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Life after the drought: temporal genetic structure of *Paracheirodon axelrodi* Schultz, 1956 (Characiformes: Characidae) in the middle Negro River

PEDRO S. BITTENCOURT[1], BRUCE G. MARSHALL[2], TOMAS HRBEK[1] & IZENI P. FARIAS[1*]

[1]Laboratório de Evolução e Genética Animal (LEGAL), Universidade Federal do Amazonas (UFAM), Av. Gen. Rodrigo Octávio Jordão Ramos, 3000 – Coroado. 69.077-000 Manaus, AM, Brazil.
[2]Norman B. Keevil Institute of Mining Engineering, University of British Columbia, 517-6350 Stores Road, Vancouver, B.C. V6T 1Z4, Canada.

**Corresponding author*: izeni@evoamazon.net

Abstract. The cardinal tetra (*Paracheirodon axelrodi*) is one of the principal species exploited by the ornamental fishery in the middle Negro River, Amazonas State, Brazil. *Paracheirodon axelrodi* has a short life cycle, between 12-16 months, reaching reproductive age in approximately 9 months, and thus is an ideal candidate species to test if severe ecological disturbances, such as droughts, leave a molecular signature. We explored this possibility through the analysis of individuals sampled from a single locality in the Negro River basin during the 2007/flood, 2007/dry, 2009/flood, 2009/dry and 2010/dry seasons. The results showed that allelic frequencies shifted significantly starting with the 2007/drought, and allelic richness diminished to the point that a significant bottleneck effect was detected, which consequently affected AMOVA results, heterozygosity and *Ne*. The dry season of 2007 was influenced by the El Niño phenomenon, which was followed by La Niña. The results suggest that *P. axelrodi* populations suffer the effects of extreme climate phenomena and that population reductions caused by droughts cause changes in genetic diversity across generations. Therefore, analyses of temporal samples can help to assess population trends and guide management strategies for the species.

Keywords: Cardinal tetra, drought, population genetics, Amazon basin.

Resumo: Vida depois da seca: estrutura genética temporal de *Paracheirodon axelrodi* Schultz, 1956 (Characiformes: Characidae) no médio Rio Negro. O cardinal tetra (*Paracheirodon axelrodi*) é uma das principais espécies exploradas pela pescaria ornamental no médio rio Negro, estado do Amazonas, Brasil. *Paracheirodon axelrodi* tem um ciclo de vida curto, entre 12-16 meses, atingindo a idade reprodutiva em aproximadamente 9 meses e, portanto, é uma espécie candidata ideal para testar se graves distúrbios ecológicos, como a seca, deixam uma assinatura molecular. Nós exploramos essa possibilidade através da análise de indivíduos amostrados de uma única localidade na bacia do rio Negro durante as estações de 2007/cheia, 2007/seca, 2009/cheia, 2009/seca e 2010/seca. Os resultados mostraram que as frequências alélicas mudaram significativamente a partir da seca de 2007. A riqueza alélica diminuiu ao ponto de se detectar um efeito significativo de redução populacional, o que consequentemente afetou os resultados de AMOVA, heterozigosidade e *Ne*. A estação 2007/seca foi influenciada pelo fenômeno de El Niño, seguido de La Niña. Os resultados sugerem que as populações de *P. axelrodi* sofrem os efeitos de fenômenos climáticos extremos e que as reduções populacionais causadas por secas causam mudanças na estrutura genética entre

gerações. Portanto, as análises de amostras temporais podem ajudar a avaliar as tendências da população e orientar nas estratégias de manejo para a espécie.

Palavras-chave: Cardinal tetra, seca, genética de populações, bacia Amazônica.

Introduction

The Amazon basin drains an area of nearly 7 million km^2. Its discharge is approximately 175,000 m^3/s, which represents close to 20% of all fresh water that flows into the oceans (Sioli 1984). Within this massive hydric network, the Negro River is the largest tributary of the Amazon in terms of annual discharge. Its basin covers an area of 0.75 million km^2 and extends over 1,700 km from the headwaters in Colombia to its confluence with the Solimões River, at which point the Amazon River is formed. It is a black water river with high concentrations of humic and fulvic acids, low pH (between 3.5-5.5) and low concentrations of dissolved nutrients (Sioli 1967). Seasonal dynamics of the Negro River create large wetlands each year, including areas of seasonally flooded forest, which locally are called *igapós*. During rising water, igapó forest adjacent to the main channel of rivers and streams becomes inundated, allowing fish to access the floodplain (Junk *et al.* 1989). In these large areas of igapó forest, there is an abundance and wide range of food sources and increased availability of aquatic habitat. Many fish species reach 80% of their annual growth during this flood period, facilitated by favorable conditions for reproduction and development of juveniles (Junk & Welcomme 1990).

The cardinal tetra *Paracheirodon axelrodi* Schultz, 1956, is a small species endemic to the tributaries of the middle and upper Negro River in Brazil and the Orinoco River in Colombia and Venezuela (Harris & Petry 2001). Each year millions of individuals of *P. axelrodi* are shipped to other parts of Brazil and exported to many countries around the world. For example, in 2007, 17.8 million of *P. axelrodi* individuals were exported internationally (IBAMA 2008). Furthermore, *P. axelrodi* represents between 76% and 89% of total annual shipments of ornamental fishes from the Amazon basin. In the middle Negro River, this extractivist activity is essential for job creation and sustenance for approximately 1,000 families (Prang 2008).

