Influence of Salinity and Temperature on the Physiology of *Limia melanonotata* (Cyprinodontiformes: Poeciliidae): A Search for Abiotic Factors Limiting Insular Distribution in Hispaniola

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ABSTRACT.—We investigated salinity and temperature effects on routine metabolic rate (RMR), temperature tolerance (CTMax, critical thermal maximum), and salinity tolerance of *Limia melanonotata*, a poeciliid fish that occurs in west-central inland waters of Hispaniola. Routine metabolic rate and CTMax were measured in fish acclimated to three salinities (0, 30, and 60 ppt) and temperatures (25°, 30°, and 35°C) for nine temperature-salinity combinations. Salinity and temperature did not significantly interact in their effect on RMR. For combined salinity acclimations, adjusted RMR (ANCOVA) was significantly lower at 25°C than either 30° or 35°C. For combined temperature acclimations, mean RMR was significantly lower at 60 ppt than either 0 or 30 ppt. Salinity and temperature had a significant interactive effect on temperature tolerance. Mean CTMax was significantly higher at 30° than 25°C at all salinities, but at 35°C was significantly higher than at 25° or 30°C only among fish acclimated in fresh water. Fish exposed to a chronic increase in salinity experienced most mortality in a salinity range of 70-107 ppt, with females exhibiting greater salinity tolerance than males. *Limia melanonotata* approaches the upper extreme in salinity and temperature tolerances known for poeciliids. Our results also suggest that *L. melanonotata* may reduce energy expenditures at environmental extremes to tolerate harsh conditions for extended periods. Despite its eurythermal and euryhaline adaptations, *L. melanonotata* has a relatively restricted inland range in Hispaniola and is unknown from inshore brackish or marine habitats. The present distribution of this species and congeners may be the result of a combination of factors that include historical zoogeography and ecological requirements.

INTRODUCTION

Poeciliid fishes of the genus *Limia* are distributed in inland waters of Hispaniola, Cuba, and Jamaica. The greatest diversity of this genus is on Hispaniola, where at least 17 species occur in rivers, springs, and inland lakes, including an endemic flock of six or more species of the subgenus *Odontolimia* in Étang de Miragôane, Tiburón Peninsula, Haiti (Burgess and Franz 1989; Rodriguez 1997). Most species of *Limia* are associated with freshwater habitats, but some are found in karst regions high in dissolved inorganic salts or in saline lakes (Fig. 1). *Limia melanonotata* (Fig. 2) is a moderately widespread Hispaniolan species with its center of distribution in the Cul-de-Sac and Valle-de-Neiba Plain region, a large tectonic valley that separates north and south regions of the island with different geological origins and biotic components (Figs. 3-4).

Limia melanonotata exhibits extreme variation in habitats occupied. These include artesian springs, headwater creeks, a hypersaline coastal lagoon, Laguna Oviedo (salinity = 55 parts per thousand [ppt]), and a central rift valley lake, Lago Enriquillo (70 ppt). The two latter habitats are also subject to high water temperatures that intensify the physiological requirements needed to survive in such environments. Only three other fish species are known to occur in these two hypersaline lakes: *Gambusia hispaniolae* (both lakes), *Cyprinodon nicholsi* (Lago Oviedo), and *C. bondi* (Lago Enriquillo).

Numerous studies have demonstrated that temperature and salinity are major physicochemical factors that can act singly or in combination to influence community structure (Sweet and Kinne 1964; Yoshikawa et al. 1993), physiology (Swanson

FIG. 1. Hypersaline habitat of *L. melanonotata*: Dominican Republic, Lago Enriquillo at Aazufaada, 4 km E of La Describierta, 9 Nov 1991.

1991; Claireaux and Lagardere 1999), and distributional patterns (Simpson and Gunter 1956; Wiederholm 1987; Stauffer and Boltz 1994) of aquatic animals. Many fishes living in extreme habitats similar to those of *L. melanonotata* have remarkable tolerance to harsh physicochemical conditions. For example, one well-studied distant relative of *L. melanonotata*, *Cyprinodon variegatus*, is widespread and among the most physiologically labile and salt-tolerant teleosts known (Nordlie and Walsh 1989; Haney 1995; Bennett and Beitinger 1997). Physiological studies of poeciliids of the genus *Gambusia* also suggest that these animals are very tolerant of temperature and salinity extremes (Meffe and Snelson 1989; Heath et al. 1993; Meffe et al. 1995). However, comparatively little is known about the physiological effects of salinity and temperature on poeciliids other than vari-

