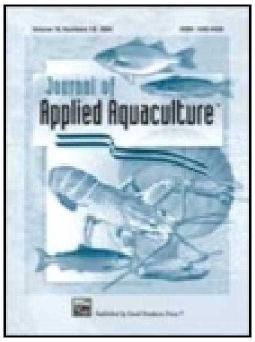

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Effects of three cold weather event simulations on early life stages of Southern Flounder (*Paralichthys lethostigma*)

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ABSTRACT

To determine the minimum age/size at which Southern Flounder, *Paralichthys lethostigma*, can safely be moved to outdoor rearing facilities in Texas, we examined survival of simulated temperature drops in two distinct life stages: premetamorphic larvae and two size classes of postmetamorphic juveniles (small = 9.8 ± 0.3 mm in TL; large = 19.7 ± 0.6 mm). Temperature was lowered by −0.33°C/h to 4°C, 7°C, or 10°C, held for 48 h and then raised at +0.33°C/h back to normal rearing temperature. Fish were monitored daily for survival. Larger postmetamorphic flounder had high survival for all temperature treatments (89%–100% survival), whereas both premetamorphic larvae and smaller postmetamorphic juveniles had low survival (<30%) for all temperature treatments.

KEYWORDS

Survival; premetamorphic; postmetamorphic; temperature tolerance

Introduction

Southern Flounder *Paralichthys lethostigma* are a heavily exploited Bothid ranging from North Carolina along the Atlantic coast and within the Gulf of Mexico to northern Mexico. In Texas, the Southern Flounder fishery has been impacted due to continued population declines (Green and Campbell 2010). Stock enhancement has been instituted to help supplement existing stocks. Current culture practices involve natural and/or strip spawning, egg incubation, and transitioning larvae diet from live rotifers to larger live brine shrimp *Artemia* spp. Fish are given the opportunity to metamorphose and are then transferred to outdoor grow-out ponds followed by pond harvest and bay stocking in the spring. The faster the juvenile flounder can be moved out of the hatchery, the more cost-efficient the system. However, in 2011, grow-out ponds harboring premetamorphic larvae experienced a cold weather event that resulted in local pond water temperatures dropping at a rate of Δ −0.5°C/h over 19 h and remaining below 4°C for a 57 h duration (R.

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Gamez, TPWD-CF, personal communication, February 2011). The limited survival of Southern Flounder in these ponds appeared to favor the larger premetamorphic fish.

Investigating the biological effects of a simulated cold weather event may give some insight into the efficiency of stocking premetamorphic Southern Flounder in grow-out ponds during winter months. Prentice (1989) compared low-temperature survival of wild-caught juvenile and adult Southern Flounder subjected to a rate of −1°C/d from 20°C until temperatures reached 1°C–2°C; juveniles (90% survival) fared much better than adults (37% survival). Taylor et al. (2000) exposed juvenile Southern Flounder to a similar temperature drop of −1°C/d from 20°C until temperatures reached 4°C (study 1) and 2°C (study 2), while keeping fish at these target temperatures for 10 d before increasing temperatures. This study found that juvenile flounder could withstand temperatures as low as 4°C with little to no mortality, whereas exposure to 2°C resulted in much higher mortality (30%–100% mortality). Kaiser et al. (2012) exposed two different feeding stages of premetamorphic larvae (stage 1: egg to rotifer diet, stage 2: *Artemia* diet) to a temperature drop at a rate of −2°C/d beginning at 18°C to target temperatures of 15°C and 12°C and then reared the larvae at target temperatures for an extended time (25 d to 35 d). This study found that stage 1 premetamorphic larvae were susceptible to the lower temperatures, having only 7.2% survival at 15°C and 0% survival at 12°C, whereas stage 2 larvae fared better, having 72% survival at 15°C and 46% survival at 12°C. Based on these reports, juveniles appear to be able to cope with these lower temperatures better than premetamorphic and adult life stages.