Paracheirodon axelrodi has a short life cycle of between 12 to 16 months and inhabits shallow, shaded and low current areas (Geisler & Annibal 1986). Different studies have shown that the reproductive cycle of this species is directly related to the flood cycle, whereby during rising water the individuals move laterally into areas of flooded forest in search of food and shelter and for reproduction (Geisler & Annibal 1986, Prang 2002, Marshall *et al.* 2008). Some *P. axelrodi* populations also migrate up to interfluvial swamps at the headwaters of small streams in the middle Negro River (Harris & Petry 2001, Prang 2002), where due to continuous flooding of some of these swamps caused by local precipitation, some fish spend their entire life cycle there (Marshall 2010, Marshall *et al.* 2011). Subsequently, during the falling water season, many individuals move out of the swamps and areas of flooded forest back down to the lower reaches of the streams, where a high density of organisms results in intra- and interspecific competition for space and limited food resources (Geisler & Annibal 1986, Marshall 2010).

Considering the short life cycle of *P. axelrodi* and its dependence on the annual hydrological cycle for survival, populations may be strongly influenced by environmental stochastic events that indirectly affect their measured genetic diversity, including allele frequencies and effective population size.

D'Assunção (2006) suggested that climatic change may end up imprinting an embedded genetic signature within *P. axelrodi* individuals. Although her study did not test this hypothesis explicitly, the author showed that *P. axelrodi* populations became re-structured following an El Niño event, followed by a decrease in population structuring during excessive flooding caused by La Niña. Typically, La Niña is characterized by higher than normal precipitation in the Amazonian region, while El Niño causes a prolonged drought, the latter of which leads to extensive mortality. Therefore, it is hypothesized here that these climactic events may cause differentiation of surviving populations due to genetic bottleneck-induced drift.

For the years sampled in this study, Climate Prediction Center records at NOAA (National Oceanic and Atmospheric Administration) reported a moderate El Niño for 2007 and a moderate La Niña for 2010. Thus, the aim of this study is to test the hypothesis that environmental stochastic phenomena such as El Niño and La Niña can cause changes in population genetic parameters of *P. axelrodi* over a short period of time.

Given the predominance of *P. axelrodi* in the ornamental fishery of the middle Negro River its

importance in providing economic livelihood for local peoples, characterization of this species' genetic diversity, distribution and seasonal variation could potentially provide relevant information to support fisheries management plans, as well as conservation of aquatic habitats critical for the life cycles of different ornamental fish species in the region.

Characterization of genetic diversity was performed using microsatellite markers developed specifically for *P. axelrodi* by Beheregaray *et al.* (2004). Microsatellite markers are highly appropriate, due to biparental, codominant and highly polymorphic properties, as well as the ability to detect population structure and demographic changes through time (Goldstein & Schlötterer 1999). Additionally, the molecular evolution of microsatellite markers is well known and many programs are available for analytical procedures.

Material and Methods

Study area and data sampling: All 143 *P. axelrodi* individuals analyzed in this study were collected from interfluvial swamps belonging to the watershed of the Tidaia stream in the middle Negro River (Figure 1), during dry and flood periods in 2007 (flood n = 13, dry n = 10), 2009 (flood n = 22, dry n = 12,) and 2010 (dry n = 86). The animals were collected with small dipnets, preserved and stored in 95% ethanol and deposited in the CTGA Animal Tissue Collection at the Federal University of Amazonas), using the following code numbers: 2007 flood (CTGA_L16609_1 – CTGA_L16609_13), 2007 dry (CTGA_L16610_1 – CTGA_L16610_10), 2009 flood (CTGA_L16611_1 – CTGA_L16611_22), 2009 dry (CTGA_L16612_1 – CTGA_L16612_12), 2010 dry (CTGA_L16613_1 – CTGA_L16613_86).

Total genomic DNA was extracted using the phenol/chloroform protocol as described by Sambrook *et al.* (1989). For amplification of microsatellite loci, five pairs of primers were used, including Pa4, Pa7, Pa13, Pa27 and Pa33 (Beheregaray *et al.* 2004), following the method described by Schuelke (2000). Polymerase chain reaction (PCR) was conducted using a final volume of 10 µL, being comprised of: 3.7µL H_2O, 1.0 µL dNTPs (10 mM), 1.0 µL 10x PCR buffer (750 mM Tris-HCI pH 8.8 a 25°C, 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) of Tween 20), 1µL $MgCl_2$ (25 mM), 1.0 µL