FIG. 2. *L. melanonotata*, male (36.0 mm SL), UF 110058 (University of Florida Ichthyology Collection), Dominican Republic, Balneario La Zurza 5 km WNW of Duverge (Lago Enriquillo drainage), 9 Nov 1991. Photograph by Noel M. Burkhead.

ous species of *Gambusia*. Thus, examination of other salt- and thermal-tolerant poeciliids is relevant to the study of the physiological and ecological requirements of species living under stressful physicochemical conditions. Such studies are also needed to better understand the distributional and zoogeographic history of these fishes throughout Central America and the circum-Caribbean area, and are important for conservation and management actions.

Our objective was to investigate the physiological basis underlying the ability of *L. melanonotata* to occupy widely diverse habitats in its native range. Because temperature and salinity are the two primary physical factors influencing the physiology of organisms like *Limia*, investigating the responses to change of one of these factors independent of the other may lead to inaccurate conclusions about their physiological effects. Thus, we examined chronic salinity tolerance, metabolic rate, and upper temperature tolerance under a combination of different salinity-temperature acclimations to assess physiological responses of this species to stressful physicochemical conditions.

METHODS AND MATERIALS

Fish were collected in 1991 from sites throughout the Barahona peninsula and rift valley of the Dominican Republic and returned alive to the U.S. Geological Survey laboratory in Gainesville, Florida, where they were propagated. Fish used in experiments were at least third-generation from original stock. Upon arrival at the laboratory individuals were initially held in aquaria (75 to 114 L) maintained at the salinity at which fish were captured. All aquaria were equipped with filtration and constant aeration, and were maintained on a 12:12 light: dark cycle at approximately 25°C. Fish were fed flake food once daily.

For metabolic and temperature tolerance studies groups of approximately 10 fish (mean mass 0.69 ± 0.31 g; mean standard length [SL] 30.7 ± 3.94 mm) were placed into experimental 38 L aquaria containing water at a salinity within 5 ppt of that in
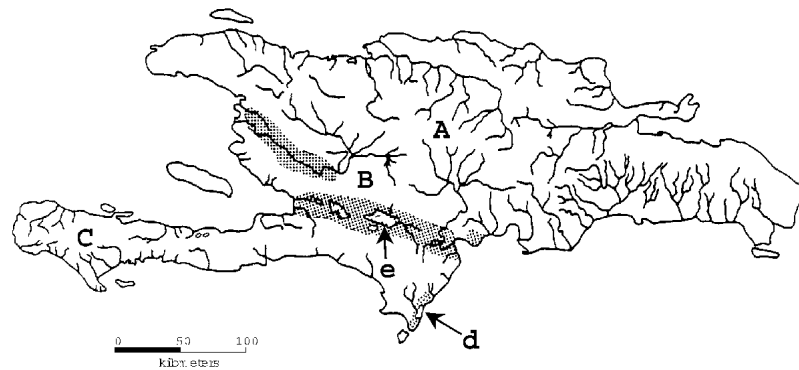
FIG. 3. Physiographic and faunal divisions of Hispaniola: north island (A); Cul-de-Sac/Valle-de-Neiba Plain (B, stippled areas); south island (C); Laguna Oviedo (D); Lago Enriquillo (E) [modified from Burgess and Franz 1989].
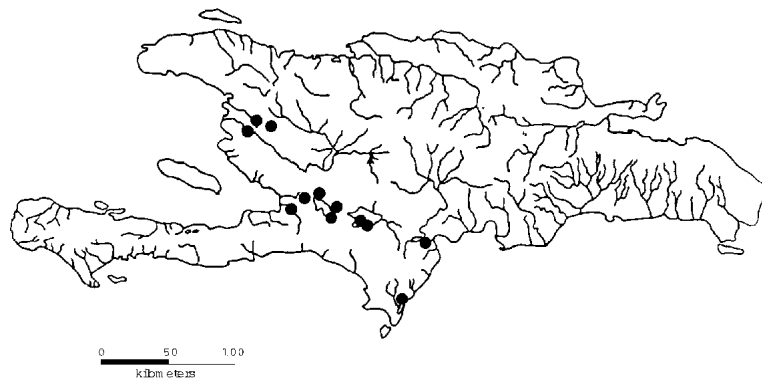

