

Hypoxia and interdemec variation in *Poecilia latipinna*

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Variation in respiratory traits was quantified between two populations of the sailfin molly *Poecilia latipinna* (one from a periodically hypoxic salt marsh, Cedar Key, and one from a chronically normoxic river site, Santa Fe River). Two suites of characters were selected: traits that may show both short-term acclimation response and interdemec variation in acclimation response (metabolic rate, critical oxygen tension and respiratory behaviour), and those that are not likely to respond to short-term acclimation but may vary among populations (gill morphometric characters). Sailfin mollies from the salt marsh, acclimated to hypoxia (1 mg l⁻¹, c. 20 mmHg) for 6 weeks, spent less time conducting aquatic surface respiration and had lower gill ventilation rates than hypoxia-acclimated conspecifics from the well-oxygenated river site. *Poecilia latipinna* acclimated to hypoxia exhibited a lower critical oxygen tension (P_c) than fish acclimated to normoxia; however, there was also a significant population effect. *Poecilia latipinna* from Cedar Key exhibited a lower P_c than fish from the Santa Fe River, regardless of acclimation. Cedar Key fish had a 14% higher mean gill surface area relative to fish from the Santa Fe River, a character that could account, at least in part, for their greater tolerance to hypoxia.

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Key words: aquatic surface respiration; dissolved oxygen; gill surface area; metabolic rate; Poeciliidae.

INTRODUCTION

A central issue in physiological ecology is understanding how animals respond physiologically to natural variation in the abiotic environment, how such responses influence distribution and abundance of populations, and their evolutionary consequences. The physical environment has a major influence on the distribution and movement of organisms. For fishes, the availability of dissolved oxygen is one abiotic factor that can exert a strong selective force affecting habitat quality, growth, survival and reproduction. Hypoxia generally occurs in systems characterized by low light and reduced mixing, such as heavily vegetated swamps (Chapman *et al.*, 1998), isolated saltmarsh pools (Timmerman & Chapman, 2004), marine or freshwater profundal zones (Beadle, 1981; Kalff, 2002), or effluents of spring heads (McKinsey & Chapman, 1998).

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Anthropogenically induced hypoxia is also becoming more common; nutrient enrichment from municipal wastes and fertilizer run-off can cause bacterial, algal and macrophytic blooms within adjacent bodies of water, depleting the water of dissolved oxygen (Jensen *et al.*, 1993). Thus, it is becoming increasingly important to understand effects of hypoxia on aquatic organisms.

Although much effort has been directed towards describing the physiological, biochemical and morphological adaptations of fishes to the oxygen-scarce conditions that occur in such habitats, little is known about the demographic consequences of hypoxic waters. Strong selection pressure for hypoxia tolerance may lead to variation among populations for species with broad habitat ranges. For example, in the African cyprinid *Barbus neumayeri* Fischer, fish from hypoxia swamp sites differ from conspecifics living in well-oxygenated waters with respect to several respiratory traits [*e.g.* respiratory behaviour (Olowo & Chapman, 1996) and total gill filament length (Chapman *et al.*, 1999)]. This interdemec variation may result from differences in the geographical origins of the populations or the developmental and environmental histories of the individuals.

From the perspective of an ecological physiologist there remain obstacles that preclude an understanding of the link between physico-chemical stressors and interdemec variation. One major issue is the fact that physiological variation occurs at multiple hierarchical levels, *i.e.* short-term acclimation or acclimatization response, developmental plasticity, genetic variation and their interactions. Historically, ecophysiological studies in fishes have primarily emphasized variation among species or acclimation effects on individuals. Studies of interdemec variation in eco-physiological characters of fishes are less common and have generally ignored the interaction between acclimation effects and fixed or developmental interdemec effects.

In this study, variation in a series of respiratory traits between two populations of the sailfin molly *Poecilia latipinna* (Lesueur) (one from a periodically hypoxic environment and one from a chronically normoxic environment) was quantified. Two suites of characters were selected: traits that may show both short-term acclimation response and interdemec variation in acclimation response (metabolic rate, critical oxygen tension and respiratory behaviour), and those that are not likely to respond to short-term acclimation but may vary among populations due to developmental plasticity, genetic effects and their interactions (gill morphometric characters). To address hierarchical levels of physiological response, interdemec variation in metabolic rate, critical oxygen tension and respiratory behaviour of sailfin mollies acclimated to normoxia or extreme hypoxia for 6 weeks were quantified. This permitted examination of geographical effects on acclimation response.

MATERIALS AND METHODS

STUDY SITE AND SPECIES

The sailfin molly is a widely distributed live-bearing species, with populations along coastal areas of the Gulf of Mexico and into the inland fresh waters of Florida (Burgess, 1980; Timmerman, 2001). This euryhaline fish is commonly found in environments characterized by periodically hypoxic conditions, including the shoreline of eutrophic

lakes, marshes and wetlands, and estuaries (Trexler & Travis, 1990). It is also found in the well-oxygenated waters of lakes and rivers and in the relatively constant conditions of many Florida springs (Trexler & Travis, 1990; McKinsey & Chapman, 1998).

