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Comparisons of Salinity Tolerances and Osmotic Regulatory Capabilities in Populations of Sailfin Molly (*Poecilia latipinna*) from Brackish and Fresh Waters

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Sailfin mollies, *Poecilia latipinna*, inhabit both fresh and brackish waters throughout their native range. In laboratory analyses, following extensive acclimation, individuals taken from freshwater populations tolerated a range of ambient salinities from fresh water through 70‰ (parts per thousand), whereas individuals from brackish water tolerated salinities ranging from fresh water through 80‰. Plasma osmotic concentrations of the two groups were not significantly different at common ambient salinities over the range from fresh water through 75‰. Isolation in nature of populations in fresh and brackish waters has not greatly altered their physiological capabilities with respect to ambient salinity.

TRULY euryhaline species make up only a small fraction of extant teleost fishes. Even rarer are teleosts that can successfully reproduce other than under narrowly restricted ranges of environmental salinity. One of these exceptions is the sailfin molly, *Poecilia latipinna* (Lesueur, 1821), a livebearing species. *Poecilia latipinna* is natively distributed over coastal areas of the Atlantic Ocean and Gulf of Mexico of North and Central America, from southeastern North Carolina to northwestern Yucatan (Burgess, 1980). This species has also been widely introduced throughout the world (Courtenay and Meffe, 1989). Sailfin mollies occupy and successfully reproduce in a variety of habitats including freshwater lakes, ponds, swamps, sluggish backwaters of freshwater streams, spring runs, salt marshes, and estuarine lagoons, but they are generally absent from turbulent waters (Herald and Strickland, 1949). The fundamental question that was addressed in this study was whether freshwater and brackishwater populations of sailfin mollies differed in osmoregulatory capabilities with respect to environmental salinities.

MATERIALS AND METHODS

Brackishwater mollies used in the study were collected from salt marshes near Cedar Key, on the west coast of Florida. Freshwater individuals were taken primarily from a creek draining into Lake Alice on the University of Florida campus. However, a few collections were taken from Biven's Arm, a nearby freshwater lake, and from a roadside ditch adjacent to State Highway 24, west of Otter Creek, Florida. Because responses did not differ significantly among these freshwater groups, results were pooled. All fishes were returned to the laboratory in water taken from the collection site. Fishes were held in this water with aeration for 24 h at room temperature (25 C). All of the brackishwater fishes and most of the freshwater fishes were held for 5–7 days in a 5-mg/liter solution of Acriflavine (Argent) before transfer to the acclimation sequence. All acclimations were carried out in filtered, aerated aquaria in a constant environment room (20 ± 1 C, 12:12 L:D cycle). All fishes were fed once a day on Tetra-min.

Fresh water used in the acclimation procedure was from Ichetucknee Springs, Florida (mean conductivity of 305 μS/cm, Ca^{++} 55 mg/liter, Mg^{++} 6.3 mg/liter, Na^{+} 3.3 mg/liter, Rosenau et al., 1977). Salinities higher than fresh water but lower than sea water were prepared by diluting filtered Atlantic Ocean water (obtained from the C. V. Whitney Laboratory of the University of Florida, Marineland, Florida) with appropriate quantities of deionized water. Salinities greater than ambient sea water were prepared by supplementing sea water with appropriate quantities of Instant Ocean. Salinities were checked daily, using an American Optical Temperature-Compensated Refractometer or Yellow Springs S-C-T Meter, and appropriately adjusted.

The acclimation sequence for freshwater

TABLE 1. SURVIVAL OF FRESHWATER AND BRACKISHWATER SAILFIN MOLLIES OVER A RANGE OF ACCLIMATION SALINITIES.