FIG. 4. Distribution of *L. melanonotata* (from Franz and Rivas 1983a).

which they were collected. Aquaria were initially maintained at 25°C and were equipped with undergravel filtration and constant aeration. Fish were then gradually acclimated to nine independent combinations of salinity (0, 30, 60 ppt) and temperature (25°, 30°, 35°C). For the 30 and 60 ppt acclimations, salinity was adjusted at a rate of 2 ppt/2 d; for the latter group salinity was increased 1 ppt/2 d after 50 ppt was reached. Salinities greater than fresh water were produced by supplementing aged tap water with appropriate amounts of synthetic sea salt (Instant Ocean®). Salinities were monitored daily with a temperature-compensated refractometer and adjusted as necessary. For the 0 ppt acclimation, salinity was decreased at 2 ppt/2 d, with fish ultimately placed into artificial medium-soft fresh water (120 mg $NaHCO_3$, 75 mg

$CaSO_4 \cdot 2H_2O$ and $MgSO_4$, 6.5 mg KCl per L deionized H_2O).

Temperature acclimations were achieved by increasing water temperature with submersible heaters by 1°C/d until temperatures of 30° or 35°C were reached. All fish were held at their experimental salinity-temperature combination for 1-2 weeks prior to metabolic rate or critical thermal maximum (CTMax) measurements. Metabolic determinations were made in sealed, opaque 54-ml glass respirometers following procedures described by Nordlie et al. (1991) and Haney and Nordlie (1997). Rate of oxygen consumption was used to measure routine metabolic rates (RMR), as animals were sequestered to minimize but not eliminate activity (Winberg 1956; Fry 1957; Chipps et al. 2000). All experiments were performed on unrestrained, post-absorp-

tive fish in a resting state. Respirometers were filled with water at the salinity and temperature of the experimental aquaria and placed in a water bath maintained at the acclimation temperature. The entire metabolic apparatus was located in a room in which no other activity took place. To allow time for fish to adjust to the respirometers, individuals were placed into aerated flasks 12 to 16 h before beginning a trial. At the beginning of the metabolic trial, aerators were removed and the flask was sealed.

Determinations of oxygen partial pressures (PO_2) were made with an ISO_2® oxygen electrode connected to a CB Sciences® automated data acquisition system. Measurements of the rate of reduction in PO_2 were continuously recorded for 3-4 hours, and continued until each fish had depleted the oxygen level to approximately 100 mm Hg. At the termination of each metabolic trial, the fish was removed from its flask, blotted dry and weighed to the nearest 0.01 g. All metabolic determinations were made between 0700 and 1200, and fish were not used in other metabolic trials or for temperature tolerance trials.

Calculation of oxygen saturation values (accounting for salinity, temperature, relative humidity, and barometric pressure) for the experimental conditions were made using the equations of Truesdale et al. (1955) and to calculate RMR (mg O_2 h^{-1}) for each fish. Oxygen consumption varies with body mass, and data for all analyses satisfied assumptions of normality and homogeneity of variance, so RMR values were mass-adjusted using analysis of covariance (ANCOVA). Log mass-independent RMR was used as the dependent variable and log mass as the covariate. Least square means derived from the ANCOVA were used as adjusted RMR values and compared using the Tukey-Kramer post hoc comparison. Values of $p \leq 0.05$ were considered significant for this and all subsequent statistical tests.

Temperature tolerance was determined using the critical thermal maximum (CTMax) endpoint as described by Becker and Genoway (1979). The CTMax was measured on individual fish at each of the nine salinity-temperature combinations described above. Each fish was placed in a 1 L beaker with 500 ml aerated water at the same salinity and temperature combination as its acclimation. Beakers were then placed in a water bath containing 10 L of water and a submersible heater connected to a programmable temperature controller (Omega Engineering) set to increase the temperature of the water bath by 0.3°C/min. A mercury thermometer calibrated to ±0.05°C was placed in each beaker and used to record the temperature (±0.1°C) at which fish lost equilibrium but recovered when placed in ambient acclimation water. All experiments were conducted between 0800 and 1200 in a lighted room, during the normal lighted portion of the acclimation period. Since test assumptions were met, a two-way analysis of variance (ANOVA) using the Tukey-Kramer post hoc comparison was performed to assess the single and interactive effects of acclimation temperature and salinity on upper temperature tolerance.

