Vertebrate Physiology Lab

Dr.Oliver

Benedict College 1600 Harden St.

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Physiology of The Mangrove Killifish

Stacy D. Mays Jr.

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Abstract

The physiology of the mangrove killifish experiment/ research paper is abstract itself due to the fact of the ability of the mangrove killifish, that you'll be further introduced to later in this paper. Now according to our data we experienced very precise results for our experiment , so readers would not and should not be discouraged by the unique and vast amount of information being displayed from abstract to conclusion. Lastly the overall rating of this experiment is a success and like wise the information in this experiment is honorable.

Introduction

In this research paper creation of other small scale experiments was conducted, most importantly we explain the physiology of the mangrove killifish. I believe most lab going scientists will find this experiment/research paper interesting because it host more than the bare minimum graphs, pictures,

data, and facts about the mangrove killifish than the average paper. Next the methods of this paper and experiment will be able to be followed and reproduced throughly because of the clear and precise steps and this is another reason that this experiment/research paper is eye catching. We hypothesize that the physiology of the mangrove killifish is or may be one of the most remarkable adaptations because of its survival and distance from extinction.

Safety

In this experiment safety was always the number one goal , to ensure the safety of the experiment lab coats and goggles were worn at all time. Closed toed shoes and pants were also worn for the protection of skin in the process of conducting this experiment and writing of this research paper. Then hair was tied back along with gloves, for safety reasons in the experiment and research paper. Now to also keep safe lab equipment and large safety structures , such was eye wash station , chemical bath , hood , chemical room , fire blanket and etc was double checked for preparation to deal with emergencies.

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Materials

A host of materials was used in the main and side experiments of the physiology of the mangrove killifish. Our most important material was the supply of mangrove killifish. The mangrove killifish was the main item. Next was the safety materials used in the experiment, with out the safety materials the completion of this experiment would not happened. Our electronic equipment was next all variables being put into our electronic materials allowed for data to be read and plugged into a graph. Now we were left with the usual / normal / beginner equipment that would be used in an experiment. Such as

measuring equipment, heating and cooling equipment, chemical and chemical glass wear, cleaning materials and etc.

Prep.

In preparation for this experiment/ research paper we use safety as the main platform. After all safety procedures were used the next thing for preparation was log in sheets for the accountability of scientists and etc. who help follow through on steps to getting our results. Then also for preparation the sanitation and cover sheets / tarpswere used for inside and outside operations. In preparation if any experiment was conducted outside then the use of a tent over cover tarps were used professionally. Also as prep garbage and toxic waste containers were always available. Item sheets were also used as a insurance of all our material was there to keep from running out.

Methods

The methods in the experiment, giving us grounds to write this paper were in a way , all the same and yet different because of a few side experiments. But as a standard the method went was following , set time and location, sign in / log in , item check / retrieval, set up / safety check , then the scientists would identify the electric equipment for the process of getting results and before hand the scientists would apply any variable the electric equipment may need to get its results . In addition the location had more to do with methods because some examples were extracted from different locations. In defense of the scientists some scientists took and used materials in different order for the completion of group experiment/ research paper.

Graphs / Results's

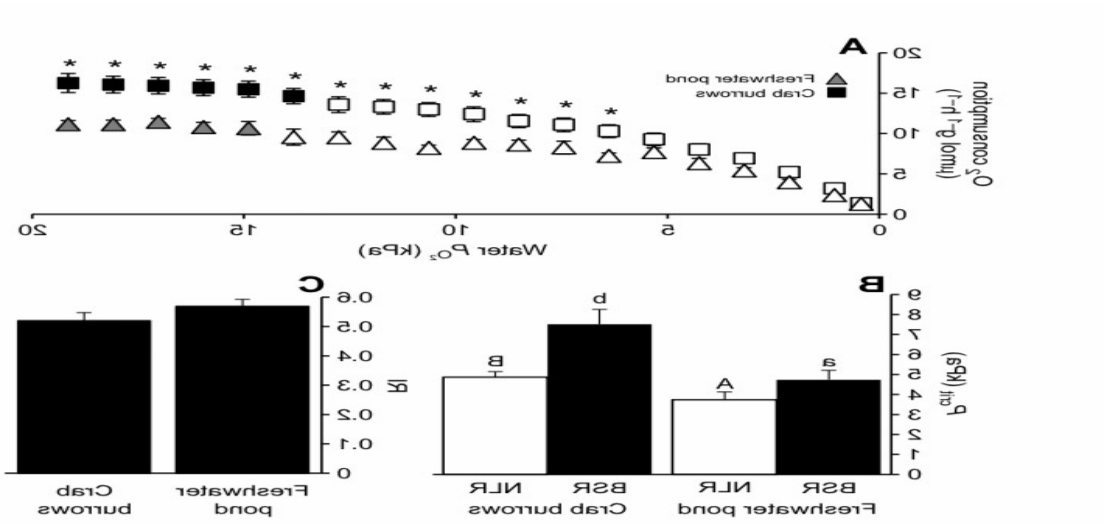

Fig. 1. Respiratory measurements of Kryptolebias marmoratus from two sites on Long Caye. (A) Oxygen consumption curves from critical O2 tension (Pcrit) trials. Asterisks above symbols denote significant differences in the rate of O consumption at a given P between collection

Freshwater pond Crab burrows locations, and open symbols denote significantly different rates of O2 consumption within a population compared with the normoxic rate at 19 kPa (two-way ANOVA, interaction $P<0.05$). (B) Calculated values of Pcrit using linear broken-stick regression (BSR) or nonlinear regression (NLR), and (C) regulation index (RI), a relative measure of whether an animal is an O2 conformer or O2 regulator. Different letters above bars denote significant differences in Pcrit between locations (lowercase letters, comparison of BSR-calculated values; uppercase letters, comparison of

