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# Intraspecific ecomorphological variations in *Poecilia reticulata* (Actinopterygii, Cyprinodontiformes): comparing populations of distinct environments

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**ABSTRACT.** Morphological variations, according to the principles of ecomorphology, can be related to different aspects of the organism way of life, such as occupation of habitats and feeding behavior. The present study sought to examine the intraspecific variation in two populations of *Poecilia reticulata* Peters, 1859, that occur in two types of environments, a lotic (Maringá Stream) and a lentic (Jaboti Lake). Due to a marked sexual dimorphism, males and females were analyzed separately. Thus, the proposed hypotheses were that the populations that occur in distinct environments present morphological differences. The morphological variables were obtained using morphometric measurements and the ecomorphological indexes. The data were summarized in a Principal Component Analysis (PCA). A Multivariate Analysis of Variance (Manova) was made to verify significant differences in morphology between the populations. Males and females showed similar ecomorphological patterns according to the environment they occur. In general the population from Maringá Stream had fins with major areas, and the Jaboti Lake population eyes located more dorsally. Additionally, others morphological differences such as wider mouth of the males from Maringá Stream, wider heads on Jaboti Lake females and more protractible mouths on males from Jaboti Lake suggest a set of environmental variables that can possibly influence the ecomorphological patterns of the populations, as the water current, availability of food resources and predation. In summary, the initial hypotheses could be confirmed, evidencing the occurrence of distinct ecomorphotypes in the same species according to the environment type.

**KEYWORDS.** Ecomorphological indexes, Poeciliidae, habitat occupation, ecomorphotypes.

**RESUMO. Variações ecomorfológicas intraespecíficas em *Poecilia reticulata* (Actinopterygii, Cyprinodontiformes): comparando populações de ambientes distintos.** Variações morfológicas, de acordo com os princípios da ecomorfologia, podem ser relacionadas a diferentes aspectos do modo de vida dos organismos, como a ocupação de hábitats e comportamento alimentar. O presente estudo buscou analisar essas variações em nível intraespecífico em duas populações de *Poecilia reticulata* Peters, 1859, que ocorrem em dois tipos de ambientes, um lótico (ribeirão Maringá) e um lêntico (lago Jaboti). Por apresentar um acentuado dimorfismo sexual machos e fêmeas foram analisados separadamente. Assim, a hipótese proposta foi de que as populações, que ocorrem em ambientes distintos, possuem diferenças morfológicas. As variáveis morfológicas foram obtidas por meio de medidas morfométricas e índices ecomorfológicos. Os dados foram sumarizados em uma Análise de Componentes Principais. Também foi feita uma Análise de Variância Multivariada para verificar se existe diferença significativa na morfologia entre as populações. Machos e fêmeas mostraram padrões ecomorfológicos semelhantes de acordo com o ambiente em que ocorrem. No geral, a população do ribeirão Maringá apresentou nadadeiras com maiores áreas e a população do lago Jaboti apresentou olhos localizados em região mais dorsal. Adicionalmente, outras diferenças morfológicas como bocas mais largas nos machos do ribeirão Maringá, cabeças mais largas nas fêmeas do lago Jaboti e bocas mais protráteis nos machos do lago Jaboti, sugerem um conjunto de variáveis ambientais que possivelmente influenciam os padrões ecomorfológicos das populações, como, por exemplo, a corrente de água, disponibilidade de recursos alimentares e predação. Em síntese a hipótese inicial pode ser confirmada, evidenciando a ocorrência de ecomorfótipos distintos da mesma espécie de acordo com o tipo de ambiente.

**PALAVRAS-CHAVE.** Índices ecomorfológicos, Poeciliidae, ocupação de hábitats, ecomorfotipos.

One of the principal concepts in ecomorphology is the relation between morphology and ecological aspects (Peres-Neto, 1999). Following this premise, it is expected that part of the ecological niche of species can be predicted by a set of morphological characteristics (Watson & Balon, 1984). In this context, inter and intraspecific morphological variations, that are considered the natural selection's raw material (Darwin, 1859), can influence the performance in the use of resources by the species and, consequently, can affect their survival (Wainwright, 1994).