Acclimation salinities ‰ / mOsm/kg	FW (7.5)	5 145	10 290	15 435	20 580	25 725	30 870	35 1015	40 1160	45 1305	50 1450	55 1595	60 1740	65 1885	70 2030	75 2175	80 2320	85 2465	90 2610
Freshwater mollies																			
Number entered into acclimation	551	540	526	487	449	388	342	321	293	264	196	158	149	118	86	42			
Number dying at salinity	0	0	27	20	41	36	12	15	9	39	24	3	25	14	36	39			
% surviving this acclimation	100	100	94.9	95.9	90.9	90.7	96.5	95.3	96.9	85.2	87.8	98.1	83.2	88.1	58.1	7.1			
Brackishwater mollies																			
Number entered into acclimation	17	28	51	399	327	290	270	259	236	222	209	176	152	135	105	85	70	51	10
Number dying at salinity	0	0	10	9	22	2	1	11	3	0	17	11	6	16	11	3	3	29	7
% surviving this acclimation	100	100	80.4	97.7	93.3	99.3	99.6	95.8	98.7	100	91.9	93.8	96.1	88.2	89.5	96.5	95.7	43.1	30.0

mollies, begun in fresh water, involved holding groups of 10–15 individuals in this medium for 14 days, at which time blood samples were taken or the group was transferred to the next higher concentration in the series [5‰ (parts per thousand)]. After transfer, a group was again held for a period of 14 days before blood sampling was carried out or the group was again transferred to the next higher salinity (10‰). This sequence was repeated, moving a group to progressively higher salinities in increments of 5‰ until a salinity was reached in which less than 50% of the individuals survived the 14-day acclimation period. The highest acclimation salinity tolerated by 50% or more of the individuals was considered to be the upper tolerance limit.

The acclimation sequence for brackishwater individuals was begun at an ambient salinity of 15‰, roughly the mean salinity of salt marsh waters where collections were made. The procedures followed were the same as those for the freshwater fishes, except that groups were acclimated to lower as well as higher concentrations, again in steps of 5‰.

The blood sampling procedures followed Nordlie and Walsh (1989). Plasma osmotic concentrations were measured on a vapor pressure osmometer (Wescor model 5100B). The necessary plasma volume of 5 μl was generally obtained from a single individual, but in a few instances, plasma from two small individuals was pooled to get a single value.

Statistical procedures followed Sokal and Rohlf (1969). Significance was accepted at the $P \leq 0.05$ level. All statistical limits presented are ± 1 SE.

RESULTS

A total of 551 individuals from freshwater populations was entered into the acclimation sequence beginning in fresh water, whereas 399 brackishwater individuals were introduced into the acclimation sequence at a salinity of 15‰, with 51 being acclimated to lower salinities and 327 moved to higher salinities. The remaining brackishwater individuals were either used in the blood sampling procedure at 15‰ (12 individuals) or did not survive the 15‰ acclimation (nine individuals).

Brackishwater and freshwater mollies were found to be fully tolerant of freshwater. Neither group (551 freshwater individuals and 17 brackishwater individuals) showed any mortality in

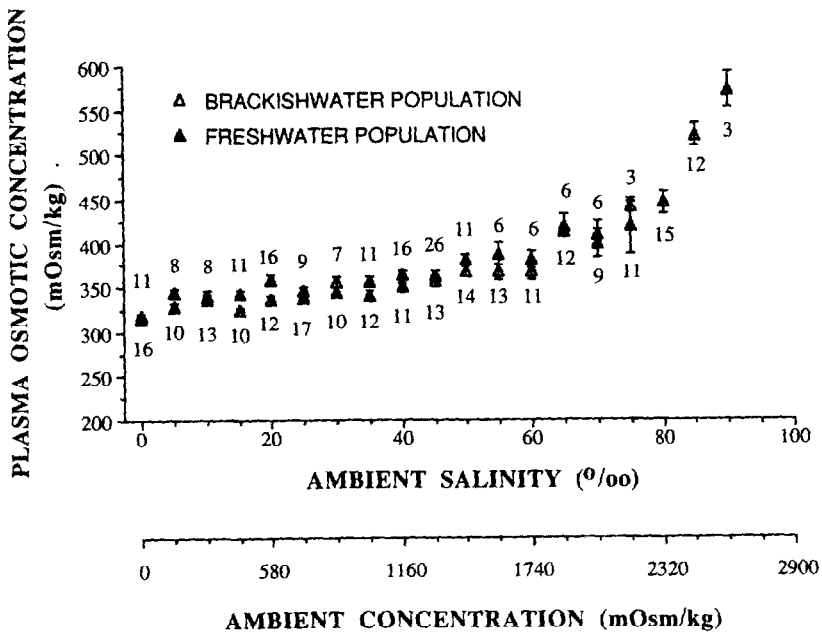

Fig. 1. Plasma osmotic concentrations as functions of acclimation salinities in brackishwater and freshwater populations of *Poecilia latipinna*. Values are means ± 1 SE. The numbers above the plasma osmotic concentrations indicate the number of plasma samples from freshwater mollies, and the numbers below indicate the number of plasma samples from brackishwater mollies.

this medium during the 14-day acclimation in Ichetucknee Springs water, a hard fresh water (osmotic concentration of approx. 7.5 mOsm/kg).