For salinity tolerance experiments, 70 fish were separated by gender and placed into two 54-L aquaria with constant aeration and filtration. Salinity adjustments were made in the same manner as metabolic acclimations with partial water changes made as needed. Salinity was measured every 2 d with a vapor pressure osmometer and adjusted accordingly by adding salt or fresh water to maintain a gradual increase at a rate of 2 ppt/day to 50 ppt, and 1 ppt/day thereafter.

Chronic salt tolerance was measured as the cumulative mortality of fish exposed to gradually increase ambient salinity. Fish were observed daily and all mortalities were recorded and dead individuals weighed (whole body mass, g), measured (SL, mm), and preserved in 10% formalin. Gender differences were analyzed using student's t-test and a three-parameter sigmoidal regression model was used to estimate salinity tolerance at the 50% mortality rate.

RESULTS

Gender had no significant effect on metabolism or temperature tolerance (t-test;

p > 0.05), so data from males and females were combined for these analyses. The overall interaction between temperature and salinity on RMR was non-significant (Table 1; p > 0.05). However, both temperature (Fig. 5) and salinity (Fig. 6) alone led to significant changes in RMR. Metabolism generally increased with increasing temperature, except in the 30 ppt acclimation, where RMR values were similar over the range of temperatures studied (Table 1). Temperature sensitivity assessed by Q_{10} values was greatest in the lower range of acclimation temperatures (from 25° to 30°C) at all salinities (Table 2). The overall Q_{10} values (25° to 35°C) indicate that temperature had the greatest effect on RMR at 60 ppt (Q_{10} = 2.52), and its lowest effect at 30 ppt (Q_{10} = 1.17). When salinities are combined, the mean RMR at 25°C was significantly lower overall than the mean RMR at either 30°C or 35°C (p = 0.001). However, mean RMR values at 30°C and 35°C did not differ. Salinity also affected RMR, with increasing salinity, especially at 30° and 35°C, leading to declines in RMR. When temperatures are combined, the mean RMR at 60 ppt was significantly lower than both 0 ppt and 30 ppt (p = 0.001). However, mean RMR values at 0 ppt and 30 ppt did not differ significantly.

Salinity, acclimation temperature, and the interaction of both significantly affected CTMax (Fig. 7; p < 0.001). As expected, mean CTMax of *L. melanonotata* increased significantly with increasing temperature acclimation over the entire thermal range at 0 ppt, but only between 25° and 30°C for groups in 30 and 60 ppt. Salinity alone had a modest impact on CTMax, with fish acclimated to 30 ppt exhibiting a slight, but significant increase in upper temperature tolerance compared to fish acclimated to 0 or 60 ppt. The most interesting results were a combined effect of temperature and salinity on CTMax. An increase in acclimation temperature from 25° to 30°C resulted in a significant increase in mean CTMax (1.64°C at 0 ppt; 1.26°C at 30 ppt; 1.33°C at 60 ppt), regardless of the acclimation salinity. However, with a further increase in acclimation temperature to 35°C only the group acclimated to 0 ppt exhibited a significant increase in mean CTMax (1.53°C); fish acclimated to 30 or 60 ppt exhibited slight but non-significant increases in mean CTMax (0.20°C and 0.24°C, respectively).

Limia melanonotata exhibited pronounced tolerance to salinities in excess of 80 ppt, with females exhibiting greater tolerance than males (Fig. 8; t-test, p < 0.001). A three-parameter sigmoid regression model of percent cumulative mortality on salinity provided a best fit of data for each sex and was used to estimate the 50% mortality rate, resulting in a calculated LC_{50} of 87.5 ppt for males and 103.9 ppt for females (SigmaPlot® ver. 8.02, SPSS, Inc., model: y = $a/[1 + e^{-(x-x_0)/b}]$). Neither body mass nor SL accounted for the difference in mortality between sexes (ANCOVA, p > 0.05).

DISCUSSION

In this study both salinity and temperature affected metabolism but did not show a clear interactive trend. Independent of temperature, RMR was relatively constant at 0 and 30 ppt, but declined in all groups at 60 ppt. At 0 and 60 ppt, RMR increased with temperature as expected; at 30 ppt, RMR did not differ across temperatures.