Among tropical freshwater fish, the identification of the relationship between morphology and ecological aspects, like the use of food and spatial resources, can be complicated by the high opportunism degree of species (Abelha *et al.*, 2001). Despite the difficulties aforementioned, recent studies have shown that there is a significant relationship between morphological variations and ecological aspects, such as feeding and habitat occupation (*e.g.* Teixeira & Bennemann, 2007; Oliveira *et al.*, 2010; Pagotto *et al.*, 2011; Santos *et al.*, 2011). From these studies, the interspecific morphological variation seems to have an important role in the ecological aspects, favoring the ecological diversification of the species, providing niche differentiation and, consequently, the coexistence of the species.

On the other hand, intraspecific morphological variations of some species also seem to have a key role in their occupation and survival over time in a particular habitat type (Violle *et al.*, 2012). Considering the ecomorphological paradigm, intraspecific morphological variations can result in subtle niche differences among the individuals, favoring the adaptation of some fish species according to changes in environmental conditions. These ideas are supported by studies conducted by Mittelbach *et al.* (1992), Robinson *et al.* (1993), Langerhans *et al.* (2003), and Sampaio *et al.* (2013).

*Poecilia reticulata* Peters, 1859 is a widespread fish species that occurs in different kinds of habitats. The high reproduction rate (Gomiero & Braga, 2007) allied to its generalist behavior, pollution tolerance (Rocha *et al.*, 2009), and phenotypic plasticity (Burns *et al.*, 2009; Torres-Dowdall *et al.*, 2012) contributed to its wide distribution (Alves *et al.*, 2000), mainly in systems where it is considered a non-native species. Therefore, investigations concerning the intraspecific morphological variation are important to identify the adaptations of this species in distinct environmental conditions.

The present study aimed to identify and analyze the intraspecific morphological variations in two populations of *P. reticulata* from different environments. Then, the formulated hypothesis was that the two analyzed fish populations' show different ecomorphological structure, and the morphological characteristics presented by each population can be related to habitat use and consequently to the environmental type they occur.

## MATERIAL AND METHODS

Samplings were carried out in Maringá Stream (51°58'8.00"W, 23°22'28.09"S) and Jaboti Lake (23°34'00"S, 51°28'26"W). Both systems are located near urban areas in state of Paraná, Brazil, belonging respectively to the Pirapó and Ivaí River basin (Fig. 1).

The Maringá Stream (located in Maringá municipality) is a third order stream. In the sampling site the margins were covered by grasses, and the bottom was composed of gravel and sand (Fig. 2). The Jaboti Lake (located in Apucarana city) is an artificial reservoir used for recreation, presenting lower current speed and muddy bottom (Fig. 3). The locations were chosen due to the occurrence of *P. reticulata* and the partial separation between the water bodies provided by a water parting. Thus, the populations can be considered partially isolated and under different environmental conditions.

Samplings were conducted in April 2010 in Jaboti Lake and May 2011 in Maringá Stream. Different methods were used in order to optimize the capture of fish in the distinct environments. Electrofishing was used in the Maringá Stream, the lotic habitat (Ac current portable generator 1KW, 200v, 3-4A), throughout a 50 m stretch with nets blocking the areas downstream and upstream. On the other hand, in the Jaboti Lake, the lentic habitat, the fish capture was made in the littoral zone with a trail-net (0.5 mm between opposing knots, 15 m length and 1.20 m height).

Collected specimens were identified according to Graça & Pavanelli (2007), separated in males and females by the analysis of external anatomy (Lucinda, 2003; Graça & Pavanelli, 2007), weighed and fixed with 10% formalin and then preserved in 70° GL ethanol. Voucher specimens (NUP13211 - Maringá Stream and NUP10010 – Jaboti Lake) were deposited in Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupelia), from Universidade Estadual de Maringá (UEM) (http://peixe.nupelia.uem.br/).

A total of 78 individuals were measured, 42 from Maringá Stream (15 males and 27 females) and 36 from Jaboti Lake (13 males and 23 females). The areas and linear measures of the body were taken: standard length, body's height and width, midline body height, caudal peduncle's length, height and width, head's length, height and width, eye's height, areas of the eye and fins (dorsal, caudal, anal, pelvic and pectoral). Detailed information about the morphological measurements is described in Gatz (1979) and Oliveira *et al.* (2010). Only adults were measured to avoid morphologic variations related to ontogeny. The linear measures were obtained using a digital caliper, and the areas from drawings of the structures previously digitalized. The software AutoCad® 2009 was used to calculate the areas.