There is a need to compare survival between the early life stages of fish (i.e., premetamorphic larvae) and metamorphed juveniles during a more natural temperature drop. Previous studies commonly expose test fish to a gradual temperature increase or decrease over a period of days. Unfortunately, this may condition fish to withstand extreme temperatures. In addition, the lengthy time it takes to subject a fish to 1°C–2°C/d temperature reduction greatly reduces the opportunity of testing a distinct size class such as premetamorphic larvae because they most likely would have undergone metamorphosis before the end of the experiment. By exposing a distinct size class of fish to a natural temperature rate change, such as a moderate cold weather event (decrease) or the warming of a hot summer day (increase), a more meaningful measurement of survival based on temperature shifts can be taken (McDonald et al. 2011). With the need to better understand cold water survival of various early life stages of Southern Flounder, the objective of this study was to monitor survival of fish exposed to three different cold weather scenarios. Here we present some preliminary cold-tolerance experiments on different life stages of Southern Flounder. Due to limited fish availability, along with the constraints of having a single environmental chamber, we were not able replicate these experiments. However, we feel that experimentation conducted in this manner would give hatchery personnel insight into the utility of using grow-out ponds under threat of winter freezes.

Materials and methods

Southern Flounder used in this study were hatchery spawned from wild-caught broodstock at two TPWD-CF operated hatcheries: Sea Center Texas, Lake Jackson, TX (SCT), and the Marine Development Center, Flour Bluff, TX (MDC). Two distinct life stages were used: premetamorphic larvae (PRE) and metamorphed juveniles (POST) (metamorphosis defined as eye migration and 90° rotation in posture; Yousson 1988). Two different size classes of postmetamorphic juveniles were tested in two of the three experiments (Experiments 1 and 2): smaller postmetamorphic (POST1): mean ± SE was 9.8 ± 0.3 mm in TL and larger postmetamorphic (POST2): 19.7 ± 0.6 mm.

Experiments to determine cold tolerance were conducted using a temperature-programmable environmental chamber (Luwa Environmental Specialties, model ES2000 C-LT-R, Raleigh, NC, USA). Recirculating systems were made up of a polypropylene reservoir (55 L) equipped with a regulated submersible water pump adjusted to deliver 0.8 L/min to an aerated shallow water bath (15 L) positioned above the reservoir, with premade seawater (40 L). Each water bath contained fourteen 500 mL polypropylene jars with flow-through holes, each with a single plexiglass wall to separate two independent fish (to prevent cannibalism) allowing for a total of 28 fish per individual recirculating system. Live *Artemia* (1 mL homogenized mixture) were added to each jar containing fish once daily throughout the experiment.

Due to the timing of spawns by state hatcheries, we were not able to conduct all trials at the same time with the same batch of fish. Experiment 1 (4°C treatment) used four independent recirculating systems consisting of 28 PRE and 28 POST1 from SCT split between two recirculating systems, along with 28 PRE and 28 POST2 from MDC split between two additional recirculating systems, all held within the environmental chamber. Controls were organized identically and monitored along with treatment. Experiment 2 (7°C treatment) used four recirculating systems holding 56 PRE from MDC divided among the four recirculating systems. Twenty-eight POST1 from MDC were split between two recirculating systems along with 28 POST2 from MDC split between the other two recirculating systems. Controls were organized identically and monitored along with treatment. Experiment 3 (10°C treatment) used two recirculating systems consisting of 14 PRE (designated as PRE1) from MDC within a single recirculating system, 14 additional PRE (designated as PRE2) from MDC within the second recirculating system, and 29 POST2 from SCT split between the two recirculating systems. Controls were organized identically and monitored along with treatment. During Experiments 2 and 3, air conditioning units malfunctioned in the room with the controls. Water temperatures in the controls reached 25.5°C for Experiment 2 and 24.6°C for Experiment 3, which exceeded lethal limits for premetamorphic larvae averaging 5.1 mm in TL (24.0°C; Kaiser et al. 2012) and attributed to low survival (<25%) among PRE control fish.