A decrease in metabolic rate at high salinities is consistent with results from other studies and may be related to reduce oxygen permeability under hypersaline conditions. For example, *C. variegatus* also exhib-
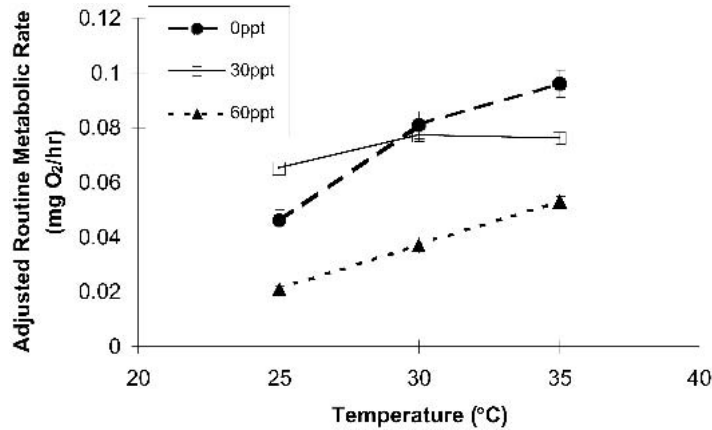
TABLE 1. Mean adjusted routine metabolic rate (±1 SE) and samples sizes (N) for each acclimation group of *L. melanonotata.* Significantly different mean routine metabolic rate values across corresponding temperature or salinity group combinations are designated by different superscript letters (ANCOVA, p < 0.05).

Temperature (°C)	Salinity (ppt)	Adjusted RMR ± SE (mg O$_2$/hr)	N
[a]25	[g]0	0.046 ± 0.004	7
[a]25	[i]30	0.065 ± 0.003	9
[b]25	[j]60	0.021 ± 0.001	6
[c]30	[h]0	0.081 ± 0.005	10
[c]30	[i]30	0.077 ± 0.002	9
[d]30	[k]60	0.037 ± 0.003	8
[e]35	[h]0	0.096 ± 0.005	10
[e,f]35	[i]30	0.076 ± 0.002	12
[f]35	[k]60	0.053 ± 0.002	7

FIG. 5. Mean adjusted routine metabolic rates (±1SE) versus temperature by salinity-acclimation groups of *L. melanonotata*. Sample sizes vary between 7-10 individuals in each temperature/salinity combination.
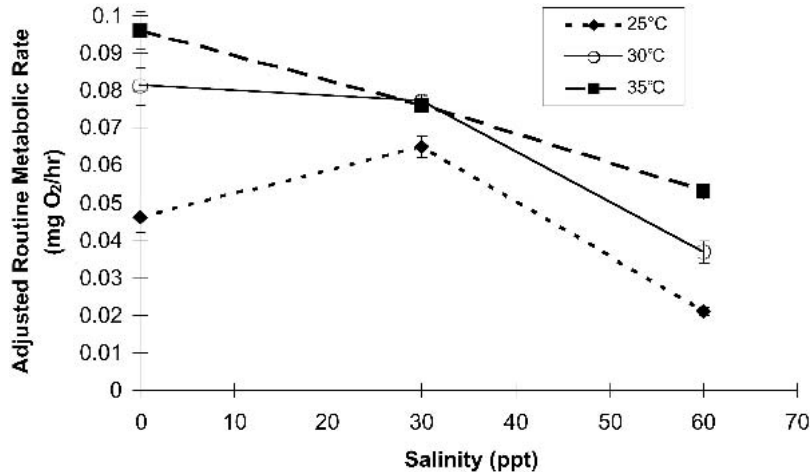

FIG. 6. Mean adjusted routine metabolic rates (±1SE) versus salinity by temperature-acclimation groups of *L. melanonotata*. Sample sizes vary between 7-10 individuals in each temperature/salinity combination.

TABLE 2. Q_{10} values over three temperature ranges for mean adjusted routine metabolic rates (ANCOVA) of *L. melanonotata* at differently salinity acclimations.

