Osmometer 5520, Logan, UT, USA) on d11–d12ph and d15–d16ph. To measure whole-body osmolality, two to eight fish from each replicate tank were sampled and gently rinsed with deionized water on a Nitex screen, then transferred to the osmometer chamber, and the measurement taken after a 30-min equilibration period.

### *Larval Buoyancy*

To determine buoyancy, approximately 20 larvae were collected from each rearing tank on d15ph. Ten larvae were placed in a 1-L beaker containing 35 g/L water, while the other 10 were placed in 24 g/L water. An anesthetic (0.3 g/L 2-phenoxyethanol) was added to the water prior to the addition of the fish to immobilize them. After the larvae were at rest in the water column (5–10 min), the vertical position of each larva was recorded as the distance from the bottom of the beaker measured to the nearest millimeter. Vertical position was converted to relative buoyancy (100% for the larvae at the surface and 0% for larvae that sink to the bottom) (Moustakas et al. 2004).

### *Water Quality*

Temperature (YSI 55, Yellow Springs, OH, USA; ±0.1 C) and salinity (refractometer; ±1 g/L) were measured daily in each tank at 1100 h. Light intensity was measured daily at the water surface of each tank with a light meter (Extech Instruments, Waltham, MA, USA). Dissolved oxygen (YSI 55; ±0.01 mg/L) was 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). Airflow to each tank was measured twice daily with a flow meter (±1 mL/min) at approximately 1000 and 1600 h and adjusted as needed. Water in each tank was exchanged (50%) daily, and tank surfaces were skimmed with paper towels to remove surface debris.

### *Analytical Methods*

Quantitative values were expressed as treatment means ± SE, with mean values from each tank considered units of observation. The effects of turbulence, salinity, and their interaction were analyzed with a two-way ANOVA. If no interaction was present, salinity treatments were combined within turbulence levels and turbulence levels were combined within salinity treatments for further analysis. The Tukey–Kramer honest significant difference test was used for multiple comparisons among means. All analyses were preformed using JMP (SAS Institute Inc., Cary, NC, USA) statistical software.

## Results

### *Water Quality*

Under both salinities, temperatures (C) were slightly, albeit significantly ($P < 0.05$), higher at 170 mL/min (19.1) than at 250 mL/min (18.6) (Table 1). A significant ($P < 0.05$) effect of turbulence on dissolved oxygen levels was observed but with no salinity or interactive effects (Table 1). Under both salinities, dissolved oxygen (mg/L) was significantly lower in the 20 mL/min (6.69) treatment compared to 90, 170, and 250 mL/min (7.00, 6.97, and 7.05) treatments throughout the study. Percent oxygen saturation ranged from 84.8% at 20 mL/min to 89.2% at 90 mL/min. Light intensity (lx) (mean = 390; range = 324–462), pH (mean = 8.0; range = 7.8–8.2), and TAN (mg/L) (mean = 0.15; range = 0.03–0.25) were not significantly ($P > 0.05$) different among treatments throughout the study.

TABLE 1. *Temperature and dissolved oxygen (mean ± SE, N = 8) under different turbulence levels (20, 90, 170, and 250 mL/min) and salinities (24 and 35 g/L).*

| Turbulence (mL/min) | Salinity (g/L) | Temperature (C) | Dissolved oxygen (mg/L) | Percent saturation (%) |
|---|---|---|---|---|
| | 35 | 18.9 + 0.05 | 6.65 + 0.06 | 0.8 |
| 20 | 24 | 18.8 + 0.03 | 6.73 + 0.05 | 82.7 |
| | 35 | 18.7 + 0.05 | 7.00 + 0.04 | 91.4 |
| 90 | 24 | 18.9 + 0.10 | 7.00 + 0.04 | 86.0 |
| | 35 | 19.5 + 0.25 | 6.90 + 0.06 | 90.1 |
| 170 | 24 | 18.7 + 0.10 | 7.04 + 0.03 | 86.5 |
| | 35 | 18.4 + 0.19 | 7.08 + 0.03 | 92.4 |
| 250 | 24 | 18.8 + 0.15 | 7.03 + 0.04 | 86.4 |

Significant ($P < 0.05$) effects of turbulence level on dissolved oxygen levels were observed.

### *Growth*

On d4ph, there was a significant effect of turbulence level ($P < 0.01$) and a significant interaction ($P < 0.01$) between turbulence and salinity on NLs (Table 2). At 35 g/L, NL (mm) generally increased with increasing turbulence levels from a minimum at 20 mL/min (2.91) to a maximum at 250 mL/min (3.41), while at 24 g/L, NLs were similar (range = 3.32–3.37) under all turbulence levels. On d8ph, NLs ranged from 3.82 to 3.95, with no significant ($P > 0.05$) treatment or interactive effects (Table 2).

