INTRODUCTION

Life History and Biology

The three leading commercially valuable species of flatfish caught in the U.S are the summer flounder (*Paralichthys dentatus)* and southern flounder (*Paralichthys lethostigma)* from the Atlantic Ocean, and the California halibut (*Paralichthys californicus)* from the Pacific Ocean. The flounder fisheries in the United States contributed 39,145 metric tons to the commercial fishing industry in 2001 (NDMFS, 2001). Southern flounder accounted for 1,597 metric tons, worth $5,635,926 from 2000 - 2001 (NDMFS, 2001). Its range extends from Albemarle Sound, NC to Corpus Christi Pass, TX, but it is absent around the southern tip of Florida (Daniels and Borski 1998). It is most commonly caught in areas of soft, organic bottoms and low salinities (Gingsburg 1952; Rogers et al. 1984; Powell and Schwartz 1997). The North Carolina Division of Marine Fisheries predicts that the decline in summer flounder populations will cause anglers to look toward the southern flounder, subsequently putting stress on this fishery as well (Waters 1999). Also, major habitat degradation in recruitment areas of the southern flounder contributes to the declining numbers caught by fishermen (Waters 1999).

The spawning season for the southern flounder is between December and February, when the photoperiod is 10 h L: 14 h D and the water temperature is between 14 and 18 C° (Henderson-Arzapalo et al. 1988; Powell and Schwartz 1977). The southern flounder is sexually dimorphic, with females growing larger than males (Smith et al. 1999). Once this species reaches sexual maturity, around two years old and between 30 and 40 cm (TL), it migrates offshore to deeper water to spawn (Daniels and Borski 1998; Smith et al. 1999). In the springtime, newly metamorphosed juveniles begin to move to the head of the estuary and toward the oligohaline zone of the riverine habitat (Burke et al. 1991). Southern flounder grow quickly at low salinities

and are most often captured in this environment (Rogers et al. 1984; Burke et al. 1991; Powell and Schwartz 1977). They stay in the estuary for the first 18 to 20 months before heading out to sea (Powell and Schwartz 1977).

Aquaculture methods for the southern flounder are advancing rapidly. The southern flounder is considered a good candidate for aquaculture because of its high market value and wide salinity tolerance at all ages (Daniels and Borski 1998). Its ability to survive in salinities of 0 ppt to +30 ppt makes it an attractive species for culture in both coastal and inland areas. The southern flounder has been successfully strip spawned when implanted with GnRHa (gonadotropin releasing hormone analog) packed in cholesterol-cellulose matrix (Berlinsky et al. 1996; Smith et al. 1999). The GnRHa treatment induces repetitive oocyte maturation and ovulation in females (Berlinsky et al. 1996; Henderson-Arzapalo 1988). GnRHa implants, along with photothermal conditioning, have also resulted in successful tank spawning with increased fertilization (Smith et al. 1999). Recently, sustained natural spawning of southern flounder broodstock has been observed with increased numbers of viable eggs and better egg quality compared to strip spawning (Watanabe et al. 2001). This was accomplished with artificial photoperiod and temperature control on an annual cycle.

Larvae

One limitation to commercial-scale culture of southern flounder fingerlings is that knowledge of optimum environmental conditions for culturing the larval stage is incomplete. New experiments and techniques are being explored each year resulting in and significant numbers of eggs and larvae being reared through juvenile stages. However, research is needed to improve these technologies and to make them cost-effective.

Temperature

The effects of temperature on survival of fish larvae cannot be overemphasized (Fukuhara 1990). There is an optimum temperature range that yields maximum growth and energetic efficiency (Brett 1979). The normal morphological development of larval fish depends on being within an optimum temperature range (Santerre 1977).

Larval temperature requirements of southern flounder have been explored by van Maaren and Daniels (2001). They found that, while 17°C was an optimum temperature for survival and growth of larvae until metamorphosis, 21°C was optimum for early juveniles.

Illumination

Henne (2003) established illumination and salinity conditions to optimize growth and survival of larval southern flounder reared through the first 15 days post hatch (dph). He found that growth was maximized at intermediate light intensities (50 and 100 lx) and minimized at extremes (5 and 1000 lx). He also found that growth rates and survival were higher at 34 compared to 24 ppt. Moustakas et al. (2004) found that growth and survival in both 24 and 34 ppt were maximized under long photoperiods of 18-24 h L compared to 12 and 6 h L.

Salinity

In salinities near natural seawater (32 ppt, 1,000 mOsm/Kg), marine fish maintain the osmolality of their body fluids (whole-body osmolality) below that of the ambient environment (Hoar and Randall 1969). Larval fish use energy to actively transport ions and regulate internal ion concentrations (Brett 1979). For example, the tissue osmolality of larval southern flounder in

full strength seawater is 400 mOsm/Kg (Moustakas 2004). In theory, rearing under iso-osmotic salinities (15 ppt) can reduce osmoregulatory costs and improve growth rates. Imsland et al. (2003) reported that growth rates in juvenile turbot (*Scophthalmus maximus*) were related to the amount of energy spent on osmoregulation, with higher growth rates at a lower salinity of 15 ppt (near iso-osmotic compared to the blood). They also found that increasing salinities increased the plasma osmolality (Imsland et al. 2003). However this concept has been challenged because of inconsistent results among species. Henne et al. (2003) reported that survival and growth rates of southern flounder larvae were higher at 34 ppt than at 24 ppt and that whole-body osmolality was lower in 24 ppt than in 34 ppt. Moustakas et al. (2004) found that in addition to survival and growth, larval body water percentage and whole-body osmolality of southern flounder were higher at 34 ppt than at 25 ppt. Summer flounder larvae were grown to larger sizes in full strength seawater (36 ppt) compared to 26 ppt (Watanabe et al. 1998), while growth of juvenile greenback flounder (*Rhombosolea tapirina*) juveniles was not affected by salinities between 15 and 45 ppt (Hart and Purser 1995). Daniels and Borski (1998) found that pre-metamorphosed southern flounder could not withstand salinities below 20 ppt; however, just days after metamorphosis these fish could handle salinities as low as 0 ppt.

Rearing salinity (Holiday and Jones 1965) and larval health (i.e., feeding success) (Sclafani et al. 1997; Sclafani et al. 2000) can affect the buoyancy of marine fish larvae. Watanabe et al. (1998) hypothesized that summer flounder larvae exhibit better growth rates in higher salinity because of the positive effect of increased salinity on buoyancy, which reduces energetic demands to maintain itself in the water column. Moustakas et al. (2004) found that southern flounder larvae raised in 34 ppt and 24 ppt were positively or neutrally buoyant at 34 ppt, but

negatively buoyant at 24 ppt. They also found that larvae reared in 34 ppt were more buoyant in salinities of 34 and 24 ppt than fish reared in 24 ppt.

Turbulence

While available evidence suggests that water turbulence affects survival of marine finfish larvae, turbulence is a factor that is seldom quantified in marine fish hatcheries. Gaignon et al. (1998) found that air flows of 30 mL/min increased larval survival of juvenile turbot when compared to 10 mL/min in full strength seawater (35 ppt). Ellis et al. (1997) compared four different turbulence levels in full strength seawater on survival of larval Nassau grouper (*Epinephelus striatus*). They found that static or high turbulence caused by aeration levels of 0.0 L/min and 0.45 L/min, respectively, led to the lower survival values (0.17% and 13.2 %, respectively) than intermediate turbulence levels of 0.15 and 0.30 L/min (39.5% and 29.5%, respectively). They also found that Nassau grouper larvae survived better in water of 32-36 ppt than at 24-28 ppt (Ellis et al. 1997). In a study on Mediterranean sea bass larvae (*Dicentrarchus labrax*) comparing turbulence levels ranging from 0.5 mL/min up to 85 mL/min in full strength seawater, lower turbulence levels produced poor survival (Barahona-Fernandes 1978). The best survival occurred at an intermediate aeration level of 40 mL/min (Barahona-Fernandes 1978). In a study on larval herring, intermediate turbulence rates (created by a vertically moving oscillation grid in the water column) resulted in a maximum attack rate of prey and a minimum swimming rate, hence saving energy and leading to higher rates of survival and growth (Utne-Palm and Stiansen 2002). The highest levels of turbulence had negative effects on the larvae, where attack rates were the lowest and swimming rates were the highest. In larval paddlefish (*Polyodon spathula*) highest turbulence levels resulted in larval mortalities of 80 to 87%, while

larvae exposed to low turbulence only showed a 3 - 13% mortality (Killgore et al. 1987). The available data suggests that turbulence has pronounced effects on growth and survival of marine fish larvae, however; the optimum turbulence level varies among species.

Based on available information, I hypothesize that the effects of turbulence on marine fish larvae may depend on buoyancy, which in turn is affected by salinity. As salinities are lowered (below full strength seawater) larvae become less buoyant, eventually reaching a point of negative buoyancy at low salinities that would cause larvae to sink in the water column. Larvae need to spend energy on swimming to maintain position in the water column at low salinities. Hence, greater turbulence of the water is required to maintain their buoyancy. Iso-osmotic salinities should reduce osmoregulatory stress.

Oxygen Consumption

Oxygen consumption is a good indicator of stress on an organism. Rates of oxygen consumption vary among species and with environmental conditions. The response to suspended particle stress in striped bass was a depression in oxygen consumption (Neumann 1982). Juvenile southern flounder showed an increase in oxygen consumption when temperature was increased from 13°C to 29°C (van Maaren et al. 2000). The highest values of oxygen consumption occurred between 21°C and 25°C (2.68 μg O_2/g fish/min) and decreased again between 25°C and 29°C (1.29 μg O_2/g fish/min) (van Maaren et al. 2000). The optimal temperature for growth was between 25°C and 29°C at salinities of 0 – 34 ppt, which was associated with the lowest oxygen consumption rate (1.29 μg O_2/g fish/min) (van Maaren et al. 2000). Taylor and Miller (2001) found that routine respiration (or oxygen consumption) increased with increasing dissolved oxygen levels in juvenile southern flounder. In larval sole

and gilt-head seabream, no significant difference in rates of oxygen consumption were found in relation to larval size, under optimal rearing conditions for these species (Parra and Yufera 2001). In southern flounder, however, rates of oxygen consumption increased with fish size (van Maaren et al. 2000). Mean oxygen consumption per individual flounder larva (*Paralihthys olivaceus*) increased with time after hatching (Kurokura et al. 1995), however, it did not change with age in larval striped mullet (*Mugil cephalus*) (10.838 μL O_2/mg dry wt/h) (Walsh et al. 1989).

Oxygen consumption also increases with abrupt exposure to different salinities. In European sea bass (*Dicentrarchus labrax*), oxygen consumption was 80% greater than the routine level when a salinity change occurred (both stepwise from 37-20-5-2-5-20-37 ppt, and abruptly from 37-50 ppt) (Dalla Via et al. 1998). It decreased to pre-exposure levels 3-10 h after the salinity change (Dalla Via et al. 1998). However, adult European sea bass showed no relationship between salinity and oxygen consumption when exposed to salinities of 30, 20, 10, and 5 ppt after an acclimation period of 10 to 15 days (Claireaux and Lagardere 1999). Also, the oxygen consumption rates of striped mullet larvae did not vary significantly among salinities of 10-35 ppt (Walsh et al. 1989). The striped mullet's habitat is the near-shore environment, so the ability to maintain stable oxygen consumption rates in a wide range of salinities may be pre-adapted for this species (Walsh et al. 1989).

Moustakas et al. (2004) found that reduced salinities (25 ppt) cause a decrease in larval buoyancy of southern flounder. We hypothesize that to counteract sinking at low salinity, the larva must spend energy to maintain its position in the water column. This increased activity can be expected to increase oxygen consumption, which may therefore be an indicator of physiological stress or an increased energy demand.

Objectives

The objectives of this study were to determine the effects of different turbulence levels, as controlled by varying rates of diffused aeration levels, on growth, survival, and whole body osmolality of larval southern flounder from d1 –16ph, reared at different salinities.

Null Hypothesis

Turbulence will have no effect on growth, survival, and whole body osmolality of larval southern flounder, reared at different salinities.

Alternative Hypothesis

The effects of turbulence on growth, survival, and whole body osmolality will vary with salinity, with optimal turbulence levels increasing at lower salinities.

METHODS

Experimental Animals

This study was conducted at the University of North Carolina Wilmington's Center for Marine Science Aquaculture Facility (Wrightsville Beach, NC, USA). Broodstock southern flounder were maintained in photothermally controlled tanks supplied with recirculating seawater. Broodstock were allowed to naturally spawn in tanks and the eggs were collected the following day. Eggs were then transferred into an egg separator (in 35 ppt seawater) where buoyant (viable) eggs were separated from sinking (non-buoyant) eggs. The positively buoyant eggs were then placed in a 155-L incubator at 35 ppt and 19°C until hatching. Fertilization rate determined 6 h before hatching was 98%.

Experimental System

The experiment was conducted in a controlled-environment laboratory. Larvae were reared in cylindrical black plastic rearing tanks (working volume = 15 L), which were placed in one of four temperature-controlled water baths (152 cm x 61 cm x 23 cm). Temperatures (19°C) in the water baths were controlled by heat pumps or submerged glass heaters. The laboratory air temperature was also held at the experimental rearing temperature (using air-conditioning).

Light was supplied to the rearing tanks by 40-W full spectrum fluorescent bulbs in light hoods suspended above each bath. 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. The light hoods were controlled by timers set to 18 h L : 6 h D beginning at 0600 h, a photoperiod found to maximize growth rates in larval southern flounder (Moustakas et al. 2004).

Aeration was supplied through diffusers (4 x 1.3 x 1.3 cm) placed at the center of each tank. The air flow was regulated by plastic valves from a PVC pipe manifold that was connected to a commercial air blower. Aeration was checked twice daily with a flow meter (Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA ± 1 mL/min) and adjusted as required.

Experimental Design

A 4 x 2 factorial experiment was conducted to determine the effects of turbulence and salinity on the growth, survival, and whole body osmolality of southern flounder larvae from day 1 post-hatching (d1ph) through d15ph. To begin the experiment, buoyant, viable embryos were stocked at a density of 50 embryos/L into thirty-two larval rearing tanks at 19°C, under four different levels of turbulence, produced by aeration rates of 20, 70, 120, and 170 mL/min. Within each

turbulence level, two different salinities of 24 and 35 ppt were maintained. There were four replicate rearing units for each treatment combination of turbulence and salinity.