Sailfin mollies from periodically hypoxic backwaters of the salt marsh of Cedar Key were compared to sailfin mollies that inhabit the clear, well-oxygenated, running waters of the Santa Fe River, Florida (29°14' N; 83°04' W). In the mudflats of the Cedar Key salt marsh, salinity typically fluctuates from *c.* 24 to *c.* 0. Dissolved oxygen levels are also highly variable (range = 0.2–10.8 mg l⁻¹), but extreme hypoxia is not unusual (Timmerman & Chapman, 2004). In the Santa Fe River sailfin mollies were collected from the edge of an area of moderate current on three occasions. In this freshwater habitat, measured oxygen levels were high and showed little variation (mean = 6.8 mg l⁻¹, range = 5.3–8.2 mg l⁻¹).

HYPOXIA ACCLIMATION

Two integrative measures of hypoxia tolerance and characters were selected that represent synergistic effects of different physiological mechanisms: oxygen consumption and respiratory behaviour. Many physiological responses to low dissolved oxygen tension such as increased haemoglobin concentration and lamellar perfusion, can decrease the oxygen tension required to maintain an individual's metabolic rate (critical oxygen tension, P_c), and thus it is not surprising that experiments of acclimation response to hypoxia in some fishes have detected a decrease in P_c (Timmerman, 2001). Behavioural response to low oxygen is another integrative measure of hypoxia tolerance. The surface film of water contains high oxygen concentration, and many fish species take advantage of this property by skimming the surface film and passing oxygen-rich water over their gills, a behaviour that is referred to as aquatic surface respiration (ASR; Kramer & Mehegan, 1981). Fishes with high physiological compensation for hypoxia tend to have relatively low thresholds (P_{O_2}) for aquatic surface respiration (ASR) and tend to spend less time at the surface once that threshold occurs (Chapman *et al.*, 2002). Other behavioural traits such as gill ventilation rate, agonism and reproductive behaviour may also change in response to hypoxic stress (Chapman *et al.*, 1995; Rosenberger & Chapman, 2000; Timmerman, 2001).

To examine acclimation effects, sailfin mollies were collected from the mudflats of Cedar Key and the shoreline of the Santa Fe River and transported to the laboratory. Four 250 l glass aquaria were divided in half with a fine plastic mesh, separating individuals from the two populations (10 fish from Cedar Key on one side and 10 from Santa Fe on the other side). Care was taken to approximate 1:1 sex ratios in the treatments and aquaria; however, the Santa Fe River collection was skewed towards females, resulting in a final ratio of three males to seven females in each Santa Fe section and five males and five females in the Cedar Key compartments. Adult sailfin mollies were selected for this study. Because mature fish tend to be larger at Cedar Key than at the Santa Fe River site, the average body mass was smaller for the Santa Fe fish. For both populations, however, it was possible to collect a good size range to permit evaluation of body mass effects and facilitate inter-population comparisons. Once fish were introduced to the aquaria, heaters were used to maintain the water temperature at 25°C. A salinity of 10 was selected to minimize osmotic stress (Evans, 1975; Nordlie *et al.*, 1992), using a mixture of filtered sea water and distilled water to make up the brackish water of the aquaria. Water temperature and salinity were monitored daily and adjusted as necessary. Two aquaria were exposed to extreme chronic hypoxia (1 mg l⁻¹ O₂, *c.* 20 mmHg), and two aquaria were held at normoxia (7.6–8.2 mg l⁻¹ O₂, *c.* 150–162 mmHg) for 6 weeks prior to collection of metabolic rate data. A 6 week acclimation period was chosen based on previous work demonstrating a trade-off between physiological and behavioural response to hypoxia in sailfin mollies. Timmerman (2001) found that 6 week exposure of sailfin mollies to extreme hypoxia facilitated acclimation response in both behavioural and haematological characters and resulted in lowered P_c . Upon completion of metabolic rate measurements in each group, fish were re-acclimated to experimental conditions for an additional 2 weeks prior to collection of behavioural data.

Fish were acclimated to a 12L:12D photoperiod and fed Tetra Min flake[®] food *ad libitum* twice daily. Fish were allowed to acclimate to these conditions for 7 days prior to beginning experiments. On the eighth day, a microprocessor-controlled aquatic oxygen maintenance unit (Timmerman, 2001), using pulses of nitrogen, progressively lowered the dissolved oxygen (DO) level in the hypoxic aquaria to $1.0 \text{ mg l}^{-1} \text{ O}_2$ (c. 20 mmHg) over 3 days. Two thirds of the surface area of hypoxic aquaria was covered by a layer of plastic bubble wrap to minimize oxygen diffusion. This DO level was chosen based on preliminary ASR experiments that showed the 10% ASR threshold for *P. latipinna* occurred near $1.0 \text{ mg l}^{-1} \text{ O}_2$.