On d12 and d16ph, significant ($P < 0.05$ and $P < 0.001$, respectively) effects of turbulence level on NLs were observed, with no significant ($P > 0.05$) salinity or interactive effects. Hence, the effects of turbulence on NL were compared by combining data from both

TABLE 2. *Notochord lengths (mm) (mean ± SE, N = 8) of southern flounder larvae on days 4, 8, 12, and 16 posthatching (dph) under different turbulence levels (20, 90, 170, and 250 mL/min) and salinities (24 and 35 g/L).*

| Age (dph) | Salinity (g/L) | Turbulence (mL/min) 20 | 90 | 170 | 250 | Salinity | Turbulence | Interaction |
|---|---|---|---|---|---|---|---|---|
| | 35 | 2.91 + 0.18 | 2.97 + 0.01 | 3.42 + 0.05 | 3.41 + 0.04 | | ** | ** |
| 4 | 24 | 3.36 + 0.06 | 3.33 + 0.03 | 3.37 + 0.08 | 3.32 + 0.06 | | | |
| | 35 | 3.85 + 0.14 | 3.89 + 0.02 | 3.85 + 0.04 | 3.89 + 0.07 | | | |
| 8 | 24 | 3.95 + 0.01 | 3.87 + 0.08 | 3.84 + 0.03 | 3.82 + 0.05 | | | |
| | 35 | 4.88 + 0.16 | 4.67 + 0.12 | 4.68 + 0.07 | 4.51 + 0.05 | | * | |
| 12 | 24 | 4.83 + 0.17 | 4.75 + 0.08 | 4.76 + 0.07 | 4.31 + 0.05 | | | |
| | 35 | 6.76 + 0.14 | 6.00 + 0.18 | 5.76 + 0.11 | 5.75 + 0.10 | | *** | |
| 16 | 24 | 6.08 + 0.26 | 5.71 + 0.24 | 5.51 + 0.08 | 5.69 + 0.05 | | | |

Asterisks indicate that the factor was significant at the <0.05 (*), <0.01 (**), or <0.001 (***) probability levels.

salinities. On d12ph, a significant ($P < 0.05$) trend toward decreasing NLs with increasing turbulence levels was observed (Table 2). NLs decreased from a maximum of 4.86 at 20 mL/min to a minimum of 4.41 at 250 mL/min. NLs were significantly ($P < 0.01$) higher at the lower turbulence levels of 20–170 mL/min than at 250 mL/min. On d16ph, a similar trend ($P < 0.001$) toward decreasing NLs with increasing turbulence levels was observed (Fig. 1). NL decreased from a maximum of 6.42 at 20 mL/min to a minimum of 5.63–5.72 at 170–250 mL/min. NL at 20 mL/min was significantly ($P < 0.05$) greater than that at 90, 170, and 250 mL/min.

When growth was expressed as wet weight, significant treatment effects were observed for all ages, although differences among treatments were pronounced after d12ph (Fig. 2). On d4ph, a significant effect of turbulence ($P < 0.01$) on wet weight was observed but with no significant effects of salinity ($P > 0.05$) and no interaction ($0.05 < P < 0.10$) between these effects. Wet weight (mg) was significantly greater at higher turbulence levels of 170 and 250 mL/min (0.25 and 0.28, respectively) compared to 20 and 90 mL/min (0.19 and 0.17, respectively).

On d8ph, significant effects of turbulence ($P < 0.05$) on wet weights were observed, while there were no significant ($P > 0.05$) salinity or interactive effects (Fig. 2). On d8ph, wet weights were higher ($P < 0.05$) at 90 mL/min (0.66) compared to 20, 170, and 250 mL/min (range = 0.31–0.40).

On d12ph, a significant effect of turbulence ($P < 0.001$) on wet weight was observed, while

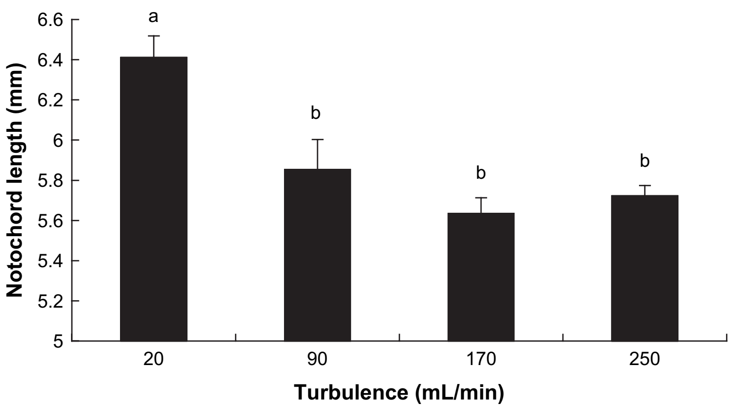


FIGURE 1. *Notochord lengths (mean ± SE, N = 8) of southern flounder larvae under different turbulence levels (20, 90, 170, and 250 mL/min) on day 16 posthatching. Data for both salinities (24 and 35 g/L) were combined under each turbulence level. Means without a letter in common are significantly different (*P *< 0.05).*