Seawater (35 ppt), obtained from the Atlantic Intracoastal Waterway adjacent to Wrightsville Beach was filtered through a 1-µm screen and treated with u.v. light before use. The 24 ppt water was prepared by diluting filtered seawater with dechlorinated fresh water, obtained from the municipal supply. Chlorine was removed by vigorous aeration for 24 h before use. The filtered (1-µm; uv-treated) seawater and brackish water (24 ppt) were stored in separate reservoirs and heated to 19°C.

Feed

Beginning d2ph, larvae were fed rotifers cultured in a "batch system". The rotifers were maintained in 150-L tanks at approximately 26°C and 19 ppt and fed a combination of preserved *Nannochloropsis oculata* and Rotimac (Aquafauna, Bio-Marine Inc., Hawthorne, CA, USA). Rotifers were enriched daily for 6 h before feeding with a commercially prepared enrichment diet (Algamac 2000, Aquafauna, Bio-Marine Inc., Hawthorne, CA, USA) to improve their nutritional value to the fish larvae.

Each morning, rotifers were harvested and thoroughly rinsed with freshwater before transfer to a 15-L incubator for enrichment. They were fed a small amount of preserved *Nannochloropsis oculata* until the enrichment was added. Before feeding, the rotifers were counted and rinsed with freshwater.

Rotifers were stocked at a density of 5 ind./mL on d2ph, approximately 36-48 h before the first-feeding stage to introduce the larval fish to their prey. The density was increased to 10 ind./mL on d3ph and maintained at this level for the duration of the experiment. The rotifer

density in each culture vessel was checked daily (using volumetric sampling methods) at 0900 h, and rotifers were added at 1100 h to maintain 10 ind./mL.

Live microalgae, cultured at the aquaculture facility, were added to the rearing tanks at a cell density of 300,000 cells/mL. Preserved *Nannochloropsis oculata* (Reed Mariculture Inc., San Jose, CA, USA) was substituted for live microalgae when contamination affected the live algal cultures on d 10 – 15 ph.

Growth, Survival, and Whole-Body Osmolality

To monitor the growth and survival, larvae were sampled from each replicate tank on d3, 6, 10, 13, and 15ph. A known volume of water was sampled from a well-mixed tank until approximately 10 larvae were removed. The sampling was initiated at approximately the same time (0730 h) on each sampling day. Survival (calculated using volumetric density data), notochord length (μm), yolk-sac length and width, and oil droplet diameter, were recorded from anesthetized (0.5 ppt 2-phenoxyethanol) fish. Living larvae were distinguished from dead ones by the presence or absence of a heartbeat, as well as opacity and appearance. Notochord length was measured (to 0.1μm) using a microscope fitted with an ocular micrometer. To determine wet weights, ten to fifteen live larvae were gently rinsed with deionized water on a Nitex screen, blotted to remove excess water, placed on a preweighed slide, and then weighed on an electrobalance (±0.01 mg) (Sartorius, Goettingen, Germany). The weight of the slide was subtracted from the weight of the larvae and the slide to determine the wet weight. The larvae and the slide were placed in a laboratory oven and dried for 72 h at 70°C and weighed again to determine the dry weight.

Survival rate was recorded by noting the number of living and dead larvae in each sample, and the feeding rate was determined by the visual examination of the gut. Percent fullness was estimated by visually estimating the gut contents of the larvae and placing them into five categories: empty, 25% full, 50% full, 75% full or full (Puvanendran and Brown 2002).

Larval whole-body osmolality was measured with a vapor pressure osmometer (Wescor Vapor Pressure Osmometer 5520, Logan, Utah, U.S.A), on d11-12ph and d15 -16ph. To measure whole-body osmolality, 2-8 fish from each replicate were sampled and gently rinsed with deionized water on a nitex screen. The larvae were quickly removed from the screen, placed on a small piece of filter paper and placed into the osmometer chamber. After a thirty-minute acclimation period, the measurement was taken and the osmolality was recorded.

Larval Buoyancy

On d15ph, an experiment was conducted to determine buoyancy of larvae from each treatment. To determine buoyancy, approximately 20 larvae were collected from each rearing tank. Ten larvae were placed in a 1-L beaker containing 35 ppt water, while the other ten were placed in 24 ppt water. An anesthetic (0.3 ppt 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 mins.), the vertical position of each larva 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 and 0% for larvae that sink to the bottom) (Moustakas et al. 2004).

Oxygen Consumption

Oxygen consumption was measured on d23 and d24 ph using a dissolved oxygen meter (YSI 57, Yellow Springs, Ohio, USA). One hundred fish were removed from selected treatments and placed in a B.O.D bottle in their salinity of origin (24 or 35 ppt). The bottle was sealed with no gaseous headspace after placing the larvae inside. After a 5 min. acclimation period, the probe was inserted into the bottle and the stirrer turned on. When the reading on the dissolved oxygen meter stabilized, it was recorded as a time zero measurement. After one hour, a second reading was taken. The difference in dissolved oxygen levels between these 2 readings and the wet weight of the larvae were used to determine the rate of oxygen consumption per fish in mg O_2/μg fish/min.

Water Quality

Temperature (YSI 55, Yellow Springs, Ohio, USA, ±0.1 C) and salinity (refractometer, ±1 g/L) were measured daily in each tank at 1100. Light intensity, also recorded daily, was measured at the water 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) was measured daily from one replicate tank per treatment and total ammonia nitrogen (TAN) was measured on alternate days (HACH DR 850, Loveland, Colorado, USA, ± 0.01 mg/L). Airflow to each tank was measured twice daily with a flow meter (Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA ± 1 mL/min), at approximately 1000 h 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

The effects of turbulence, salinity and their interaction were analyzed with a two-way analysis of variance. If no interaction was present, salinity treatments were combined within turbulence levels and turbulence levels were combined within salinity treatments for further analysis. Significant treatment effects were detected by the Tukey-Kramer Honest Significant Difference test for multiple comparisons among means. Analysis was preformed using JMP (SAS Institute Inc, Cary, NC) statistical software. F-max tests were preformed on all data sets to test for homogeneity and data was adjusted as needed.

RESULTS

Water Quality

A significant effect ($P < 0.05$) of turbulence on dissolved oxygen levels was observed, but with no salinity or interactive effects (Table 1). Under both salinities, dissolved oxygen was significantly lower in the 20 mL/min (6.69 mg/L) treatment compared to 90, 170, and 250 mL/min (7.00, 6.97, and 7.05 mg/L, respectively) throughout the study. Percent oxygen saturation ranged from 84.8% at 20 mL/min to 89.2% at 90 mL/min. Under both salinities, temperatures were slightly, albeit significantly higher ($P < 0.05$) at 170 mL/min (19.1ºC) compared to 250 mL/min (18.6ºC) (Table 2). Light intensity (mean = 390 lux, range = 324 - 462), pH (mean = 8.0, range = 7.8 - 8.2), and total ammonia nitrogen (TAN) (mean = 0.15 mg/L, range = 0.03 – 0.25 mg/L) were not significantly ($P > 0.05$) different among treatments throughout the study.

Growth: Notochord Length

Larvae in all treatments showed positive growth over the duration of the study (Fig. 1). On d4ph, there was a significant effect of aeration level ($P < 0.05$) and a significant interaction ($P < 0.05$) between turbulence and salinity on notochord lengths (Table 3; Fig. 2). At 35 ppt, notochord length generally increased with increasing turbulence levels from a minimum at 20 mL/min (2.9 mm) to a maximum at 250 mL/min (3.41 mm), while at 24 ppt, notochord lengths were similar (range = 3.31 – 3.37 mm) under all turbulence levels. On d8ph, the notochord lengths ranged from 3.82 to 3.95 mm, with no significant treatment or interactive effects observed (Table 3, Fig. 1).

On d12ph and d16ph, significant ($P < 0.05$ and $P < 0.001$, respectively) effects of turbulence level on notochord lengths were observed, with no significant ($P > 0.05$) salinity or interactive effects. Hence, the effects of turbulence on notochord length were compared by combining data from both salinities. On d12ph, a significant ($P < 0.05$) trend toward decreasing notochord lengths with increasing turbulence levels was observed (Fig. 3). Notochord lengths decreased from a maximum of 4.86 mm at 20 mL/min to a minimum of 4.41 mm at 250 mL/min. Notochord lengths 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 notochord lengths with increasing turbulence levels was observed (Fig. 4). Notochord length decreased from a maximum of 6.42 mm at 20 mL/min to a minimum of 5.63 – 5.72 mm at 170 – 250 mL/min. Notochord length at 20 mL/min was significantly ($P < 0.05$) greater than at 90, 170, and 250 mL/min.

Wet Weight

When growth was expressed as wet weight, significant treatment effects were observed for all ages, although differences among treatments were pronounced after d12ph (Table 4; Fig. 5). On d4ph, a significant effect of turbulence ($P < 0.01$) on wet weight was observed but with no significant ($P > 0.05$) effects of salinity and no interaction between these effects ($0.05 < P < 0.10$) (Table 4; Fig. 6). Wet weight was significantly greater at higher turbulence levels (170 and 250 mL/min) compared to 20 and 90 mL/min.

On d8ph, significant effects of turbulence ($P < 0.05$) on wet weights were observed, while there were no significant salinity ($P > 0.05$) or interactive effects ($P > 0.05$) (Table 4). The effects of turbulence levels were compared across both salinities. On d8ph, wet weights were higher ($P < 0.05$) at 90 mL/min (mean = 659 μg) compared to 20, 170 and 250 mL/min (range = 310 – 402 μg).

On d12ph, a significant effect of turbulence ($P < 0.001$) on wet weight was observed, while there were no salinity or interactive effects (Table 4). Under both salinities, a clear trend toward decreasing wet weights with increasing turbulence levels was observed (Table 4; Fig. 7). Wet weights were significantly ($P < 0.05$) higher at 20 mL/min (1,068 μg) than at 170 (819 μg) and 250 mL/min (650 μg).

On d16ph, there were significant effects of turbulence ($P < 0.001$) and salinity ($P < 0.05$) on wet weight, but there was no significant ($0.05 < P < 0.10$) interaction between these effects (Table 4; Fig. 8a). Under both salinities, a trend toward decreasing wet weights with increasing turbulence levels was observed. Wet weights were higher ($P < 0.05$) at 20 and 90 mL/min (2,656 and 2,378 μg, respectively) than at 170 and 250 mL/min (1,681 and 1,676 μg, respectively).

Under all turbulence levels, wet weights were significantly higher ($P < 0.05$) at 35 ppt than at 24 ppt (Fig. 8b).

Dry Weight

When growth was expressed in terms of dry weight, no significant differences among treatments were observed until d16ph (Table 5; Fig. 9). On d4ph, dry weights ranged from 31.9 – 34.9 μg among treatments. On d12ph, dry weights ranged from 141 – 178 μg 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 (Fig. 10). Dry weights were significantly greater at 20 and 90 mL/min compared to 170 and 250 mL/min.

Survival

Survival showed marked differences among treatments from d4ph, continued to decline through d8ph and then decreased slightly thereafter (Table 6; Fig. 11). On d4ph, there was a significant ($P < 0.05$) effect of turbulence on survival, while the salinity and interactive effects were not significant ($P > 0.05$) (Fig. 12). The highest turbulence treatments of 170 and 250 mL/min experienced significantly greater survival than 20 and 90 mL/min.

On d8ph, significant effects of turbulence ($P < 0.001$) and salinity ($P < 0.05$) on survival were observed, with no interactive effects ($P > 0.05$) (Table 6). 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 (Fig. 13a). Survival under all aeration levels was greater under 24 ppt (49.3%) compared to 35 ppt (36.1%) (Fig. 13b).

On d12ph, there was a significant effect of turbulence ($P < 0.001$) on survival, with no significant ($P > 0.05$) salinity or interactive effects (Table 6). As seen on d8ph, a clear trend toward higher survival with increasing aeration levels was observed from 22.5 % at 20 mL/min to 64.5% at 250 mL/min (Fig.14).

On d16ph, there was a significant effect of turbulence ($P < 0.01$) on survival, but with no salinity or interactive effects ($P > 0.05$) (Table 6). 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. 15).

Percent Body Water

Percent body water varied significantly among treatments over the course of the study. On d4ph, highly significant effects of aeration ($P < 0.001$) and salinity ($P < 0.001$) on percent body water and a significant interaction ($P < 0.01$) between these effects were observed (Table 7; Fig. 16). Under 35 ppt, percent body water increased with increasing turbulence from 72.3 - 72.8% at 20 and 90 mL/min to 85.6 - 87.3% at 170 and 250 mL/min. Under 24 ppt, percent body water was similar (range = 84.3 – 87.8%) over all levels of turbulence. On d8ph, percent body water ranged from 93.3 to 96.3% among treatments, and there were no significant ($P > 0.05$) treatment or interactive effects observed (Table 7).

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 (Fig. 17; Table 7). A clear trend toward lower percent body water with increasing turbulence levels was observed from a maximum of 84.6% at 20 mL/min to a minimum of 77.5% 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 percentage body water were observed (Fig. 18; Table 7), with no significant salinity effects ($P > 0.05$). Fish in the 35 ppt treatments showed a similar percentage body water (range = 88.8 – 90.6%) over all the turbulence levels, while fish in 24 ppt showed lower percent body water at the higher turbulence levels. At 24 ppt, percent body water decreased from 92.3 and 91% at 20 and 90 mL/min to 88% and 89.7% at 170 and 250 mL/min.

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. Osmolality increased with increasing turbulence levels from 381 mOsm/kg at 20 mL/min to 427 mOsm/kg at 250 mL/min (Fig. 19).

Buoyancy

On d14ph, there were no significant effects of turbulence level and salinity on larval buoyancy, but a significant interaction ($P < 0.05$) between these effects was observed (Fig. 20). Under 35 ppt, buoyancy increased with aeration level to a maximum at 170 - 250 mL/min. Under 24 ppt, buoyancy increased to a maximum at 90 – 250 mL/min. Fish reared at 35 ppt were less buoyant than fish reared at 24 ppt in the 1 L beaker filled with 35 ppt water.