NLRcalculated values; t-test, P<0.05). Freshwater pond, n=11; crab burrow, n=10. Error bars represent plus or minus s.e.m

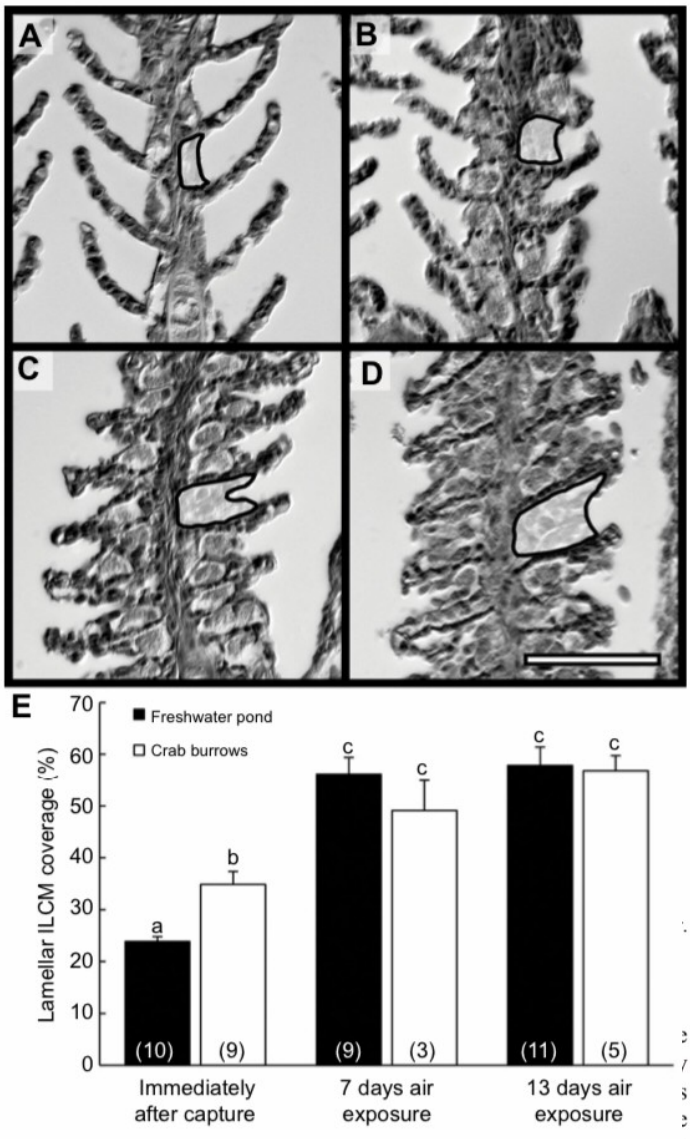

Fig. 2. Gill morphology of *K. marmoratus* from Long Caye. Representative photographs of gills from fish taken immediately after capture from (A) a freshwater pond or (B) crab burrows. Terrestrial acclimation induced gill remodelling in fish from both (C) the freshwater pond and (D) crab burrows. An example of an interlamellar cell mass (ILCM) in each panel is outlined in black. Scale bar: 50 μm. (E) ILCM coverage of gill lamellae in fish collected directly from the wild or terrestrially acclimated after capture. Different letters above bars denote significant differences between groups (two-way ANOVA, interaction $P<0.05$). Sample sizes are given in brackets at the base of each bar. Error bars represent ±s.e.m.

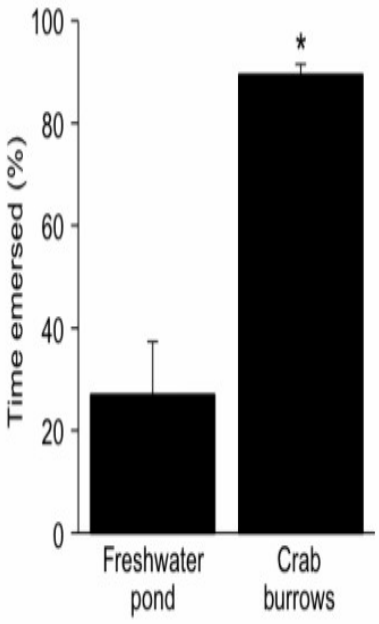

Fig. 3. Emersion behaviour of *K. marmoratus* from Long Caye. Proportion of time fish spent stuck to the side of a plastic sample container above the water. The asterisk denotes a significant difference between sites (*t*-test, $P=0.001$). Freshwater pond, $n=10$; crab burrow, $n=10$. Error bars represent ±s.e.m.

Table 3. Statistical results from phylogenetically controlled analysis of correlations between degree of heterozygosity and respiratory function

Phylogeny	P_{crit} calculation	λ	*F* statistic	R^2	*P*
NJ	BSR	ML (=0)	0.53	0.02	0.47
NJ	BSR	1	3.31	0.10	0.08
NJ	NLR	ML (=0)	0.27	0.01	0.61
NJ	NLR	1	1.11	0.04	0.30
UPGMA	BSR	ML (=0.15)	0.02	0.001	0.89
UPGMA	BSR	0	0.46	0.02	0.50
UPGMA	BSR	1	1.33	0.04	0.26
UPGMA	NLR	ML (=0.02)	0.13	0.004	0.72
UPGMA	NLR	0	0.24	0.01	0.63
UPGMA	NLR	1	3.16	0.10	0.09

Phylogenies were created using neighbour-joining (NJ) or unweighted pair group method with arithmetic mean (UPGMA) methods. Critical O_2 tension (P_{crit}) was calculated using broken-stick regression (BSR) or nonlinear regression (NLR) approaches. Values of λ, the importance of branch lengths, were set to 0, 1 or the calculated maximum likelihood (ML) value.