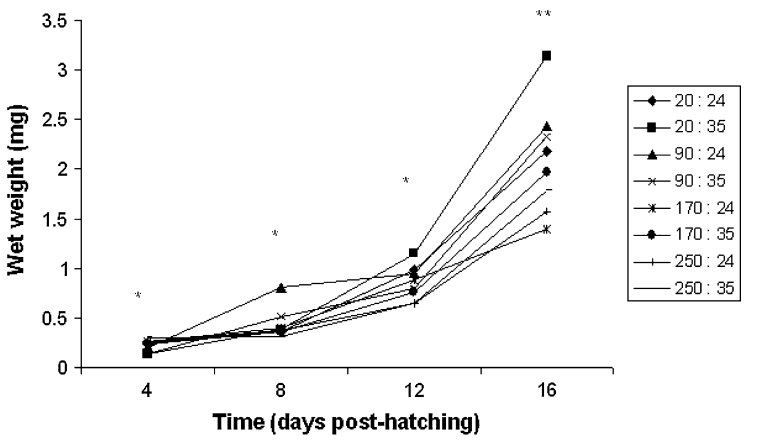


FIGURE 2. *Growth (wet weights) of southern flounder larvae under different turbulence levels (20, 90, 170, and 250 mL/min) and salinities (24 and 35 g/L). Plotted points represent means (N = 8). Asterisks indicate significant treatment (*) or interactive (**) effects observed on that sampling day (*P $< 0.05$*, two-way ANOVA).*

there were no salinity or interactive effects (Fig. 2). Under both salinities, a clear trend toward decreasing wet weights with increasing turbulence levels was observed. Wet weights were significantly ($P < 0.05$) higher at 20 mL/min (1.1) than at 170 mL/min (0.82) and 250 mL/min (0.65).

On d16ph, there were significant effects of turbulence ($P < 0.001$) and salinity ($P < 0.05$) on wet weight, with no significant ($0.05 < P < 0.10$) interaction between these effects (Fig. 3A). Under both salinities, a trend toward decreasing wet weights with increasing turbulence level was observed. Wet weights were higher ($P < 0.05$) at 20 mL/min (2.7) and 90 mL/min (2.4) than at 170 and 250 mL/min (1.7). Under all turbulence levels, wet weights were significantly ($P < 0.05$) higher at 35 g/L (2.3) than at 24 g/L (1.9) (Fig. 3B).

When growth was expressed in terms of dry weight, no significant differences among treatments were observed until d16ph (Table 3). On d4ph, dry weights (μg) ranged from 31.9 to 34.9 among treatments. On d12ph, dry weights ranged from 141 to 178 among treatments. On d16ph, significant effects of turbulence ($P < 0.01$) on dry weights were observed, with no significant salinity ($0.05 < P < 0.10$) or interactive effects ($P > 0.05$) (Fig. 4). Dry weights were significantly greater at 20 and 90 mL/min (243 and 236, respectively) compared to 170 and 250 mL/min (167 and 175, respectively).

### *Survival*

Survival showed marked differences among treatments from d4ph, continued to decline through d8p, and then decreased slightly thereafter (Fig. 5). On d4ph, there was a significant effect of turbulence ($P < 0.05$) and a significant interaction ($P < 0.05$) between turbulence and salinity on survival, with no significant salinity effects ($P > 0.05$). Survival (%) was higher under the higher turbulence levels of 170 and 250 mL/min (66.1 and 65.7, respectively) than at 20 and 90 mL/min (38.8 and 44.1, respectively).

On d8ph, significant effects of turbulence ($P < 0.001$) and salinity ($P < 0.05$) on survival were observed, with no interactive effects ($P > 0.05$) (Fig. 5). Under both salinities, survival increased with aeration from 22.1 at 20 mL/min to 59.2 and 55.5 at 170 and 250 mL/min, respectively. Survival under all turbulence levels was greater under 24 g/L (49.3) compared to 35 g/L (36.1).

On d12ph, there was a significant effect of turbulence ($P < 0.001$) on survival, with no significant ($P > 0.05$) salinity or interactive effects (Fig. 5). As seen on d8ph, a trend toward higher survival with increasing turbulence levels was