Oxygen Consumption

On d23 - d24ph, there were no significant treatment or interactive ($P < 0.05$) effects observed on oxygen consumption (Fig. 21). Oxygen consumption ranged between 2.11 and 4.62

mg/L/μg/min. 90 and 250 mL/min treatments were selected for analysis because of high larval densities compared to 20 mL/min.

Rotifer Concentration

Mean rotifer concentration (ind./mL) measured 24 hours after delivery to rearing tanks, varied significantly among treatments. There was a significant ($P < 0.01$) effect of turbulence on the mean rotifer concentration after 24 hours (Fig. 22), while the effects of salinity ($0.05 < P < 0.10$) and interactive effects ($0.05 < P < 0.10$) were not significant. Mean rotifer concentrations (24 hours post-feeding) were significantly higher ($P < 0.05$) in the low turbulence treatments of 20 (4.48 ind./mL) and 90 mL/min (4.23 ind./mL) than in the high turbulence treatments of 170 and 250 mL/min (2.28 and 2.45 ind./mL, respectively).

DISCUSSION

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

On d4ph, there was a significant interaction between turbulence and salinity on notochord lengths. This was probably related to acclimation to experimental conditions from d1 – d4ph. All eggs were incubated at 35 ppt and 19°C until hatching and then abruptly transferred to the experimental tanks at 24 and 35 ppt. Turbulence levels were adjusted to treatment conditions (20 – 250 mL/min) within several hours. Fish that were placed in the 35 ppt water showed increased notochord lengths with increasing turbulence levels. However, fish that were placed in 24 ppt water did not exhibit similar growth trends.

On d4ph wet weights were higher at 170 and 250 mL/min compared to 20 and 90 mL/min. On d12ph these trends were reversed and wet weights were higher at 20 and 90 mL/min compared to 170 and 250 mL/min. On d10ph larvae begin to develop fin rays and the caudal fin becomes more apparent. Larvae in low turbulence levels, where prey densities were higher, actively sought after prey items and growth increased at these low turbulence levels on d12ph. At the high levels of turbulence (170 and 250 mL/min) larvae experienced high swimming activity and low prey ingestion rates causing lower growth rates in these treatments.

In this study, the largest decline in larval survival occurred within the first 4 dph (yolk-sac stage larvae). Survival continued to decline considerably during the first-feeding stage through d8ph and then generally stabilized through the end of the study on d16ph. Survival was lower at the lower turbulence levels of 20 and 90 mL/min. Lower turbulence levels probably allowed these yolk-sac stage larvae to sink to the bottom and die, whereas higher turbulence levels maintained the larvae in the water column. However, at the lower turbulence levels, larvae in 24 ppt were larger than those in 35 ppt on d4ph (Fig. 2). At the lower turbulence levels, larvae may have gained an osmoregulatory advantage in 24 ppt (closer to isoosmotic salinity), allowing for better growth. At higher turbulence levels, growth (notochord lengths and wet weights) to d4ph

was similar in 24 and 35 ppt (Figs. 2 and 6), possibly because the yolk-sac larvae spent less energy in swimming, allowing more available energy for growth.

In this study, survival to d16ph was improved by increasing turbulence within the range of 20-250 mL/min in 15-L tanks. This was primarily related to the marked differences in survival among treatments during the yolksac and first-feeding stages (d1-d8ph), since survival under all treatments stabilized after d8ph when larvae were feeding on rotifers. Precise comparisons of studies dealing with the effects of turbulence levels on marine finfish larvae are difficult due to the variations in experimental conditions used, particularly tank size and configuration. However, other studies have also reported improved survival of larval marine finfish with increasing turbulence levels. In larval turbot *Psetta maxima* (Gaignon et al. 1998), survival to d9ph in 150-L tanks increased with increasing turbulence rates within a range of 0.5 to 85 mL/min. Battaglene and Talbot (1993) also found higher survival (86.8%) of Australian bass *Morone saxatilis* larvae to d10ph at higher turbulence levels (> 1,000 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 salinities of 15 – 35 ppt.

The results of this study revealed that larval southern flounder are tolerant of much higher levels of turbulence than those typically used for this species. For example, in this study, turbulence levels as high as 250 mL/min did not impair survival of southern flounder larvae to d16ph in 15-L tanks. Henne et al. (2003) and Moutakas et al. (2004) reared larval southern flounder until d15ph using turbulence levels of only 30 mL/min and 50 mL/min, respectively, in 15-L tanks. Such low levels of turbulence were used to avoid damaging the delicate early larval stages. Barahona-Fernandes (1978) obtained highest survival of sea bass *Dicentrarchus labrax* embryos and larvae at an aeration rate of 40 mL/min (vs 0.5 – 85 mL/min) in 150-L tanks. Ellis

et al. (1997) reported that survival of larval Nassau grouper *Epinephelus striatus* was higher under a relatively low turbulence level of 150 mL/min (39.5%) compared to 450, 300, and 0 mL/min in 500-L tanks (13.2, 29.5, and 0.17%, respectively). These authors suggested that, under lower turbulence levels, the larvae were evenly dispersed in the water column instead of congregating on the walls of the tank as under static conditions, whereas excessive turbulence levels increased contact between larvae and the tank walls, resulting in injury (Ellis et al. 1997).

The results of this study indicate southern flounder larvae were neutrally or positively buoyant in water of 35 ppt and negatively buoyant in water of 24 ppt. Therefore, fish reared in 24 ppt may have exhibited lower growth rates because they needed to continually swim and expend energy to maintain position in the water column (Moustakas 2004). The increased survival seen at high turbulence levels may be related to the absence of a swim bladder, as was reported for winter flounder larvae, which do not possess a swim bladder and turbulence may help maintain buoyancy in larval rearing tanks (Litvak 1999). This could be especially important within the first few days after hatching when their swimming ability is poorly developed and they depend mainly on environmental conditions within the culture tank for buoyancy. On the other hand, a marine finfish larva with a fully developed swim bladder could regulate its position in the water column without having to depend on highly turbulent conditions for survival. It is important to note that after d10ph, the larvae begin to develop the caudal fin, which improves their swimming ability, making highly turbulent conditions unfavorable for locating and capturing prey as well as growth.

Whereas survival of yolksac and first-feeding stage larvae (d1-d8ph) improved with increasing turbulence levels in this study, growth rates decreased at higher turbulence levels. Water turbulence affects prey capture success and feeding rates in fish larvae (MacKenzie et al.

1994). Finfish larvae begin life with a limited supply of yolk and need to quickly learn to capture food to survive (Strickler 1994). It is thought that first-feeding and weak larvae benefit from small-scale turbulence (MacKenzie and Leggett 1991). Higher turbulence would generate more mixing, breaking up patches of prey, and make the distribution of larvae and prey more homogenous within the culture vessel, thereby increasing chance of contact between the two (Barahona-Fernandes 1978). Prey encounter rates and the probability of successful capture increase with increasing turbulence (MacKenzie et al. 1994). Rothschild and Osborn (1988) found that even small-scale turbulence can increase encounter rates of planktonic predators and their prey. A dome-shaped relationship between turbulence and prey ingestion rates in larval fish was suggested, with maximal ingestion rates at intermediate levels of turbulence (MacKenzie 1994; Gallego et al. 1996). Attack rate (on prey) may increase at median turbulence levels and begins to show a negative effect (low attack rates and high swimming rates) at high turbulence levels (Utne-Palm and Stiansen 2002). Hence, slower growth at higher turbulence levels in this study could be a product of the negative effects of too much turbulence on energy expenditure. At the high levels of turbulence (170 and 250 mL/min), prey encounter rates for larvae were high, but larvae probably spent more energy swimming against the currents than capturing food.

Higher growth rates at low turbulence levels may have also been related to higher prey/larvae ratios, caused by lower larval densities at low turbulence levels. From d3ph through the end of the study, prey were added each morning to each rearing tank to maintain a concentration of 10 ind./mL, even as larval survival declined over the course of 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 levels (170 and 250

mL/min) where survival was better. This was supported by significantly higher average daily prey concentrations in the lower turbulence treatments (Fig. 22). Hence, greater prey concentrations may have also contributed to higher feeding and growth rates at lower turbulence levels (Figs. 4 and 8a). Larval bay anchovies (*Anchoa mitchilli*) exhibited higher growth rates when fed medium (1,500 ind./mL) and high prey concentrations (3,000 ind/mL) compared to 500 ind./L (Saksena and Houde 1972). Greater growth and survival were also seen in larval sea bream (*Archosargus rhomboidalis*) when fed at high prey (copepods) concentrations (500 ind./L) compared to median and low levels (25 and 50 ind./L) (Dowd and Houde 1980). Also, Japanese flounder larvae grew significantly larger when fed at high *Artemia* densities (3,000 and 5,000 ind./L) compared to low densities of 1500 ind./L (Dou et al. 2003).

In terms of both wet and dry weights, growth of southern flounder larvae was higher in 35 ppt seawater than in 24 ppt brackish water, consistent with what was previously reported for this species (Henne et al. 2003, Moustakas et al. 2004). Summer flounder also showed higher growth rates in full-strength seawater (36 ppt) compared to 26 ppt (Watanabe et al. 1998). Moustakas (2004) concluded that early southern flounder larvae are not entirely euryhaline. Euryhalinity in this species appears to be acquired after metamorphosis as these fish transition from the pelagic to a benthic mode of existence (Moustakas et al. 2004).

In this study, larval whole-body osmolality increased with increasing turbulence. On d15ph, larvae under the highest turbulence level (250 mL/min) had significantly higher osmolality values (427 mOsmol/ kg) than those reared at the lowest turbulence level (20 mL/min) (382 mOsmol kg) and may indicate greater osmotic stress on the larvae at the higher turbulence level. This was related to lower body water percentage with increasing turbulence levels, which is also consistent with osmoregulatory failure in a hyperosmotic (24-36 ppt) environment. Lower food

availability (i.e., prey densities) and higher energy expenditures in swimming at higher turbulence levels may have caused inadequate allocation of energy for osmoregulation. The energetic cost of maintaining an internal osmolality within normal ranges, may have led to poor growth seen at the higher turbulence levels, where whole-body osmolality values were the highest. High osmotic stress within an organism leads to high-energy expenditure (Bashamohideen and Parvatheswararao 1976). In juvenile turbot (*Scophthalmus maximus*) sub-optimal temperature (10°C) and salinity (33.5 ppt) combinations produced higher internal osmotic pressure values (336 mOsm/kg) than normal (324 mOsm/kg) (Imsland et al. 2003).

In contrast to the findings of this study which showed no differences in whole-body osmolality for fish raised in 24 or 35 ppt, Henne et al. (2003) and Moustakas et al. (2004) found that southern flounder had a lower internal osmotic concentration (288 - 304 mOsm/kg) at 25 ppt, a salinity closer to isosmotic than 35 ppt (322 - 343 mOsm/kg). In the present study, both higher turbulence levels (250 vs. 20-50 mL/min) and higher prey densities (10 vs. 5 ind./mL) were used. These differences in environmental conditions may have masked the effects of salinity on osmoregulation that were observed in previous studies with southern flounder. Different rearing salinities, prey densities, and environmental conditions may have contributed to the higher internal osmolality seen in this study compared to values recorded by Henne (2003) and Moustakas et al. (2004).

Oxygen consumption is considered an indicator of metabolic rate within an organism (Kurohura et al. 1995). An increase in metabolic rate will lead to higher energy expenditure. So, whereas increased levels of oxygen consumption were expected at the higher turbulence levels, no significant differences were observed. However, it is likely that high replicate variability did

not allow these differences to be resolved. Further study is needed to ascertain the effects of turbulence and salinity on oxygen consumption in southern flounder.

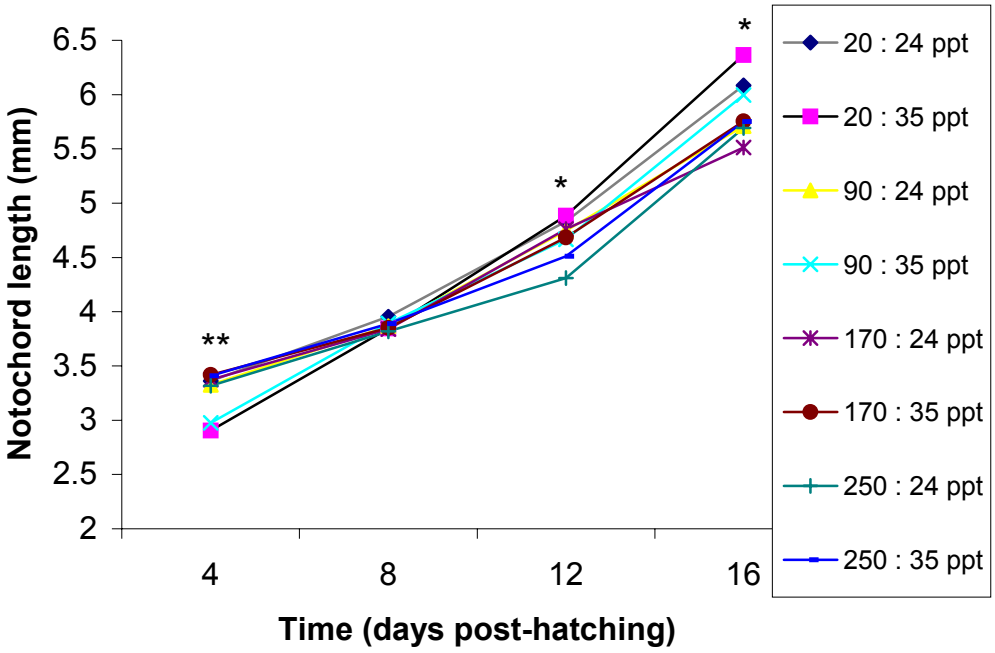
In summary, the results of this study demonstrated that growth of southern flounder larvae in 15-L tanks was maximized at lower turbulence levels in the range of 20 – 90 mL/min, while survival was maximized at higher turbulence levels of 170 - 250 mL/min. At the yolk-sac larval stage, where the larvae were developing their eyesight and fins and were dependent on their yolk reserves, high turbulence enhanced the buoyancy of these fish (which lack a swim bladder) and kept them from sinking to the bottom of the culture vessel and hence, improving survival in these treatments. In fact, the major mortalities occurred during this pre-feeding period. At the first-feeding stages, where acquisition of food is very important to the early survival of larvae, higher turbulence levels would promote an even distribution of food. This would allow more larvae to encounter prey and successfully feed, thereby improving survival. Higher turbulence levels would also increase larval buoyancy in these larvae, which possess a poorly-developed swim bladder. At lower turbulence levels, larvae and prey would congregate in certain areas of the tanks and prey encounter rates would be lower, causing lower survival.