METABOLIC RATE AND CRITICAL OXYGEN TENSION

The metabolic rate and P_c of each fish was estimated using closed respirometry on post-absorptive fish. Metabolic rate was determined as routine oxygen consumption [R_r ; rates during random movement under experimental conditions (Nordlie *et al.*, 1991) for a range of body sizes]. An average of six fish was selected for measurement from each group and trial of the long-term acclimation experiment; fish were selected to maximize the range of body sizes analysed. Total R_r was calculated for each fish using data collected at least 30 min after the container was sealed with the probe and prior to estimated P_c .

Each fish was placed in an opaque Nalgene container (130–210 ml) with an air stone and held for 24 h before the trial at 25° C. At the start of each experiment, the chamber was flushed and sealed with a DO probe (YSI Model 600). The meter was programmed to measure water temperature and DO at 10 min intervals and to display plotted values on a portable computer throughout the trial. Once the P_c was detected on the computer-generated plots, the experiment was terminated, and the water in the chamber was aerated and quickly returned to normoxia. Following each trial, the wet mass (M) of the fish was recorded. The P_c was determined using the BASIC programme by Yeager & Ultsch (1989) that is designed to fit two regression lines to a data set and calculate P_c as the point of intersection of these two lines. The R_r in the 6 week acclimation experiment were measured over a period of 14 days subsequent to the acclimation period with measurements alternated daily between the hypoxia-acclimated and normoxia-acclimated fish.

Analysis of covariance (ANCOVA) on \log_{10} - \log_{10} -transformed data revealed no significant trial effects within treatments and populations for R_r . Therefore, trials were combined for further analyses. ANCOVA was used to test for effects of population and treatment on total R_r . Adjusted mean R_r were calculated for treatments and populations from the ANCOVA (sample means adjusted for a common mean body M and a common regression line). Timmerman & Chapman (2003) found that females in late gestation show an elevated mass-specific R_r relative to other females and males. Although it was not possible to dissect females to identify those in late gestation immediately after metabolic runs (because of subsequent behavioural observations), females were visually scored as gestating or non-gestating. Results were similar with and without inclusion of gestating females, therefore analyses were combined for all fish. ANCOVA on \log_{10} - \log_{10} -transformed data was used to test for effects of M on P_c for each treatment within each trial and population. No significant body size effects were detected. t -tests were then used to detect trial effects on P_c within treatments and populations. As none were detected, trials were combined for further analyses. Population and treatment effects on P_c were detected using two-way ANOVA.

BEHAVIOURAL COMPARISONS

Behavioural data were collected for the long-term acclimation experiment only using a scan method. Once the P_c and R_r data were collected from all groups, fish were allowed to re-acclimate to experimental conditions for another 2 weeks. Behavioural data were therefore collected after 10 weeks of acclimation. The characters selected included behavioural traits for which specific predictions with respect to treatment and population effects, based on previous study of effects of hypoxia on the behaviour of *P. latipinna* (Timmerman,

2001), were available. A depression in reproductive behaviour (*i.e.* courtship displays and copulation attempts) and agonism (*i.e.* chasing and nipping at fins) under hypoxia and an increase in both ASR and gill ventilation rate were predicted. It was also predicted that Santa Fe fish would engage in ASR more frequently, have higher gill ventilation rates, and a greater depression in reproductive behaviour when acclimated to hypoxia than fish from Cedar Key. Each aquarium was scanned at 5 s intervals over a 10 min period, and the number of individuals engaging in a specific behaviour was counted. This process was repeated for each behavioural category. The number of individuals conducting ASR was counted, as well as the number of fish involved in agonistic, courtship or copulatory encounters. The number of courtship and copulatory encounters were counted at once and combined to represent the frequency of reproductive behaviour. There was no differentiation between whether the monitored individual was the initiate or the recipient of courtship, copulatory or agonistic encounters. As the scan approach integrates information across many individuals, it reduces the number of zero scores in the data set, increasing the likelihood of observing rare behaviours. At the end of each 10 min session, individual ventilation rates were assessed by timing the duration required for 20 opercular ventilations for five individuals within each chamber. This entire behavioural data collection process was repeated seven times for each compartment over a period of 10 days. To minimize effects of dependence among trials, each observation period was separated by at least 24 h, and results were averaged for each 10 min trial.

Subsequent to R_r analyses, a bleach spill resulted in mortality in one normoxia tank. Thus, behavioural data were collected on fish in one normoxia tank (control) and two hypoxia aquaria (treatments). ASR, agonism and reproductive data exhibited non-normal distributions. Therefore, medians and ranges are reported, and the data were analysed using non-parametric statistics. The Mann–Whitney U -test indicated no trial effects for either population within the hypoxia treatment, so these two trials were combined for further analyses. The Kruskal–Wallis test to detect differences among the four groups (Cedar Key hypoxia, normoxia; Santa Fe hypoxia, normoxia), and Mann–Whitney U -tests were used for *a posteriori* comparisons. Four sets of *a posteriori* tests were used: Cedar Key normoxia (CKN) *v.* Cedar Key hypoxia (CKH); Santa Fe normoxia (SFN) *v.* Santa Fe hypoxia (SFH); CKH *v.* SFH; and CKN *v.* SFN. The Bonferroni correction factor was used to adjust the probability value of significance for multiple comparisons to 0.013, and one-tailed tests were used to calculate P values. Gill ventilations were not quantified in normoxia-acclimated fish because opercular movement in normoxia was too subtle to permit an accurate count. Gill ventilations were compared between populations acclimated to hypoxia using a t -test.

