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LACK OF SALINITY SELECTION BY FRESHWATER AND BRACKISH POPULATIONS OF JUVENILE BLUEGILL, *LEPOMIS MACROCHIRUS* RAFINESQUE

Mark S. Peterson[1], Natalie J. Musselman[1], Jay Francis[2], Geoffrey Habron[2] and Karen Dierolf[3]

[1] *Department of Biological Sciences*
P.O. Drawer GY
Mississippi State University
Mississippi State, Mississippi 39762

[2] *Department of Wildlife and Fisheries*
P.O. Drawer LW
Mississippi State University
Mississippi State, Mississippi 39762

[3] *Mississippi Museum of Natural Science*
111 North Jefferson Street
Jackson, Mississippi 39202

Abstract: Salinity preferences of juvenile bluegill (*Lepomis macrochirus*) from a freshwater pond in northeastern Mississippi and a brackish bayou in coastal Mississippi held at 0 ‰ were tested at 26.5 ± 1.0°C in salinity-gradient chambers (0, 2, 4, 6, 8, and 10 ‰) under a 12L:12D photoperiod cycle. Juvenile bluegill from both sites did not show any statistically significant preference for any of the salinity options and were not significantly different from controls. These data suggest that juvenile bluegill from either locale fail to show any differential short-term (1.5 h) behavioral effects that might influence their perception and use of saline habitats. These data and data from previous studies suggest that bluegill are better able to physiologically and behaviorally tolerate elevated salinity relative to other centrarchids, particularly *Micropterus* spp.

Key Words: behavioral plasticity, Centrarchidae, salinity, wetlands.

INTRODUCTION

The use of any habitat by fish requires some level of tradeoff between conflicting abiotic and biotic factors. Abiotic factors and physiological responses to those factors have been shown to be an important and often overlooked mechanism in community organization (Dunson and Travis 1991). Species that occupy habitats of different salinities may show different physiological abilities that can influence conspecific distribution patterns. For example, Nordlie *et al.* (1992) determined that sailfin molly (*Poecilia latipinna* Lesueur) from a freshwater habitat showed lower salinity tolerance and regulated their plasma osmotic concentration at a lower range than those from a brackish water habitat. They did not, however, address the fundamental question of whether the differences noted were genetically based or simply the result of an irreversible, nongenetic adaptation to salinity (*sensu* Kinne 1962).

The vast majority of studies addressing distribution or tolerance of fishes to salinity used euryhaline species. The family Centrarchidae, a stenohaline freshwater fish group, contains species able to tolerate salinities approaching 20 ‰. Peterson (1988) and Meador and Kelso (1990) have postulated that physiological abilities among the species within this family may account for observed differences in salinity tolerance. Bluegill (*Lepomis macrochirus* Rafinesque), are found abundantly in saline wetlands and are one of the most saline-tolerant of the centrarchids (Peterson 1988, Peterson and Ross 1991), but very little is known about the influence of salinity on their life history. Results from earlier experiments on bluegill have suggested that they are able to detect slight changes in ambient salinity and are able to use the rate of salinity change as a directional cue to move into and out of low salinity marshes (Peterson *et al.* 1987). Thus, bluegill may be one of the most plastic of all centrarchids in their response to salinity.

We were interested in evaluating salinity selection of juvenile bluegill from brackish and freshwater locations (965 km apart) and the role it plays in their use of saline habitats. A number of studies have evaluated teleost responses to salinity using salinity selection experiments as behavioral descriptors of long-term (hrs to days) exposure to different salinities (Reynolds and Thomson 1974, Meador and Kelso 1989). However, we chose to evaluate behavioral differences and similarities in salinity selection over a short time frame (1.5 hrs). This was done for two reasons: 1) behavioral responses are the first in a suite of responses by metazoans when confronted with an environmental perturbation (Slobodkin and Rapoport 1974), and thus, behavior should be elicited quickly and may play an important initial role in habitat selection; and 2) long-term exposure to salinity may elicit physiological responses (Peterson 1988, Meador and Kelso 1990) that could modify or mask behavioral responses (*sensu* Slobodkin and Rapoport 1974) when bluegill first contact saline media.