As the larvae grew and their swimming and hunting abilities developed, the higher turbulence levels would improve encounters between larvae and prey because of the heavy mixing in the culture tanks; however, this also cost them energy for continual swimming that would have been available for growth. Stress of increased swimming and hunting activity and lower prey densities at higher turbulence levels was accompanied by increased osmoregulatory stress and tissue osmotic concentrations in the hyperosmotic rearing environment. Fish in the less turbulent treatments encountered higher prey availability (i.e. prey/larvae ratios) because larval densities were lower due to high early mortality.

The results of this study did not support our hypothesis that the effects of turbulence on growth, survival, and whole body osmolality will vary with salinity, with optimal turbulence levels increasing at lower salinities. Under salinities of 24 and 35 ppt, lower turbulence levels promoted growth, while higher turbulence levels increased survival. In 15L tanks, we recommend moderate turbulence levels of 100 – 150 mL/min to maximize growth rates (seen at lower turbulence levels) and survival (seen at higher turbulence levels). This technique could be used for most species of flounder however fish with a swim bladder may experience different results. We also recommend that southern flounder larviculturists determine turbulence levels in their specific systems, since these may vary with tank size and configuration. These turbulence levels should maintain buoyancy and survival in early yolk-sac (pre-feeding stage) larvae and optimize prey encounters and feeding efficiency in feeding stage larvae. Based on the results of this study, turbulence levels should be maintained relatively high during pre-feeding and early feeding stages and then decreased for mid to late feeding and pre-metamorphic stage larvae.

In the future, it would be practical to study higher turbulence levels to determine where the dome-shaped relationship of (growth vs. energy spent) would begin to show negative effects of too much turbulence on survival. Studies using lower salinities closer to isosmotic (12-14 ppt) would also help to determine if greater turbulence would increase growth and survival at these reduced salinities where buoyancy would be even less. Also, an experiment where available food levels are increased, especially at the higher turbulence level, would help to determine if the energetic disadvantage of high turbulence levels could be overcome under conditions of high food availability.

Fig. 1



Figur

e 1. Growths (notochord lengths) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Plotted points represent means ($N = 8$). Asterisks indicate significant treatment (*) or interactive (**) effects observed on that day ($P < 0.05$).

Fig. 2

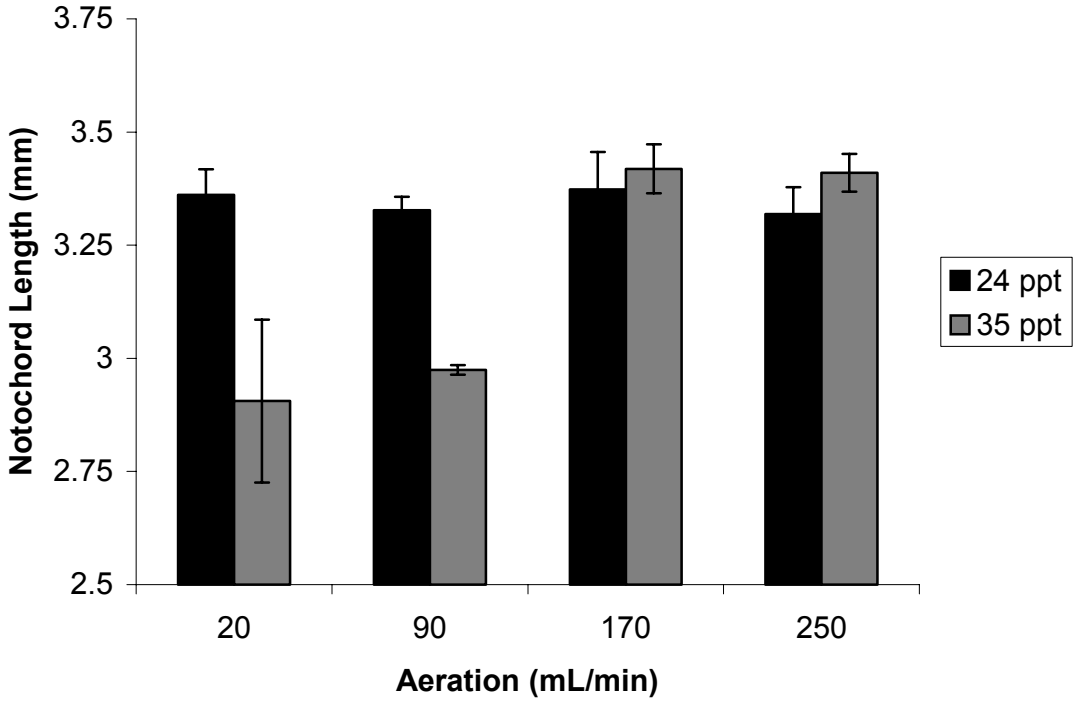


Figure 2. Notochord lengths (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt) on day 4 post-hatching ($P < 0.05$).

Fig. 3

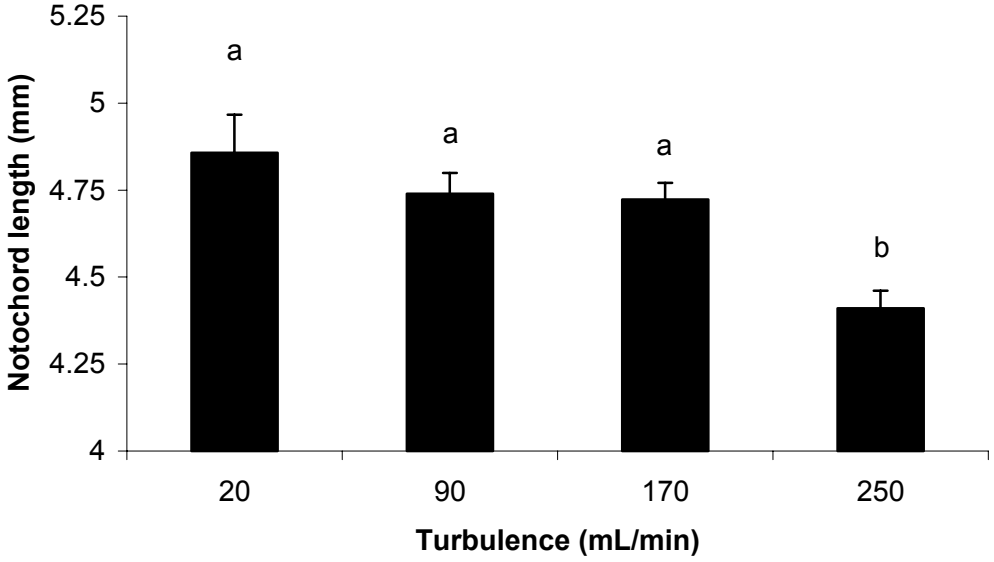


Figure 3. Notochord lengths (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 12 post-hatching. Data for both salinities (24 and 35 ppt) were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 4

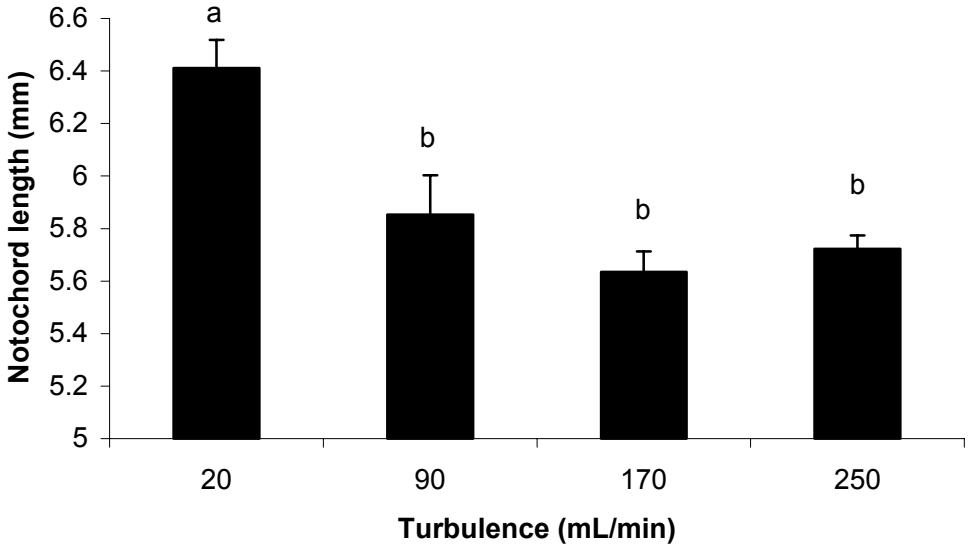


Figure 4. Notochord lengths (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 16 post-hatching. Data for both salinities (24 and 35 ppt) were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 5

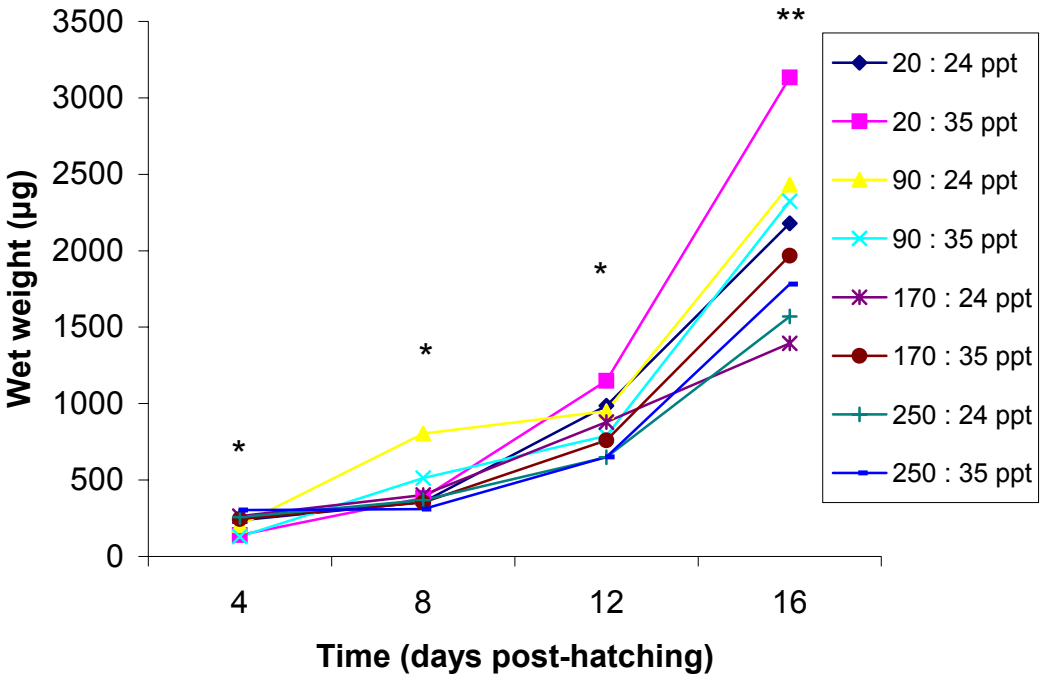


Figure 5. Growth (wet weights) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Plotted points represent means ($N = 8$). Asterisks indicate significant treatment (*) or interactive (**) effects observed on that sampling day ($P < 0.05$).

Fig. 6

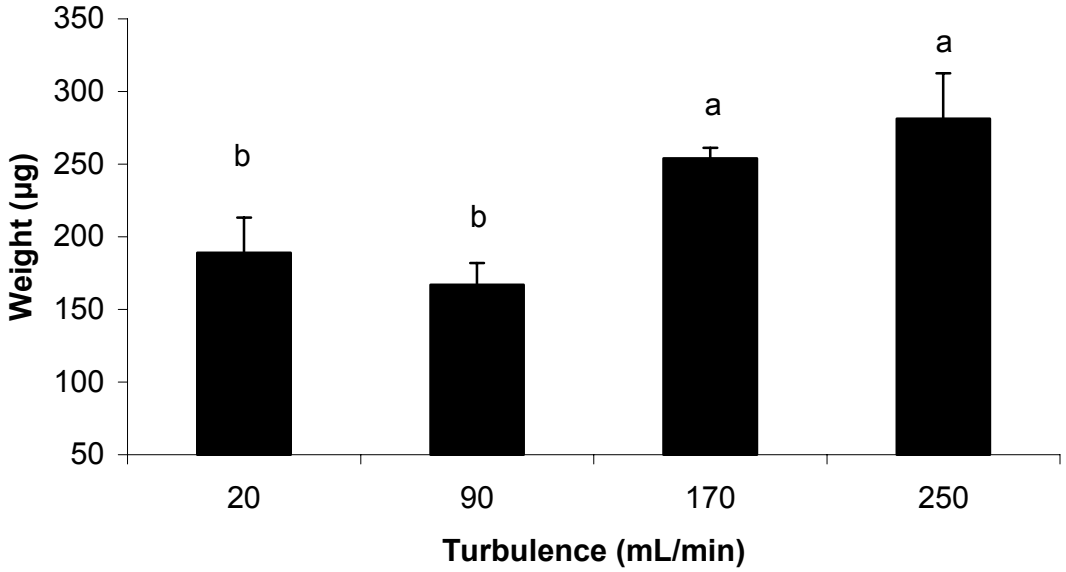


Figure 6. Wet weights (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt) on day 4 post-hatching ($P < 0.05$).