GILL MORPHOMETRY

Fish from each population were preserved in paraformaldehyde (35 g l⁻¹). Total gill filament length was measured using standard methods modified after Muir & Hughes (1969) and Hughes (1984). For each fish, the left branchial basket was removed, and the four gill arches were separated. For each hemibranch, the length of every fifth gill filament was measured. Two successive measurements along a hemibranch were averaged and multiplied by the number of filaments in the section between the two filaments. Filament lengths were summed for the four hemibranchs and multiplied by two to produce an estimate of total gill filament length (L_{TGF}). Lamellar density was measured in the dorsal, middle and ventral parts of every tenth filament of the second gill arch on the left side. The total number of lamellae (on one side of the filament) and average lamellar density (D_L) were estimated using a weighted mean method that takes into account the difference in length of various filaments (Muir & Hughes, 1969; Hughes & Morgan, 1973). For every 10th or 20th filament (depending on the number of filaments), the lengths of the secondary lamellae were measured a number of times over the filament (Galis & Barel, 1980). Lamellar height was also measured on specimens where it could be accurately determined. Average values of these characters for each filament were converted to estimates of lamellar area using a regression determined through the dissection of 30 to 60 lamellae from various sections of the second gill arch from two or more

specimens from each population. The sum of the total lamellar area for all sections of the second arch was divided by the total number of lamellae and multiplied by two to produce a weighted average bilateral surface area on one side of the filament (A_{LA}). Total gill surface area (A_{TGS}) was determined using the formula: $A_{TGS} = 2L_{TGF}D_L A_{TG}$.

ANCOVA was used to compare L_{TGF} and A_{TGS} between the Santa Fe and Cedar Key populations of *P. latipinna* with M (total mass of preserved specimens) as the covariate. All variables were \log_{10} -transformed. Adjusted means (sample means adjusted for a common mean M and a common regression line) were calculated from the ANCOVA analysis.

RESULTS

METABOLIC RATE AND CRITICAL OXYGEN TENSION

Slopes of the bilogarithmic relationship between R_r and M did not differ between treatments or populations ($P > 0.118$). There was no significant effect of population, acclimation treatment or their interaction on the intercepts of the bilogarithmic relationship between R_r and M (population, $P = 0.763$; DO, $P = 0.105$, population \times DO, $P = 0.225$). Thus, for a fish of a given M , R_r did not differ between fish held under normoxia *v.* hypoxia (mean R_r for normoxia-acclimated fish = $0.22 \text{ mg O}_2 \text{ h}^{-1}$; hypoxia = $0.26 \text{ mg O}_2 \text{ h}^{-1}$, adjusted to a mean M of 1.48 g , $n = 45$) or between populations (Cedar Key = $0.25 \text{ mg O}_2 \text{ h}^{-1}$; Santa Fe = $0.23 \text{ mg O}_2 \text{ h}^{-1}$).

The P_c differed among populations and treatments (ANOVA, population: $F_{1,35}$, $P = 0.001$; DO, $F_{1,35}$, $P = 0.002$; population \times DO: $F_{1,35}$, $P = 0.403$). Overall, acclimation to chronic hypoxia induced lower P_c values (mean antilogged P_c : normoxia = 25.2 mmHg ; hypoxia: 19.0 mmHg , Fig. 1). Cedar Key sailfin mollies, however, appeared better able to cope with extreme hypoxia and had P_c values consistently lower than Santa Fe River fish regardless of acclimation (mean antilogged P_c : Cedar Key = 18.7 mmHg ; Santa Fe = 25.6 mmHg , Fig. 1).

BEHAVIOUR

Poecilia latipinna from Cedar Key were consistently more aggressive than their Santa Fe conspecifics (CKN *v.* SFN, $P = 0.005$; CKH *v.* SFH, $P < 0.001$, Fig. 2). Acclimation to chronic hypoxia had no significant effect on the level of aggression in either population (CKN *v.* CKH, $P = 0.052$; SFN *v.* SFH, $P = 0.072$, Fig. 2). Reproductive behaviours were less frequent in the Santa Fe groups than Cedar Key groups (CKN *v.* SFN, $P = 0.003$; CKH *v.* SFH, $P < 0.001$, Fig. 2). In addition, acclimation to chronic hypoxia reduced the frequency of reproductive behaviours in both populations (CKN *v.* CKH, $P = 0.005$; SFN *v.* SFH, $P = 0.008$, Fig. 2). Aquatic surface respiration (ASR) did not occur in normoxia for fish from either population. The median frequency of ASR, however, was higher for hypoxia-acclimated fish from the Santa Fe River than for those from Cedar Key (SFH *v.* CKH, $P = 0.003$, Fig. 2). Under hypoxic conditions, Santa Fe River individuals had a much higher gill ventilation rate than Cedar Key fish (Santa Fe: 74.7 ± 2.2 gill ventilations per 20 s; Cedar Key: 48.1 ± 7.8 gill ventilations per 20 s, $P < 0.001$).