For each collected species, we calculated the mean value of each morphological measurement, and subsequently calculated the ecomorphological indices. This procedure allows evaluating information restricted to differences between shapes and promotes the independence of analysis as to the size of specimens (Gatz, 1979; Winemiller, 1991). Although the body size is admittedly an important factor in the ecological relationships between fish, significant differences in body size may conduct the results to a trend in a variation related exclusively to specimens' size. Thus, the index use nullifies the chance of analyzes being dominated by a single variable (Winemiller, 1991). Despite the controversy about this procedure in ecomorphological studies (Peres-Neto, 1999), some authors have used index in their analysis (Gatz, 1979; Watson & Balon, 1984; Winemiller, 1991; Willis *et al*., 2005; Casatti & Castro, 2006; Oliveira *et al*., 2010). These authors assume that, by expressing the shape of morphological structures, the indices are able to reveal their ecological roles. Therefore, aware of the limitations of this approach, but at the same time, considering it an important alternative in the attempt to express the ecology of the species, in the present study ecomorphological indexes were calculated combining the morphological measures and areas: compression index; depression index; relative length, height and width of the caudal peduncle; relative length, height and width of the head; vertical eye position, relative area of the eye and fins (dorsal, anal, caudal, pectoral and pelvic). The indexes were selected according to Gatz (1979), Winemiller (1991), Mazzoni *et al.* (2010), Oliveira *et al.* (2010), and Pagotto *et al.* (2011).

All ecomorphological indexes were standardized according to $X'_{ik}=(X_{ik} - {}_{k})/SD$, where $X_{ik}$ is the value of the index $k$ to de specimen $i$, ${}_{k}$ is the mean value of the index $k$