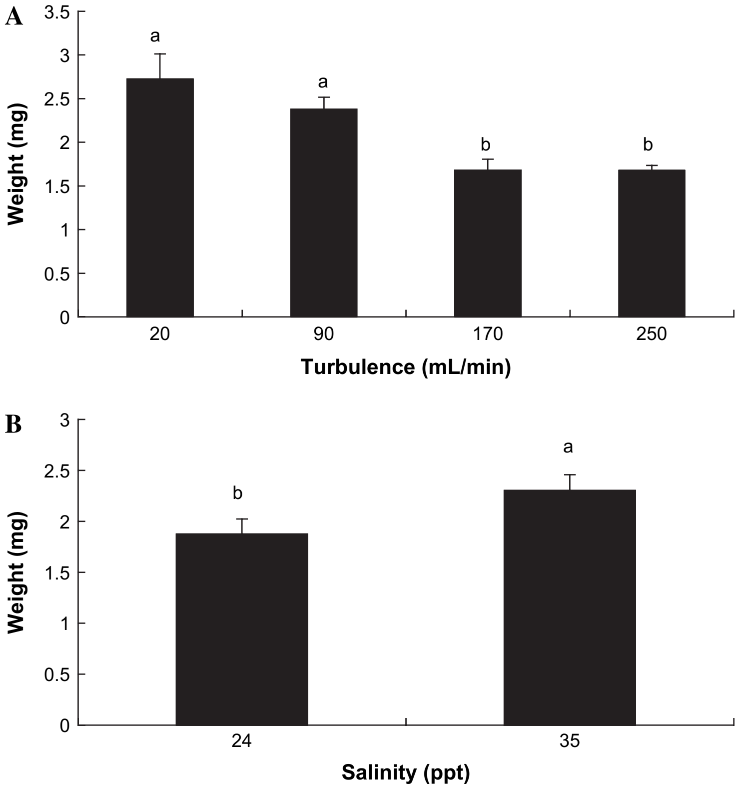


FIGURE 3. *Wet weights (mean ± SE, N = 8) of southern flounder larvae under different turbulence levels (20, 90, 170, and 250 mL/min) (A) and under different salinities (24 and 35 g/L) on day 16 posthatching (B). In (A), data for both salinities were combined under each turbulence level. In (B), data for all turbulence levels were combined under each salinity. Means without a letter in common are significantly different (*P *< 0.05).*

observed from 27.9 at 20 mL/min to 65.7 at 250 mL/min (Fig. 5).

On d16ph, there was a significant effect of turbulence ($P < 0.01$) on survival but with no salinity or interactive effects ($P > 0.05$) (Fig. 5). Under both salinities, survival generally increased with increasing turbulence and was significantly higher at 170 and 250 mL/min (57.9 and 54.0, respectively) than at 20 and 90 mL/min (21.4 and 26.2, respectively) (Fig. 6).

### *Percent Body Water*

On d4ph, highly significant effects of turbulence ($P < 0.001$) and salinity ($P < 0.001$) on percent body water and a significant interaction

TABLE 3. *Dry weight (μg) (mean ± SE, N = 8) of southern flounder larvae on days 12 and 16 posthatching (dph) under different turbulence levels (20, 90, 170, and 250 mL/min) and salinities (24 and 35 g/L).*

| Age (dph) | Salinity (g/L) | Turbulence (mL/min) 20 | 90 | 170 | 250 | Salinity | Turbulence | Interaction |
|---|---|---|---|---|---|---|---|---|
| | 35 | 178 + 7.62 | 162 + 6.54 | 162 + 6.54 | 141 + 12.0 | | | |
| 12 | 24 | 148 + 19.0 | 164 + 4.66 | 159 + 9.87 | 149 + 2.39 | | | |
| | 35 | 293 + 13.7 | 254 + 15.1 | 187 + 14.2 | 174 + 13.7 | | ** | |
| 16 | 24 | 177 + 64.0 | 217 + 8.74 | 163 + 12.0 | 160 + 6.34 | | | |

Asterisks indicate that the factor was significant at the <0.05 (*), <0.01 (**), or <0.001 (***) probability levels.

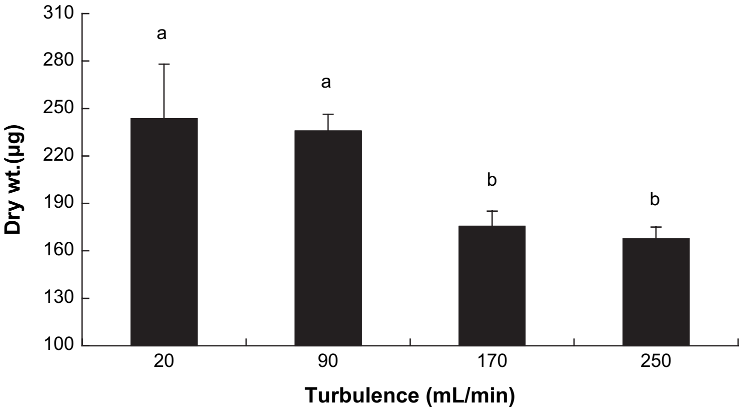


FIGURE 4. *Dry weight (mean ± SE, N = 8) of southern flounder larvae under different turbulence levels (20, 90, 170, and 250 mL/min) on day 16 posthatching. Means without a letter in common are significantly different (*P *< 0.05).*

($P < 0.01$) between these effects were observed (Table 4). Under 35 g/L, percent body water increased with turbulence from 72.4 at 20 mL/min to 87.3 at 250 mL/min. Under 24 g/L, percent body water was similar (range = 84.3–87.9) at all levels of turbulence. On d8ph, percent body water ranged from 93.3 to 96.3 with no significant ($P > 0.05$) treatment or interactive effects (Table 4).

On d12ph, a significant effect of turbulence ($P < 0.05$) on percent body water was observed, with no significant ($P > 0.05$) salinity or interactive effects (Table 4). Percent body water decreased with increasing turbulence from 84.4 at 20 mL/min to 77.6 at 250 mL/min.