Experimental fish were first kept at the start temperature (18°C) for 24 h so that any immediate mortalities caused by transportation could be replaced with acclimated reserve fish prior to the actual experiment. After this 24 h monitoring period, the temperature within the environmental chamber was programmed to decrease $\Delta = -0.33$°C/h to a single target temperature of 4°C, 7°C, or 10°C (each an independent experiment) and were kept at this temperature for a period of 48 h. Temperatures were then increased $\Delta = +\ 0.33$°C/h back to the original start temperature 18°C and maintained for an additional 24 h. Control water temperatures were maintained at around 18°C using ambient room temperature controlled by the laboratory central air conditioner. Fish were monitored for survival one to two times daily.

Water quality was recorded daily with the use of a handheld meter (YSI Pro-plus with quatro probe YSI, Yellow Springs, OH, USA); water parameters consisted of temperature (°C), salinity (ppt), dissolved oxygen (mg/L), saturated dissolved oxygen (%), and pH. Un-ionized ammonia was also monitored daily using a colorimeter (Colorimeter II Hach, Loveland, CO, USA). If ammonia levels were greater than 0.01 mg/L in any recirculating system, a recommended dosage of ammonia remover (Amquel® Kordon LLC, Hayward, CA, USA) was added to each system.

All statistical analyses were performed using the Statistical Analysis System (SAS) version 9.2 (SAS Institute, Inc., Cary, NC, USA), and statistical significance was considered to occur at $\alpha = 0.05$. Initial size differences were tested using a subsample of fish ($n = 10$, per batch), prior to experimentation, using a generalized linear model (Proc GLM) within experiments and among experiments using Tukey's HSD tests. Our analysis included right-censored data (individuals that did not experience death) because we had survival in each experiment. Survival curves comparing life stages were created using the Survival Distribution Function (Proc LIFETEST) along with a comparison of mortality between life stages using a Log rank test with the Šidák multiple-comparison adjustment.

Results

Life stage comparisons differed for all three experiments; Experiments 1 and 2 compared PRE, POST1, and POST2; whereas Experiment 3 compared two PRE stages along with POST2. PRE did not fare well among the temperature

Table 1. Life stages (PRE = premetamorphic; POST1 = smaller postmetamorphic juveniles; POST2 = larger postmetamorphic juveniles) of test batches and survival percentages for control and experimental tanks.

Treatment	Life stage	Control survival (%)	Experiment survival (%)
4°C	PRE	96%	0%
	POST1	100%	14%
	POST2	96%	96%
7°C	PRE	25%	23%
	POST1	64%	7.1%
	POST2	100%	89%
10°C	PRE1[1]	21%	14%
	PRE2[1]	71%	28%
	POST2	100%	100%

[1] We designated PRE in this experiment as PRE1 and PRE2 for two separate batches in this individual experiment.

treatments (0%–28% survival), smaller postmetamorphic fish also had low survival (7.1%–14% survival); whereas the larger POST2 withstood all three water temperature treatments (89%–100% survival: Table 1). Cold water survival was greater as temperature treatments increased (4°C → 10°C) for the premetamorphic larvae; the one exception is PRE1 (MDC) in the 10°C resulting in 14% survival in the treatment.

Log-ranked tests of survival curves detected no differences between PRE and POST1 in treatments 4°C ($P = 0.79$) and 7°C ($P = 0.99$) (Figure 1). Survival of POST2 was greater than the other life stages for each experiment ($P < 0.01$) (Table 1).