The behavior associated with initial selection of saline habitats is unknown. We theorized that behavioral responses to initial salinity contact would be dependent upon history of salinity exposure and thus geographic location. Therefore, we tested the hypothesis that individuals from freshwater populations of bluegill would select lower salinities than individuals from brackish populations of bluegill that are consistently exposed to saline media.

MATERIALS AND METHODS

Experimental Fish and Laboratory Protocol

Juvenile bluegill from a brackish population were collected in water varying from 0–2 ‰ salinity from Old Fort Bayou, Mississippi (30° 25′ lat and 88° 45′ long), where salinity can range between 0 ‰ and 10 ‰ and bluegill are quite abundant across this range of salinity (Peterson and Ross 1991). Juvenile bluegill from a freshwater population were collected from a pond on the Mississippi State University campus (33° 27′ lat and 88° 49′ long). All fish were collected with a beach seine between late September and early October 1991.

Fish from each population were maintained separately in the laboratory at 0 ‰ and 26.5 ± 1.0°C under a 12L:12D photoperiod in 375 l polyethylene tanks for at least 4 weeks prior to experimentation. Fish were fed Tetramin flake food *ad libitum* 3 times per day. Fish from both populations were held in 0 ‰ because Meador and Kelso (1989) determined that salinity selection by young-of-the-year (YOY) largemouth bass, (*Micropterus salmoides* Lacepede) was not influenced by acclimation to 0 ‰ or 5 ‰. We were not interested in how acclimation of juvenile bluegill to different salinities influences behavior associated with salinity selection, but only in behavior associated with individuals from environments with different histories of salinity exposure.

Four Staaland chambers consisting of six test compartments each (with horizontally staggered upper and lower vertical partitions) were constructed and used as described by Fivizzani and Spieler (1978). This chamber design only allows a continuous linear gradient of salinity based on density. Seawater was made with Instant Ocean Sea Salts (Mentor, Ohio) and dechlorinated tapwater. Two control chambers were filled with dechlorinated tapwater (0 ‰ salinity). The six compartments of the remaining two chambers were filled to the top of the lower partition with water ranging from 0 ‰ to 10 ‰ salinity in 2 ‰ increments. Freshwater was then siphoned into the 0 ‰ salinity chamber at 750 ml/min and allowed to overflow onto the 2 ‰ water in the next compartment. The 2 ‰ water in that compartment was pushed up (due to weight of lower density surface water) and on top of the 4 ‰ water of the next compartment. This was continued until all compartments were filled to a depth of 25.2 cm. By minimizing mixing of the overflowing freshwater and the saline water in the test compartments, a linear salinity gradient was created.

During the experiments, juvenile bluegill were able to move above the lower partitions of the test compartments and descend into adjacent compartments. During each replicate (fish were used only once), a single bluegill was placed into the 0 ‰ salinity end of the treatment and corresponding control chambers and allowed 1 hour to explore the chamber. Camcorders were used to document fish position for an additional 1.5 hours. Individual location of bluegill was recorded at 1-min intervals, producing 90 observations per fish. Position of all chambers within the laboratory and direction of the salt gradient (0 ‰ to 10 ‰) were reversed between replicates to reduce chamber and room effects. Chambers were emptied and rinsed between each replicate. We completed nine replicates (treatment and control) with freshwater bluegill and nine (control) and eight (treatment) replicates with brackish bluegill. Salinity and temperature were recorded immediately after filling the chambers and at the end of each experiment with a YSI S-C-T meter. Fish standard length (SL, mm) was recorded at the end of each experiment.