On d16ph, a significant effect of turbulence ($P < 0.05$) and a significant interaction ($P < 0.05$) between turbulence and salinity on percent body water were observed (Table 4), with no significant salinity effects ($P > 0.05$). Fish in the 35 g/L treatments showed similar percent body water (range = 88.8–90.6) at all turbulence levels, while fish in 24 g/L showed lower percent body water at the higher turbulence

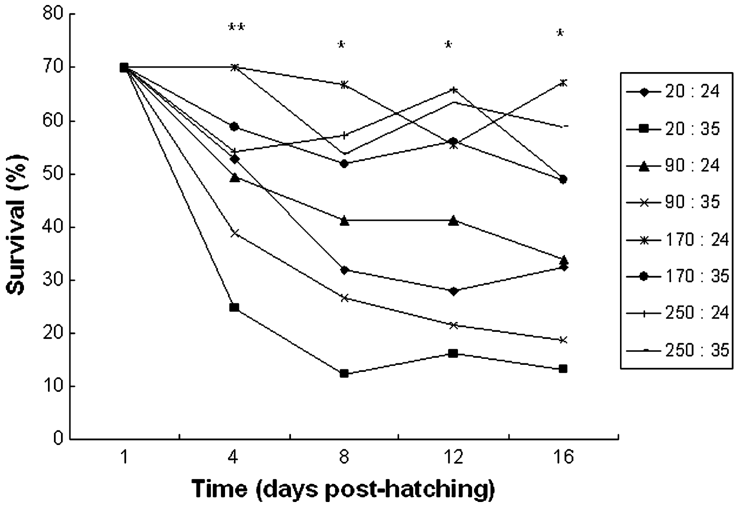


FIGURE 5. *Survival of southern flounder larvae under different turbulence levels (20, 90, 170, and 250 mL/min) and salinities (24 and 35 g/L). Plotted points represent means (N = 8). Asterisks indicate significant treatment (*) or interactive (**) effects observed (*P *< 0.05, two-way ANOVA).*

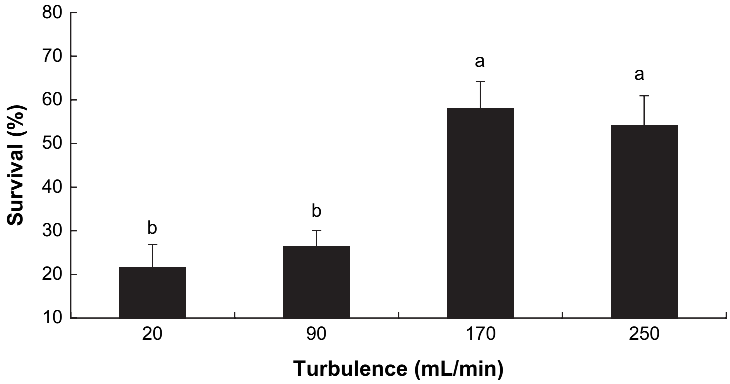


FIGURE 6. *Survival* (mean ± *SE, N* = 8) *of southern flounder larvae under different turbulence levels (20, 90, 170, and 250 mL/min) on day 16 posthatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different (*P < 0.05*).*

levels, decreasing from 92.3 and 91 at 20 and 90 mL/min to 88.0 and 89.8 at 170 and 250 mL/min, respectively.

### *Whole-Body Osmolality*

On d15ph, a significant effect of turbulence ($P < 0.05$) on whole-body osmolality was observed but with no interactive or salinity effects ($P > 0.05$). Whole-body osmolality (mOsm/kg) increased ($P < 0.05$) with increasing turbulence from 381 at 20 mL/min to 427 at 250 mL/min (Fig. 7).

### *Buoyancy*

On d14ph, there were no significant effects ($P > 0.05$) of turbulence and salinity on larval buoyancy, but a significant interaction ($P < 0.05$) between these effects was observed (Fig. 8). Under 35 g/L, relative buoyancy (%) increased with turbulence level from a minimum at 20 mL/min (19) to a maximum at 170–250 mL/min (41–43). Under 24 g/L, relative buoyancy increased from a minimum at 20 mL/min (22) to a maximum at 90–250 mL/min (55–58). Larvae reared at 35 g/L were less buoyant than those reared at 24 g/L (Fig. 8).

### *Rotifer Concentration*

Mean rotifer concentration (measured 24 h postfeeding) varied significantly among treatments. There was a significant ($P < 0.01$) effect of turbulence on the mean rotifer concentrations (Fig. 9), while the effects of salinity ($0.05 < P < 0.10$) and interactive effects ($0.05 < P < 0.10$) were not significant. Mean rotifer concentrations (individuals/mL) were significantly

