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Influence of salinity on the rates of oxygen consumption in two species of freshwater fishes, *Phoxinus erythrogaster* (family Cyprinidae), and *Fundulus catenatus* (family Fundulidae)

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Abstract

A dye process in a textile plant in southern Kentucky (USA) produces large quantities of saline wastewater which eventually enter Lake Cumberland via a municipal sewage treatment plant on Lily Creek. The impact of hypersaline conditions on two fish species native to the Cumberland River drainage system, redbelly dace (*Phoxinus erythrogaster*) and northern studfish (*Fundulus catenatus*), was assessed. These species were subjected to salinities of 0, 4, and 10‰ after which routine oxygen consumption values were determined. Significant correlations of salinity with oxygen consumption were demonstrated for both species with *P. erythrogaster* showing greater overall impact of salinity on metabolic rate.

Introduction

Many freshwater fish species experience naturally saline conditions in what Bayly (1972) has termed athalassic waters. Among North American freshwater fishes are those with no phylogenetic affinity with the marine environment, termed 'primary' species, and those whose marine affinities cause them to be termed 'secondary' species (Berra, 1981). It would be of particular interest to compare adaptation to saline conditions in primary versus secondary freshwater species. Tolerance to saline conditions has been investigated in primary species such as members of the families Cyprinidae (Matthews & Hill, 1977; Kilambi & Zdinak, 1980; Vigg, 1982; Kraiem & Pattee, 1988; Frain, 1987), Catostomidae (Wilkes & McMahon, 1986a; 1986b; Walker *et al.*, 1989), and Centrarchidae (Peterson, 1988) as well as secondary freshwater families such as the Fundulidae (Duff & Fleming, 1972; Ahokas & Duerr, 1975), Poeciliidae (Al-Dahan & Bhatti, 1977; Chervinski, 1984), Gasterosteidae (Nelson, 1968) and Cichlidae (cf. Stickney, 1986).

Human-induced salinization of inland waters has become an environmental problem of global magnitude. Studies on the salinization of freshwater systems usually have focused on large-scale impacts of agricultural practices such as deforestation and irrigation (Hart *et al.*, 1990; Williams, Taafe & Boulton, 1991). Salinity increases resulting from industrial wastewater release are significant for the localized, sometimes severe, impact they have on freshwater biota. In assessing the tolerance of freshwater fishes to salinization of their habitat, it would be of particular interest to consider comparative adaptive capacities of primary vs secondary species.

Among the industrial processes that result in the production of large quantities of saline wastewater is the dying of textiles. The Union Underwear Company in Jamestown, Kentucky currently uses over 30 000 kg of sodium chloride per day to fix dyes on clothing (Commonwealth of Kentucky, 1989). The wastewater has been treated along with other municipal wastewater at the Jamestown Sewage Treatment Plant before being released into Big Lily Creek which drains into Lake Cumberland.

Rather than relying upon standard assays of critical responses to toxicants, a precise, more humane methodology would be the analysis of physiological responses to sublethal toxicant levels. This would also be a more realistic appraisal as habitat quality is not ultimately determined by its potential for lethality but by the extent of physiological stress it imposes on resident species. A method that may lend itself to rapid yet accurate field analysis of pollution impact is the evaluation of metabolic response to environmental conditions by measurement of dissolved oxygen consumption. This approach is used here in the assessment of the effect of salinity on the oxygen uptake of two freshwater fish species native to the Cumberland River drainage of the southeastern United States, the redbelly dace, *Phoxinus erythrogaster*, and the northern studfish, *Fundulus catenatus*. As a cyprinid, *Phoxinus* is considered a primary species, while *Fundulus*, as a member of the family Fundulidae, is a secondary species. Utilizing these two species will enable a consideration of the role of phylogenetic history in determining the adaptive capacity of freshwater species to environments subject to industrial salinization.

Materials and methods

The study site

The Lily Creek system comprises two streams – Big Lily Creek, which receives treated effluent from the Jamestown Sewage Treatment Plant, and Little Lily Creek which is relatively unaffected by pollutants. Waterfalls (about 15 m in height) at the lower reaches of both streams act as barriers to upstream dispersal of fishes. The two streams join at the base of these falls and eventually empty into Lake Cumberland 3 km downstream (Fig. 1). A profile of the capacity for the stream system to dilute saline effluents was obtained by measuring conductivity with a Fisher model no. 152 conductivity/salinity meter along the length of the streams at the sites indicated in Fig. 1. A limited amount of field sampling of fish fauna was done using a backpack mounted electroshocker.