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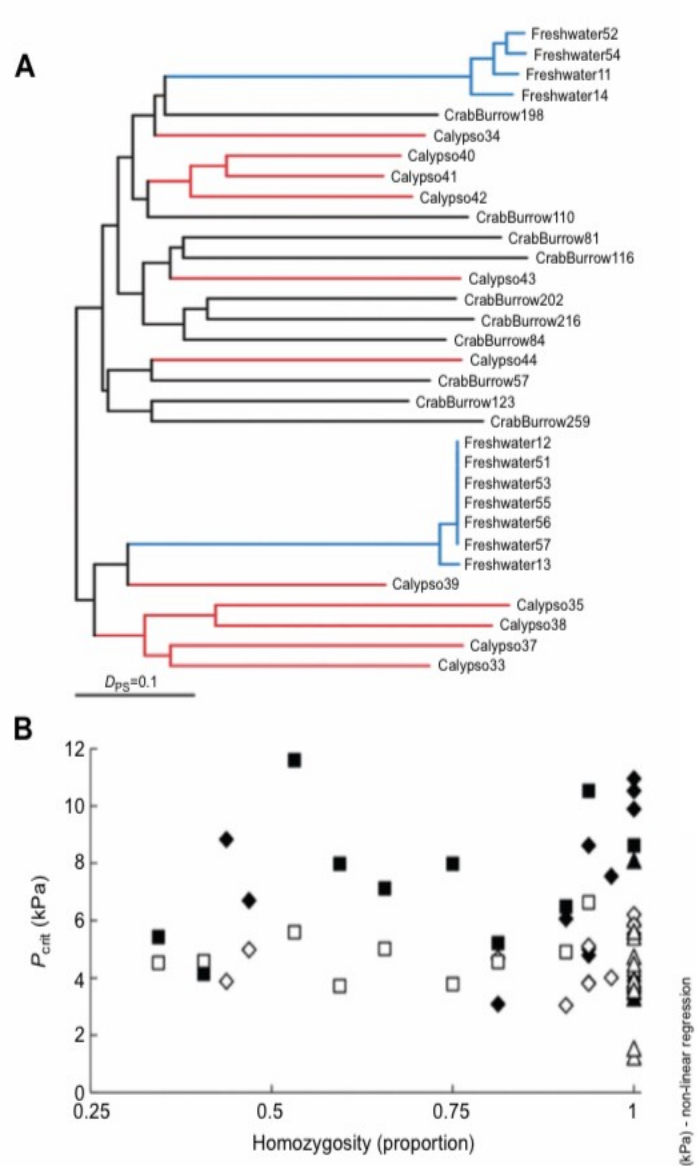

Fig. 4. Genetic relationships between Long Caye *K. marmoratus* and consequences for respiratory function. (A) Neighbour-joining tree of *K. marmoratus* sampled from three sites on Long Caye. Fish captured from crab burrows are labelled in black, fish from the freshwater pool are labelled in blue and fish from the brackish pool Calypso are labelled in red. (B) Heterozygosity did not significantly explain the respiratory function (P_{crit}) calculated using broken-stick regression (black symbols, R^2=0.015, P>0.05) or nonlinear regression (open symbols, R^2=0.001, P>0.05). Square symbols represent fish from crab burrows, triangles represent those from the freshwater pool and diamonds represent those from Calypso.

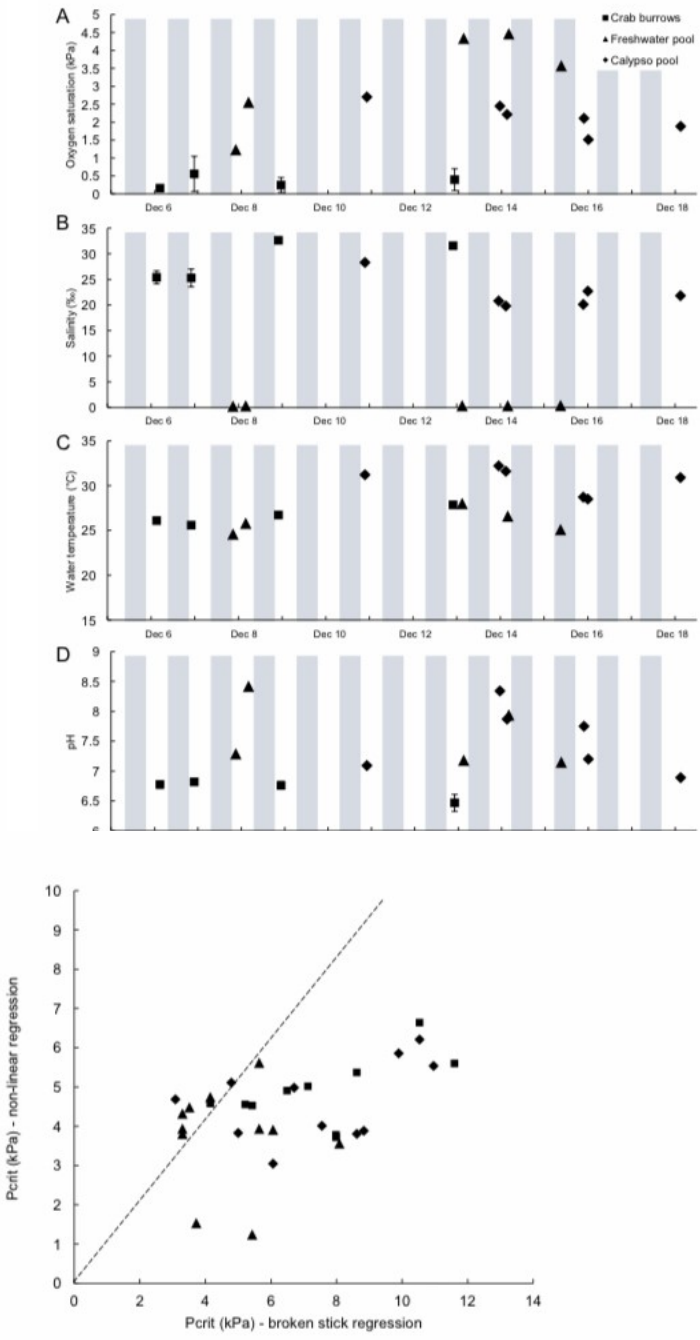

Fig. S3. There was a significant relationship between calculations of P_{crit} using broken stick regression and non-linear regression (R^2= 0.21, P<0.01). Fish from crab burrows are represented with square symbols, from the freshwater pool with triangles, and from Calypso with diamonds. The dashed diagonal line indicates equivalency.