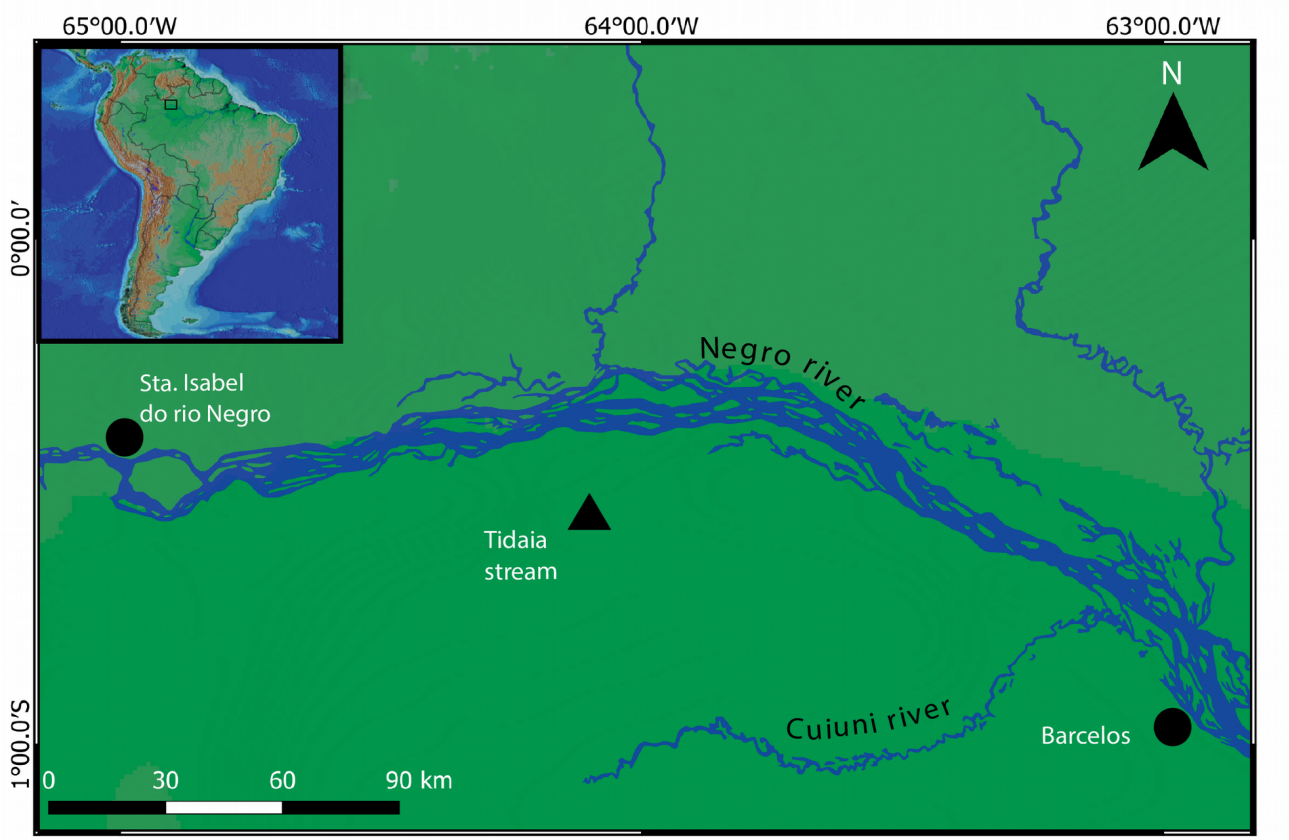

Figure 1. Location of *P. axelrodi* specimens sampled in the interfluvial region of Tidaia Stream (black triangle) in the middle Negro River. Black dots represent the cities of Barcelos and Santa Isabel do Rio Negro.

reverse primer (2 µM), 0.5 µL forward primer (2 µM), 0.5 µL M13 primer (2 µM), 0.3 µL Taq DNA Polymerase (1 U/µL), and 1.0 µL of total DNA. The genotyping reactions, containing a mix of ROX fluorophore and formamide HI-DI™, were injected into the automatic sequencer ABI 3130XL (Applied Biosystems®), according to the manufacturer's protocol. The presence of alleles, their size and genotyping quality were checked in each individual using the Gene Mapper 4.0 software (Applied Biosystems®), and allele size were exported for subsequent analyses.

Data analyses: Population genetic analyses were performed using the program Arlequin 3.5 (Excoffier & Lischer 2010). The genetic variability parameters obtained included: the number of alleles per locus; heterozygosity (observed and expected); and genetic diversity estimated for each population group. As these estimates are affected by sample size (Leberg 2002), a rarefaction analysis was implemented in the program HP-Rare (Kalinowski, 2005). This procedure removes the effect of unequal sample size and allows for unbiased comparison of the number of allelic and alleles richness among samples. In comparison, heterozygosity estimates are less influenced by sample size (Nei & Roychoudhury 1974). The effective population size (Ne) was estimated by using the molecular co-ancestry method in the program NeEstimator v2 (Do *et al.* 2014). In order to verify whether there was a significant reduction in effective population size, the program BOTTLENECK (Piry *et al.*1999) was used, which identifies populations that have suffered a recent reduction in effective population size (*Ne*) or bottleneck. The analyses were implemented using three different mutation models: the stepwise mutation model, SMM (Ohta & Kimura 1973); the two-phase model, TPM (Di Rienzo *et al.* 1994); and the infinite alleles model, IAM (Estoup *et al.* 1995). Subsequently, the Wilcoxon and standardized differences test were applied for each one of the models.