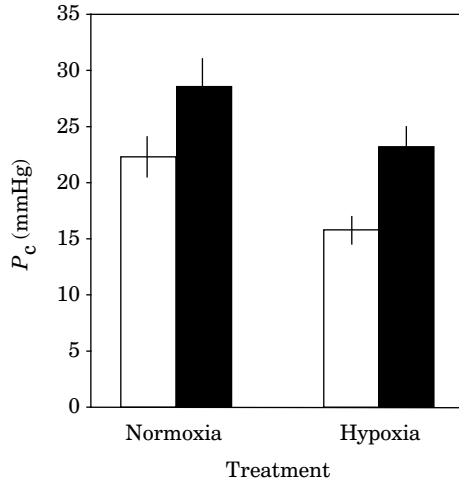


FIG. 1. Mean \pm S.E. critical oxygen tension for sailfin mollies from the periodically hypoxic Cedar Key salt marsh (\square) and from the well-oxygenated Santa Fe River (\blacksquare), acclimated to either normoxia or chronic hypoxia (1 mg l^{-1} , $c.$ 20 mmHg). Both population (two-way ANOVA, $P=0.001$) and treatment ($P=0.002$) had significant effects on P_c .

GILL MORPHOMETRY

Gill characters were measured on eight fish from each population selected to maximize range in M (Cedar Key, mean $M=2.08 \text{ g}$, range = 0.92 to 5.00 g ; Santa Fe, mean = 2.09 g , range = 1.06 to 4.87 g). Gill measurements permitted estimation of the following characters: L_{TGF} , D_{L} , A_{LA} and A_{TGS} .

For the two populations, L_{TGF} , D_{L} and A_{TGS} were positively correlated with M (Table I). Average lamellar density was negatively related to M in both groups (Table I). ANCOVA indicated no difference in the slopes of the bilogarithmic relationships between individuals of the Cedar Key and Santa Fe River populations for any of the gill characters examined (Table I). Intercepts differed, however, for two of the four gill morphometric characters (Table I). When adjusted for M , L_{TGF} was greater in fish from the periodically-hypoxic waters of the Cedar Key salt marsh than in fish from the well-oxygenated waters of the Santa Fe River [$F_{1,13}$, $P=0.007$; Table I and Fig. 3(a)]. The longer gill filament length among individuals in the Cedar Key population was reflected in larger gill surface area. The A_{TGS} was 14% greater in fish from the Cedar Key salt marsh population than in those from the Santa Fe River population [$F_{1,13}$, $P=0.033$; Table I and Fig. 3(b)]. Neither lamellar density ($P=0.123$) nor average lamellar area ($P=0.545$) differed between the two populations.

DISCUSSION

In fishes, plastic behavioural and physiological responses to hypoxia may be critical for species living in habitats characterized by strong spatial and temporal variation in dissolved oxygen content. In addition, strong selection pressure for hypoxia tolerance in oxygen-scarce habitats may lead to interdemographic variation among populations with broad habitat ranges. This interdemographic