Fig. 7

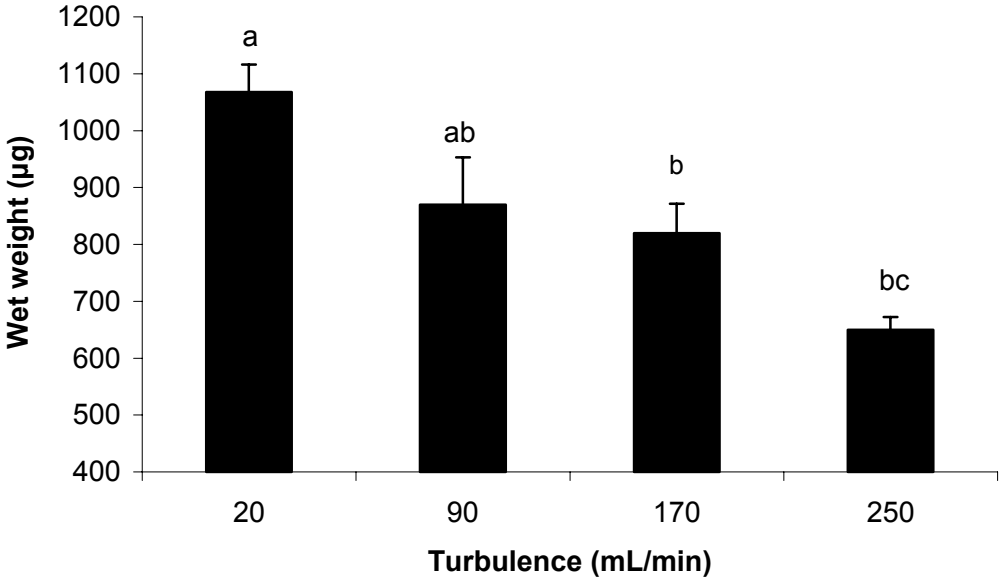


Figure 7. Wet weights (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 12 post-hatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 8a

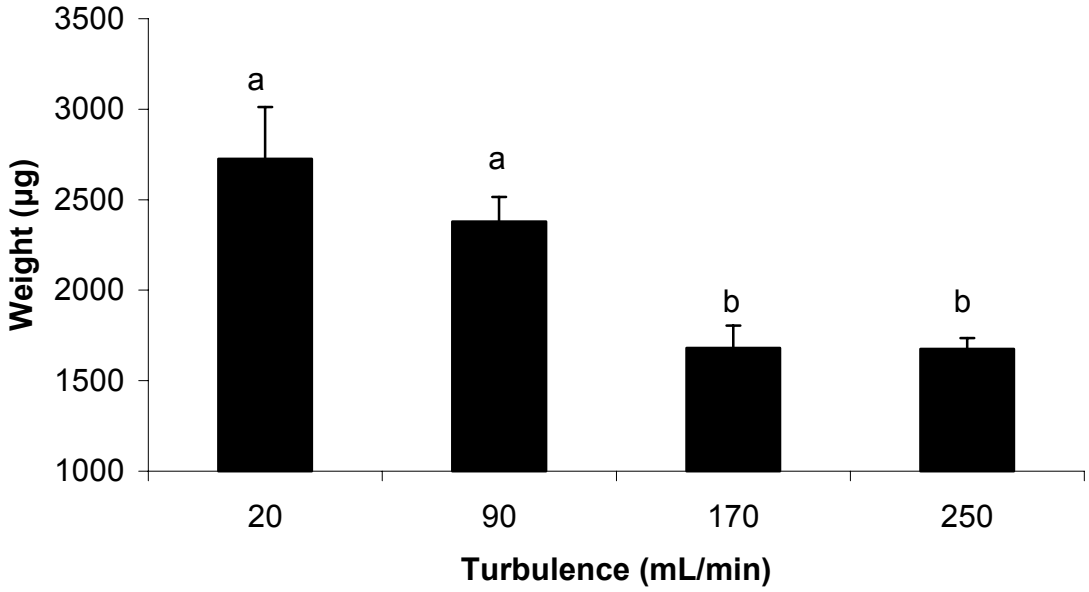


Fig. 8b

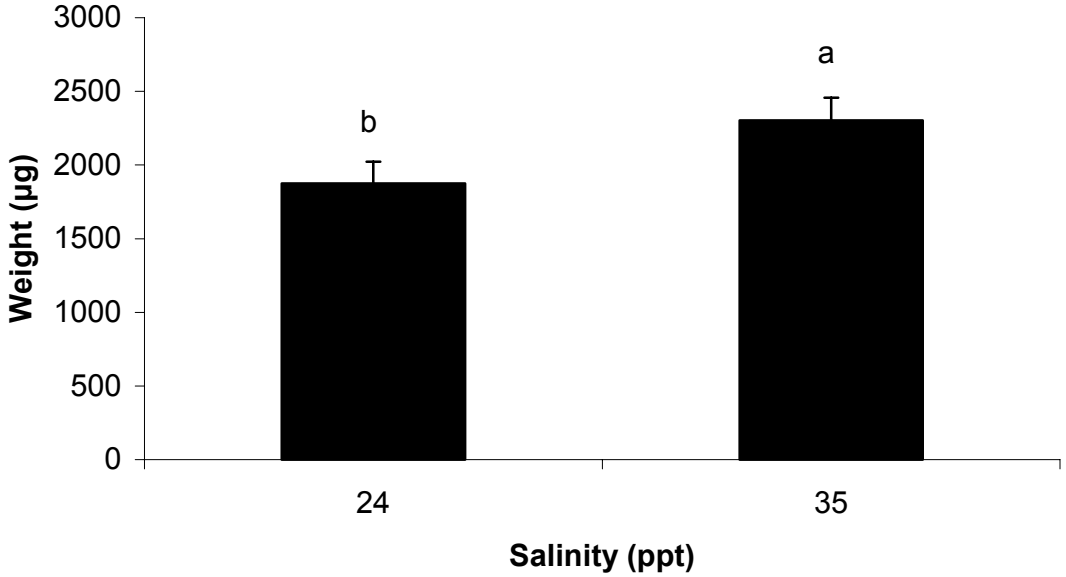


Figure 8a. Wet weights (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 16 post-hatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Figure 8b. Wet weights (mean ± SE, $N = 8$) of southern flounder larvae under different salinities (24 and 35 ppt) on day 16 post-hatching. Data for all turbulence levels were combined under each salinity. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 9

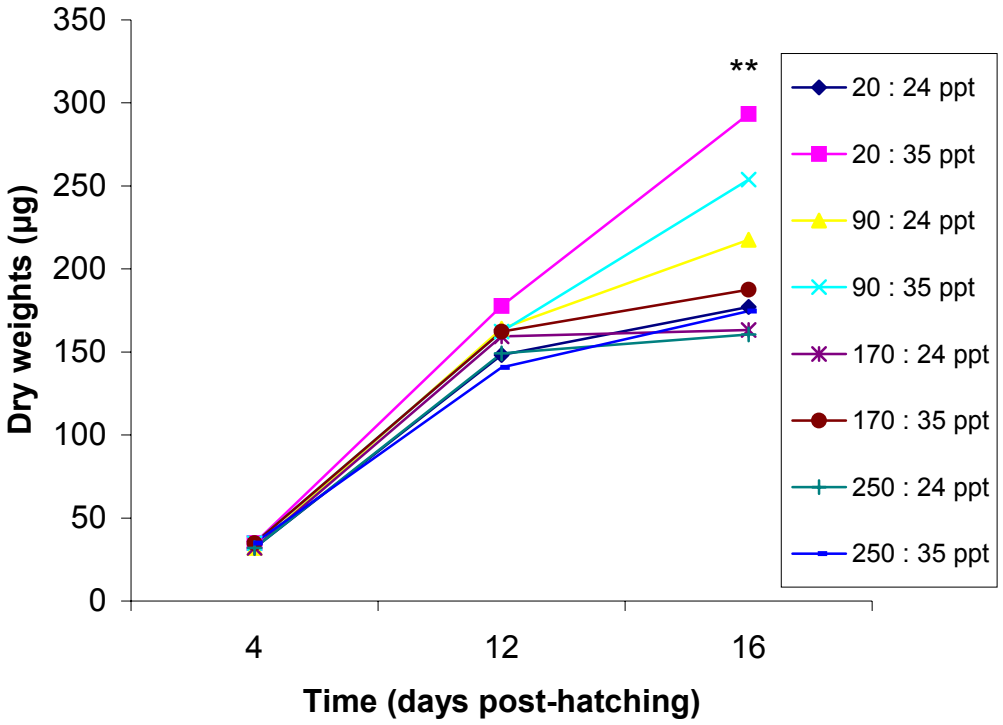


Figure 9. Dry weight of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Plotted points represent means ($N = 8$). Asterisks (**) indicate significant interactive effects observed on that sampling day ($P < 0.05$).

Fig. 10

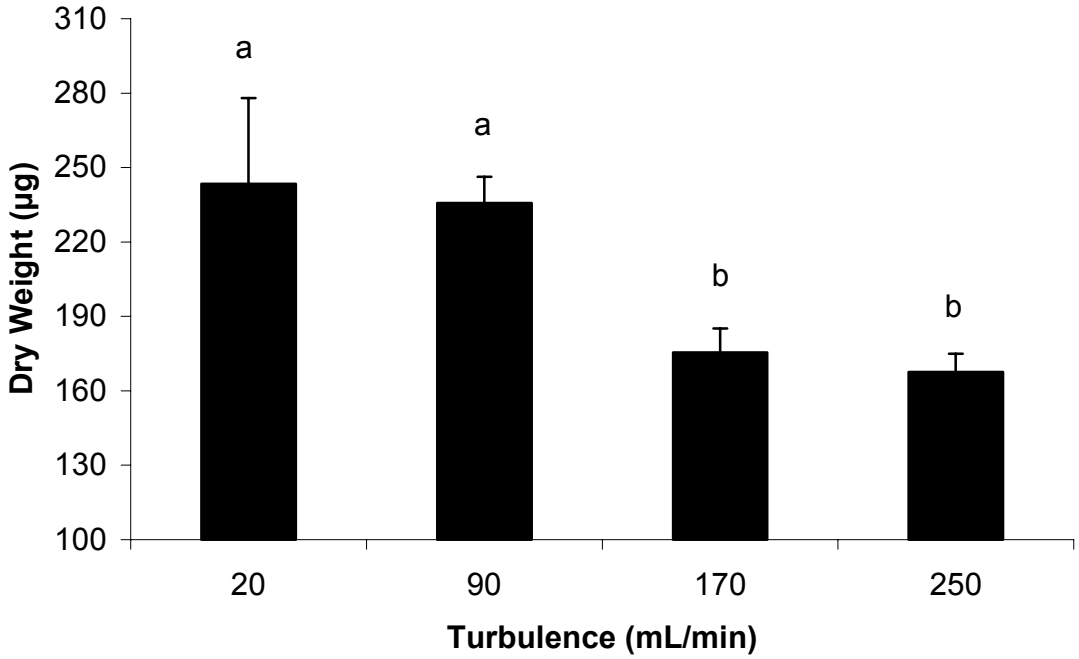


Figure 10. Dry weight (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 16 post-hatching ($P < 0.05$).

Fig. 11

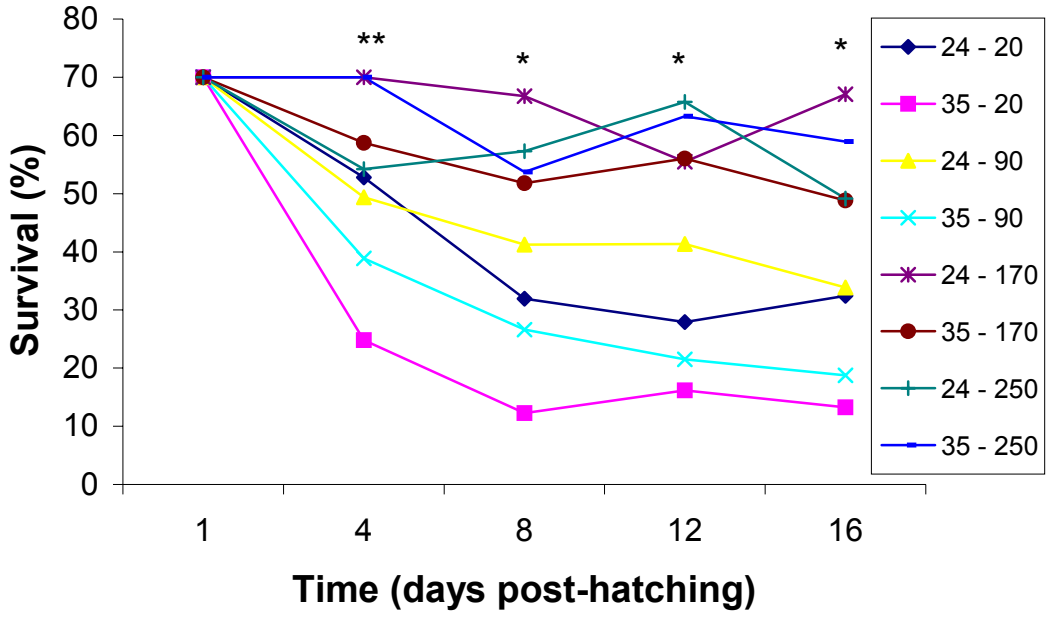


Figure 11. Survival of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Plotted points represent means (N = 8). Asterisks indicate significant treatment (*) or interactive (**) effects observed ($P < 0.05$).