The upper salinity tolerance limit of the freshwater mollies was established at 70‰ following our definition, as 58% (50 of 86) of the individuals entered into the acclimation of 70‰ survived the 14-day acclimation period, but only 7% (three of 42) survived at 75‰. The upper salinity tolerance limit for the brackishwater group was established at 80‰, as 96% (67 of 70) of the individuals entered into acclimation at 80‰ survived the acclimation period, whereas only 43% (22 of 51) of the individuals survived the 85‰ acclimation. Freshwater mollies showed generally greater mortality in acclimations to elevated salinities than did brackishwater individuals, though there was significant mortality among the brackishwater individuals in the move from the 15‰ acclimation to 10‰. Data on salinity tolerances are summarized in Table 1.

The responses in plasma osmotic concentration to altered ambient concentration between the freshwater and brackishwater groups are not significantly different (two-way analysis of variance with unequal sample sizes, $P > 0.05$) from one another at common ambient salinities from fresh water through 75‰ (Fig. 1). Plasma osmotic concentrations ranged from 320 ± 2 mOsm/kg in fresh water to 418 ± 31 mOsm/kg at 75‰ for the freshwater group, and from 315 ± 4 mOsm/kg in fresh water to 440 ± 6 mOsm/kg at 75‰ in the brackishwater group. Both groups showed gradual increases in plasma osmotic concentrations at ambient concentrations from fresh water through 60‰, with plasma osmotic concentrations increasing slightly more rapidly as ambient salinities were increased from 60‰ to 75‰ (80‰ in the brackishwater group). Plasma concentrations in the brackishwater group were dramatically higher at salinities of 85 and 90‰ (521 ± 13 and 571 ± 21 mOsm/kg, respectively), salinities above the upper lethal salinity levels as defined above. There was no similar sharp increase found in plasma osmotic concentration of the freshwater group when the acclimation salinity was raised from 70‰ to 75‰ (409 ± 15 mOsm/kg to 418 ± 31 mOsm/kg) with the latter ambient salinity above the upper salinity tolerance level determined for that group.

Discussion

The hypotheses that we tested, which were based on our previous general conclusions regarding salinity responses in euryhaline fishes (Nordlie, 1985; Nordlie and Walsh, 1989), were (1) mollies from brackish water will tolerate higher ambient salinities than individuals from freshwater; (2) mollies from brackish water will tolerate fresh water; (3) mollies from fresh water, when acclimated to this medium, will maintain their plasma osmotic concentration at a level roughly equivalent to that of other freshwater cyprinodontoids; and (4) mollies from brackish water will maintain plasma osmotic concentrations at levels similar to freshwater mollies, and lower than those typical of marine teleosts, when values are compared following acclimation to common salinities within mutually tolerated ranges. Our results support all four hypotheses presented above. Both groups of mollies tolerate fresh waters, but the brackishwater fishes tolerate a higher salinity (80‰) than do the freshwater group (70‰). Mollies, especially freshwater mollies, seem generally to be less tolerant of abrupt salinity changes than, for example, several species of cyprinodontids (Nordlie and Walsh, 1989). In nature, sailfin molly populations are widely established in Florida freshwater rivers (Hellier, 1967) and spring runs (Herald and Strickland, 1949). Moreover, they are a dominant species in salt marshes, being among the most abundant fishes in the salt marshes at Cedar Key and Bayport on the Florida Gulf of Mexico coast (Kilby, 1955). Salinities of salt marsh waters from which mollies were collected at Cedar Key ranged from 1.2 to 37.6‰, with the largest percentage captured at moderate salinities of 20.0 to 24.9‰, whereas in Bayport salt marshes, mollies were found to be most abundant in waters no more saline than 4.9‰ (Kilby, 1955). However, Renfro (1960) took sailfin mollies from waters of salinities as high as 52.2‰, and Simpson and Gunter (1956) collected the species from waters as saline as 53.9‰, both at locations along the Texas coast.