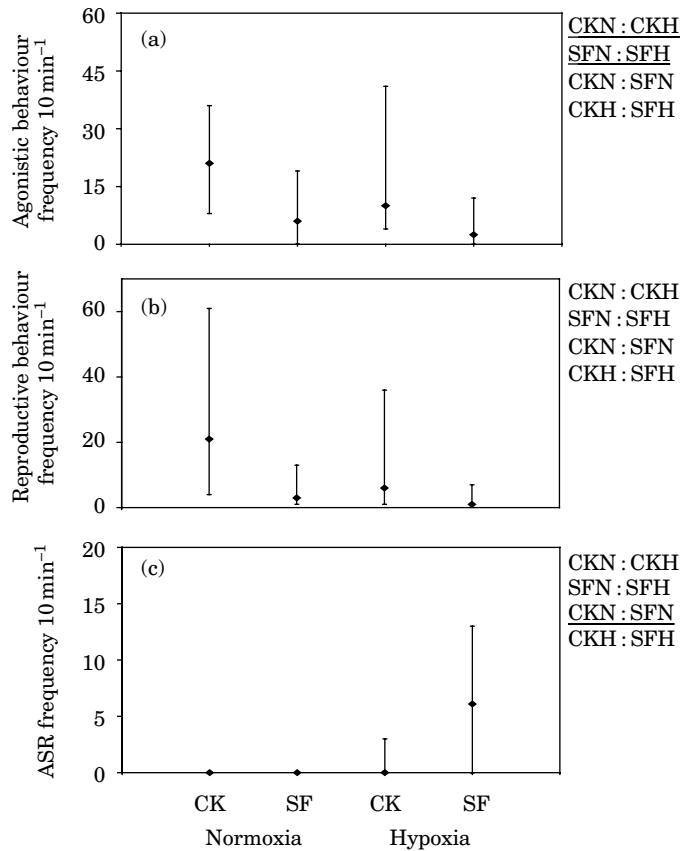


FIG. 2. Median (\blacklozenge) and range for the frequency of (a) agonistic behaviour, (b) reproductive behaviour and (c) aquatic surface respiration for sailfin mollies from the Cedar Key salt marsh (CK; periodically hypoxia) and the well-oxygenated waters of the Santa Fe River (SF). Fish were acclimated to normoxia or chronic hypoxia (1 mg l^{-1} , *c.* 20 mmHg). Underlined comparisons are not significantly different (Mann-Whitney *U*-tests with Bonferonni correction, $P < 0.013$).

variation may derive from differences in the geographical origins of the populations or the developmental and environmental histories of the individuals within the populations. The results of this study of *P. latipinna* demonstrate short-term acclimation response to hypoxia, interdemec variation in acclimation response, and interdemec variation in gill morphometry. The source of the observed interdemec variation is unknown but supports a strong role of hypoxic waters in physiological diversification.

SHORT-TERM ACCLIMATION RESPONSE

Acclimation to chronic hypoxia indicated short-term compensatory response, but also demonstrated interdemec variation in the acclimation response. Individuals from both populations spent more time conducting ASR under hypoxia. Sailfin mollies from the Cedar Key salt marsh, however, spent less time conducting ASR and had lower gill ventilation rates than conspecifics from the

TABLE I. Summary of linear regression ANCOVA of relationships between gill morphometric characters and body mass (*M*, g) for sailfin mollies from the Cedar Key salt marsh (CK; periodic hypoxia) and the well-oxygenated waters of the Santa Fe River (SF). Both gill characters and *M* were log₁₀ transformed. The mean values represent antilogged adjusted means calculated from the ANCOVA analyses (sample means adjusted for a common mean *M* of 2.08 g and a common regression line)

Character	Site	Slope		Intercept	<i>r</i>	<i>P</i>	ANCOVA		Slope	ANCOVA		Intercept	Adjusted means
		Slope	Intercept				<i>F</i>	<i>P</i>		<i>F</i>	<i>P</i>		
Total gill filament length (cm)	CK	0.488	2.037	0.967	<0.001	0.094	0.765	10.186	0.007	155.96			
	SF	0.518	1.978	0.972	<0.001					138.36			
Lamellar density (number per mm)	CK	-0.084	1.693	0.930	0.001	1.358	0.267	2.717	0.123	46.34			
	SF	-0.059	1.677	0.818	0.013					45.50			
Lamellar area (mm ²)	CK	0.196	-2.496	0.850	0.007	4.101	0.066	0.387	0.545	0.0037			
	SF	0.080	-2.469	0.788	0.020					0.0036			
Total gill surface area (cm ²)	CK	0.611	0.831	0.924	0.001	0.436	0.522	5.653	0.033	10.62			
	SF	0.529	0.791	0.959	0.000					9.12			

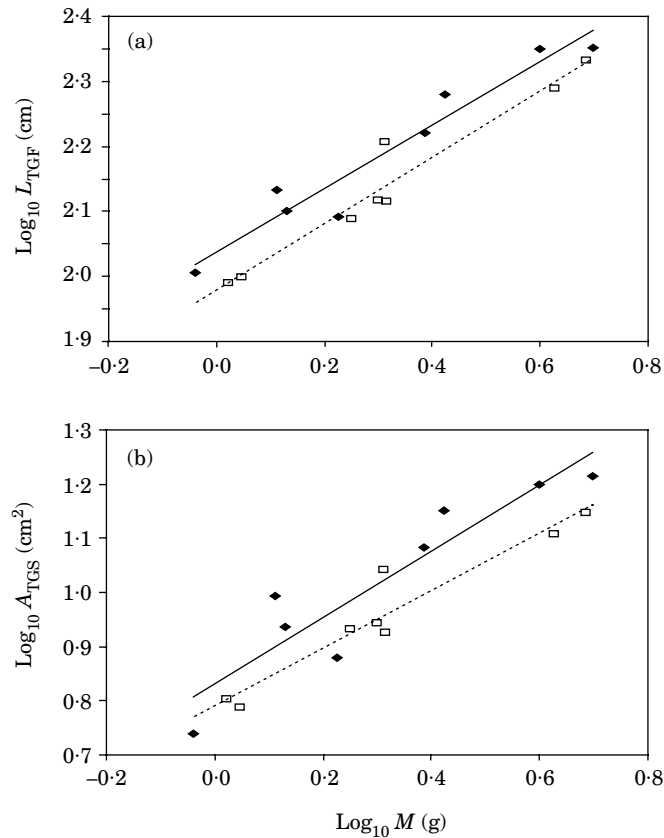


FIG. 3. Bilogarithmic plots of (a) the relationship between total gill filament length and (b) total gill surface area and body mass for sailfin mollies from the periodically hypoxic waters of the Cedar Key salt marsh (—) and the well-oxygenated waters of the Santa Fe River (.....).