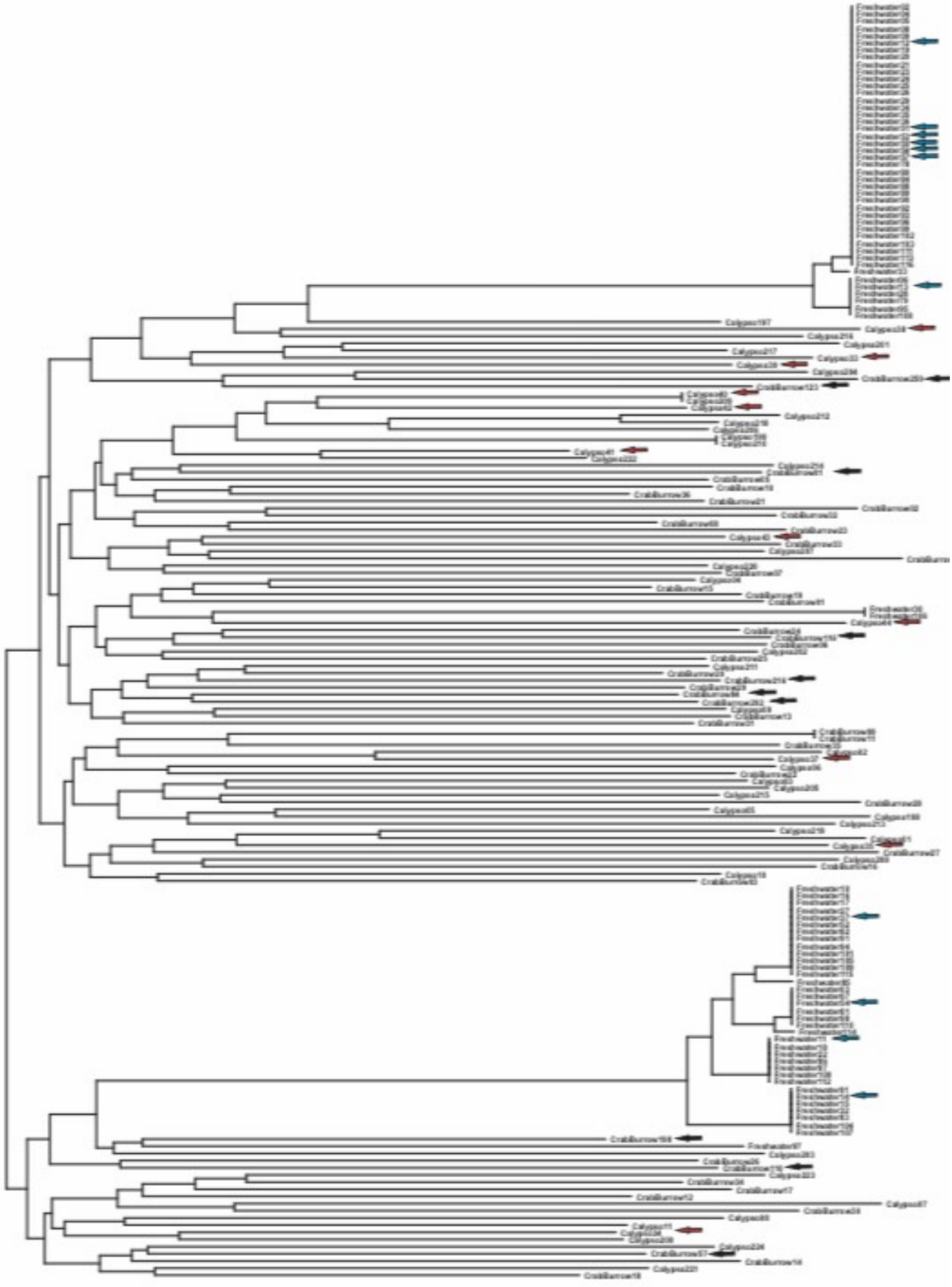

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Fig. S4. Neighbour-joining tree showing microsatellite-based genetic similarity of 178 Kryptolebias marmoratus collected from three sites on Long Caye, Belize. Arrows point fish used in respiration experiments (n=32 individuals); black for crab burrows, blue for freshwater pond, and red for Calypso pond.

Main Body Paragraph

Respiration and critical O2 tension

Aquatic O2 consumption of size-matched fish was measured using closed glass respirometry chambers (~50 ml, 28°C), as described previously (Rodela and Wright, 2006; Turko et al., 2012). Briefly, dissolved O2 (DO) was continually monitored using Clark-type electrodes (Vernier DO-BTA and LabPro, Vernier Software and Technology, Beaverton, OR, USA) connected to a computer running Vernier LoggerPro 3.8 software. Fish were acclimated to the respirometry chambers for 30 min, as preliminary experiments indicated that 30 min was sufficient to allow metabolic rates to stabilize. Chambers were then sealed and O2 consumption was recorded over a 2–4 h period until DO dropped below 2% of saturation. Background O2 consumption was measured in chambers without fish for approximately 1 h immediately after each experiment and this value was subtracted from the O2 consumption values of the fish. Oxygen consumption of freshwater fish (n=11) was measured in well-aerated water obtained from the pond where they were captured. Fish collected from crab burrows (n=10) and the Calypso site (n=11) were tested in relatively clean, well-aerated seawater (34‰). High background rates of O2 consumption in crab