Laboratory studies

Adult individuals of *P. erythrogaster* and *F. catenatus* were collected from Lily Creek and neighboring streams, transported in carboys to the University of Kentucky Aquatic Research Facility and acclimated in a controlled environment room held at 16 °C with a photoperiod of 14 h light, 10 h dark. Fish were fed Tetramin flake flood *ad lib* during the two week initial acclima-

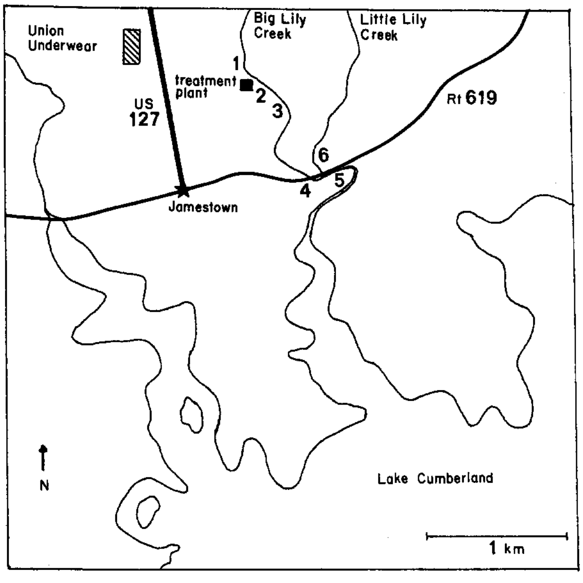

Fig. 1. Map of study area. Numbers indicate sample sites.

tion period. Saline exposure conditions were established using several 38 l (10 gal) aquaria each with its own under-gravel filtration and aeration device. Desired salinities were created using Instant Ocean synthetic sea salts mixed with tap water and vigorously aerated for one day. Two experimental tanks were used for each salinity tested – one for each species. Twenty five individuals of each species were directly introduced into the tanks with salinities of 0 and 4‰. Gradual exposure to 10‰ was achieved by directly introducing the fishes to 4‰ and increasing the salinity in 2‰ increments every four days. Fishes were permitted to acclimate to the experimental salinity for at least one week prior to measurement of oxygen consumption. In the case of those individuals subjected to the more gradual acclimation to 10‰, the duration of acclimation before measurements were made was three or four days after the final salinity adjustment. Fishes were not fed for 24 hours preceding measurement of oxygen consumption. Ten individuals were used in each experimental trial with two replicates for each salinity and species combination. Oxygen consumption was therefore measured for a total of 20 individuals at each salinity and species combination. Each individual was placed in a BOD bottle containing water of the respective acclimation salinity and temperature. Each bottle was shielded with black plastic to minimize external disturbances of the fishes contained therein. Fishes were permitted to adjust to their new surroundings for a period of three hours during which time oxygen saturation was maintained by gently bubbling air into the bottles. Initial dissolved oxygen values (mg l^{-1}) were obtained using an Extech (model EX 401) dissolved oxygen probe and pH/mV meter. Each bottle was then topped up and sealed for one hour after which a second oxygen measurement was made. At this time, each individual was removed, blotted dry and weighed. Experimental subjects were only used once; all fishes were eventually returned to the sites from which they were collected. This technique has proven useful for the determination of weight-corrected oxygen consumption rates in other small fish species (cf. Barton & Barton, 1987; Barton & Elkins, 1988). Data were statistically analyzed using ANOVA techniques available in the SAS software.

Results

Measurements of stream conductivity were made during a period of peak flow in 1989 (Table 1). Marked fluctuation of flow is characteristic of headwaters of streams in this area with flows becoming intermittent during the seasonal drought period of late summer-early fall. During this time, streamflow in Big Lily Creek may be maintained solely by the discharge from the treatment plant which releases approximately 132 000 m^3 of wastewater per day (Commonwealth of Kentucky, 1989). The significance of the waterfalls as a barrier to dispersal is most apparent in comparing the number of species collected from Little Lily Creek above the falls with collections made below the falls (Table 1). Three species were recorded in Big Lily Creek above the sewage treatment plant. The only individuals collected between the outfall of the treatment plant and the point of confluence of Big and Little Lily Creeks

Table 1. Conductivity and fish species recorded at each sample site.

Sample site	Conductivity (μmho cm^{-1} cm^{-2})	Species collected*
1	10.5	*Rhinichthys atratulus* *Campostoma oligolepis* *Semotilus atromaculatus*
2	180.0	None collected
3	108.0	None collected
4	59.5	*Rhinichthys atratulus*
5	56.2	*Semotilus atromaculatus* *Campostoma oligolepis* *Rhinichthys atratulus* *Lepomis megalotis* *Micropterus punctulatus* *Funulus catenatus*
6	5.2	*Rhinichthys atratulus* *Campostoma oligolepis* *Semotilus atromaculatus* *Phoxinus erythrogaster*

* In descending order of abundance.

were the occasional strays recovered within a few meters of the point of confluence. No fishes were observed from the outfall to the waterfalls on Big Lily Creek. Electroshocking in this area was hindered by the excessive conductivity of the water, however.

Laboratory studies

Mean oxygen consumption values and their respective 95% confidence intervals were calculated for each species at each salinity (Fig. 2). Both species demonstrated increased consumption rates as salinity increased with *P. erythrogaster* showing a greater overall metabolic rate and a more pronounced response to increased salinity when compared with *F. catenatus* (Fig. 2). In an analysis of the combined effects of salinity and species on oxygen consumption, both the salinity level and the species tested were demonstrated to have a significant impact on mean oxygen consumption ($F = 17.98$ and 93.52 respectively; $P < 0.0001$). While the salinity impact on oxygen consumption was significant for both species, the effect was greater for *P. erythrogaster* ($F = 12.35$; $P < 0.0001$) than for *F. catenatus* ($F = 7.91$; $P < 0.0009$).