Santa Fe River. In fact, time allocated to ASR was extremely low in the Cedar Key population, even under extreme hypoxia. This may reflect selection pressure to minimize time spent at the surface under hypoxia and the development of other mechanisms to cope with hypoxic stress.

Poeciliids seem well-suited to survive under hypoxia, having a head morphology that facilitates efficient skimming of the oxygen rich-water surface layer while maintaining a horizontal swimming position (McKinsey & Chapman, 1998). Short-term experiments have shown that, without access to the surface, sailfin mollies sustain high mortality rates at low oxygen levels (Peterson, 1990), suggesting that ASR is a primary mechanism for coping with hypoxia. Aquatic surface respiration is clearly a widespread behavioural response to extreme aquatic hypoxia in fishes (Kramer & McClure, 1982; Chapman *et al.*, 2002). It has been shown, however, to have high associated energetic costs and can increase risk of aerial predation (Kramer *et al.*, 1983). Therefore, fishes adapted to periodic or chronic hypoxia may have developed other physiological, morphological or biochemical mechanisms to reduce the need for ASR or to lower their ASR threshold. Timmerman (2001) reported a decrease in the ASR

frequency in *P. latipinna* from the Cedar Key salt marsh over a 6 week acclimation period, suggesting that there are costs to the behavioural response that favour physiological compensation. Lower allocation to ASR in the Cedar Key population, when compared to the Santa Fe River population, may reflect the greater development of other mechanisms to cope with hypoxia, such as their larger gill surface area, or perhaps haematological adjustments (Timmerman, 2001).

Acclimation to hypoxia also reduced the frequency of reproductive behaviours in individuals of both populations. Cedar Key individuals exhibited higher rates of reproductive behaviours than Santa Fe River individuals, possibly reflecting interdemec variation in reproductive behaviour, which has been shown to occur among other populations of this species (Farr & Travis, 1989; Ptacek & Travis, 1998). Since there were fewer males in the Santa Fe River groups, however, differences in sex ratio between the experimental groups cannot be ruled out as a factor influencing the reproductive behaviour rate. Similarly, the higher rates of agonism in the Cedar Key fish may reflect the higher proportion of males.

Sailfin mollies acclimated to hypoxia exhibited a lower critical oxygen tension than fish acclimated to normoxia; however, there was also a significant population effect. *Poecilia latipinna* from Cedar Key exhibited a lower P_c than fish from the Santa Fe River, thus demonstrating interdemec variation in acclimation response. Timmerman (2001) measured critical oxygen tension of *P. latipinna* from the Cedar Key salt marsh within 6 days of capture and reported a mean P_c of 19 mm, intermediate to the acclimation P_c averages for *P. latipinna* from Cedar Key reported in this study. This might be expected because sailfin mollies were exposed to chronic hypoxia in this experiment but experience only periodic hypoxia in the field at Cedar Key.

Although studies of interdemec variation in physiological characters are uncommon, population level differences have been demonstrated in oxygen consumption e.g. *Astyanax fasciatus* (Cuvier) (Hueppop, 1986) and *Gadus morhua* L. (Nelson *et al.*, 1996). Experiments examining variation among populations in hypoxia tolerance or physiological characters affecting hypoxia tolerance, such as blood oxygen capacity, in fishes are extremely rare, but have provided evidence for interdemec effects e.g. *Carassius auratus* (L.) (Bel'Chenko & Kel, 1992) and *Perca flavescens* (Mitchill) Nelson *et al.*, 1988). In comparing two populations of *Gambusia affinis* (Baird & Girard) known to differ in their pesticide resistance, McCorkle *et al.* (1979) found that the pesticide resistant population was more vulnerable to hypoxia. It is possible that characters leading to superior hypoxia tolerance, such as increased gill surface area, increase pesticide susceptibility due to increased uptake through the gills, thus selecting against more hypoxia-tolerant individuals.

In this study of *P. latipinna*, the source of the interdemec variation in acclimation response characters was not identified. Interdemec variation could be genetically based, fixed by environmental pressures at a critical period of ontogeny, or simply be a phenotypic response that remains labile throughout the lifetime of an individual. A study of clonal strains of *Daphnia pulex* from a eutrophic pond showed genotypic effects on characters affecting hypoxia tolerance, including metabolic rate and haemoglobin concentration (Weider &

Lampert, 1985). Nordlie *et al.* (1992) demonstrated salinity tolerance in the sailfin molly is a plastic character, but that population-level differences were still maintained between freshwater and estuarine populations after several weeks of acclimation to increasing salinity. Similar experiments on *P. latipinna* have shown that populations living in either mildly brackish or freshwater habitats are distinct in their response to temperature and salinity. Travis & Trexler (1987) suggested that these differences have a genetic basis, but emphasized the need for quantitative data. In the current study of *P. latipinna*, chronic hypoxia induced a decrease in critical oxygen tension; however, population level differences were still maintained after 6 weeks of acclimation. This suggests that, although P_c is a plastic character, there are other developmentally plastic or genetic differences between the two populations. A rigorously controlled growth experiment would be useful in teasing apart the relative environmental and genetic contributions to interdemec variation in P_c .

