FUNDULUS HETEROCLITUS DISTRIBUTION IN NORTHEAST CREEK: SEX AND SIZE CLASS CHANGES ALONG A SALINITY GRADIENT

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Estuarine species such as the killifish, *Fundulus heteroclitus*, are exposed to a wide range of salinities (1 ppt to about 35 ppt). Killifish can survive indefinitely without pre-acclimation in water with salinities between about 1 ppt and 120 ppt (Griffith, Copeia 1974: 319-331, 1974). The impressive mechanisms for physiological osmoregulation by killifish have clearly been demonstrated, but it has also been hypothesized that they can behaviorally osmoregulate by seeking salinities isotonic to their blood plasma (Kidder, Bull. Mt. Desert Island Biol. Lab 36:69, 1997). Behavioral osmoregulation helps fish to minimize the energy requirements otherwise necessary for changes in expression of ion transport and water channel proteins in gills, kidneys, and intestines. Behavioral osmoregulation occurs in the lab, with individuals seeking isoosmotic regions within a salinity gradient (Kidder, Bull. Mt. Desert Island Biol. Lab 36:69, 1997). In addition, field observations suggest that individuals move with changing salinity by following incoming tides (Findlay et al. Bull. Mt. Desert Island Biol. Lab 41:99, 2002). The purpose of this investigation is to determine if *F. heteroclitus* densities in a natural population are correlated with salinity. Additionally, we explored whether different sexes or size classes are distributed differently with regard to salinity.

Field observations were carried out from late May to early August 2002 in Northeast Creek on Mount Desert Island, ME. The site is a small estuary 7 km long crossed by a bridge 2 km from the ocean. Surface water in the estuary is typically low in salinity but brackish water extends for about 3 km above the bridge. *Fundulus heteroclitus* is abundant in this estuary along with American eels (*Anguilla rostrata*), sticklebacks (*Apeltes quadracus* and *Pungitius pungitius*), and Atlantic silversides (*Menidia menidia*). Overall, data were collected at 18 sites within the estuary over the season. These sites within the estuary were also lumped into four large areas during data analysis, with the first representing all upstream sites more than 0.5 km above the bridge at Rt.1, the second the portion immediately upstream of the bridge, the third immediately below the bridge, and the fourth all areas from 0.2 km below the bridge to the ocean. At each collection site we collected samples of fish as well as data on salinity, temperature, and depth.

Individuals were collected by seines (3.5 m and 8.5 m) and non-baited minnow traps in waters up to 1.5 m deep left for approximately 2 hours. Size classes were determined by a natural break (42 mm) in a sample size distribution (n=1893) although the size classes were changed mid-July to account for individual growth during the season (break was at 45 mm). Sex was determined by coloration. Virtually all males were identified by the presence of a black ocellus on the first dorsal fin; males also tended to have darker and thicker bars and brighter yellow to green bodies ventrally. Females tended to have no distinct markings and were grey in color. Individuals that were questionable were identified by gentle squeezing to extrude sperm or eggs.

Killifish were found throughout the estuary in a wide range of salinities, temperatures, and depths. *Fundulus heteroclitus* were found to be abundant in a wide variety of salinities ranging from < 1 - 34.1 ppt. To examine fish abundance and distribution vs. salinity, we compared water salinities by dividing salinities associated with collections into three ranges, hypoosmotic (< 7.5 ppt), relatively isoosmotic (7.5 – 15 ppt), and hyperosmotic (> 15ppt). Summing over all sites, the category of salinity had no effect on the total number of killifish collected (Kruskal-Wallis tests, traps: $p = .42$, $n = 80$; seine: $p = .97$, $n = 61$). The same absence of a salinity effect on total number of individuals captured could be seen when the most intensively sampled site, the channel in Area 3, was examined ($n = 42$ minnow trap samples, $p = .36$). We did observe large shoals of individuals moving upstream with the incoming tide as previously reported (Findlay et al. Bull. Mt. Desert Island Biol. Lab 41:99, 2002). However, this flux of individuals did not affect the apparent densities of fish in various salinities as seen in our collections.

It is possible that the absence of a salinity effect on the number of individuals collected is an artifact of the collecting methods. If traps and seines are highly stochastic collecting devices, this might obscure real differences in density. However, a separate experiment done in the fall of 2002 support traps as a viable, repeatable measure. In this experiment, colleagues (S. Salinas, C. Tremblay & D. Lofland, COA) showed that paired minnow traps had similar catch rates (Spearman rank correlation, $r = .78$, $p= .005$), and that traps with 5 individuals present collected similar numbers compared with empty traps (paired t-test, $p=.47$). Thus, at least for trap data, results are repeatable and small numbers of conspecifics do not have strong negative or positive effects on the capture of additional individuals.

There were distinct patterns of killifish distribution with respect to sex. Individual samples often showed substantial biases away from the estimated population sex ratio. Of the 89 samples with greater than 20 individuals, 73% (26 of 35 seine samples, 39 of 54 trap samples) had a statistically significant bias in the sex ratio compared to the overall population sex ratio of 52:48 (male:female). This suggests that sexes are not randomly distributed in the environment, but tend to associate with same-sexed individuals. We examined this pattern more closely by looking at the effects of salinity on the sex ratio of collections.

The percentage of males in a collection was significantly higher in relatively isoosmotic waters, both when the entire data set was examined (all samples with $\geq$ 20 individuals - traps: $p = .022$, $n = 44$; seine $p = .006$, $n = 31$) and when the most intensively examined site (the large channel at Area 3) was analyzed separately (trap only: $p = .02$, $n = 29$). Sex ratio averaged 68% for isoosmotic samples, but ranged near 50% for collections with hyperosmotic and hypoosmotic salinities with both methods.

The reasons for the sex differences in overall distribution could be related to several factors. If reproduction is restricted to a subset of the adult habitat, males may spend longer times at these sites, generating the observed pattern in sex ratio. Alternatively, there may be some physical or biological factor that varies with isoosmotic waters that produces more favorable habitat for males.

There were also distinct patterns of killifish distribution with respect to individual size. Specifically, smaller individuals were more common in the upstream portions of the estuary, which tended to be hypoosmotic. Looking at all collections with at least 10 individuals, the percentage of the small size class differed significantly with salinity ($p = .031$, $n = 99$ samples), and was highest in hypoosmotic conditions (82 % small fish), and lowest in isoosmotic conditions (56 % small fish). The higher proportion of small individuals upstream appears to be due, at least in part, to the larger areas of shallow habitat that exist in the upstream portions of this estuary. However, either salinity or a variable that was correlated with salinity appeared to affect size distribution of individuals within a site in a similar way. Using data from the site with the most collections (Area 3), minnow trap samples taken during hypoosmotic salinities had a higher median percentage of small individuals (80%) than when the same site had isoosmotic salinities (55% small individuals). The size related pattern of distribution was also reflected when data were analyzed independently for each sex.

One important point is that although these results reveal statistically significant patterns, individuals of both sexes and size classes are found over a broad range of physical factors, including salinity, in the estuary. Within these broad ranges, however, there are real differences in the tendencies of how individual fish respond to changes in salinity depending on their sex and size class.

These results demonstrate that males and females have different probabilities of occurring in waters of differing osmolality during the summer months. This suggests that studies of osmoregulation might benefit by including sex and size of individuals as a covariate in any study, especially when data are taken from individuals recently collected from the field. In particular, physiological studies often use large individuals from a population, and in this case that would represent individuals found more commonly in relatively isoosmotic conditions compared with smaller conspecifics. (Supported by NSF C-RUI 011186 to RLP, GWK and CWP).