Salinity (ppt)	25° to 30°C	30° to 35°C	25° to 35°C
0	3.10	1.41	2.09
30	1.40	1.00	1.17
60	3.10	2.05	2.52

its a decline in RMR at salinities exceeding 40 ppt (Nordlie et al. 1991; Haney and Nordlie 1997). We believe that such a depression in metabolism at high salinities is likely related to permeability changes of the gill membrane and/or integument. If osmotic permeability of the gills is reduced under hypersaline conditions to help offset ionic and osmotic fluxes, the potential for oxygen uptake may be reduced as well (Davenport and Sayer 1993). Evidence for this hypothesis comes from several studies. Kultz and Onken (1993) found that overall *in vitro* permeability of the opercular membrane of the cichlid *Oreochromis mossambicus* was reduced in hypersaline media, with a simultaneous reduction in passive ion fluxes. Studies by Bindon et al. (1994a, b) and Perry et al. (1996) on the rainbow trout,
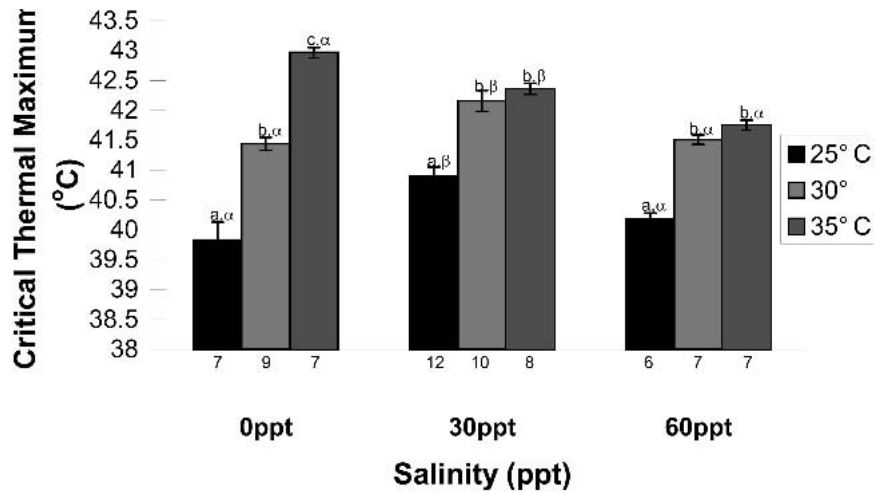
FIG. 7. Mean (± 1SE) critical thermal maximum (CTMax, °C) versus salinity by temperature-acclimation groups of *L. melanonotata*. Significantly different mean CTMax values within each salinity acclimation are designated by different Roman letters above the error bars and significantly different mean CTMax values within each temperature acclimation are designated by different Greek symbols above the error bars (two way ANOVA, $p < 0.05$). Sample sizes are given below each data bar.
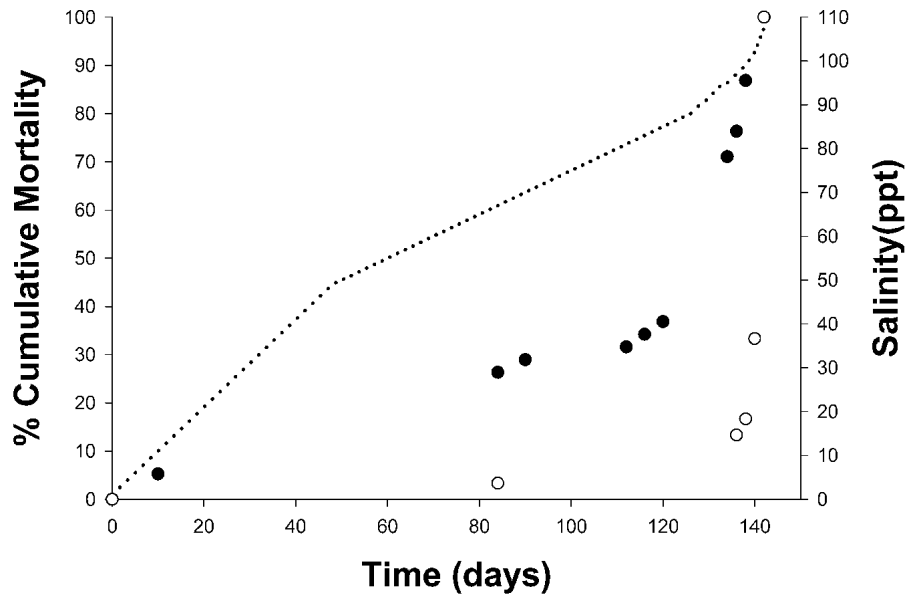

FIG. 8. Time of exposure and mortality (left scale and symbols) of *L. melanonotata* subjected to chronic increase in salinity (right scale). Dotted line = salinity; closed circles = males (n = 38; including values at 0 and 100% cumulative mortality); open circles = females (n = 30).