Population structure was tested using an Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992), which was used to estimate differentiation of sampled groups among seasons and years. In addition, the number of population groups was estimated by applying Bayesian analysis using the program STRUCTURE 2.3.3 (Pritchard *et al.* 2000). The program assumes that a biological population is a group of individuals where observed genotypic frequencies deviate minimally from expected genotypic frequencies, and where linkage disequilibrium within a population is minimized, while between populations is maximized (the 'correlated-allelic-frequencies' model). A total of 20 independent runs were performed for each predetermined number of biological groups (K = 1 to 6), with each run consisting of 1,000,000 MCMC after having discarded the first 100,000 chains as burn-in. The 'admixture' and 'correlated-allelic-frequency' models were used with a location prior, whereby it is assumed that individuals sampled in the same locality are likely to belong to the same biological population, while the admixture model allows individuals to have multiple ancestries. These results were processed using the STRUCTURE HARVESTER script (Earl & VonHoldt 2012), whereby the most likely number of biological groups was determined by the method developed by Evanno *et al.* (2005). The 20 independent runs were summarized in the program CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and the results were visualized in the program DISTRUCT 1.1 (Rosenberg 2004).

In order to verify changes in heterozygosity parameters over time, the proportion of heterozygous loci (*PHt*) in each individual was obtained using the following formula: *PHt* = number of heterozygous loci/number of genotyped loci. The mean heterozygosity was then calculated for each sample. The Analysis of Molecular Variance (ANOVA), Levene's Test and Tukey's HSD test were used to verify the statistical differences in the means and homogeneity of variances among groups. All analyses were conducted using the R software (R Development Core Team, 2008).

Considering that a large number of samples were collected in the dry season of 2010, the effect of this difference was tested by randomly reducing the sample number to n = 21 for all analyses. For comparison purposes, the 2010/dry sample was then renamed 2010/dryR.

Results

The 143 samples were genotyped for all five loci and subdivided into six groups, according to the year of collection: 2007/flood; 2007/dry; 2009/flood; 2009/dry; 2010/dry, and 2010/dryR. All population genetic parameters can be visualized in Table I. The genetic diversity and observed heterozygosity (H_O) diminished between 33% to 48% after 2007/flood and the following years. These parameters of genetic diversity are measured mainly by alleles present in the population and their frequencies (Templeton, 2006). The removal of individuals due to stochastic

Table I. The main populational genetic parameters of *P. axelrodi* collected in different seasons and years. Statistically significant probabilities are in bold. Note: n= sample size; NA = Average number of Alleles; ARA = Alleles Richness over loci, PAR = Private Allelic richness over loci; Ne = Effective population size, inf = infinity; p_stdv_SMM = p value of Bottleneck Analysis using the method of Standardized Differences Test with the SMM model of evolution.

Sampling	N	NA	A_{RA}	P_{AR}	*Ne* (95% CIs)	p_stdv_SMM	Gene Diversity	Ho	He	F_{IS}
2007/flood	13	3.6	3.07	0.36	inf (290.8–inf)	0.1315	0.50 ± 0.32	0.71	0.52	-0.4136
2007/dry	10	3.0	2.87	0.05	11.0 (1.0–inf)	0.4284	0.46 ± 0.31	0.48	0.50	-0.0206
2009/flood	22	4.0	3.15	0.32	3.1 (1.5–inf)	**0.0174**	0.44 ± 0.28	0.45	0.47	0.0093
2009/dry	12	3.6	3.24	0.43	3.2 (1.2–inf)	**0.0457**	0.43 ± 0.27	0.37	0.46	0.1448
2010/dry	86	5.6	3.24	0.42	inf (inf–inf)	**<0.0001**	0.48 ± 0.29	0.44	0.50	0.0947*
2010/dryR	21	5.6	3.15	0.40	492.0 (8.2–inf)	**0.0006**	0.46 ± 0.29	0.44	0.46	0.0416

events can lead to the disappearance of many alleles, thus causing changes in allelic frequencies, which may ultimately induce modifications in *Ne*, heterozygosity, and bottle bottleneck events. Indeed, standardized differences tests suggested a bottleneck signal under the SMM model for the seasons after 2007. Population size (*Ne*) was another parameter that diminished between 2007 and 2009, and then increased again in 2010 (Table I).

Values of observed (H_O) and expected (H_E) heterozygosity per locus per sample of *P. axelrodi* in different years and seasons under Hardy-Weinberg equilibrium (HWE) expectations can be visualized in Supplementary Table I (Table S-1**)**. As the defined groups had unequal sample sizes, the Levene's test was used to verify whether there were significant deviations from the assumption of homogeneity of variances (p = 0.3477, α = 0.05) for the values of observed heterozygosity. The ANOVA results showed that means of different groups were significantly different (p = 0.00286, α = 0.01). A *post hoc* Tukey HSD test was applied to detect which pairs of groups were significantly different from each other. All comparisons involving the 2007/flood group were statistically significant (Table II), except for the pair 2007dry-2007flood (p = 0.079). Reducing the samples of the 2010/dry group (from 86 to 21) did not change the overall results of the observed heterozygosity parameters (Figure 2).

Similar results were obtained for the AMOVA analysis, which showed that samples from the 2007/flood were significantly different from all of the other sampled seasons (F_{ST} = 0.047, p < 0.001). The global Analysis of Molecular Variance (AMOVA) indicated significant population structure (F_{ST} = 0.049, p < 0.001), where the greatest variation was observed within the different populations (93.93%) rather than among the populations (2.54%), or among populations within groups (2.43%). All pairwise comparisons of F_{ST} values involving the 2007/flood were significantly different (Table III). In contrast to the structure observed in the F_{ST}-type analyses, the STRUCTURE program analyses identified only one biological group (ln Pr (X|K = 1) = -1423.4400. The discordance between these two analyses are due to the fact that F_{ST}-type analyses are based on variance in allelic frequencies between/among groups, while STRUCTURE tests for the shared system of mating among individuals of the same group. Although the results clearly show a significant difference in variance in allelic frequencies of the different temporal samples, individuals comprising these samples belong to only one biological group.