Statistical Analyses

Statistics were processed using either SPSS PC+ (Ver. 4.0; SPSS Inc., Chicago, IL) or SAS (Statistical Analysis Software; Research Triangle Park, NC) and all values

Table 1. Comparison of initial and final salinity and water temperature for freshwater and brackish population control and treatment experiments. BT = brackish treatment; BC = brackish controls; FT = freshwater treatment; FC = freshwater controls. Initial and final values of each parameter were analysed by a paired t-test. Values are reported as X ± SD.

Salinity (‰)	Brackish Treatment		
	Initial	Final	P
0	0.0 ± 0.0	0.0 ± 0.0	0.35
2	1.9 ± 0.2	1.7 ± 0.2	0.12
4	3.9 ± 0.2	3.7 ± 0.3	0.17
6	5.9 ± 0.2	5.6 ± 0.2	0.01
8	8.1 ± 0.3	7.8 ± 0.3	<0.01
10	10.2 ± 0.4	9.9 ± 0.6	0.12
Salinity (‰)	Freshwater Treatment		
	Initial	Final	P
0	0.0 ± 0.0	0.1 ± 0.1	0.19
2	1.8 ± 0.1	1.7 ± 0.2	0.12
4	3.8 ± 0.2	3.8 ± 0.2	1.00
6	5.8 ± 0.2	5.7 ± 0.3	0.13
8	8.0 ± 0.3	7.7 ± 0.4	0.01
10	10.1 ± 0.4	9.8 ± 0.5	0.01

Treatment	Water Temperature (°C)		
	Initial	Final	P
BT	25.4 ± 0.7	23.3 ± 0.3	<0.01
BC	25.3 ± 0.9	22.9 ± 0.8	<0.01
FT	25.0 ± 1.5	23.1 ± 0.6	<0.01
FC	25.2 ± 0.6	22.9 ± 0.7	<0.01

were declared significant at the $P < 0.05$ level. We used a Split-plot Analysis of Variance (GLM; main plot= treatment, subplot= salinity) and a Fisher's PLSD (protected least significant difference) to 1) evaluate differences in mean time spent in each salinity or compartment within each treatment or control; and 2) compare mean time spent per salinity or compartment among the control and treatment groups. A one-way Analysis of Variance (ONEWAY) was used to compare fish SL among the control and treatment groups for bluegill from both locations. A paired t-test was used to compare water temperature and salinity before and after each replicate.

RESULTS

Mean fish lengths of the four groups ranged between 43.7 (freshwater treatment) and 46.8 (freshwater control) mm SL with no significant differences in length among any control or treatment groups (ONEWAY, $P > 0.05$). Overall, salinity between the initial and final values of the treatment compartments was not significantly different ($P > 0.05$). However, in the coastal bluegill experiments, final salinity values were lower in the 6 ‰ and 8 ‰ salinity chambers ($P \leq 0.01$; Table 1), whereas the final values were lower in the 8 ‰ and 10 ‰ salinity chambers for the freshwater bluegill experiments ($P \leq 0.01$). Reduction in salinity in these exceptions were < 0.5 ‰ on average (Table 1). There were minor (about 2°C on average) but statistically significant differences between initial and final water temperatures in the control and treatment chambers (paired t-test, $P < 0.01$; Table 1).

Table 2. Split-plot ANOVA summary table of the influence of treatment (mainplot) and salinity (subplot) on the amount of time spent in each salinity (treatments) or compartments (controls) for freshwater and brackish populations of juvenile bluegill.

Source	DF	Type III SS	MS	F	P
Treatment (Trt)	3	10.9	3.6	1.2	0.3167
Error (a)	31	92.4	3.0		
Salinity (Sal)	5	3476.4	695.3	3.3	0.0074
Sal*Trt	15	4699.6	313.3	1.5	0.1161
Error (b)	155	32663.6	210.7		
Total	209	40795.2			