TABLE 4. *Body water (%) (mean ± SE, N = 8) of southern flounder larvae on days 4, 8, 12, and 16 posthatching (dph) under different turbulence levels (20, 90, 170, and 250 mL/min) and salinities (24 and 35 g/L).*

| Age (dph) | Salinity (g/L) | Turbulence (mL/min) | | | | Salinity | Turbulence | Interaction |
|---|---|---|---|---|---|---|---|---|
| | | 20 | 90 | 170 | 250 | | | |
| | 35 | 72.4 + 4.49 | 72.8 + 1.53 | 85.6 + 0.57 | 87.3 + 2.14 | *** | *** | ** |
| 4 | 24 | 86.3 + 1.05 | 84.3 + 0.58 | 87.9 + 0.44 | 87.3 + 1.12 | | | |
| | 35 | 95.1 + 8.22 | 94.9 + 0.80 | 94.6 + 1.12 | 93.3 + 1.26 | | | |
| 8 | 24 | 95.1 + 3.83 | 96.3 + 1.21 | 94.2 + 0.82 | 93.5 + 0.57 | | | |
| | 35 | 84.4 + 0.83 | 80.1 + 0.80 | 77.3 + 3.47 | 78.4 + 1.31 | | * | |
| 12 | 24 | 84.4 + 2.22 | 82.6 + 1.21 | 81.8 + 1.29 | 76.7 + 1.71 | | | |
| | 35 | 90.6 + 0.23 | 88.8 + 0.75 | 90.5 + 0.33 | 90.2 + 0.35 | | * | * |
| 16 | 24 | 92.3 + 1.48 | 91.0 + 0.14 | 88.0 + 1.45 | 89.8 + 0.10 | | | |

Asterisks indicate that the factor was significant at the <0.05 (*), <0.01 (**), or <0.001 (***) probability levels.

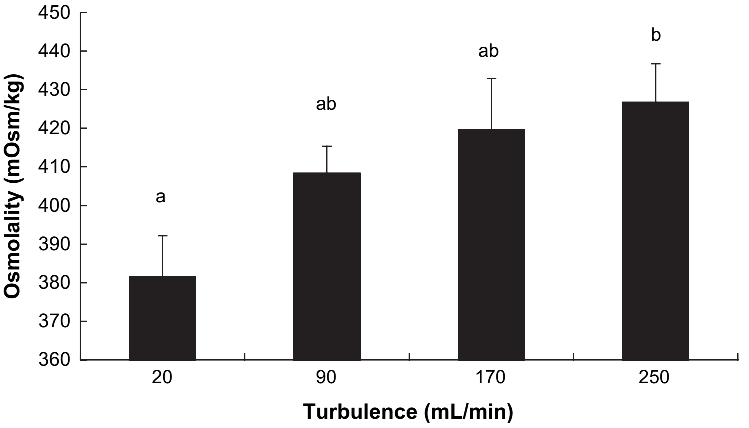


FIGURE 7. *Whole-body osmolality* (mean ± *SE, N* = 8) *of southern flounder larvae under different turbulence levels (20, 90, 170, and 250 mL/min) on day 15 posthatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different (*P < *0.05).*

($P < 0.05$) higher in the low-turbulence treatments of 20 mL/min (4.48) and 90 mL/min (4.23) than in the high-turbulence treatments of 170 and 250 mL/min (2.28 and 2.45 individuals/mL, respectively).

## Discussion

Temperature and dissolved oxygen in all treatments were within the optimal ranges for southern flounder larvae. Differences among treatments were very small and were not likely to have influenced the results of this study. Temperature was slightly lower at 250 mL/min (18.6 C) than at 170 mL/min (19.1 C). However, these values are within the optimum temperature range (17–21 C) for culture of southern flounder larvae up through metamorphosis (van Maaren and Daniels 2001). Dissolved oxygen levels were slightly lower at 20 mL/min (6.7 mg/L) than at higher turbulence levels (range = 6.33–7.29 mg/L); however, oxygen saturation was >82% in all treatments throughout the study. At dissolved oxygen concentrations >5 mg/L, no adverse effects on the larvae would be expected (Stickney 2000).

On d4ph, there was a significant interaction (Table 2) between turbulence and salinity on NLs. This was probably related to acclimation to experimental conditions from d1 to d4ph. All eggs were incubated at 35 g/L and 19 C until hatching and then abruptly transferred to

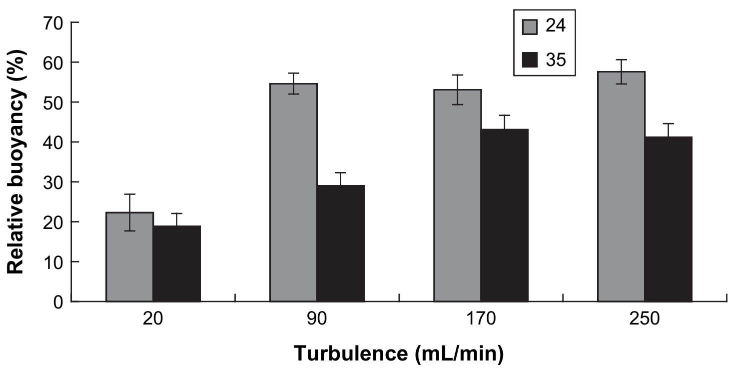


FIGURE 8. *The relative buoyancy (mean ± SE, N = 8) of southern flounder larvae reared under different turbulence levels (20, 90, 170, and 250 mL/min) and salinities (24 and 35 g/L) on day 14 posthatching.*