Discussion

Results from this study suggest that larger juvenile POST2 Southern Flounder averaging ~16 mm in TL are able to tolerate a cold weather simulation (i.e., rapid temperature drop (–0.33°C/h) to 4°C for 48 h). Previous low-temperature work exposed larger juvenile postmetamorphic fish (≥27 mm in TL, Prentice 1989; weights ≥ 1 g, Taylor et al. 2000) to a gradual temperature drop of 1°C–2°C/d before reaching low temperatures. We believe our study gives a more realistic scenario of a cold weather event, thus confirming the resilience of this species at the larger postmetamorphic life stage. Contrary to the POST2, the POST1 and PRE did not tolerate the cold weather event well. Unlike the POST1, the PRE were expected to follow the same trend of decreased survival as temperatures decreased as indicated in the Kaiser et al. (2012) study. The susceptibility of the POST1 may be due to the amplified stress and increased aerobic and anaerobic metabolism, as demonstrated in many species of flatfish undergoing metamorphosis (De Jesus et al. 1991; Schreiber and Specker 1999; Amara and Galois 2004; Ishibashi et al. 2007; Wang et al. 2015). In nature, the act of metamorphosis in Southern Flounder occurs at the same time larvae migrate from offshore to the shallow, more

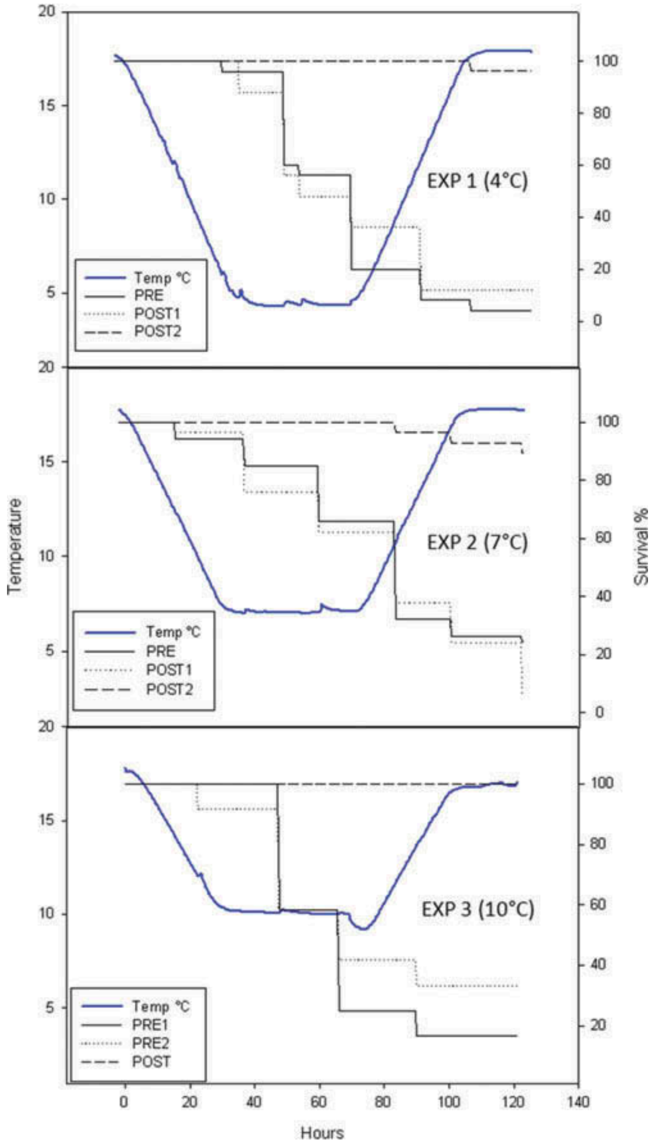

Figure 1. Survival curves of different life stages (PRE = premetamorphic larvae; POST1 = smaller postmetamorphic juveniles; POST2 = larger postmetamorphic juveniles) of Southern Flounder exposed to three different water temperature treatments (4°C, 7°C, and 10°C) with water temperature (°C) data.

variable estuarine systems (Smith, Denson, et al. 1999; Smith, McVey et al. 1999; Yamashita et al. 2001; Wang et al. 2015). The low-temperature tolerance observed in larger postmetamorphic juveniles in this study show that these life stages are well suited to handle the highly variable environments found in an estuarine system. The differences in survival between the POST1 life stage as compared to POST2 at 4°C and 7°C suggests that low-temperature tolerance vastly improves beyond metamorphosis and may improve as the fish grows.