Discussion

Extreme environmental events are expected to leave signatures on the genetic diversity of species, and these effects have been observed in few recent studies of North America flora (Welt *et al.* 2015) and fauna (Vandergast *et al.* 2016). The present study is the first to demonstrate that extreme environmental events also effect Amazonian fauna, and that they can be detected using standard molecular data and analyses. In our study we were able to detect an

Table II. Tukey multiple comparisons of means (95% confidence level) of the observed heterozygosity parameter over time. Significant p-values are in bold.

Pairs of Comparisons	diff	lower	upper	p adj
2007/dry-2007/flood	-0.232820546	-0.4819379	0.01629682	0.0790762
2009/flood-2007/flood	-0.234790210	-0.4419773	-0.02760308	**0.0177641**
2009/dry-2007/flood	-0.304487179	-0.5415806	-0.06739378	**0.0047242**
2010/dry-2007/flood	-0.247316637	-0.4235583	-0.07107502	**0.0015003**
2009/flood-2007/dry	-0.001969664	-0.2278484	0.22390911	0.9999999
2009/dry-2007/dry	-0.071666633	-0.3252568	0.18192358	0.9356745
2010/dry-2007/dry	-0.014496091	-0.2123744	0.18338220	0.9996211
2009/dry-2009/flood	-0.069696970	-0.2822412	0.14284723	0.8940214
2010/dry-2009/flood	-0.012526427	-0.1540286	0.12897574	0.9991992
2010/dry-2009/dry	0.057170543	-0.1253387	0.23967982	0.9088555

effect of an extreme drought on the genetic diversity of a small aquatic vertebrate. Our results indicated that allelic frequency spectrum shifted significantly starting with the 2007/drought, and allelic richness diminished to the point that a significant bottleneck effect was detected (Table I). The question, therefore, becomes: how do these extreme environmental events leave a molecular signature in *P. axelrodi* populations?

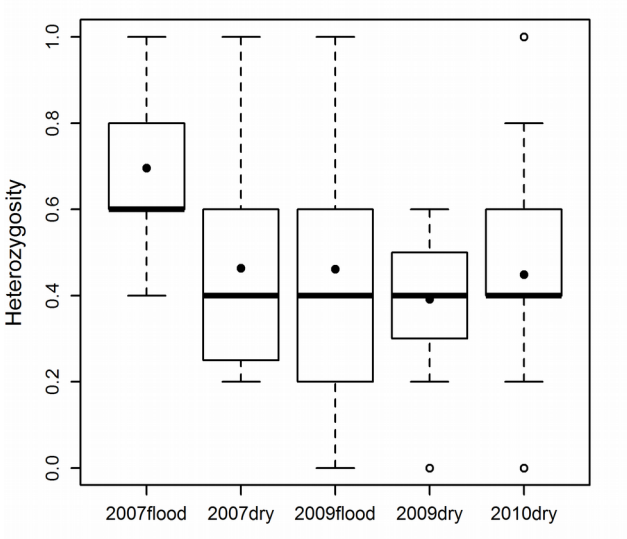

Figure 2. Distribution of individual heterozygosity in *P. axelrodi* over time. Black dots are the mean values.

As mentioned previously, the life cycle of *P. axelrodi* is directly related to the flood cycle, whereby during the rising water and peak flood periods, the fish access the flooded forest and interfluvial swamps to reproduce, find refuge from predators and take advantage of a greater array of food resources. In comparison, during the dry season, populations of *P. axelrodi* are confined to shallow streams, with individuals competing for fewer food resources.

The 2007/dry season was characterized by a period of drought caused by an El Niño (http://enos.cptec.inpe.br/), while 2009-2010 brought one of the greatest floods ever recorded and caused by La Niña, where the water level of the Negro River rose to a historic high of 29.77 meters (CPRM 2009). As seen in this study, values of many population genetic parameters inferred for *P. axelrodi* changed drastically after the 2007 drought. Previous ecological and biological studies of *P. axelrodi* populations suggest that this species suffers indirectly from the seasonal fluctuations caused by El Niño/La Niña. Prang (2001, 2002) reported that the extreme El Niño-induced drought of 1997-1998 affected reproduction cycles of *P. axelrodi* through a lack of food resources, thereby compromising population stability of this species. Most likely, extreme droughts caused the fatality of many *P. axelrodi* individuals, in turn resulting in drastic reductions of population sizes. Similarly, Chao (2001, p 171) reported that there was a decrease in the number of individuals collected during the 1997-1998 El Niño, again likely resulting from increased mortality.

Other studies globally have reported population crashes via ecological disturbance such as wildland fires, floods, and droughts (Banks *et al.* 2013, Davies *et al*. 2016). It is already established that population crashes cause genetic bottlenecks,

which often result in drastic changes in allelic frequencies (Templeton 2006). Allelic frequency shifts might be detectable in neutral genetic markers

Table III. Pairwise Fst (bellow) x Nm (above) for cardinal sampled in different years.