Oncorhynchus mykiss, demonstrate that pro-liferation of mitochondria-rich cells impairs respiratory gas transfer. Finally, Haney et al. (2003) observed qualitatively that the surface involved in ionic exchange seems to proliferate at the expense of gill epithelia involved in gas exchange in *C. variegatus.*

Increases in salinity and temperature also confront fishes with the necessity of satisfying oxygen requirements under con-

ditions of reduced oxygen availability. While fishes likely exploit multiple strategies to optimize blood oxygen transport under such conditions, one solution would also be to reduce energy expenditures. Decreased metabolism significantly reduces energetic expenditures at elevated salinities, with this response effectively increasing the time animals can tolerate adverse conditions. This type of metabolic response to salinity fits the concept of scope for survival as described by Hochachka (1990). This is a pattern of metabolic response to environmental stressors characterized by a depression in metabolism, sometimes below maintenance levels. The advantage of such a reduction in energy expenditure is essentially the slowing of "biological time," enabling survival despite the temporarily imposed physiological stressor.

Similar to our results, Cech et al. (1994) observed higher Q_{10} values (3.2-4.1) at low to moderate temperatures in the northern pikeminnow (*Ptychocheilus oregonensis*) and lower Q_{10} values (1.8) at the highest acclimation temperature they studied. Muller-Feuga et al. (1978) recorded a similar pattern in the rainbow trout (*O. mykiss*). Since the higher acclimation temperatures of 30° and 35°C are closer to the preferred, and likely field, temperatures for other poeciliids studied, this reduction in temperature dependence on RMR at higher acclimation temperatures may represent an energy saving measure for *L. melanonotata* over the range of temperatures most commonly encountered. Previously reported temperature preferences for mosquitofish (*Gambusia affinis*) range from 28-29°C (Bacon and Neill 1967), to 31°C (Winkler 1979), to as high as 35°C (Cherry et al. 1976), depending upon acclimation temperature. Thus, exposure to an increase in temperature without a significant rise in RMR may lead to an overall energetic gain for *L. melanonotata* under these conditions. *Limia melanonotata* exhibited metabolic temperature independence at all acclimation temperatures in the 30 ppt salinity acclimation. The reason for this observation is unclear, but 30 ppt may be the least stressful of the three salinities studied here; future studies may

be informative in determining if this is the case.

Salinity and prior acclimation temperature both affected upper temperature tolerance; acclimation temperature had the greatest effect. As expected, mean CTMax of *L. melanonotata* increased with increasing temperature acclimation. Upper temperature tolerance values recorded here for *L. melanonotata* range from 39.8°C to 43°C, and are among the highest CTMax values recorded for a poeciliid fish. Only *G. affinis* acclimated to 35°C (CTMax = 43.5°C; Otto 1973) and several species of fish within the family Cyprinodontidae were reported to exhibit higher mean CTMax (Beitinger et al. 2000). The next highest recorded values for any poeciliids are for *Gambusia yucatana* (CTMax = 42.4°C; Heath et al. 1993) and *G. holbrooki* (CTMax = 40.1°C; Meffe et al. 1995).

Salinity had a more modest impact on CTMax than did acclimation temperature. Fish acclimated to 30 ppt exhibited a slight but significant increase in CTMax compared to fish acclimated to either 0 or 60 ppt. However, salinity and acclimation temperature had a synergistic effect on upper temperature tolerance. Mean CTMax of *L. melanonotata* increased significantly with temperature acclimation over the entire thermal range at 0 ppt, but only so between 25° and 30°C for groups in 30 and 60 ppt. At 35°C and 60 ppt salinity, CTMax values declined significantly compared to values obtained in freshwater conditions. Since RMR also decreases at 60 ppt, these results could be further indications of the depression in metabolic scope under hypersaline conditions.

Limia melanonotata demonstrated remarkable tolerance to a chronic increase in salinity. Both male and female *L. melanonotata* survived salinities in excess of 85 ppt (females in excess of 100 ppt), higher values than reported for any poeciliid other than *P. sphenops* (135 ppt, Kristensen 1969; Meffe and Snelson 1989), and approaching the salinity tolerances of related cyprinodontid fishes. Most poeciliids occur primarily in fresh water, and thus exhibit modest osmoregulatory abilities and salinity tolerances. Nordlie and Mirandi (1996), for example,

reported an upper tolerance limit of only 25 ppt for freshwater acclimated *G. holbrooki*. However, as a family the poeciliids are considered broadly euryhaline (Meffe and Snelson 1989) and several species exhibit tolerance to hypersaline conditions. These include the sailfin molly, *Poecilia latipinna*, which can tolerate salinities up to 80 ppt (Nordlie et al. 1992), the guppy, *P. reticulata*, which can survive salinities in excess of 43 ppt (Arai et al. 1998), and *G. yucatana*, which can tolerate salinities up to at least 34 ppt (Carter 1981).