Site

Freshwater pond Crab burrows Calypso pool
GPScoordinates 17°13.24′N, 87°35.53′W
17°13.08′N, 87°35.65′W 17°13.17′N, 87°35.45′W
PO2 (kPa) 3.23±0.61 (1.23–4.47)
0.29±0.13 (0–1.95) 2.14±0.17 (1.5–2.7)
Salinity(‰) 0.33±0.02 (0.24–0.37)
28.1±0.7 (24.0–33.8) 22.3±1.3 (19.8–28.3)
Temperature(°C) 26.0±0.60 (24.6–28.0)
26.4±0.1 (25.6–27.3) 30.5±0.6 (28.5–32.2)
pH 7.60±0.25 (7.15–8.42)
6.77±0.05 (6.44–7.19) 7.52±0.23 (6.89–8.34)
Data are presented as means±s.e.m. of 4–6 measurements per site; ranges are given in parentheses.
2

Table 2. Body size and condition of Kryptolebias marmoratus used for behaviour and respiratory function experiments.

Emersion behavior

Emersion rates were quantified for individual fish by measuring the proportion of time each individual spent stuck to the side of the holding container above the water. Fish collected from either crab burrows or the freshwater pond were placed in translucent 100ml plastic containers) containing 60 ml well-aerated water obtained from the collection site. After a 1h acclimation period, fish were video recorded for an additional 1 h period. Each container was surrounded by white paper on three sides to prevent fish from observing each other, and care was taken not to disturb the fish for the duration of

the acclimation and recording period. The video files were subsequently used to quantify the duration of each emersion event to the nearest minute.

Heterozygosity and genetic relationships

Experimental fish were either killed or lightly anaesthetized) with 500 mg l−1 tricaine methanesulphonate buffered to neutral with sodium bicarbonate, and a small piece of caudal fin was collected using an ethanol-cleaned razorblade. Fin clips were immediately stored in DNA preservative for transport to the University of California, Irvine. Besides genotyping fish used in the respiration experiments, we sampled and genotyped fin clips from additional fish in the same locations. The combined samples were as follows: freshwater pond, crab burrows site, and Calypso pond. These large sample sizes allowed us to evaluate how well experimental fish represented the genetic diversity in our field site. Genetic relatedness and heterozygosity were estimated with 32 nuclear microsatellite loci developed previously for K. marmoratus . DNA preparations, the genotyping protocol and the binning of alleles followed. Individual heterozygosity was calculated by summing the number of heterozygous loci in an individual and dividing the sum by the total number of loci. The number of heterozygous loci for each individual was counted using Microsatellite Analyser v. 4.05. Note that the average of individual heterozygosities corresponds to the observed heterozygosity of a population. Genetic differences between individuals were estimated with the distance metric based on the proportion of shared alleles. Values of DPS can range from zero to one. Calculations of DPS were done using Microsatellite Analyser. The genetic relationships among individuals were summarized with the neighbour-joining trees constructed in PHYLIP software ver. 3.695.

Statistical analysis

We compared Pcrit, RI and emersion behaviour between the crab burrow and freshwater populations of K. marmoratus using Student's t-tests to understand the effect of the environment on these traits. Two-way repeated measures ANOVA and post hoc

Site
Freshwater pond Crab burrows
Standard length (mm)
37.5±1.1 39.1±1.5
Mass (g)
0.83±0.07 0.77±0.10
Condition (Fulton's K)

Burrow and Calypso pond water due to large amounts of organic matter would have prevented accurate measurements of metabolic rate. Immediately after each trial, fish were killed and a fin clip was taken for determination of heterozygosity).

Critical O2 tension was calculated from the O2 consumption curves using two methods: broken-stick regression and nonlinear regression. The traditional BSR approach estimates Pcrit as the intersection of the two linear regression lines that best fit the PO2 versus O2 consumption plot. BSR estimates of Pcrit were calculated using REGRESS, Jeffrey Muday's software , which uses the algorithm described in Yeager and Ultsch. The newer NLR technique was designed to calculate Pcrit in cases when the transition between O2 regulation and O2 conformation is not a clear break-point; instead of using linear regression, this method estimates Pcrit from the best nonlinear function that describes the PO2 versus O2 consumption relationship. Briefly, six separate curves were fitted to a normalized O2 consumption curve for each fish, and the curve with the lowest corrected Akaike's information criterion score was chosen. Pcrit was then calculated from the derivative of the chosen function using the equations in Marshall et al. or using Maple software to solve Weibull functions.

The regulation index is a complementary measure to Pcrit that describes the extent to which an organism regulates O2 uptake at low environmental PO2. The RI is calculated from the same O2 consumption versus PO2 trace used to calculate Pcrit, and represents the relative area under the curve but above the line of equality. Thus, a perfect oxyconformer has an RI value of zero, whereas a hypothetically perfect O2 regulator maintains O2 consumption even in near-anoxic conditions.

Gill morphology

Tissue samples were taken immediately from fish collected from both saltwater crab burrows and the freshwater pond. To determine whether the gill morphology of wild-caught fish was phenotypically plastic, additional fish from each of these habitats were acclimated to a terrestrial environment for 7 or 13 days before being killed. Fish were kept out of water on moist filter paper above a cotton ball reservoir soaked with either seawater or freshwater , as described previously. Whole heads were fixed in 10% buffered formalin for 24 h, decalcified! For 1 h, and then transferred to 70% ethanol for storage and shipment to Guelph, ON, Canada. Tissues were routinely paraffin embedded, sectioned in 5µm increments, and stained with haematoxylin and eosin.