Fig. 12

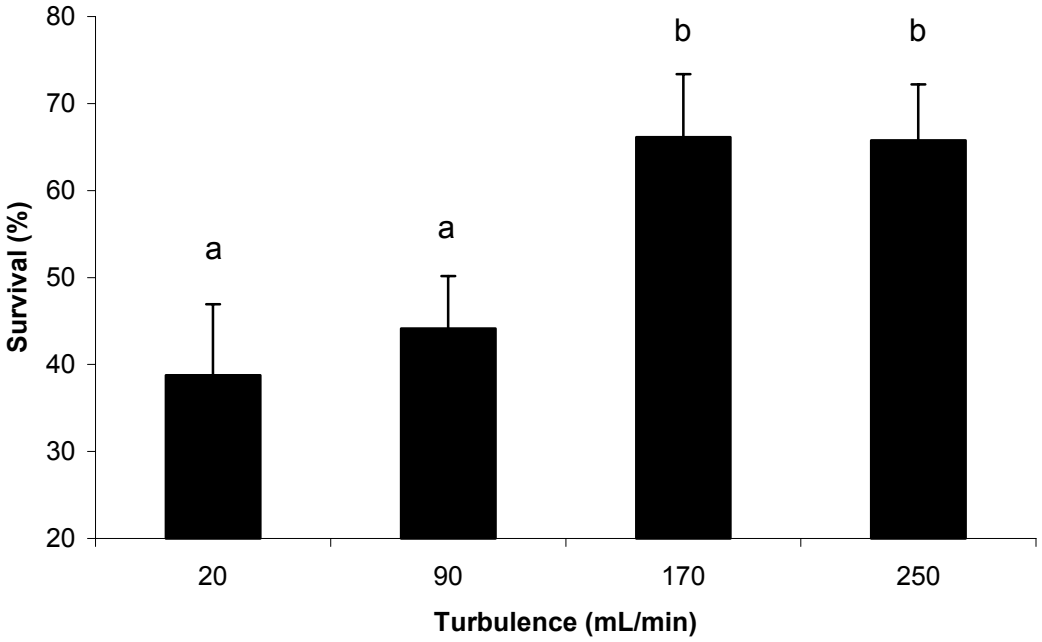


Figure 12. Survival (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 4 post-hatching ($P < 0.05$).

Fig. 13a

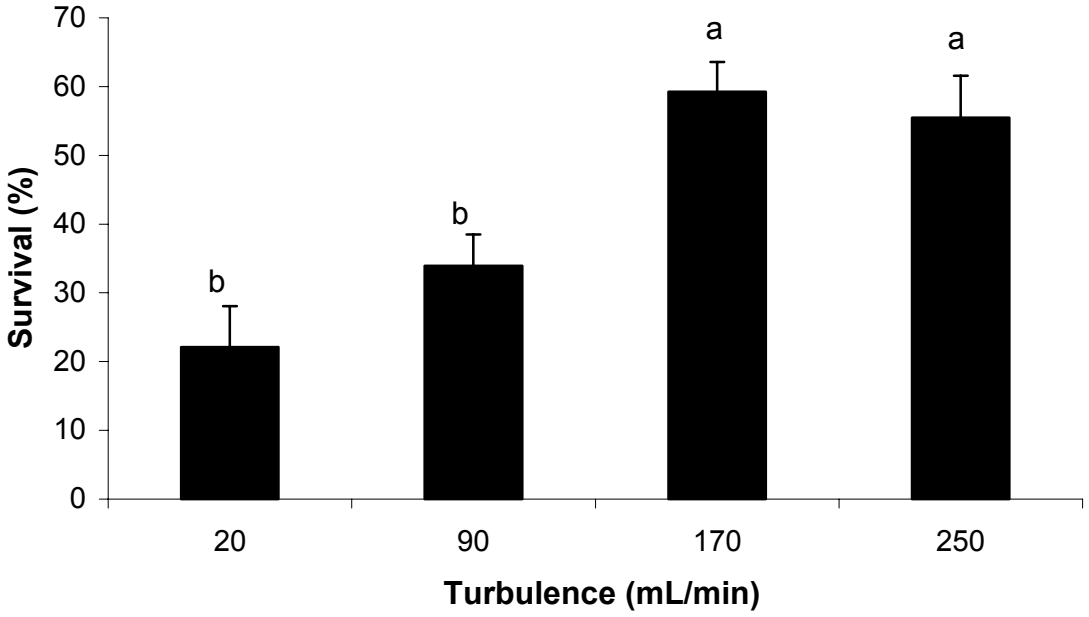


Figure 13a. Survival (mean ± SE, *N* = 8) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 8 post-hatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 13b

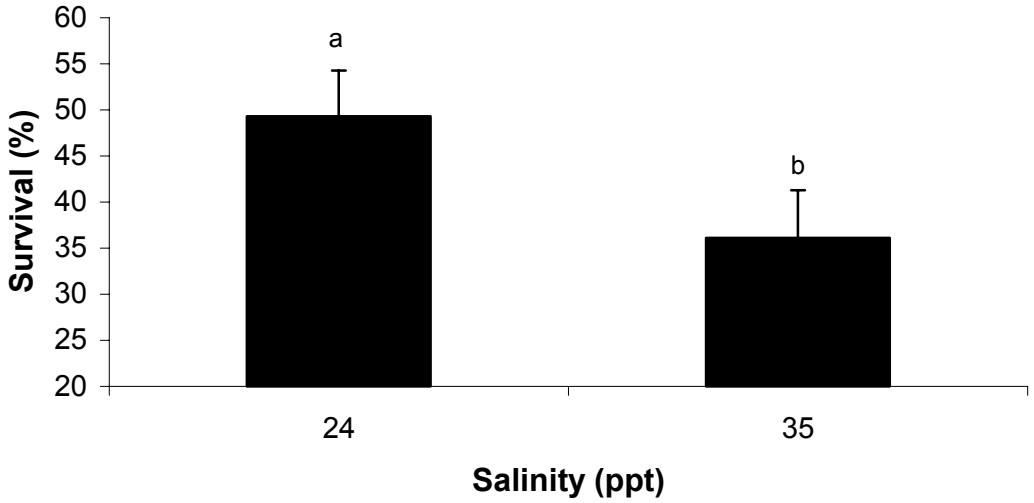


Figure 13b. Survival (mean ± SE, *N* = 8) of southern flounder larvae under different salinities (24 and 35 ppt) on day 8 post-hatching. Data for all aeration rates was combined under both salinities. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 14

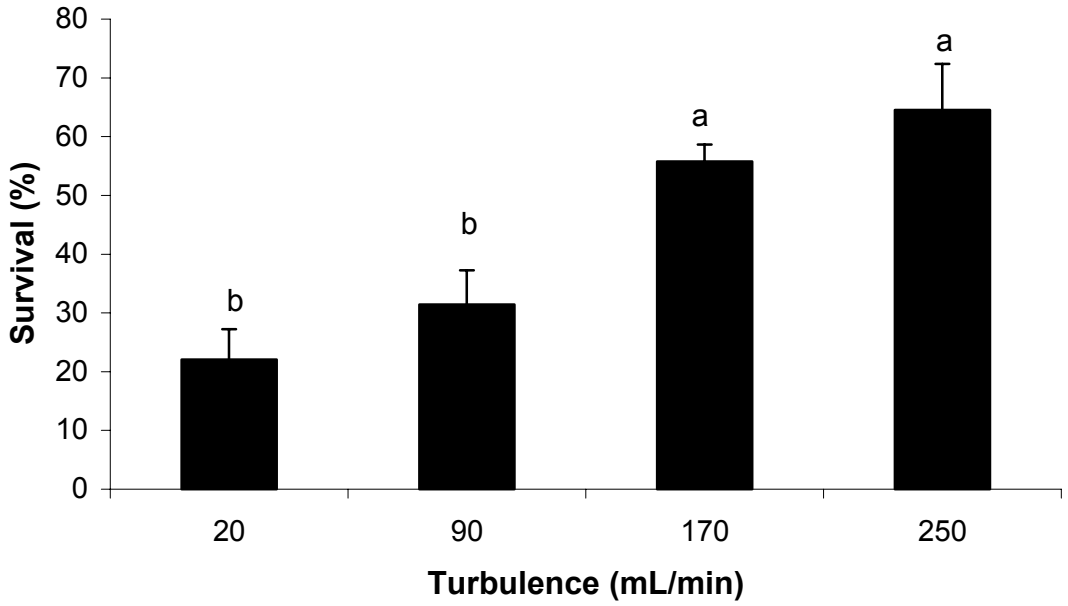


Figure 14. Survival (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 12 post-hatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 15

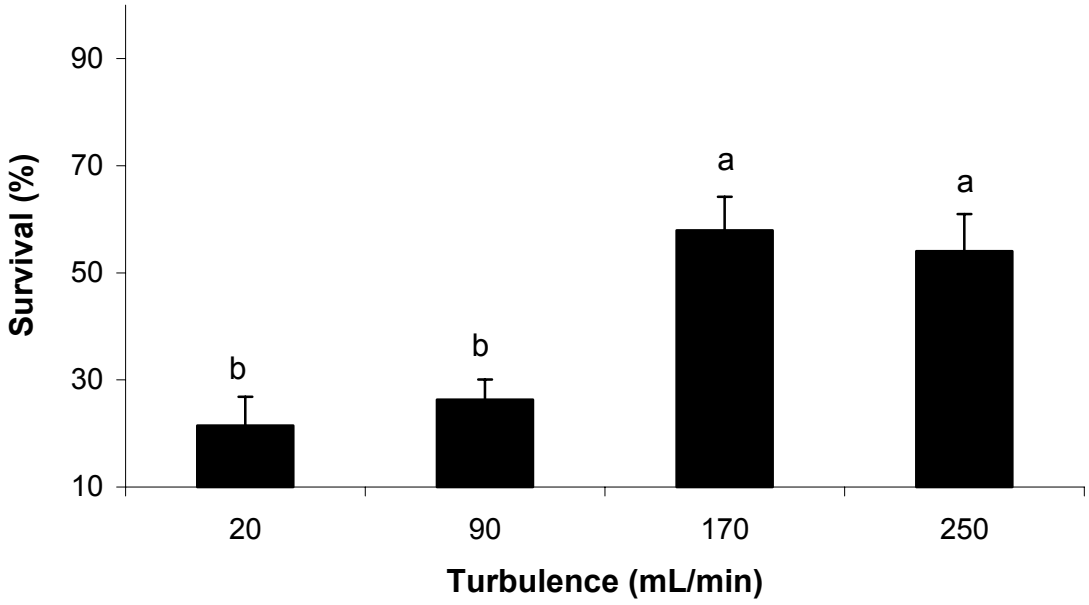


Figure 15. Survival (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 16 post-hatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 16

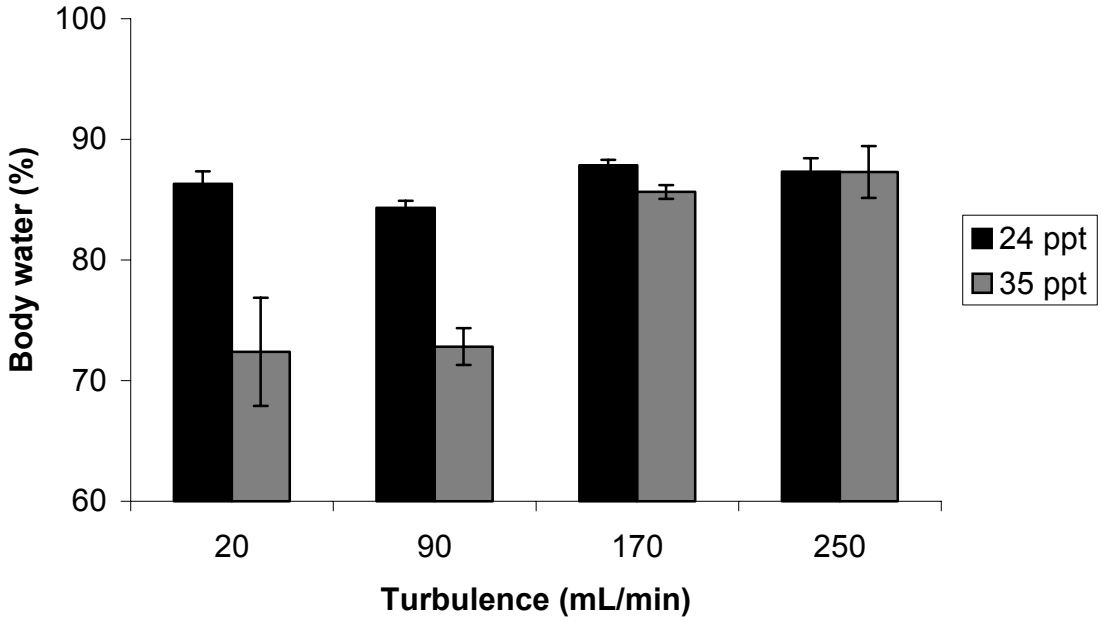


Figure 16. Body water percentage (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt) on day 4 post-hatching ($P < 0.05$).

Fig. 17

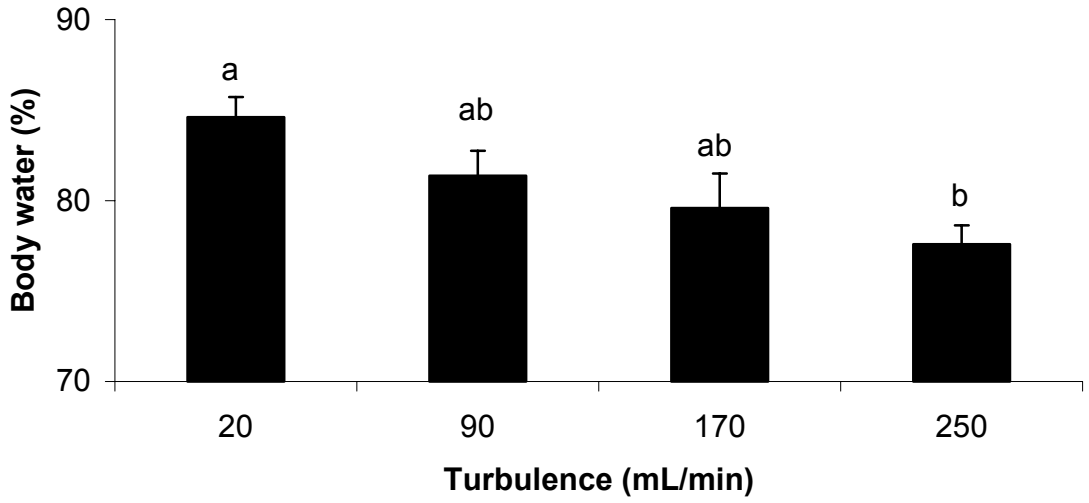


Figure 17. Body water percentage (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 12 post-hatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 18

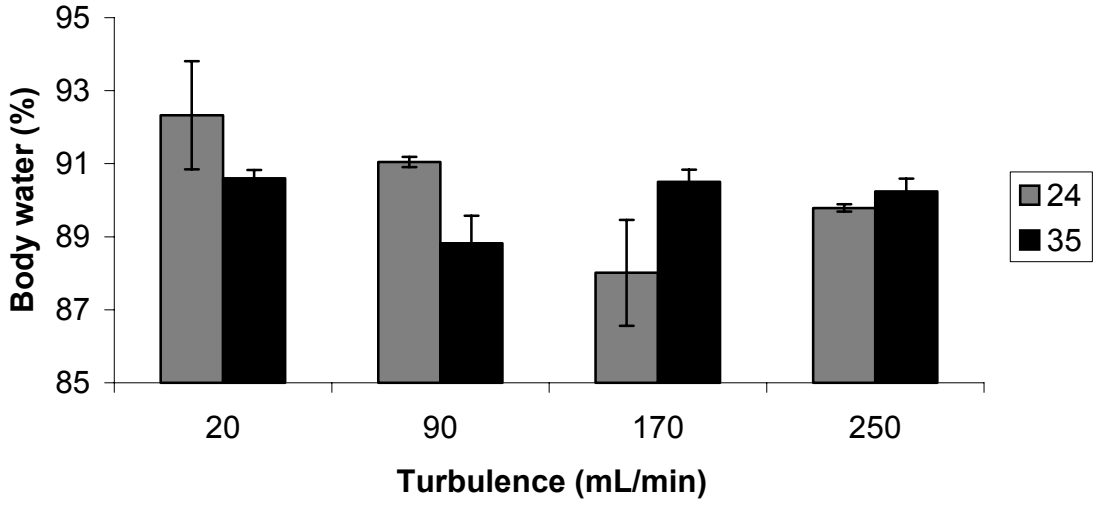


Figure 18. Body water percentage (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt) on day 16 post-hatching ($P < 0.05$).