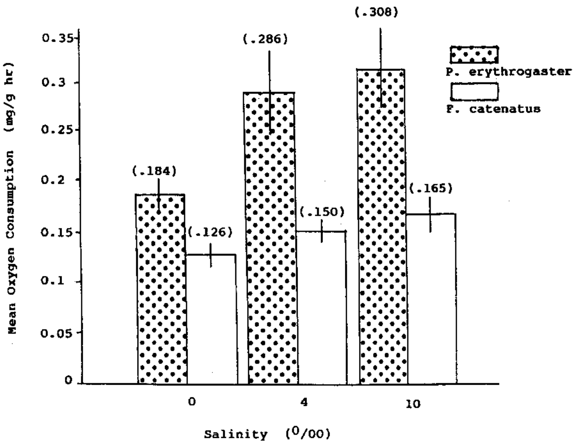

Fig. 2. Mean oxygen consumption of *Phoxinus erythrogaster* and *Fundulus catenatus* at three salinities. Oxygen consumption values are reported in parentheses above each bar. Vertical lines indicate 95% confidence intervals calculated as two standard errors about the mean.

Discussion

Several primary and secondary freshwater fish species have been found in naturally occurring saline environments in North American inland waters. A number of cyprinid species inhabit saline lakes and streams in the northern Great Plains, including *Pimephales promelas* (Held & Peteranka, 1974), *Hybognathus placitus*, *Hybopsis placitus*, *H. aestivalis*, *Notropis bairdi*, *N. lutrensis*, *N. percobromus* (Echelle *et al.*, 1972; Matthews & Hill, 1977). Naturally occurring saline conditions are also frequently encountered in the arid Great Basin region. The relict dace, *Relictus solitarius*, has evolved a tolerance to high levels of salinity and alkalinity here (Vigg, 1982) as have several cyprinodontid species (cf. Naiman & Stoltz, 1981). Williams & Williams (1991) have studied the salinity tolerance of Australian freshwater fish species potentially impacted by agriculturally related salinization of inland waters.

There has been comparatively little documentation of the biotic impact of localized release of saline effluents from industrial sources. Brine effluents from oil fields are the subject of most of the reports on such salinity impacts (Clemens & Jones, 1954; Shipley, 1991). The net effect of release of saline effluent into Lily Creek has been to render several kilometers of of the stream uninhabitable by fishes.

This study substantiates the general perception that secondary freshwater species, specifically members of the family Fundulidae, tolerate a greater range of salinities by virtue of their phylogenetic association with brackish water environments. This observation applies even to fundulids, such as *Fundulus catenatus*, that are restricted to headwater environments. Studies have suggested that freshwater fishes exposed to slightly elevated salinities respond by decreasing their metabolic rate owing to diminishing osmoregulatory demands under more isosmotic conditions (Prosser, 1973). This has been demonstrated for the primary cyprinid species, *Pimephales promelas* (Igram & Wares, 1979) and *Ctenopharyngodon idella* (Maceina *et al.*, 1980). Small quantities of salt are often added when transporting bait minnows as it has been claimed that it has a calming effect (Montgomery, 1985). Some authors (cf. Chervinski, 1977; Stickney, 1986) have suggested that such metabolic depression may prove useful in the rearing of freshwater species in brackish water for aquacultural purposes. In the present study, however, no such decrease in metabolism was recorded. Rather, the lowest oxygen consumption rates were observed in freshwater. Studies by Stuenkel & Hillyard (1981) and Peterson (1989) suggest that the pattern of osmoregulatory response to increased salinity is more complex than orinally perceived.

It is important to recognize that any impact that salinity may have on metabolic capacities is not operating independently of other environmental variables. Environmental salinity alters the relation between temperature and assimilation efficiency in *Cyprinodon macularis* (Kinne, 1960). Individuals of *Cyprinodon salinus* acclimated to 25 °C showed increased standard metabolic rate with salinity while those acclimated to 30 °C showed no significant differences when exposed to half strength seawater versus full strength seawater (Stuenkel & Hillyard, 1981). As mentioned earlier, late summer drought conditions may result in the only flow through Big Lily Creek being that contributed by the sewage treatment plant. It is probably at this time that the combination of high salinities and temperatures exerts its greatest impact on the biota. Excessively saline conditions may negatively impact the capacities of freshwater fishes to acclimate to the prevailing thermal regime. Also, it is possible that responses to other pollutants released as a consequence of the dye process, or originating elsewhere in the watershed, may be affected by environmental salinity (cf. Dyer *et al.*, 1989). The saline nature of the effluent discharged into Big Lily Creek may combine with other toxic substances to exert a synergistic effect on the metabolic capacities of the resident fish fauna.

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