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Holm–Šidák tests were used to compare rates of O2 consumption between habitats and across environmental PO2, and two-way ANOVA was used to compare coverage of the gill lamellae between crab burrow and freshwater pond fish directly from the field and after 7 or 13 days of air exposure.
To determine whether homozygosity influenced respiratory function, we used fish collected from all three sites. We first performed simple linear regression analyses with either BSR or NLR estimates of Pcrit as the dependent variable and individual heterozygosities as the independent variable. To account for any possible influence of phylogenetic autocorrelation on our results, we also analysed the data using the phylogenetic generalized least squares function in the R package caper). These analyses were performed using both NJ and unweighted pair group method with arithmetic mean (UPGMA)

phylogenies that were built using the 32 microsatellite markers with a Brownian model for evolution at λ values (importance of branch lengths) of 0, 1 or the 'maximum likelihood' value calculated by caper. RStudio (https://www.rstudio.com/) was used to perform the pgls analysis, and SigmaPlot 11 (Systat Software, San Jose, CA, USA) was used for all other analyses (critical α=0.05). Throughout the text, values are given as means±s.e.m.

RESULTS

Respiration and gill morphology

In normoxic water, the rate of O2 consumption by mangrove rivulus from the freshwater pond was significantly lower than in fish collected in crab burrows, and this difference persisted at all values of PO2 above 6 kPa (two-way repeated measures ANOVA interaction, $F_{1,18}=7.633$, $P<0.001$; Fig. 1A). Fish from the freshwater pond had significantly lower Pcrit values than those of crab burrow fish, although the magnitude of this effect varied, depending on the technique used to estimate the break-point of the O2 consumption curve. Using the traditional BSR approach, the Pcrit of crab burrow fish was ∼60% higher than that of freshwater pond

fish ($t_{19}=3.220$, $P=0.005$; Fig. 1B), but using NLR calculations the Pcrit of crab burrow fish was only ∼30% higher than that of freshwater pond fish ($t_{19}=2.338$, $P=0.030$; Fig. 1B). Estimates of Pcrit using BSR and NLR were significantly correlated (linear regression, $R^2=0.216$, $F_{1,31}=8.522$, $P=0.006$) but differed by an average of 2.55±0.30 kPa and by as much as 5.98 kPa (Fig. S3). There was no difference in the RI between fish collected at different locations ($t_{19}=1.333$, $P=0.20$; Fig. 1C).

We observed a significant interaction between habitat and acclimation (two-way ANOVA interaction, $F_{2,41}=3.957$, $P=0.027$; Fig. 2) on gill coverage by the ILCM. Fish sampled immediately after capture from crab burrows had significantly more of the interlamellar space covered by an ILCM than fish from the freshwater pond ($t=2.786$, $P=0.008$). After 7 days of air exposure, the ILCM had significantly enlarged in both the crab burrow ($t=2.476$, $P=0.018$) and freshwater pond fish ($t=8.153$, $P<0.001$; Fig. 2C). The ILCM

did not enlarge further after an additional 6 days in air in either group . There was no difference in ILCM coverage between freshwater and crab burrow fish after 7 days or 13 days of terrestrial acclimation. Fish collected from crab burrows spent almost 90% of the recording period out of water, significantly more than freshwater pond fish, which emersed ∼30% of the time.

Heterozygosity and respiratory function

Phylogenies using both UPGMA and NJ methods revealed a similar population structure among mangrove rivulus on Long Caye. NJ trees summarizing genetic similarity among fish are shown in Fig. 4A and Fig. S4 . Both trees illustrate high genetic diversity, evidenced by long branches, although some fish are genetically identical. There were two major lineages of fish present in the freshwater pond. Fish within each of these lineages were highly genetically similar. Experimental fish are randomly and evenly scattered among branches of the full genetic tree. Furthermore, the lack of geographic clustering suggests that the physiological patterns described above are not due to idiosyncrasies of particular genetic lineages, but are the result of the environmental conditions in which fish were collected. However, the high degree of relatedness between some individuals within the freshwater pond site suggests that, when working with self-fertilizing hermaphroditic species, it is possible to take a series of replicate measurements on individuals of the same genotype inadvertently. Thus, caution must be exercised when interpreting results. Heterozygosity did not significantly explain the variation in Pcrit calculated using either BSR or NLR, when tested with simple linear regressions. Phylogenetically corrected statistical models similarly found no relationship between heterozygosity and either measure of Pcrit..

were identical at all 32 loci examined, and another seven fish were distinct at one locus only. There was slightly more genetic diversity in the other freshwater lineage, although they were nonetheless highly similar, being distinct from each other at no more than three of the 32 loci examined. Notably, divergence between the two freshwater lineages) is of the same order as the divergence between any

random pair of fish captured in crab burrows or the Calypso pool. The crab burrow and Calypso fish effectively form a genetic mosaic with almost no indication of geographic pattern. These fish were far less homozygous than freshwater fish.

acclimated to aquatic hypoxia for 7 days in the laboratory had significantly increased gill surface area compared with normoxic controls, but these fish were forcibly submerged for the duration of the acclimation period. In wild mangrove rivulus, however, we observed the opposite pattern – fish collected from nearly anoxic crab burrows had significantly enlarged ILCMscompared with fish from the freshwater pool. Thus, in wild mangrove rivulus, it appears that the relationship between aquatic PO2 and gill morphology is mediated by emersion behaviour, such that aquatic hypoxia ultimately results in mangrove rivulus with gills largely covered by an ILCM.

We found that mangrove rivulus captured from crab burrows spent almost 90% of their time out of water, triple the amount of time spent by fish from the freshwater pond. In the laboratory, gill remodelling in mangrove rivulus is caused both by prolonged acclimation out of water and by frequent voluntary emersions interspersed with periods submerged in water . In two isogenic populations of laboratory fish), we previously quantified individual variation in emersion tendencies and found that some mangrove rivulus never left water, whereas others spent 78% of the week-long recording period out of water. The size of the ILCM in these fish was positively correlated with the amount of time each fish spent out of water, but this relationship disappeared after we prevented fish from emersing for a further week, strongly indicating that emersion behaviour causes enlargement of the ILCM Therefore, we think that relatively high rates of emersion in mangrove rivulus from crab burrows versus those from the freshwater pond caused the differences in gill surface area we measured.