This has been shown in species with similar life cycles where larger-sized juveniles outperformed smaller-sized juveniles exposed to critically low temperatures (Malloy and Targett 1991; Lankford and Targett 2001). It is also worth noting that low-temperature tolerance decreases as juveniles transform to adults, which coincides with the migratory nature of adults (Prentice 1989). Adults emigrate offshore to warmer thermally stable waters during winter months and immigrate back inshore to bay systems once water temperatures warm back up in the spring season.

Previous research has demonstrated that temperature and salinity both greatly influence survival of premetamorphic larvae and fish undergoing metamorphosis (Daniels et al. 1996; Mangino and Watanabe 2006; Kaiser et al. 2012). Our current research in conjunction with survival data of the 2011 Texas freeze of Southern Flounder in hatchery grow-out ponds further suggests the vulnerability of PRE or POST1 once stocked in grow-out ponds. Therefore, some variety of thermal protection may help protect this species during cold weather events (e.g., pond cover or external heat source) (Rafferty 1991; Baird et al. 1993). Current TPWD-CF stock enhancement goals include meeting a production quota as in other sportfish that are currently produced (e.g., Red Drum *Sciaenops ocellatus* and Spotted Seatrout *Cynoscion nebulosus*). However, Southern Flounder have a limited spawning time (winter months) and seem to be more vulnerable to abiotic extremes than Red Drum and Spotted Seatrout, which are spawned from spring through fall months. If thermal protection is not practical for grow-out pond production, an alternative would be to retain the fish in thermally stable indoor hatcheries until weather conditions improve or fish increase to a mean size of 16 mm in TL. Hatchery biologists from TPWD-CF have observed growth averages around 0.5 mm in total length a day shortly after metamorphosis (unpublished data); this would correspond to an additional 2 weeks of additional growth within the indoor hatchery. Kaiser et al. (2012) determined that maintaining water temperatures at around 18°C was necessary for optimum growth and survival for larval Southern Flounder. Hatchery costs associated with this additional time holding fish within indoor tanks are minimal (i.e., only artemia cysts and labor); the issue would be the utilization of tanks that may potentially be used for other batches of larval-sized flounder. This research does help strengthen recent TPWD-CF efforts in raising Southern Flounder to larger sizes within indoor hatcheries for the limited time they are spawned.

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References

Amara, R., and R. Galois. 2004. Nutritional condition of metamorphosing sole: Spatial and temporal analyses. *Journal of Fish Biology* 64:72–88. doi:10.1111/jfb.2004.64.issue-1.

Baird, C. D., R. A. Bucklin, C. A. Watson, and F. A. Chapman. 1993. Heat pump for heating and cooling water for aquacultural production. *Florida Energy Extension Service Series: Circular* 1096.

Daniels, H. V., D. L. Berlinsky, R. G. Hodson, and C. V. Sullivan. 1996. Effects of stocking density, salinity, and light intensity on growth and survival of Southern Flounder *Paralichthys lethostigma* larvae. *Journal of the World Aquaculture Society* 27(2): 153–159. doi:10.1111/j.1749-7345.1996.tb00264.x.

De Jesus, E. G., T. Hirano, and Y. Inuia. 1991. Changes in cortisol and thyroid hormone concentrations during early development and metamorphosis in the Japanese flounder, *Paralichthys olivaceus*. *General and Comparative Endocrinology* 82(3): 369–376. doi:10.1016/0016-6480(91)90312-T.

Green, L., and P. Campbell. 2010. Trends in finfish landings of sport-boat anglers in Texas marine waters, May 1974–May 2008. *TPWD-Management Data Series* 257.

Ishibashi, Y., T. Kotaki, Y. Yamada, and H. Ohta. 2007. Ontogenic changes in tolerance to hypoxia and energy metabolism of larval and juvenile Japanese flounder *Paralichthys olivaceus*. *Journal of Experimental Marine Biology and Ecology* 352:42–49. doi:10.1016/j.jembe.2007.06.036.