Fig. 19

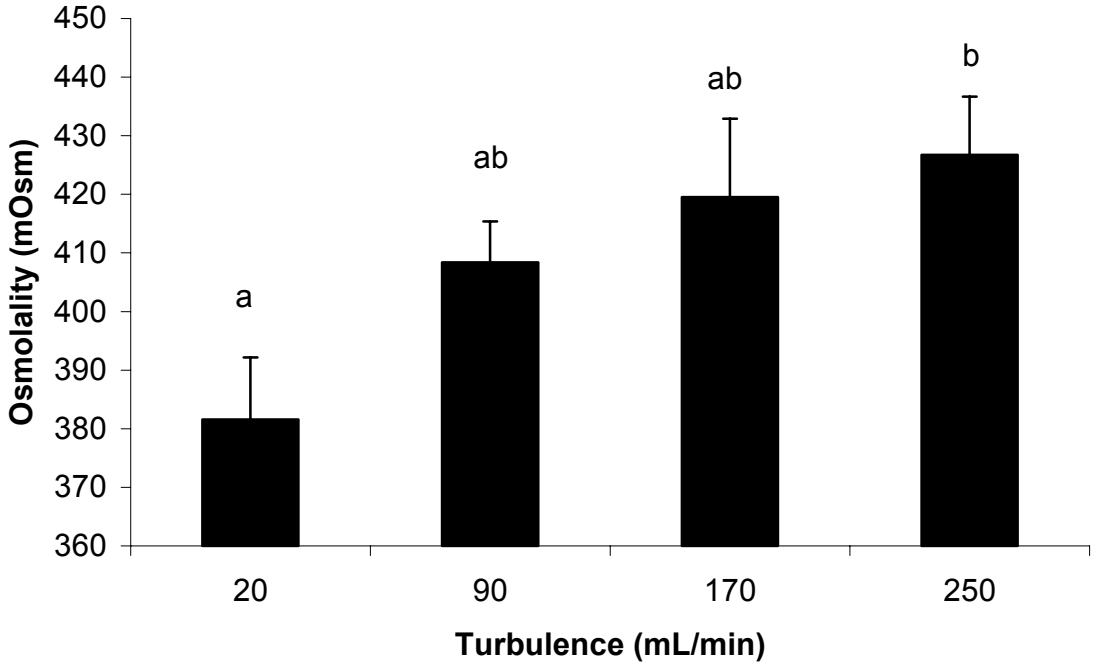


Figure 19. Whole body osmolality (mean ± SE, $N = 8$) of southern flounder larvae under different turbulence levels (20, 90, 170 and 250 mL/min) on day 15 post-hatching. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Fig. 20

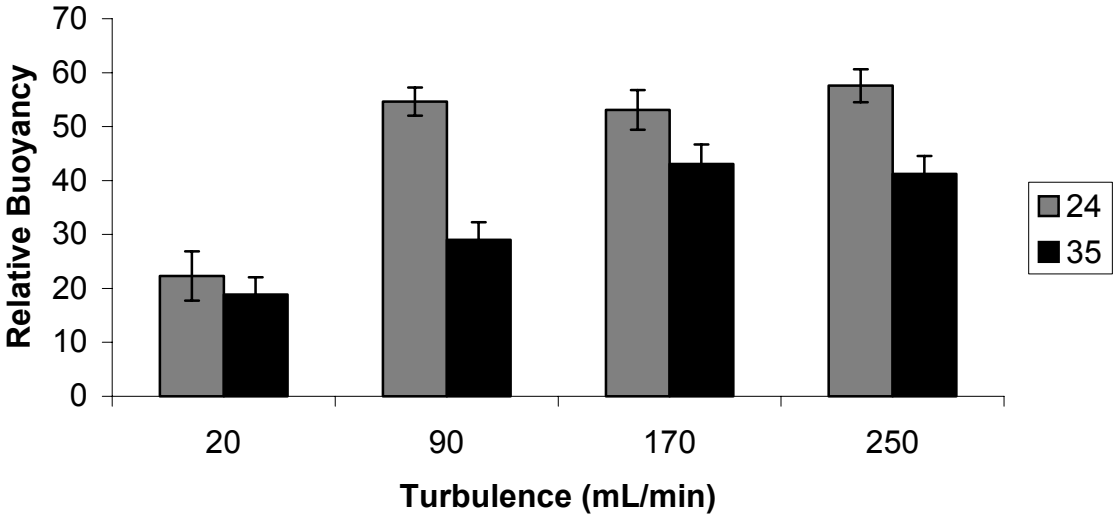


Figure 20. The relative buoyancy (mean ± SE, $N = 8$) of southern flounder larvae reared under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt) on day 14 post-hatching ($P < 0.05$).

Fig. 21

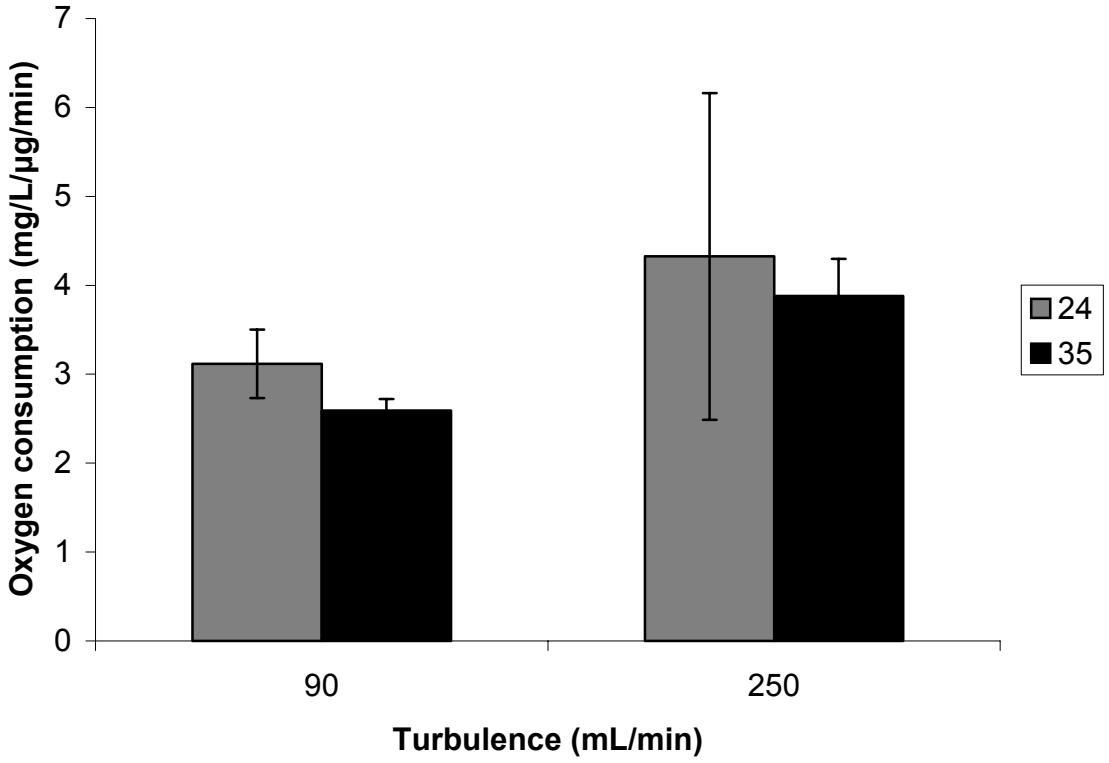


Figure 21. Oxygen consumption (mean ± SE, $N = 6$) of southern flounder larvae reared under different turbulence levels (90 and 250 ml/min) and salinities (24 and 35 ppt) on day 22 - 23 post-hatching ($P > 0.05$).

Fig. 22

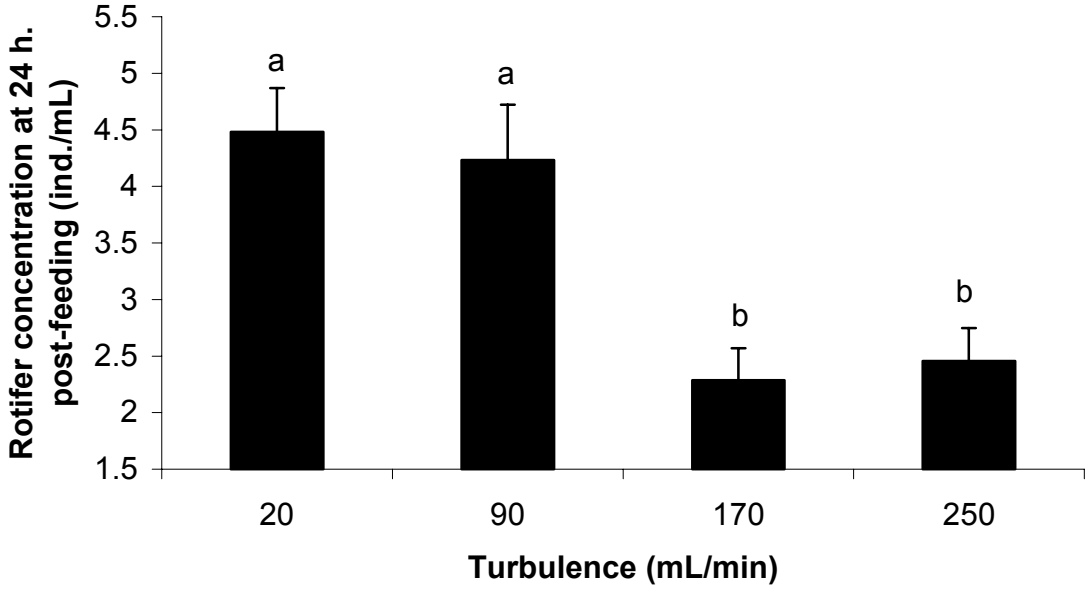


Figure 22. Mean rotifer concentrations (ind./mL) 24 hours after introduction to rearing tanks under different turbulence levels (20, 90, 170 and 250 mL/min). Values represent daily means ± SE ($N = 8$) from d4 -16ph. Data for both salinities were combined under each turbulence level. Means without a letter in common are significantly different ($P < 0.05$).

Table 1. Dissolved oxygen (mg/L) (mean ± SE, N = 8) under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Significant ($P < 0.05$) effects of turbulence level on dissolved oxygen levels were observed.

Turbulence (mL/min)	Salinity (ppt)	Dissolved Oxygen (mg/L)	Percent Saturation (%)
20	35	6.65 + 0.06	86.8
	24	6.73 + 0.05	82.7
90	35	7.00 + 0.04	91.4
	24	7.00 + 0.04	86.0
170	35	6.90 + 0.06	90.1
	24	7.04 + 0.03	86.5
250	35	7.08 + 0.03	92.4
	24	7.03 + 0.04	86.4

Table 2. Temperature (ºC) (mean ± SE, N = 8) under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Significant ($P < 0.05$) turbulence level effects on temperature were observed.

Turbulence (mL/min)	Salinity (ppt)	Temperature (°C)
20	35	18.9 + 0.05
	24	18.8 + 0.03
90	35	18.7 + 0.05
	24	18.9 + 0.10
170	35	19.5 + 0.25
	24	18.7 + 0.10
250	35	18.4 + 0.19
	24	18.8 + 0.15

Table 3. Notochord lengths (mm) (mean ± SE, $N = 8$) of southern flounder larvae on days 4, 8, 12, and 16 post-hatching (dph) under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Asterisks indicate that the factor was significant at the $P < 0.01$ levels (**).

Age (dph)	Salinity (ppt)	Turbulence (mL/min) 20	90	170	250	Sal.	Tur.	Int.
4	35	2.91 + 0.18	2.97 + 0.01	3.42 + 0.05	3.41 + 0.04		**	**
	24	3.36 + 0.06	3.33 + 0.03	3.37 + 0.08	3.32 + 0.06			
8	35	3.85 + 0.14	3.89 + 0.02	3.85 + 0.04	3.89 + 0.07			
	24	3.95 + 0.01	3.87 + 0.08	3.84 + 0.03	3.82 + 0.05			
12	35	4.88 + 0.16	4.67 + 0.12	4.68 + 0.07	4.51 + 0.05		*	
	24	4.83 + 0.17	4.75 + 0.08	4.76 + 0.07	4.31 + 0.05			
16	35	6.37 + 0.14	6.00 + 0.18	5.76 + 0.11	5.75 + 0.10		*	
	24	6.08 + 0.26	5.71 + 0.24	5.51 + 0.08	5.69 + 0.05			

Table 4. Wet weight (μg) (mean ± SE, $N = 8$) of southern flounder larvae on days 4, 8, 12, and 16 post-hatching (dph) under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Asterisks indicate that the factor was significant at the < 0.05 (*), < 0.01 (**), or < 0.001 (***) levels.