Sampling	2007/flood	2007/dry	2009/flood	2009/dry	2010/dry	2010/dryR
2007/flood	0	6.60	3.60	2.89	2.78	2.88
2007/dry	0.070**	0	inf	inf	inf	inf
2009/flood	0.121**	-0.0265	0	inf	76.88	310.51
2009/dry	0.147**	-0.0180	-0.0133	0	inf	inf
2010/dry	0.152**	-0.0150	0.0064	-0.0014	0	-
2010/dryR	0.148**	-0.0122	0.0016	-0.0046	-	0

Note: **=significant at 0.005; inf= infinity.

such as microsatellite markers, when stressful conditions end up causing a population decline (Hoffmann & Willi, 2008). Although there are only a few published studies to date, drought-induced population crashes have been studied in lizards (Vandergast *et al.* 2016) and plants (Welt *et al.* 2015), whereby the authors in both cases were able to detect strong genetic signatures in the studied species. From the current results, it appears that the El Niño drought of 2007 caused variations in the genetic parameters of *P. axelrodi* populations in the middle Negro River. In comparison with the 2007 sample (the El Niño sample), the genetic signatures of samples from other years showed signs of genetic disturbance. However, due to *P. axelrodi* having a short life cycle with high fecundity, population numbers can rebound very rapidly. Additionally, the recovery of *P. axelrodi* populations in the study area may also be due to significant contributions from individuals from other nearby locations through recolonization, refuges or in situ survival. Indeed, D'Assunção (2006), investigating *P. axelrodi* populations collected over various years, including samples collected before and after the El Niño of 1997-98, observed that the large and significant differentiation among localities observed during the El Niño event diminished in each subsequent year, due to geneflow among localities.

It appears that *P. axelrodi* may be "pre-adapted" to life in a stochastic and unpredictable environment by possessing an explosive breeding strategy, which allows its populations to recover quickly from ecological disturbances. However, other species with lower fecundity, especially apex species or those with K reproductive strategies, may have much greater difficulty rebounding from severe events and will therefore suffer disproportionately the effects of climate change. Ultimately, understanding how different aquatic organisms respond to large climatic oscillations that lead to periods of drought and flooding will be important in guiding regional management and conservation strategies, especially for exploited species of great economic importance.

Conservation lessons from an exploited species: The individuals of *P. axelrodi* collected in this study were sampled in a remote region that is almost never subject to ornamental fishing activity. Furthermore, as the area was not visited by commercial fishermen during the course of this study, the observed effects cannot be explained by anthropogenic activities. However, this does not signify that the removal of millions of individuals every year does not cause any effect. Typically, fish are collected predominantly during the dry season, when they are concentrated in small streams and are easy to capture. Removal of adult fish specimens during normal climatic conditions largely represents a culling of excess individuals, which would normally not survive until the next reproductive season. Indeed, this is typical of all *r* life-history strategist species (Stearns 1977). However, the removal of millions of individuals for export during a drought, such as that caused by El Niño in the Amazon, ends up amplifying the impact of the large-scale die-off provoked by the drought.

Although *P. axelrodi* has the biological capability to rebound from both intense fishing and environmental stochasticity, the present study shows that population disturbances could alter population genetic parameters relatively quickly in this species. As environmental variance increases, extreme

drought events are likely to occur at greater frequency, which together with intensive extraction of millions of individuals could end up inducing genetic bottlenecks that over time may compromise the long-term viability of this species, and other species of the Negro River basin with similar population dynamics.

In conclusion, the results obtained in this study should serve as an indication that monitoring of *P. axelrodi* populations in the middle Negro River is greatly needed to improve the information base regarding population dynamics of the species. Consequently, monitoring will be able to better track effective population sizes (*Ne*) and levels of genetic diversity, so that any negative impacts can be adequately mitigated, ensuring the long-term sustainability of an important commercial Amazonian fish species.

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References

Banks, S. C., G. J. Cary, A. L. Smith, I. D. Davies, D. A. Driscoll, A. M. Gill, D. B. Lindenmayer & Peakall, R. 2013. How does ecological disturbance influence genetic diversity? **Trends in Ecology & Evolution**, 28(11): 670-679.

Beheregaray, L. B., L. M. Möller, T. S. Schwartz, N. L. Chao & Caccone, A. 2004. Microsatellite markers for the cardinal tetra *Paracheirodon axelrodi*, a commercially important fish from central Amazonia. **Molecular Ecology Notes**, 4: 330-332.

Chao, N. L., P. Petry, G. Prang, L. Sonneschien & Tlusty, M. 2001. **Conservation and Management of Ornamental Fish Resources of the Rio Negro Basin, Amazonia, Brazil - Project Piaba**. Editora da Universidade do Amazonas, Manaus, Brazil, 303p.

CPRM. 2009. Relatório da Cheia Manaus 2009. **Companhia de pesquisa de recursos minerais**. Available from: http://www.cprm.gov.br/rehi/manaus/pdf/rel_final_2009.pdf (Date of access – 05 August 2016).