The highest salinities from which *P. latipinna* has been taken within its native range are well below the upper salinity tolerance limits found by us. It is noteworthy that sailfin mollies were not among the species taken from hypersaline waters (≥45‰) of the Laguna Madre of the Texas coast (Simmons, 1957; Gunter, 1967). However, Herre (1929) found introduced individuals of *P. latipinna* to be abundant in salt ponds around Manila Bay, Philippine Islands, where salinities ranged from 32 to 87‰. Herre suggested that the latter salinity constituted an upper tolerance limit, because mollies were absent from ponds where the salinity had risen to 94‰. The highest salinity at which Herre found mollies is close to the salinity tolerance limit determined here for brackishwater individuals. A closely related species, *P. sphenops vandepolli*, is tolerant of even higher salinities, because individuals were reported living at a salinity of 135‰ in a lagoon in the Netherlands Antilles (Kristensen, 1970).

Plasma osmotic concentrations of both freshwater and brackishwater groups, when acclimated to fresh waters, were maintained at levels typical of many freshwater cyprinodontoids (i.e., at relatively low plasma osmotic concentrations, near 300 mOsm/kg). This is generally true of the most euryhaline of brackishwater species (reviewed in Holmes and Donaldson, 1969). Previous comparisons of several species of teleosts (Spargaaren, 1976; Nordlie, 1985; Nordlie and Walsh, 1989) showed that marine fishes (near-shore and open-water species, including some cyprinodontoids that are intolerant of freshwater or dilute brackish waters) have consistently higher plasma osmotic concentrations at all tolerated ambient salinities than do the more euryhaline of brackishwater species. Euryhaline species regulate their plasma osmotic concentrations at consistently lower levels when subjected to ambient salinities ranging from fresh water through sea water, and in some cases into the hypersaline range.

The above comparisons were among species, not between populations of a single species, as in the present study. In a study of another group of poeciliids from Belize, individuals of *Gambusia yucatana* from marine and mainland populations were found to have plasma osmotic concentrations that were virtually identical to one another and which were little increased by increases in ambient salinities (Carter, 1981). This response differed from the situation in two other sympatric species of *Gambusia*, *G. luma* and *G. sexradiata*, both exclusively freshwater species, which showed dramatic increases in plasma osmotic concentrations when subjected to increased ambient salinities. The *G. yucatana* groups from marine and mainland populations responded very similarly to the two groups of sailfin mollies compared in the present study.

Gustafson (1981) compared groups of mollies from the same brackishwater and freshwater populations used in the present study. He found that plasma osmotic concentrations in the brackishwater individuals were less altered by variations in ambient salinities than were those of freshwater individuals when concentrations were measured at common ambient salinities. Gustafson's fishes were acclimated to a series of salinities ranging from <1.0 to 37.9‰, with the brackishwater group consistently maintaining lower plasma osmotic concentrations than the freshwater group over the entire salinity range. His results thus differed significantly from those we obtained. Moreover, plasma osmotic concentrations of both groups in our study are lower than those reported by Gustafson. Gustafson used a rapid acclimation procedure, increasing or decreasing the ambient salinity by 120 mOsm/kg (4.14‰) per day, though he stated that he frequently used a slower acclimation for freshwater mollies. Once the desired ambient salinity level was reached, he allowed an additional nine-day acclimation at that salinity before blood sampling. Thus, his procedure required a minimum of 19 days to acclimate freshwater mollies to sea water. Our procedure required 112 days for the same acclimation. It is likely that Gustafson's acclimation protocol did not allow experimental fishes to achieve plasma equilibrial levels prior to blood sampling at each ambient salinity. The implications of differences between Gustafson's results and those of the present study are that a group of freshwater mollies moving into saline waters or a group of brackishwater mollies moving into fresh waters will immediately differ from "native" individuals in plasma osmotic concentration. However, if these new arrivals remain in their new environments, they will acclimate and their plasma concentrations will gradually become equal to those of the long-term residents.