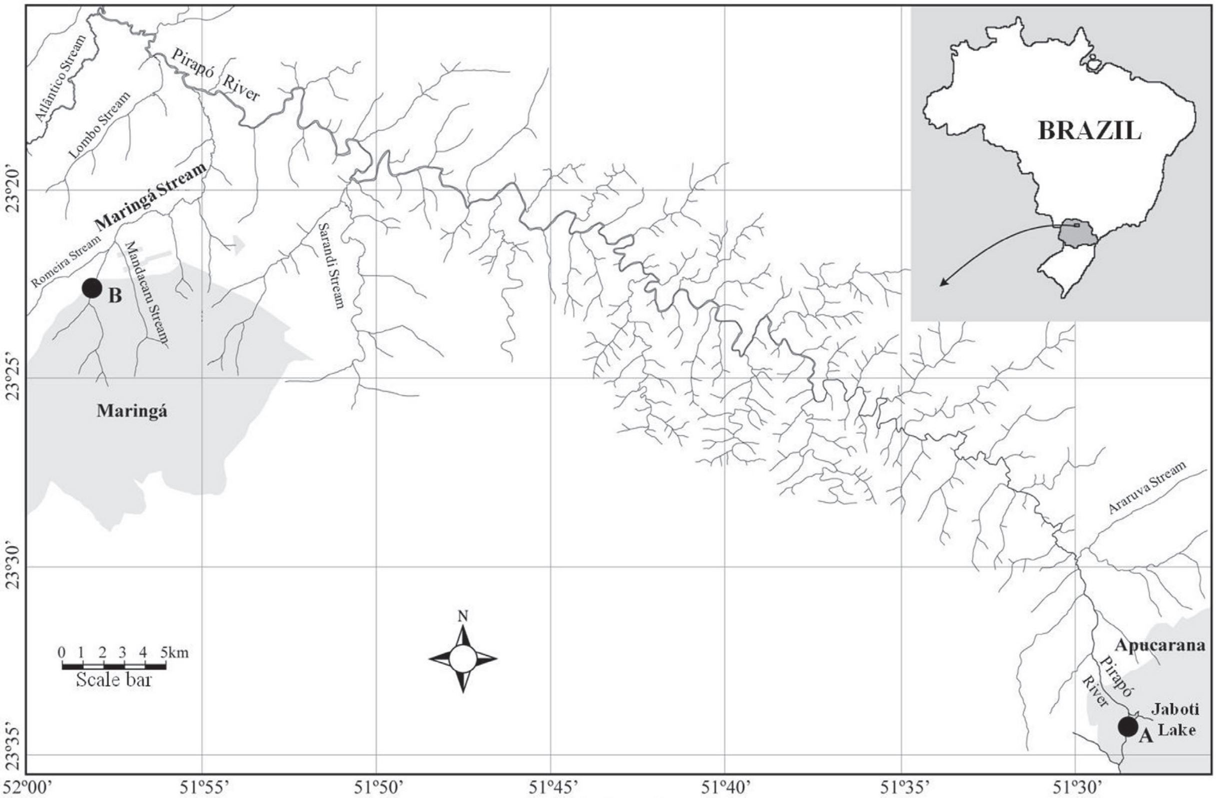


Fig. 1. Location of the sampling sites: Jaboti Lake (A) and Maringá Stream (B), state of Paraná, Brazil.

Fig. 2. Sampling site in Maringá Stream, municipality of Maringá, state of Paraná, Brazil.

Fig. 3. Sampling site in Jaboti Lake, Apucarana city, state of Paraná, Brazil.

of the specie and SD is the standard deviation of the mean. In order to summarize the data from the ecomorphological indexes and identify trends in the morphology, a Principal Component Analysis (PCA) was performed using a correlation matrix. The Broken-Stick criterion was adopted to select the significant axis for interpretation (Jackson, 1993). In complement to the PCA, a Multivariate Analysis of Variance (Manova) was performed to test significant differences of the ecomorphological indices between populations. As an alternative to control the influence of the morphological characteristics related to the sexual dimorphism in the analysis, males and females were analyzed separately, since males and females presents distinct morphology (Lucinda, 2003).

The PCA was performed on PcOrd® 5.0 software (McCune & Mefford, 1999), and the Manova was conducted on R software (R Core Team, 2013), using the *stats* package.

## RESULTS

The PCAs results are summarized on Table I. According to the Broken-stick criterion only one axis was retained for interpretation for males, and two for the females. The tendencies of morphological differentiation between the populations on the first axis were similar, with the Maringá Stream population presenting negative scores and the Jaboti Lake population positive scores (Fig. 4). Also, in both ordinations the morphological indices that separated the populations were significant according to the Manova (Tab. I). Then, individuals that occur on the lotic environment can be characterized by having fins and eyes with major areas, and the individuals from the lotic environment can be characterized by having eyes positioned more dorsally.

For males, the Maringá Stream population showed wider mouths, and the Jaboti Lake had mouths with grater protrusion capacity. On the other hand, for the females, considering the first axis, the Jaboti Lake population was differentiated by having wider heads. On the second axis only one individual was separated in the Jaboti Lake population with major values of compression index and wider caudal peduncles, but according to the Manova only significant differences were evidenced for the relative width of the caudal peduncle.

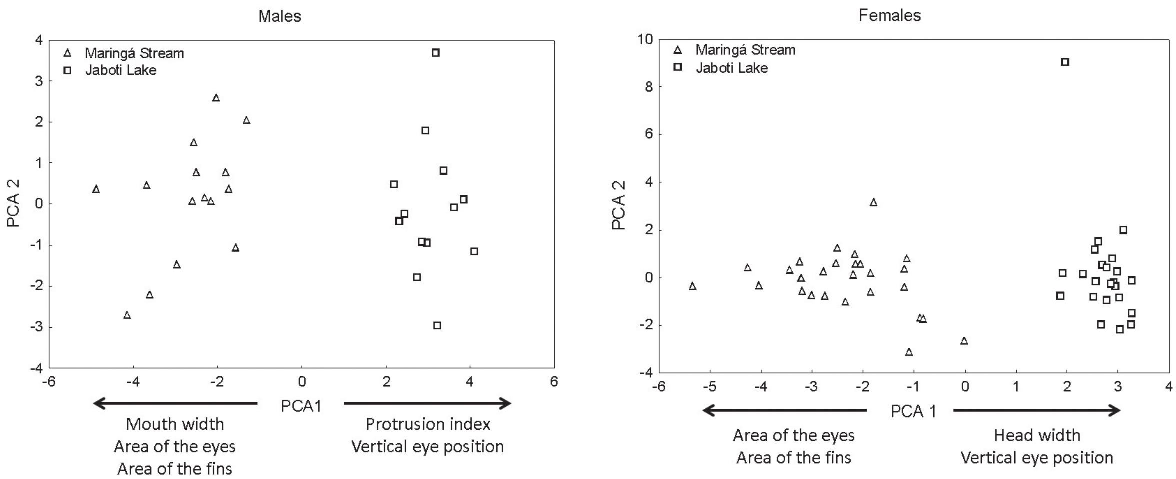


Fig. 4. PCA's ordination scatterplot for males and females, showing the ecomorphological indexes correlated with each axis.