Male and female *L. melanonotata* had significantly different upper salinity tolerances, with females exhibiting higher tolerance than males. This is the first report of a gender difference in salinity tolerance among poeciliids. Our *a priori* assumption was that this was attributable to gender-specific size or condition factors. However, we found no significant differences in mortality rate related to size factors (length or mass). Additional research is needed to examine possible size-related and/or sexually dimorphic variation in salinity tolerance in this and other poeciliid species. Another possible explanation for the observed variation in gender-specific mortality rates may have been a tank effect as a result of differences in water quality (e.g., buildup of waste metabolites). It is also possible that some other unmeasured biological parameter may be a factor.

Adaptations to extreme physicochemical conditions are inadequately studied in most poeciliids exclusive of the genus *Gambusia*. Among poeciliids that are primarily distributed in freshwater habitats, *L. melanonotata* is one of the most euryhaline and eurythermal, as evidenced by its natural occurrence in freshwater streams, springs, and two inland hypersaline lakes. Based on our results, *Limia melanonotata* approaches the upper extreme in salinity and temperature tolerances known for the family. The apparent absence of this species and most congeners in inshore brackish or marine habitats and their limited distribution throughout coastal rivers of Hispaniola is enigmatic. Distribution and dispersal of these species may be regulated by unknown biotic factors, physiography, vicari-

ant history, or there may be an absence of records from such habitats as a result of inadequate sampling effort. Other Central American and Antillean poeciliid species (e.g., *Poecilia vivipara*, *P.* cf. *sphenops*, *Gambusia* spp.) occur in coastal lagoons and mangrove areas (Kristensen 1969; Meffe and Snelson 1989) and are relatively widespread. Among congeners, *Limia caymanensis* is known from brackish water in coastal mangrove lagoons of Grand Cayman Island (Rivas and Fink 1970), *L. vittata* was reported from estuaries, coastal lagoons, and mangrove swamps throughout Cuba and the Isle of Pines (Franz and Rivas 1983b), and *L. rivasi* was described from specimens collected from a tidal creek in a red mangrove swamp of Ile de la Gonave, where it was suspected to occur in nearby mangrove areas and possibly on the mainland of Hispaniola (Franz and Burgess 1983). Based on distribution of other species and results of this study, there is no known phylogenetic or physiological basis precluding the occurrence of *L. melanonotata* in coastal habitats or preventing it from establishing a more widespread distribution via dispersal through marine or brackish waters. Burgess and Franz (1989) discuss distributions of the Antillean freshwater fish fauna in detail, and invoke a zoogeography model that combines elements of dispersal and geophysical history (vicariance). In the case of *L. melanonotata*, they postulated that dispersal occurred from the Cul-de Sac Plain to the lower Rivière l'Artibonite via low-elevation stream captures, rather than dispersal through precipitous coastal corridors around the western fringe of the Chaines dux Matheux. However, our results suggest that there is no physiological barrier preventing *L. melanonotata*, and presumably most other congeners, from occurring in more widespread habitats than currently occupied.

A combination of historical zoogeography and ecology may be required to explain the current distribution patterns of *L. melanonotata* and most other cyprinodontoids in Hispaniola and other Antillean islands. We are unaware of any published field studies on the general ecology or natural history of *Limia*, although some species

have been the subject of laboratory studies on reproductive biology and behavior. It is possible that species of *Limia* have very specific microhabitat requirements, and/or unknown biotic factors such as competition or predation that may combine to regulate the distributions of these species primarily to inland habitats. Studies that address ecological requirements of *Limia* are critically needed, as is additional research to further explore the remarkable range of physiological adaptations among poeciliids. A better understanding of the distributional patterns of the diverse Antillean freshwater fish fauna requires investigation of many species using multidisciplinary approaches that link ecology, physiology, phylogenetic systematics, genetics, and behavior.

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