There was no significant difference (Table 2; $P > 0.31$) in terms of time spent within each salinity or compartment among either the two controls or treatments. There was, however, a significant salinity (or compartment) effect ($P < 0.01$) within each of the four groups (Table 2 and 3). There was not a significant interaction [treatment (or control) × salinity (or compartment)] effect (Table 2; $P > 0.11$). Individuals from the freshwater pond did not show a significant preference for any salinity or compartment (Table 3; all $P > 0.09$). Both control and treatment individuals from brackish water spent significantly more time in the two end-compartments when compared to the middle compartments (Table 3; all $P \leq 0.05$). Comparison of the mean time spent in each salinity or compartment among all groups indicated that brackish control individuals spent significantly more time in the 0 ‰ (or compartment 1) than freshwater control individuals (Table 4; $P < 0.05$). Brackish treatment individuals spent significantly more time in 0 ‰ than individuals from freshwater (Table 4; $P \leq 0.01$). No other significant pairwise comparisons were found (Table 4).

DISCUSSION

Results from these experiments indicated that there were no differences in salinity selection between brackish and freshwater juvenile bluegill. Therefore, juvenile

Table 3. Number of minutes spent by bluegill in each of six salinities or compartments for the brackish control and treatment and freshwater control and treatment. Data were analysed by a split-plot ANOVA (treatment = main plot; salinity = subplot) and a Fisher's PLSD (protected least significant difference) with the GLM procedure of SAS. Values are reported as X ± SD.

Freshwater Control

Compartment #	Time (min)	Significance Matrix	1	2	3	4	5	6
1	11.4 ± 11.9	1		.94	.39	.82	.86	.09
2	11.9 ± 9.0	2			.43	.87	.91	.10
3	17.3 ± 12.6	3				.53	.50	.39
4	13.0 ± 7.3	4					.96	.14
5	12.7 ± 8.1	5						.12
6	23.2 ± 21.8	6						

Freshwater Treatment

Salinity (‰)	Time (min)	Significance Matrix	0	2	4	6	8	10
0	13.9 ± 11.2	0		.55	.60	.91	.36	.97
2	9.8 ± 7.1	2			.26	.48	.13	.53
4	17.4 ± 13.4	4				.68	.70	.63
6	14.7 ± 11.2	6					.43	.93
8	20.1 ± 13.1	8						.38
10	14.1 ± 21.1	10						

Brackish Control

Compartment #	Time (min)	Significance Matrix	1	2	3	4	5	6
1	21.0 ± 12.2	1		.08	.05	.21	.20	.35
2	9.1 ± 4.5	2			.81	.65	.65	<.01
3	7.4 ± 3.7	3				.47	.49	<.01
4	12.4 ± 9.5	4					.97	.03
5	12.2 ± 4.7	5						.03
6	27.4 ± 24.9	6						

Brackish Treatment

Salinity (‰)	Time (min)	Significance Matrix	0	2	4	6	8	10
0	31.5 ± 22.4	0		<.01	<.01	<.01	<.01	.12
2	11.6 ± 8.1	2			.60	.36	.85	.24
4	7.9 ± 6.8	4				.69	.74	.09
6	5.0 ± 4.5	6					.47	.04
8	10.2 ± 9.5	8						.17
10	20.2 ± 23.1	10						

bluegill from either locale should be able to spend an equal amount of time in salinities ranging from 0 ‰ to 10 ‰ with little or no apparent short-term behavioral responses that might influence their selection and use of saline habitats. However, the end-compartment effect (probably an artifact of the chamber design) documented for the brackish population makes differences in actual salinity selection within and between groups difficult to interpret. The fact that all juvenile bluegill from brackish water spent more time in 0 ‰ (compartment 1) and 10 ‰ salinity (compartment 6) compared to intermediate salinities (or compartments) indicates that 10 ‰ salinity is not avoided by bluegill for short (< 1.5 hrs) periods of time. Peterson and Ross (1991) documented considerable numbers of sunfish in salinity up to 10 ‰. Energetics associated with osmoregulation are influenced by increasing ambient salinity up to the isosmotic point (about 8–9 ‰; Peterson 1988, Meador and Kelso 1990); beyond that salinity, fish will dehydrate and eventually die.