High rates of emersion in the crab burrow fish were probably stimulated by the nearly anoxic conditions in this habitat. In the laboratory, acute exposure to aquatic hypoxia induces emersion and, once the fish are out of water, respiration occurs across the skin and bucco-opercular cavity. High concentrations of

hydrogen sulphide and CO2 have also been shown to induce emersion in mangrove rivulus. Given the stagnant conditions within the crab burrows, it is plausible that these gasses may have further contributed to the high rates of emersion we observed. In addition to our behavioural assay, we remotely video recorded a crab burrow and observed 11 instances of emersion by mangrove rivulus within 30 min . Taylor also reported frequent observations of emersed fish near crab burrows, suggesting that this behaviour is a regular occurrence in the field and not an artefact of the artificial plastic containers used for our behavioural experiments. Anecdotally, fish were never observed emersed at the freshwater pond site when water was present. However, we did not measure emersion behaviour or dissolved O2 in the freshwater pond at night, when algal respiration may have caused extreme hypoxia and possibly triggered emersion behaviour. If this occurred, presumably the fish did not spend enough time out of water to experience ILCM growth.

The functional benefits of ILCM enlargement during air exposure are unclear. One hypothesis is that the ILCM supports the lamellae, and prevents collapse and possible fusion of the epithelial tissue. Support of gill arches and filaments in terrestrially acclimated mangrove rivulus is also provided by collagen deposition.). In contrast, we found that wild K. marmoratus captured from nearly anoxic crab burrows had higher rates of O2 consumption, increased Pcrit and reduced total gill surface area relative to fish in a less hypoxic freshwater pool. The probable explanation for these results is that amphibious K. marmoratus emerse far more frequently in poor water conditions, and air exposure is the dominant determinant of gill remodelling and metabolic status in this species. Finally, we found no evidence that Pcrit was affected by high or complete homozygosity, indicating the absence of inbreeding depression in these environments.

Gill remodelling.

Gill remodelling is one mechanism used by some fully aquatic fishes to increase total gill surface area and improve O2 uptake in response to hypoxic environments but bony or cartilaginous tissues are not

present in lamellae. Proliferation of the ILCM may thus provide an alternative structural mechanism in the absence of buoyant support from water. Alternatively, large ILCMs may reduce evaporative water loss across the gills. Considering that rates of bucco- opercular ventilation are low during air exposure and mangrove rivulus only survive in highly humid terrestrial habitats, it seems improbable that the primary role of the ILCM is water conservation.

Respiratory function

Mangrove rivulus collected from nearly anoxic crab burrows had significantly higher Pcrit and rates of O2 consumption compared with fish collected from the freshwater pond. These differences are opposite to the typical pattern observed in fully aquatic fishes and indicate that amphibious behaviour may shape these aspects of aquatic respiratory function. One possibility is that access to O2-rich air during frequent emersions enables a larger 'metabolic engine' in crab burrow fish which causes the relatively high aquatic metabolic rates we observed. Increased O2 demand in these fish may also result from physiological costs associated with terrestrial acclimation, such as maintaining enlarged cutaneous ionocytes. Crab burrow fish were also in significantly worse body condition than freshwater pond fish, suggesting that differences in body composition or relative organ masses between the populations may also cause the observed difference in whole-animal metabolic rate. Alternatively, the worse body condition of crab burrow fish may reflect smaller energy reserves caused by the higher metabolic rate. Finally, the relatively high metabolic rate of crab burrow fish may have been caused by larger ionoregulatory demands in seawater than in freshwater. However, acclimation to water of different salinities did not change rates of O2 consumption in laboratory-reared mangrove rivulus.

Relatively high rates of normoxic O2 consumption in crab burrow fish occurred despite reduced gill surface area in this population. This is not surprising, as respiratory gas transfer in fish at rest is thought to be perfusion limited and thus does not depend on gill surface area. At rest, only about 60% of the gill lamellae are perfused with blood in rainbow trout, Oncorhynchus mykiss . Furthermore, mangrove

rivulus can maintain routine rates of O_2 consumption with gill ventilatory frequency as low as five opercular movements per minute. Finally, O_2 transfer across the skin may also contribute to total O_2 uptake in mangrove rivulus under aquatic conditions.

In moderately hypoxic water, fishes can maintain O_2 uptake by increasing gill ventilation and perfusing more gill lamellae with blood but, under severe hypoxia, respiratory gas transfer becomes diffusion limited, and large total gill surface area becomes beneficial. The enlarged ILCMs of mangrove rivulus from crab burrows may thus impair O_2 uptake under hypoxic aquatic conditions and increase Pcrit, as has been demonstrated in Carassius carassius and Fundulus heteroclitus. Similarly, enlarged ILCMs in laboratory-reared mangrove rivulus, induced with acclimation to either soft water or terrestrial conditions, were linked to higher Pcrit than in control fish with small ILCMs. In these earlier experiments, there were no differences in normoxic rates of O_2 consumption between treatments. However, in the current study, crab burrow mangrove rivulus had higher normoxic O_2 consumption rates than freshwater pond fish. It is, therefore, possible that the higher values of Pcrit we measured in crab burrow fish simply resulted from higher overall O_2 demand, and the enlarged ILCMs of these fish did not meaningfully impair branchial gas exchange. Consistent with this view, the rate of O_2 consumption in crab burrow fish at any environmental PO_2 was never lower than that in freshwater pond fish, despite the difference in ILCM coverage. If enlarged ILCMs impaired branchial gas exchange, absolute rates of O_2 uptake should have been lower in crab burrow fish under hypoxic conditions, all else being equal. One possibility is that compensatory responses help to maintain O_2 uptake, such as increased haemoglobin concentrations and higher O_2 binding affinity of haemoglobin. Ultimately, both increased O_2 demand and enlarged ILCMs probably contributed to the increased Pcrit of crab burrow mangrove rivulus. This was the case in a phylogenetically controlled study of sculpins, where large gill surface area, low metabolic rate and high haemoglobin affinity for O_2 influenced Pcrit.