Age (dph)	Salinity (ppt)	Turbulence (mL/min) 20	90	170	250	Sal.	Tur.	Int.
4	35	141 + 30.6	129 + 7.33	244 + 9.69	304 + 60.2		**	
	24	237 + 16.9	204 + 7.24	264 + 9.35	258 + 24.9			
8	35	384 + 84.3	514 + 117	354 + 32.3	310 + 67.4		*	
	24	355 + 8.24	804 + 179	402 + 30.9	367 + 25.0			
12	35	1149 + 66.2	790 + 156	760 + 99.1	650 + 80.7		**	
	24	987 + 45.9	950 + 60.1	879 + 20.2	650 + 50.9			
16	35	3134 + 208	2324 + 267	1967 + 82.0	1781 + 80.7	*	***	
	24	2179 + 482	2432 + 118	1395 + 101	1571 + 50.9			

Table 5. Dry weight (μg) (mean ± SE, N = 8) of southern flounder larvae on days 4, 12, and 16 post-hatching (dph) under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Asterisks indicate that the factor was significant at the < 0.05 (*), < 0.01 (**), or < 0.001 (***) levels.

Age (dph)	Salinity (ppt)	Turbulence (mL/min) 20	90	170	250	Sal.	Tur.	Int.
4	35	34.9 + 0	34.9 + 0	34.9 + 0	34.9 + 0			
	24	31.9 + 0	31.9 + 0	31.9 + 0	31.9 + 0			
12	35	178 + 7.62	162 + 6.54	162 + 6.54	141 + 12.0			
	24	148 + 19.0	164 + 4.66	159 + 9.87	149 + 2.39			
16	35	293 + 13.7	254 + 15.1	187 + 14.2	174 +13.7		**	
	24	177 + 64.0	217 + 8.74	163 +12.0	160 + 6.34			

Table 6. Survival (%) (mean ± SE, N = 8) of southern flounder larvae on days 4, 8, 12, and 16 post-hatching (dph) under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Asterisks indicate that the factor was significant at the < 0.05 (*), < 0.01 (**), or < 0.001 (***) levels.

Age (dph)	Salinity (ppt)	Turbulence (mL/min) 20	90	170	250	Sal.	Tur.	Int.
4	35	24.8 + 4.57	38.9 + 3.32	58.7 + 6.04	70.0 + 3.60		*	
	24	52.7 + 12.7	49.4 +11.8	70.0 + 13.1	54.2 + 9.62			
8	35	12.3 + 3.78	26.6 + 3.94	51.8 + 6.44	53.7 + 8.00	*	***	
	24	31.9 + 9.34	41.2 + 6.75	66.7 + 2.96	57.3 + 10.3			
12	35	16.2 +4.58	21.5 + 4.81	56.0 +4.32	63.3 + 13.2		**	
	24	27.9 + 9.01	41.3 + 8.41	55.5 + 4.54	65.7 + 10.5			
16	35	13.2 + 5.22	18.7 + 3.63	48.8 + 3.52	58.9 + 13.7		**	
	24	32.5 + 6.46	33.8 + 3.83	67.0 + 10.8	49.1 + 4.32			

Table 7. Body water (%) (mean ± SE, $N = 8$) of southern flounder larvae on days 4, 8, 12, and 16 post-hatching (dph) under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Asterisks indicate that the factor was significant at the < 0.05 (*), < 0.01 (**), or < 0.001 (***) levels.

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

Table 8. Osmolality (mOsm) (mean ± SE, N = 8) of southern flounder larvae on day 15 (dph) under different turbulence levels (20, 90, 170 and 250 mL/min) and salinities (24 and 35 ppt). Asterisks indicate that the factor was significant at the < 0.05 (*), < 0.01 (**), or < 0.001 (***) levels.

Age (dph)	Salinity (ppt)	Turbulence (mL/min) 20	90	170	250	Sal.	Tur.	Int.
15	35	388 + 13.8	402 + 11.9	438 + 12.9	423 + 3.59		*	
	24	372 + 18.1	415 + 7.78	401 + 20.9	430 + 20.9			

LITERATURE CITED

Barahona-Fernandes, M.H. 1978. Effect of aeration on the survival and growth of sea bass (*Dicentrarchus labrax*) larvae: a preliminary study. Aquaculture. 14:67-74.

Bashamohideen M. and V. Parvatheswararao. 1976. Adaptations to osmotic stress in the fresh-water euryhaline teleost, *Tilapia mossambica.* Zool. Anz. Jena. 197:47-56.

Battaglene, S.C. and R.B. Talbot. 1993. Effects of salinity and aeration on survival of and initial swim bladder inflation in larval Australian bass. The Progressive Fish-Culturist. 55:35-39.

Berlinsky, D.L., King, W.V., Smith, T.I.J., Hamilton, J., Sullivan, C.V. 1996. Induced ovulation of southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. Journal of the World Aquaculture Society. 27:143-152.

Brett, J.R. 1979. Environmental Factors and Growth. Fish Physiology. 8:599-675.

Burke, S.D., J.M. Miller, D.E. Hoss. 1991. Immigration and settlement pattern of *Paralichthys dentatus* and *P. lethostigma* in an estuarine nursery ground, North Carolina, U.S.A. Netherlands Journal of Sea Research. 27:393-405.

Claireaux, G. and J.P. Lagardere. 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. Journal of Sea Research. 42:157-168.

Daniels, H.V. and R.J. Borski. 1998. Effects of low salinity on growth and survival of southern flounder (*Paralichthys lethostigma*) larvae and juveniles. UJNR Technical Report 26:187-191.

Dalla Via, J. et al. 1998. Oxygen consumption in sea bass fingerling *Dicentrarchus* l*abrax* exposed to acute salinity and temperature changes: metabolic basis for maximum stocking density estimations. Aquaculture. 169:303-313.

Dou, S., Masuda, R., Tanaka, M., and K. Tuskamoto. 2003. Identification of factors affecting the growth and survival of the settling Japanese flounder larvae, *Paralichthys olivaceus*. Aquaculture. 218:309-327.

Dowd, C.E. and E.D. Houde. 1980. Combined effects of prey concentration and photoperiod on survival and growth of larval sea bream, *Archosargus rhomboidalis*. Marine Ecology – Progressive Series. 3:181-185.

Ellis, E.P. W.O. Watanabe, S.C. Ellis, J. Ginoza, and A. Moriwake. 1997. Effects of turbulence, salinity, and light intensity on hatching rate and survival of larval Nassau Grouper, *Epinephelus straitus*. Journal of Applied. Aquaculture. 7:33-43.

Fukuhara, O. 1990. Effects of temperature on yolk utilization, initial growth, and behavior of unfed marine fish-larvae. Marine Biology. 106:169-174.

Gallego, A., M.R. Heath, E. McKenzie, and L.H. Cargill. 1996. Environmentally Induced short-term variability in the growth rates of larval herring. Marine Ecology Progressive Series. 137:11-23.

Gaignon, J.L., B. Petton. 1998. Hydrodynamics effects in larval rearing tanks on survival and growth of turbot (*Psetta maxima*). Bull. Fr. Peche Piscic. 350-351:303-323.

Ginsburg, I., 1952. Flounders of the genus *Paralichthys* and related genera in American waters. Fish. Bull. 52:267-351.

Hart, P.R., and G.J. Purser. 1995. Effects of salinity and temperature on eggs and yolk sac larvae of the greenback flounder (*Rhombosolea tapirina*). Aquaculture. 136:221-230.

Henderson-Arzapalo, A., R.L. Colura, A.F. Maciorowski. 1988. Temperature and photoperiod induced maturation of southern flounder. Management data series number 154, Texas Parks and Wildlife Dept., Austin, TX. 1-20.

Henne, J.P. and W.O. Watanabe. 2003. Effects of light intensity and salinity on growth, survival and whole-body osmolality of larval southern flounder *Paralichthys lethostigma*. Journal of the World Aquaculture Society. 34(4): 450-465.

Hoar, W.S., and D.J. Randall. 1969. Fish Physiology. 3:177-252.

Holliday, F.G.T., and M.P. Jones. 1965. Osmotic regulation in the embryo of the herring (*Clupea harengus*). Journal of Marine Biology Ass. 45:305-311.

Holliday, F.G.T., and M.P. Jones. 1967. Some effects of salinity on the developing eggs and larvae of the plaice (*Pleuronectes platessa*). Journal of Marine Biology Ass. 47:39-48.

Imsland, A.K., S. Gunnarsson, A. Foss, S. Stefansson. Gill Na, K-ATPase activity, plasma chloride and osmolality in juvenile turbot (*Scophthalmus maximus*) reared at different temperatures and salinities. Aquaculture. 218:671-683.

Killgore, K.J., A.C. Miller, and K.C. Conley. 1987. Effects of turbulence on yolk-sac larvae of paddlefish. Transactions of the American Fisheries Society. 116:670-673.

Kurokura, H., T. Matsumoto, K. Hamba, and S. Aoki. 1995. Oxygen consumption of larval flounder *Paralichthys olivaceus* measured by an improved water bottle method. Fisheries Science. 61:7-10.

Litvak, M.K. 1999. The development of winter flounder (*Pleuronectes americanus*) for Aquaculture in Atlantic Canada: current status and future prospects. Aquaculture. 176:55-64.

MacKenzie, B.R. and W.C. Leggett. 1991. Quantifying the contribution of small-scale turbulence to the encounter rates between larval fish and their zooplankton prey: Effects of wind and tide. Marine Ecology Progressive Series. 73:149-160.

MacKenzie, B.R., T.J. Miller, S. Cyr, and W.C. Leggett. 1994. Evidence for a dome-shaped relationship between turbulence and larval fish ingestion rates. Limnology and Oceanography. 29:1790-1799.

Moustakas, C.Th., W.O. Watanabe, and K.A. Copeland. 2004. Effects of light intensity and salinity on growth, survival, and osomregulatory ability of southern flounder larvae *Paralichthys lethostigma.* Aquaculture. (in press).

Neumann, D.A., et al. 1982. Respiratory response of striped bass (*Morone saxatilis*) to suspended solids. Estuaries. 5:28-39.

Parra G., and M. Yufera. 2001. Comparative energetics during early development of two marine fish species, *Solea senegalensis* and *Sparus aurata*. The Journal of Experimental Biology. 204:2175-2183.

Personal communication from the National Marine Fisheries Service, Fisheries Statistics and Economics Division, Silver Spring, MD. 2001.

Powell, A.B., F.J. Schwartz. 1977. Distribution of paralichthid flounders (Bothidae: *Paralichthys*) in North Carolina estuaries. Chesapeake Science 18:334-339.

Puvanendran, V. and J.A. Brown. 2002. Foraging, growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. Aquaculture. 214:131-151.

Rogers, S.G., T.E. Targett, S.B. Van Sant. 1984. Fish-nursery use in Georgia salt marsh estuaries: the influence of springtime freshwater conditions. American Fisheries Society. 113:595-606.

Rothschild, B.J. and T.R. Osborn. 1988. Small-scale turbulence and plankton contact rates. Journal of Plankton Research. 10:465-474.

Santerre, M.T. 1977. Some effects of temperature and salinity on laboratory-reared eggs and larvae of *Polydactylus sexfilis* (Pisces: *Polynemidae*). Aquaculture. 10:341-351.

Sclafani, M., G. Stirling, and W. Leggett. 1997. Osmoregulation, nutritional effects and buoyancy of marine larval fish: a bioassay for assessing density changes during the earliest life history changes. Marine Biology. 129:1-9.

Sclafani, M., G. Stirling, and W. Leggett. 2000. Osmotic condition, buoyancy change and mortality in larval cod *Gadus morhua*. A bioassay for assessing near-term mortality. Marine Ecology Progressive Series. 193:157-166.

Smith, T.I.J. et al. 1999. Broodstock management and spawning of southern flounder, *Paralichthys lethostigma*. Aquaculture. 176:87-99.

Stickney, R.R. 2000. Encyclopedia of Aquaculture. John Wiley & Sons, Inc. New York, New York.

Strickler, J.R. 1994. Suction prey capture by clownfish larvae (*Amphiprion perideraion*). Copeia. 1:242-246.

Taylor, J.C. and J.M. Miller. 2001. Physiological performance of juvenile southern flounder, *Paralichthys lethostigma*, in chronic and episodic hypoxia. Journal of Experimental Marine Biology and Ecology. 258:195-214.

Utne-Palm, A.C., and J.E. Stiansen. 2002. Effect of larval ontogeny, turbulence and light on prey attack rate and swimming activity in herring larvae. Journal of Experimental Marine Biology and Ecology. 268:147-170.

van Marren, C.C. and H.V. Daniels. 2001. Effects of temperature on egg hatch, larval growth and metamorphosis for hatchery-cultured southern flounder, *Paralichthys lethostigma*. Journal of Applied Aquaculture. 11:21-33.

van Maaren, C.C. et al. 2000. Spawning and maturation of aquaculture species: proceedings of the twenty-eighth UNJR aquaculture panel symposium. UJNR Technical Report; no 28.

Walsh, W.A. et al. 1989. Oxygen consumption by eggs and larvae of the striped mullet, *Mugil cephalus*, in relation to development, salinity, and temperature. The Fisheries Society of the British Isles. 35:347-358.

Watanabe, W.O., and M.W. Feeley, and S. C. Ellis, and E. P. Ellis. 1998. Light intensity and salinity effects on eggs and yolk sac larvae of the summer flounder. The Progressive Fish-Culturist 60:9-19.

Watanabe, W.O., P.M. Carroll. 2001. Sustained, natural spawning of southern flounder *Paralichthys lethostigma* under an extended photothermal regime. World Aquaculture Society. 32:153-166.

Waters, E.B. 1999. Flounder aquaculture and stock enhancement in North Carolina: issues, opportunities and recommendations. North Carolina Sea Grant Publication. UNC-SG-99-02, Raleigh, North Carolina.