D'Assunção, A. A. A. 2006. Estudo da variabilidade genética do cardinal (Ostariophysi: Characiformes: *Paracheirodon axelrodi*) na bacia do rio Negro. **Master Thesis**, Programa de Pos-graduação em Genética, Conservação e Biologia Evolutiva, Instituto Nacional de Pesquisas da Amazônia (INPA) and Universidade Federal do Amazonas (UFAM), Manaus, AM, Brazil, 67 p.

Davies, I. D., G. J. Cary, E. L. Landguth, D. B. Lindenmayer & Banks, S. C. 2016. Implications of recurrent disturbance for genetic diversity. **Ecology and Evolution**, 6: 1181-1196.

Di Rienzo, A., Peterson, A. C., Garza, J. C., Valdes, A. M., Slatkin, M., Freimer, N. B. 1994. Mutational processes of simple-sequence repeat loci in human populations. **Proceedings of the National Academy of Sciences of the United States of America**. 91:3166–3170.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett & Ovenden, J. R. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. **Molecular Ecology Resources**, 14: 209-214.

Earl, D. A., & VonHoldt, B. M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. **Conservation Genetics Resources**, 4: 359-361.

Estoup, A., Tailliez, C., Cornuet, J.-M. & Solignac, M. 1995. Size homoplasy and mutational processes of interrupted microsatellite in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). **Molecular Biology and Evolution** 12:1074-1084.

Evanno, G., S. Regnaut, & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. **Molecular Ecology**, 14: 2611-2620.

Excoffier, L. & Lischer, H. E. L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. **Molecular Ecology Resources**, 10: 564-567.

Excoffier, L., P. E. Smouse & Quattro J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. **Genetics**, 131: 479-491.

Geisler, R. & Annibal, S. R. 1986. Ecology of the cardinal-tetra *Paracheirodon axelrodi* (Pisces, Characoidea) in the river basin of the rio Negro/ Brazil as well as breeding-related factors. In: Bittner, A. (Ed.). **Animal research and development. A biannual collection of recent German contributions concerning development through animal research**, 23: 7-39.

Goldstein, D. B. & Schlötterer. C. 1999. **Microsatellites: Evolution and Applications**. Oxford University Press, New York, NY, 343p.

Harris, P. M. & Petry, P. 2001. Preliminary report on the genetic population structure and phylogeography of cardinal tetra (*Paracheirodon axelrodi)* in the rio Negro Basin. Pp. 205-225. In: Chao, N. L., P. Petry, G. Prang, L. Sonneschien & M. Tlusty (Eds.). **Conservation and Management of Ornamental Fish Resources of the Rio Negro Basin, Amazonia, Brazil - Project Piaba**. Editora da Universidade do Amazonas, Manaus, Brazil, 303 p.

Hoffmann, A. A. & Willi, Y. 2008. Detecting genetic responses to environmental change. **Nature Review Genetics** 9: 421–432

IBAMA. 2008. **Diagnóstico geral das práticas de controle ligadas a exploração, captura, comercialização exportação e uso de peixes para fins ornamentais e de aquariofilia**. Brasília. Available from: http://www.ibama.gov.br/phocadownload/recursos_pesqueiros/diagnostico_completo.pdf (Date of Access – 15 July 2016).

Jakobsson, M. & Rosenberg, N. A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. **Bioinformatics**, 23: 1801-1806.

Junk, W. J., Bayley, P. B. & Sparks, R. 1989. The flood pulse concept in river-floodplain systems. **Canadian Special Publication of Fisheries and Aquatic Sciences**, 106: 110-127.

Junk, W. J. & Welcomme, R. L. 1990. Floodplains. In: Patten B. C. (Ed.). **Wetlands and Shallow Continental Water Bodies: Case studies**. Pp. 491–524. The Hague, The Netherlands, SPB Academic Publishing.

Kalinowski, S. T. 2005. Hp-Rare 1.0: a computer program for performing rarefaction on measures of allelic richness. **Molecular Ecology Notes**, 5: 187-189.

Leberg, P. L. 2002. Estimating allelic richness: Effects of sample size and bottlenecks. **Molecular Ecology**, 11: 2445-2449.

Marshall, B. G., Forsberg, B. R. & Thomé-Souza, M. J. F. 2008. Autotrophic energy sources for *Paracheirodon axelrodi* (Osteichthyes, Characidae) in the middle Negro River, Central Amazon, Brazil. **Hydrobiologia**, 596: 95-103.

Marshall, B. G. 2010. Fatores que influenciam a variação espacial e temporal nas fontes autotróficas de energia e nível trófico do *Paracheirodon axelrodi* (Osteichthyes, Characidae) num sistema interfluvial do médio rio Negro. **PhD. Thesis**, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM, Brazil, 170 p.

Marshall, B. G., Forsberg, B. R., Hess, L. L. & Freitas, C. E. 2011. Water temperature differences in interfluvial palm swamp habitats of *Paracheirodon axelrodi* and *P. simulans* (Osteichthyes: Characidae) in the middle Rio Negro, Brazil. **Ichthyological Exploration of Freshwaters**, 22: 377-383.

Nei, M. & Roychoudhury, A. K. 1974. Sampling variances of heterozygosity and genetic distance. **Genetics**, 76: 379-390.

Ohta, T. & Kimura, M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. **Genetics Research**. 22:201–204.