Kaiser, J. B., C. K. Faulk, E. A. Williamson, and G. J. Holt. 2012. Natural spawning and larviculture of Southern Flounder *Paralichthys lethostigma*. *World Aquaculture* 43(1): 48–54.

Lankford, T. E., and T. E. Targett. 2001. Low-temperature tolerance of age-0 Atlantic croakers: Recruitment implications for U.S. mid-Atlantic estuaries. *Transactions of the American Fisheries Society* 130:236–249. doi:10.1577/1548-8659(2001)130<0236:LTTOAA>2.0.CO;2.

Malloy, K. D., and T. E. Targett. 1991. Feeding, growth and survival of juvenile summer flounder *Paralichthys dentatus*: Experimental analysis of the effects of temperature and salinity. *Marine Ecology Progress Series* 72:213–223. doi:10.3354/meps072213.

Mangino, A., and W. O. Watanabe. 2006. Combined effects of turbulence and salinity on growth, survival, and whole-body osmolality of larval Southern Flounder. *Journal of the World Aquaculture Society* 37(4): 407–420. doi:10.1111/jwas.2006.37.issue-4.

McDonald, D. L., P. D. Cason, and B. W. Bumguardner. 2011. The critical thermal maximum of juvenile red drum reared for out-of-season stocking in Texas. *North American Journal of Aquaculture* 73:462–467. doi:10.1080/15222055.2011.633690.

Prentice, J. A. 1989. Low-temperature tolerance of Southern Flounder in Texas. *Transactions of the American Fisheries Society* 118:30–35. doi:10.1577/1548-8659(1989)118<0030:LTOSFI>2.3.CO;2.

Rafferty, K. 1991. Hot spots for cold fish: Geo-heat center participates in aquaculture research. *GHC Bulletin* April 1991:14–15.

Schreiber, A. M., and J. L. Specker. 1999. Metamorphosis in the summer flounder, *Paralichthys dentatus*: Thyroidal status influences salinity tolerance. *Journal of Experimental Zoology* 284:414–424. doi:10.1002/(ISSN)1097-010X.

Smith, T. I. J., M. R. Denson, L. D. Heyward, W. E. Jenkins, and L. M. Carter. 1999. Salinity effects on early life stages of Southern Flounder *Paralichthys lethostigma*. *Journal of the World Aquaculture Society* 30:236–244. doi:10.1111/j.1749-7345.1999.tb00870.x.

Smith, T. I. J., D. C. McVey, W. E. Jenkins, M. R. Denson, L. D. Heyward, C. V. Sullivan, and D. L. Berlinsky. 1999. Broodstock management and spawning of Southern Flounder, *Paralichthys lethostigma*. *Aquaculture* 176:87–99. doi:10.1016/S0044-8486(99)00053-8.

Taylor, W. E., J. R. Tomasso, C. J. Kempton, and T. Smith. 2000. Low-temperature tolerance of Southern Flounder *Paralichthys lethostigma*: Effect of salinity. *Journal of the World Aquaculture Society* 31(1): 69–72. doi:10.1111/jwas.2000.31.issue-1.

Wang, Y., Q. Guo, H. Zhao, and W. Lu. 2015. Larval development and salinity tolerance of Japanese flounder (*Paralichthys olivaceus*) from hatching to juvenile settlement. *Aquaculture Research* 46(8): 1878–1890. doi:10.1111/are.12343.

Yamashita, Y., M. Tanaka, and J. M. Miller. 2001. Ecophysiology of juvenile flatfish in nursery grounds. *Journal of Sea Research* 45:205–218. doi:10.1016/S1385-1101(01)00049-1.

Yousson, J. H. 1988. First metamorphosis. In *Fish physiology*, ed. W. S. Hoar, and D. J. Randall, Vol. 11B, 135–196. San Diego, CA: Academic Press, Inc.