Plasticity versus genetic differences

Phenotypically plastic responses to emersion are one probable cause of the differences we observed between fish from crab burrows and those from the freshwater pond, but genetic differences between these sites could also be involved. In support of the plasticity hypothesis, we found that emersion behaviour in wild fish was correlated with ILCM size, and emersion behaviour drove plastic changes in gill morphology as described above . Furthermore, we found that both freshwater pond and crab burrow fish responded to 1 and 2 weeks of forced air exposure by increasing ILCM coverage to the same final magnitude. These data suggest that the two wild populations had similar scopes for plasticity in ILCM size, consistent with the hypothesis that the variation we observed in wild fish is due to plasticity. Finally, the freshwater pond fish comprised two distinct lineages, and emersion behaviour, metabolic rate, Pcrit and gill morphology were similar in the two, as would be expected if phenotypic differences between them were the result of plasticity rather than of genetic divergence. However, it is premature to rule out the hypothesis that genetic differentiation between populations also contributed, as selection could have simply favoured similar phenotypes in each habitat . Genetically based phenotypic differences are known to exist between other populations of K. marmoratus , and cause differences in gill morphology in the closely related F. heteroclitus . Common garden and/or reciprocal transplant experiments would be valuable to disentangle environmental and genetic effects.

Self-fertilization and inbreeding depression. We were surprised to find that there was no relationship between heterozygosity and Pcrit in wild K. marmoratus. As one of only two vertebrate species with a mixed-mating system that results in nearly homozygous or heterozygous mangrove rivulus are an excellent model system for understanding the evolution of sexual reproduction. Typically, heterozygous offspring are assumed to have higher fitness than that of inbred homozygotes . However, guaranteed reproductive success from self-fertilization may outweigh the costs of inbreeding depression, especially when individual animals regularly colonize new habitats.. We found no evidence of inbreeding depression with respect to Pcrit, suggesting that multiple generations of selfing and subsequent

selection may have purged deleterious alleles from mangrove rivulus populations. Our ability to detect an inbreeding effect may have been restricted by limited statistical power, considering the relatively small sample size but, because there was no hint of a relationship between heterozygosity and either measure of Pcrit in our data we do not think that this is the case. Thus, self-fertilization may allow single mangrove rivulus to colonize unoccupied and diverse habitats within the mangrove forest without paying a respiratory penalty.

Respiratory function in hypoxia is determined by complex phenotypic traits spanning the respiratory cascade . One might, therefore, expect that heterozygosity provides a greater diversity of alleles that could, in turn, enhance respiratory function, but this was not the case in our study. Consistent with our results, homozygosity did not impair developmental stability in nine populations of mangrove rivulus. However, more heterozygous K. marmoratus have lower parasite loads, suggesting a cost to inbreeding in some circumstances resistance to parasites is conferred by only a small set of major histocompatibility complex genes . In contrast, the complexity of the respiratory system may protect against inbreeding . For example, mangrove rivulus can modify gill surface area, gill ventilation, blood flow (Cooper et al., 2012), haemoglobin concentration and binding affinity (Turko et al., 2014), and/or O2 delivery (Brunt et al., 2016). Modifications at one or several of these steps of the O transport cascade may be able to compensate for deleterious effects of inbreeding at any of the other levels. Furthermore, even highly inbred fish (as estimated using microsatellites or known from the pedigree of the laboratory lineages are not completely homozygous when the whole genome is considered. For example, showed that genomes of inbred K. marmoratus lineages harbour 0.031–0.055% heterozygous sites, which amounts to tens of thousands of single nucleotide polymorphisms. At least some of this heterozygosity may be physiologically important and preserved by natural selection.

Conclusion

In Conclusion to the experiment about the the mangrove killifish having the adaptation of breathing air and escaping harm due to changes in its body , the answer is a success due to the fact of our hypothesis being true and our facts being evident. If we could go back and re-do the experiment the only thing I would certainly do better would be the time and the time it would take to complete the experiment. Again the results of the physiology of mangrove killifish with the information we have about the mangrove killifish together give us another conclusion of true.

Interesting Fact

1. A**killifish** grows up 2 to 3 inches. Therefore, 1 **killifish** requires 3 gallons of **aquarium** water.

2. **Killifish** are carnivores. Depending on the size of your **fish**, frozen foods such as brine shrimp, newly hatched brine shrimp nauplii, daphnia, mysis shrimp, mosquito larvae and bloodworms are all good choices.
3. Sexually mature as early as 17 days from hatching, female **killifish** can **lay** between 10 and 20 **eggs** a day with a recorded maximum of 100
4. **killifish** fish are not schooling fish and **do** not show any type of schooling behavior.
5. **Killifish** are known for **jumping** from puddle to puddle, which seems necessary, as puddles typically don't last forever. Some survive the leap, for others it's a kamikaze endeavor.

Visuals

Work Cited/References

The following references were given and were used in the making of this paper to ensure that the content of this paper was accurate. The following references came from the research article, ***" Emersion behaviour underlies variation in gill morphology and aquatic respiratory function in the amphibious fish Kryptolebias marmoratus"*** In addition, I would like to thank my school for the platform to conduct the creation of this paper . Next I would like to honor the individuals who could not make the list but still was apart of the experiment process. Also I want to show appreciation to everyone else who gives this reference list it's credited acknowledgment.

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