Piry, S., G. Luikart & Cornuet, J. 1999. BOTTLENECK: a computer program for detecting recent reduction in the effective size using allele frequency data. **Journal of Heredity**, 90: 502-503.

Prang, G. 2001. Ornamental fish and the relations of production in the extractive economy of the rio Negro, Brazil: implications for sustainable resource use. Pp. 43-73. In: Chao, N. L., P. Petry, G. Prang, L. Sonneschien & M. Tlusty (Eds.). **Conservation and Management of Ornamental Fish Resources of the Rio Negro Basin, Amazonia, Brazil - Project Piaba**. Editora da Universidade do Amazonas, Manaus, Brazil, 303p.

Prang, G. 2002. A caboclo society in the middle rio Negro Basin: ecology, economy, and historyof an ornamental fishery in the State of Amazonas, Brazil. **PhD. Thesis**, Wayne State University, Detroit, Michigan, 270 p.

Prang, G. 2008. An industry analysis of the freshwater ornamental fishery with particular reference to the supply of Brazilian freshwater ornamentals to the UK market. **Uakari**, 3: 7-51.

Pritchard, J. K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. **Genetics**, 155: 945-959.

R Development Core Team. 2008. **R: A language and environment for statistical computing**. R Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R-project.org.

Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. **Molecular Ecology Notes**, 4: 137-138.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. **Nature Biotechnology**, 18: 233-234.

Sioli, H. 1967. Studies in Amazonian waters. Atas do simpósio sobre a biota amazônica. **Limnologia**, p. 39-50.

Sioli, H. 1984. The Amazon and its main affluents: hydrography, morphology of the river courses and river types. Pp. 127-165. In: Sioli, H. (Ed.). **The Amazon. Limnology and Landscape Ecology of a Mighty Tropical River and its Basin**. Springer Verlag, New York, NY, 800p.

Stearns, S. C. 1977. The evolution of life history traits: A critique of the theory and a review of the data. **Annual Review of Ecology and Systematics**, 8: 145–171.

Templeton, A. **Population Genetics and Microevolutionary Theory**. John Wiley & Sons. New York, NY, 720p.

Vandergast, A. G., Wood, D. A., Thompson, A. R., Fisher, M., Barrows, C. W. & Grant, T. J. 2016. Drifting to oblivion? Rapid genetic differentiation in an endangered lizard following habitat fragmentation and drought. **Diversity and Distributions**, 22: 344-357.

Welt, R. S., A. Litt & Franks, S. J. 2015. Analysis of population genetic structure and gene flow in an annual plant before and after a rapid evolutionary response to drought. **AoB Plants**, 7: plv026

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Life after the drought: temporal genetic structure of *Paracheirodon axelrodi* Schultz, 1956 (Characiformes: Characidae) in the middle Negro River

PEDRO S. BITTENCOURT[1], BRUCE G. MARSHALL[2], TOMAS HRBEK[1] & IZENI P. FARIAS[1*]

[1]Laboratório de Evolução e Genética Animal (LEGAL), Universidade Federal do Amazonas (UFAM), Av. Gen. Rodrigo Octávio Jordão Ramos, 3000 – Coroado. 69.077-000 Manaus, AM, Brazil.
[2]Norman B. Keevil Institute of Mining Engineering, University of British Columbia, 517-6350 Stores Road, Vancouver, B.C. V6T 1Z4, Canada.

*Corresponding author: izeni@evoamazon.net

Supplementary Material

Table S-I. Observed (H_O) and expected (H_E) heterozygosity per locus per sampling of *P. axelrodi* in different years and seasons under Hardy-Weinberg equilibrium (HWE) expectations.

2009/dry	**Pa4**	**Pa7**	**Pa13**	**Pa27**	**Pa33**
Na	2	4	3	3	6
H_O	1.000	0.538	1.000	0.154	0.846
H_E	0.520	0.495	0.567	0.283	0.732
p	**0.0007**	0.1862	**0.0035**	0.080	1.000
2007/dry					
Na	3	2	3	2	5
H_O	0.333	0.400	0.571	0.300	0.800
H_E	0.307	0.442	0.626	0.394	0.773
p	1.000	1.000	0.5180	0.4803	1.000
2009/flood					
Na	3	4	5	2	6
H_O	0.263	0.500	0.350	0.410	0.727
H_E	0.243	0.481	0.515	0.333	0.782
p	1.000	0.8608	0.0675	0.5383	0.2700
2009/dry					
Na	2	4	4	2	6

2009/dry	**Pa4**	**Pa7**	**Pa13**	**Pa27**	**Pa33**
H_O	0.272	0.583	0.091	0.091	0.833
H_E	0.246	0.561	0.402	0.246	0.820
p	1.000	0.4938	**0.0022**	0.1436	0.9295
2010/dry					
Na	3	8	7	3	6
H_O	0.139	0.488	0.595	0.232	0.764
H_E	0.132	0.545	0.625	0.457	0.710
p	1.000	0.1088	0.5742	**0.0000**	0.1678
2010/dryR					
Na	3	4	6	3	5
H_O	0.095	0.523	0.619	0.143	0.810
H_E	0.094	0.579	0.594	0.298	0.716
p	1.000	0.7979	0.550	**0.004**	0.8851

Note: Na=Number of alleles; p= Exact test of HWE p-values. Significant statistical probabilities are in bold.