The physiological and behavioural interdemec variation observed in the acclimation response of sailfin mollies suggests that there are other developmentally plastic or genetic differences between populations in characters compensating for hypoxia tolerance. The 14% larger gill surface area in fish from the Cedar Key population is one character that may contribute to the observed pattern, as a larger gill surface area should increase oxygen uptake capacity.

Travis & Trexler (1987) determined that *P. latipinna* exhibits a trend toward increased genetic differentiation with distance between populations, conforming to a model of isolation by distance. The distance between Cedar Key and the site where sailfin mollies were collected on the Santa Fe River (*c.* 78 km) may be sufficient to prevent significant genetic mixing and allow for the development of habitat-associated genetic distinctions, although many other factors, not measured, may also be involved.

INTERDEMIC VARIATION IN GILL MORPHOMETRY

Studies of interdemec variation in morphological characters of fishes have focused on jaw, skull and body morphology, and have demonstrated significant morphological differences between or among populations (Wimberger, 1992; Motta *et al.*, 1995; Holopainen *et al.*, 1997; Cutwa & Turingan, 2000; Sediva *et al.*, 2000). Studies of interdemec variation in gill morphometry are few but also support variation in gill size among populations of some species. For example, Chapman *et al.* (1999) found a negative relationship between total gill filament length and dissolved oxygen concentration among populations of the African cyprinid *B. neumayeri*. Chapman & Hulen (2001) recorded a greater total gill surface area in a swamp-dwelling population of the African mormyrid *Gnathonemus victoriae* (Worthington) than for fish from an open-water population, and similar results were documented in the African cichlid, *Pseudocrenilabrus multicolor victoriae* Seegers (Chapman *et al.*, 2000). The degree to which interdemec variation in gill morphometry represents phenotypic plastic and genetic differences in these species and for *P. latipinna* is unknown. Chapman *et al.* (2000), however, raised *P. multicolor* from a single brood under normoxic and hypoxic conditions. Total gill surface area was 18% greater in the hypoxia-acclimated group,

demonstrating plasticity in gill size, but the difference between field populations was greater (38%) and suggesting both genotypic and phenotypic effects. Saroglia *et al.* (2002) reported a negative relationship between the gill surface area in sea bass *Dicentrarchus labrax* (L.) and the oxygen partial pressure of the water in which they were reared for 3 months, again providing evidence of phenotypic plasticity in gill characters. Developmental plasticity in gill characters is not unique to fishes. A study of the decapod *Munida quadrispina* showed that gill size is developmentally plastic and responds to exposure to chronic hypoxia. Populations of *M. quadrispina* subjected to chronic severe hypoxia nearly doubled their ratio of gill dry mass to dry body mass compared to counterparts in normoxic waters (Burd, 1988). Plasticity in gill size in response to hypoxia has also been reported for bullfrog *Rana catesbeiana* larvae (Bond, 1960) and salamander, *Salamandra maculosa* and *Ambystoma jeffersonianum*, larvae (Burggren & Mwalukoma, 1983).

It seems likely that the increased gill surface area seen in individuals from the Cedar Key population is related to dissolved oxygen availability. The two sites in question, however, differ not only in oxygen availability but also in salinity, among other characters. The Cedar Key population is exposed to salinity values ranging from near zero (on rare occasions) to 24 (daily), while the Santa Fe River population is in a spring-fed (hardwater) river. Differences in osmoregulatory requirements between the two populations might also be a factor in the observed gill size differences. Under osmotically stressful conditions, the surface area of the gill devoted to chloride cells may increase (Evans, 1993). An increase in the gill surface area devoted to chloride cells may impede respiratory uptake of oxygen (Bindon *et al.*, 1994). In one study of *Oncorhynchus mykiss* (Walbaum) exposed to tap water, sea water, or ion-poor water, chloride cell proliferation increased in the ion-poor water (Bindon *et al.*, 1994). It is possible sailfin mollies could react similarly, with essentially no difference in the number or size of chloride cells between hard freshwater populations and estuarine populations. Alternatively, the osmotically variable environment of the estuary may induce an increase in gill surface area allocated to chloride cells, resulting in a compensatory increase in gill size for estuarine sailfin mollies. This is apparently supported by Palzenberger & Pohla (1992) who summarized that the gill areas of freshwater fishes are smaller than those of comparable marine species, a trend that could not be adequately explained by activity level alone. A controlled growth experiment using salinity and hypoxia as treatments would be helpful in quantifying the relative contribution of each of these factors to variation in gill morphometric characters. In addition, future studies that include measurement of morphometric diffusing capacity (Kisia & Hughes, 1992), which is a good metric for summarizing the overall adaptation of a gas exchange surface, will be useful in understanding more fully the response of *P. latipinna* gills to different oxygen environments.

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