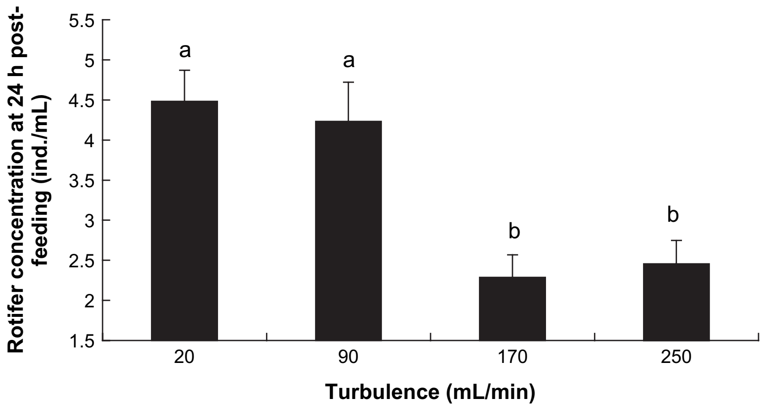


FIGURE 9. *Mean rotifer concentrations (individuals/mL) 24 h after introduction to rearing tanks under different turbulence levels (20, 90, 170, and 250 mL/min). Values represent daily means* ± *SE (N* = *8) from d4– to 16ph. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different (*P < *0.05).*

the experimental tanks at 24 and 35 g/L. Turbulence levels were adjusted to treatment conditions (20–250 mL/min) within several hours. Fish maintained at 35 g/L showed increased NLs with increasing turbulence levels, while fish transferred to 24 g/L showed similar growth at all levels of turbulence.

In this study, the largest decline in larval survival was seen in yolk-sac-stage larvae during the first 4 d posthatching (Fig. 5), with lower survival at the lowest turbulence levels of 20 and 90 mL/min. Lower turbulence levels probably allowed the yolk-sac-stage larvae to sink to the bottom where they were exposed to low dissolved oxygen and to bacterial attack, whereas higher turbulence levels maintained the larvae in the water column. However, at the lower turbulence levels, larvae in 24 g/L (closer to isosmotic salinity) were larger than those in 35 g/L on d4ph, indicating that these larvae may have gained an osmoregulatory advantage, allowing better growth. At higher turbulence levels, growth to d4ph was similar at 24 and 35 g/L, possibly because high turbulence allowed yolk sac larvae to spend less energy in swimming and more on growth.

In this study, survival continued to decline significantly in early-feeding-stage larvae from d4 through d8ph (Fig. 5) and then generally stabilized through d16ph, when a clear trend toward higher survival with increasing turbulence within the range 20–250 mL/min was observed. This was primarily related to the marked treatment effects on survival during the yolk sac and first-feeding stages (d1–d8ph), because survival generally stabilized after d8ph when larvae were feeding on rotifers. In earlier studies, larval southern flounder were reared in 15-L tanks using low turbulence levels of only 30–50 mL/min to avoid damaging the delicate early larval stages (Henne and Watanabe 2003; Moustakas et al. 2004). Larval southern flounder evidently require much higher levels of turbulence, because survival in 15-L tanks was markedly improved by increasing turbulence as high as 250 mL/min.

Precise comparisons among studies dealing with the effects of turbulence levels on marine finfish larvae are difficult as a result of variations in experimental conditions used, particularly tank size and configuration. However, the available data suggest strong species-specific differences in the effects of turbulence on larval performance. Improved survival of larval marine finfish with increasing turbulence levels has been reported for some species. In larval turbot, *Psetta maxima* (Gaignon et al. 1998), survival to d9ph in 150-L tanks increased with increasing turbulence within a range of 0.5–85 mL/min. Survival of Australian bass, *Macquaria novemaculeata*, larvae to d10ph was higher (86.8%) at higher turbulence levels

(>1000 mL/min) compared to low turbulence levels (<50 mL/min; 75.8%) or static conditions (0 mL/min; 76.1%) in 60-L tanks at 15–35 g/L (Battaglene and Talbot 1993).

In other species, high turbulence levels were found to impair larval survival. In sea bass, *D. labrax*, highest survival of embryos and larvae in 150-L tanks was obtained at a moderate aeration rate of 40 mL/min within a range of 0.5–85 mL/min (Barahona-Fernandes 1978). Survival of larval Nassau grouper, *E. striatus*, was best under a relatively low turbulence level of 150 mL/min (39.5%) compared to 450, 300, and 0 mL/min (13.2, 29.5, and 0.17%, respectively) in 500-L tanks (Ellis et al. 1997). These workers suggested that larvae were evenly dispersed in the water column under lower turbulence levels, instead of congregated on the walls of the tank as under static conditions. Excessive turbulence levels, however, increased contact between larvae and the tank walls, resulting in injury (Ellis et al. 1997).