A further conclusion is that, although the brackishwater group can become acclimated to fresh waters, the freshwater group will have an upper salinity tolerance limit slightly lower than that of the brackishwater group. A fundamental question that remains unanswered is whether the difference in upper salinity tolerance noted here between the two experimental fish groups is genetically based or simply the result of an irreversible (at least through extended acclimation) nongenetic adaptation induced by ambient salinity as described by Kinne (1962).

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LITERATURE CITED

BURGESS, G. H. 1980. *Poecilia latipinna* (Lesueur), p. 549. *In:* Atlas of North American freshwater fishes. D. S. Lee, C. R. Gilbert, C. H. Hocutt, R. E. Jenkins. D. E. McAllister, and J. R. Stauffer, Jr. (eds.). Pub. No. 1980 12 North Carolina Biological Survey, North Carolina State Museum of Natural History, Raleigh.

CARTER, H. J. 1981. Aspects of the physiological ecology of species of *Gambusia* from Belize, Central America. Copeia 1981:694–700.

COURTENAY, W. R., JR., AND G. K. MEFFE. 1989. Small fishes in strange places: a review of introduced poeciliids, p. 319–331. *In:* Ecology and evolution of livebearing fishes (Poeciliidae). G. K. Meffe and F. F. Snelson, Jr. (eds.). Prentice Hall, Englewood Cliffs, New Jersey.

GUNTER, G. 1967. Vertebrates in hypersaline waters. Publications Institute of Marine Science Univ. of Texas 12:230–241.

GUSTAFSON, D. L. 1981. The influence of salinity on plasma osmolality and routine oxygen consumption in the sailfin molly, *Poecilia latipinna* (Lesueur), from a freshwater and an estuarine population. Unpubl. Master's thesis, Univ. of Florida, Gainesville.

HELLIER, T. R., JR. 1967. The fishes of the Santa Fe river system. Bull. Fla. State Museum 11:1–46.

HERALD, E. S., AND R. R. STRICKLAND. 1949. An annotated list of the fishes of Homosassa Springs, Florida. Quart. J. Florida Acad. Sci. 11:99–109.

HERRE, A. W. T. C. 1929. An American cyprinodont in Philippine salt ponds. Philippine J. Sci. 38:121–127.

HOLMES, W. N., AND E. M. DONALDSON. 1969. The body compartments and distribution of electrolytes, p. 1–79. *In:* Fish physiology. Vol I. W. S. Hoar and D. J. Randall (eds.). Academic Press, New York, New York.

KILBY, J. D. 1955. The fishes of two Gulf coastal marsh areas of Florida. Tulane Studies in Zoology 2:175–247.

KINNE, O. 1962. Irreversible nongenetic adaptation. Comp. Biochem. Physiol. 5:265–282.

KRISTENSEN, I. 1970. Competition in three cyprinodont fish species in the Netherlands Antilles. Studies on the Fauna of Curacao and other Caribbean Islands 32:82–101.

NORDLIE, F. G. 1985. Osmotic regulation in the sheepshead minnow *Cyprinodon variegatus* Lacepède. J. Fish Biol. 26:161–170.

———, AND S. J. WALSH. 1989. Adaptive radiation

in osmotic regulatory patterns among three species of cyprinodontids (Teleostei: Atherinomorpha). Physiol. Zool. 62:1203–1218.

RENFRO, W. C. 1960. Salinity relations of some fishes in the Aransas River, Texas. Tulane Studies in Zoology 8:83–91.

ROSENAU, J. C., G. L. FAULKNER, C. W. HENDRY, JR., AND R. W. HULL. 1977. Springs of Florida. Bull. No. 31 (Rev.). Florida Department of Natural Resources, Bureau of Geology and Bureau of Water Resource Management, Florida Department of Environmental Regulation, Tallahassee.

SIMMONS, E. G. 1957. An ecological survey of the upper Laguna Madre of Texas. Publications Institute of Marine Science, Univ. of Texas 4:156–200.

SIMPSON, D. G., AND G. GUNTER. 1956. Notes on habitats, systematic characters and life histories of Texas salt water cyprinodontes. Tulane Studies in Zoology 4:115–134.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freeman and Company, San Francisco, California.

SPAARGAREN, D. H. 1976. On osmotic and ionic regulation in blood, bile, and tissues of three *Pleuronectes* species. Netherlands J. Sea Res. 10:479–490.

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