Comparable data on the influence of salinity on centrarchid behavior is based on studies by Peterson *et*

Table 4. Comparison of amount of time spent in each of the six salinities or compartments per group. Data were analysed by a split-plot ANOVA (treatment = main plot; salinity = subplot) and a Fisher's PLSD (protected least significant difference) with the GLM procedure of SAS. Abbreviations as in Table 1. All values are reported as X ± SD. NS = no significant comparisons.

Salinity (‰) or Compartment	Brackish Control (BC)	Brackish Treatment (BT)	Freshwater Control (FC)	Freshwater Treatment (FT)	P
0/1	21.0 ± 12.2	31.5 ± 22.4	11.4 ± 11.9	13.9 ± 11.2	BC > FC BT > FT
2/2	9.1 ± 4.5	11.6 ± 8.1	11.9 ± 9.0	9.8 ± 7.1	NS
4/3	7.4 ± 3.7	7.9 ± 6.8	17.3 ± 12.6	17.4 ± 13.4	NS
6/4	12.4 ± 9.5	5.0 ± 4.5	13.0 ± 7.3	14.7 ± 11.2	NS
8/5	12.2 ± 4.7	10.2 ± 9.5	12.7 ± 8.1	20.1 ± 13.1	NS
10/6	27.4 ± 24.9	20.2 ± 23.1	23.2 ± 21.8	14.1 ± 21.1	NS

al. (1987) and Meador and Kelso (1989). Peterson *et al.* (1987) indicated that juvenile bluegill can use gradual salinity fluctuations (± 1 ‰/hr) as directional cues to move into and out of low-salinity habitats. The increased activity under gradual salinity change (increase or decrease) documented in Peterson *et al.* (1987) is similar to the activity noted for bluegill when moving among salinity compartments in this study. Meador and Kelso (1989) examined two size classes of largemouth bass. They determined that YOY largemouth bass (65–90 mm TL) from freshwater and saline habitats selected freshwater when given a choice, whereas adult largemouth bass (190–250 mm TL) selected 3 ‰. This indicates a size-dependent response to salinity in largemouth bass that the authors determined was not influenced by acclimation to 0 ‰ or 5 ‰. In contrast, juvenile bluegill from fresh and brackish waters did not select one salinity over another when given a choice. Because the freshwater bluegill population had never been exposed to salt water and individuals of the brackish bluegill population had been exposed to salt water (possibly to 10 ‰), we suggest that there is no geographic variation in salinity selection by bluegill. Perhaps juvenile bluegill are more plastic in their behavior and physiology relative to salinity than other *Lepomis* and *Micropterus* spp. (Peterson *et al.* 1987, Peterson 1988, Meador and Kelso 1990).

We believe that the decreases in water temperature and salinity noted during the course of this study did not influence salinity selection in bluegill. The average reduction in salinity was only 0.5 ‰, which is within the range of accuracy of the salinometer. Although seasonal water temperature changes influence salinity selection in the Gulf killifish (*Fundulus grandis* Baird & Girard) (Miller *et al.* 1983), the 2°C drop in water temperature over the 2.5-hr period of the experiment was most likely not important; it should also be noted that all bluegill were exposed to the same decrease in water temperature.

Bluegill are one of the most abundant centrarchids in low-salinity habitats (Bailey *et al.* 1954, Peterson and Ross 1991) and may rival the abundance of freshwater ictalurids in saline media (Stickney and Simco 1971). Peterson (1988, 1991) suggested that *Lepomis* spp. were better able to use saline habitats than *Micropterus* spp. based on physiological and growth data. The fact that juvenile bluegill from both freshwater and brackish locales spent considerable time in all salinities supports this assertion and suggests that juvenile bluegill may be one of the most adaptable to saline media of the stenohaline freshwater family Centrarchidae. Based on this and other studies, it seems that bluegill may be able to tolerate moderate saltwater intrusion, although more long-term studies are required that include different life stages of bluegill and higher salinity treatments.

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