The results of this study showed that southern flounder larvae were neutrally or positively buoyant at 35 g/L and negatively buoyant at 24 g/L. Therefore, fish reared in 24 g/L may have exhibited lower growth rates because they needed to expend energy to swim and maintain position in the water column (Moustakas et al. 2004). As reported in winter flounder, *Pleuronectes americanus* (Litvak 1999), increased survival of southern flounder larvae at higher turbulence levels may be related to their lack of a swim bladder, because turbulence helps maintain buoyancy in rearing tanks. This could be especially important within the first few days after hatching when larvae are weak swimmers and they depend mainly on environmental conditions for buoyancy. In contrast, a marine finfish larva with a fully developed swim bladder could regulate its position in the water column without relying on turbulent conditions for buoyancy.

Whereas survival of yolk-sac- and first-feeding-stage larvae (d1–d8ph) improved with increasing turbulence levels in this study, growth rates decreased at higher turbulence levels in older feeding-stage larvae. First-feeding and weak fish larvae benefit from small-scale water turbulence, because higher turbulence generates mixing, making the distribution of larvae and prey more homogenous within the culture vessel and increasing prey encounter rates and probability of successful capture (Barahona-Fernandes 1978; Rothschild and Osborn 1988; MacKenzie and Leggett 1991; MacKenzie et al. 1994). A dome-shaped relationship between turbulence and prey ingestion rates in larval fish was suggested, with maximal attack and ingestion rates at intermediate levels of turbulence (MacKenzie 1994; Gallego et al. 1996). Highest levels of turbulence, however, increase swimming rates and reduce attack rates and produce negative effects on growth, as was observed in larval herring (Utne-Palm and Stiansen 2002). Because swimming ability in southern flounder improved markedly around d10ph when the process of notochord flexion began and the fin rays and the caudal fin were developed, highly turbulent conditions may have caused excessive swimming and impaired feeding and growth.

Lower growth rates at higher turbulence levels were also related to lower prey/larvae ratios, caused by high larval densities at high turbulence levels. Starting from d3ph, prey was added each morning to maintain a concentration of 10 individuals/mL, even as larval survival declined during the study. So, fish held under low-turbulence conditions (e.g., 20 and 90 mL/min) where survival was poor encountered higher prey concentrations (prey/larvae ratios) than fish held under high-turbulence conditions (170 and 250 mL/min) where survival was better (Fig. 9). Hence, lower prey availability at higher turbulence levels may have also decreased feeding and growth. Increased larval growth at higher prey densities has been reported in a number of marine fish species, including bay anchovies (*Anchoa mitchilli*; Saksena and Houde 1972), sea bream (*Archosargus rhomboidalis*; Dowd and Houde 1980), and Japanese flounder (*Paralichthys olivaceus*; Dou et al. 2003).

Lower growth rates at higher turbulence levels may have also been related to crowding (as a result of higher survival at higher turbulence levels), causing reduced availability of space and consequent endocrine responses or disruption of feeding efficiency (Pankhurst and Van der Kraak 1997; Schreck et al. 1997). A negative relationship between larval stocking density and growth was reported in summer flounder, *Paralichthys dentatus* (King et al. 2000), and in sea bream, *A. rhomboidalis* (Houde 1975).

Growth (wet and dry weights) of southern flounder larvae was higher in 35 g/L than in 24 g/L, consistent with what was previously reported for this species (Henne and Watanabe 2003; Moustakas et al. 2004). In southern flounder, euryhalinity is acquired after metamorphosis as these fish transition from a pelagic to a benthic mode of existence (Moustakas et al. 2004).

Larval southern flounder under the highest turbulence level (250 mL/min) had significantly higher osmolality values (427 mOsm/kg) than those reared at the lowest turbulence level (20 mL/min; 382 mOsm/kg), indicating greater osmotic stress on the larvae at the higher turbulence level. This was related to lower percent body water with increasing turbulence levels, which is also consistent with osmoregulatory failure in a hyperosmotic (24–36 g/L) environment. Lower food availability (i.e., prey densities) and higher swimming activity at higher turbulence levels caused inadequate allocation of energy for osmoregulation. In juvenile turbot (*S. maximus*), suboptimal temperature (10 C) and salinity (33.5 g/L) combinations produced higher internal osmotic pressure (336) than normal (324) (Imsland et al. 2003).

To summarize, survival of southern flounder larvae in 15-L tanks was maximized at higher turbulence levels of 170–250 mL/min, while growth was maximized at lower turbulence levels of 20–90 mL/min. During the yolk sac stage, higher turbulence enhanced buoyancy and improved survival. At the first-feeding stage, higher turbulence levels promoted an even distribution of larvae and prey, prey encounter rates, and successful feeding. During the feeding stages, when larval swimming and hunting abilities were well developed, increased swimming activity and lower prey densities at higher turbulence levels caused osmoregulatory stress and reduced growth.

Based on these results, we recommend turbulence levels be maintained relatively high during the prefeeding and early-feeding stages and then decreased for feeding- and premetamorphic stage larvae. While specific turbulence levels will vary with tank size and configuration, these levels should maintain buoyancy and survival in early yolk sac (prefeeding stage) larvae and optimize prey encounters and feeding efficiency in feeding-stage larvae.

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