Tab. I. PCA loadings, with grater values, positive and negative, used for interpretation in bold. The variation explained by each axis and the eigenvalues are also presented. The Manova results are also presented with significant values in bold (p<0.05).

| Indices | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | PCA 1 | PCA 2 | p (Manova) | PCA 1 | PCA 2 | p (Manova) |
| Compression index | -0.508 | -0.805 | **0.025** | -0.006 | **0.759** | 0.811 |
| Depression index | 0.210 | 0.041 | 0.583 | -0.339 | 0.506 | 0.057 |
| Relative length of the caudal peduncle | -0.416 | 0.197 | **0.031** | 0.122 | 0.091 | 0.834 |
| Relative height of the caudal peduncle | 0.437 | 0.277 | 0.091 | 0.307 | 0.182 | 0.074 |
| Relative width of the caudal peduncle | -0.351 | -0.729 | 0.185 | 0.285 | **0.796** | **0.049** |
| Relative length of the head | -0.343 | 0.041 | 0.168 | 0.469 | -0.410 | **0.014** |
| Relative height of the head | -0.068 | 0.522 | 0.740 | 0.175 | 0.359 | 0.257 |
| Relative width of the head | 0.539 | -0.640 | **0.003** | **0.566** | 0.626 | **<0.001** |
| Relative height of the mouth | -0.619 | 0.009 | **0.004** | -0.595 | 0.374 | **0.001** |
| Relative width of the mouth | **-0.881** | -0.297 | **<0.001** | -0.510 | 0.545 | **0.001** |
| Protrusion index | **0.705** | -0.289 | **<0.001** | 0.171 | -0.385 | 0.555 |
| Vertical eye position | **0.790** | -0.298 | **<0.001** | **0.524** | -0.415 | **0.002** |
| Relative area of the eye | **-0.957** | 0.015 | **<0.001** | **-0.948** | -0.104 | **<0.001** |
| Relative area of the dorsal fin | **-0.895** | -0.038 | **<0.001** | **-0.925** | -0.072 | **<0.001** |
| Relative area of the caudal fin | **-0.969** | 0.020 | **<0.001** | **-0.974** | -0.092 | **<0.001** |
| Relative area of the anal fin | **-0.961** | 0.033 | **<0.001** | **-0.946** | -0.081 | **<0.001** |
| Relative area of the pectoral fin | **-0.899** | 0.161 | **<0.001** | **-0.959** | -0.057 | **<0.001** |
| Relative area of the pelvic fin | **-0.962** | -0.042 | **<0.001** | **-0.926** | -0.059 | **<0.001** |
| Eigenvalues | 8.796 | 2.271 | | 7.168 | 2.990 | |
| Eigenvalues (Broken-stick) | 3.495 | 2.495 | | 3.495 | 2.495 | |
| Explained variability (%) | 48.868 | 12.617 | | 39.820 | 16.613 | |
| Accumulated variability (%) | 48.868 | 61.485 | | 39.820 | 56.433 | |

## DISCUSSION

The two analyzed populations showed distinct ecomorphological structure regardless the morphological differences related to sexual dimorphism, since males and females presented similar morphological characteristics according to the environmental pressures. The fins areas, with major values on the Maringá Stream population, are important morphological features related to swimming ability, and its role in the performance of movement on aquatic systems are well documented on literature (Gosline, 1971; Gatz, 1979; Watson & Balon, 1984; Kerfoot & Schaefer, 2006).

On intraspecific level, Kerfoot & Schaefer (2006) verified that in *Cottus* specimen individuals that occur on different environmental pressures also presents different morphological characteristics. Coupling field and laboratory studies, these authors found that longer fins and larger eyes occurs on individuals on high water current environment, and that these morphological features (mainly larger fins) optimize the performance in this kind of habitat. Then, the morphological tendencies with fins with greater areas found on the studied *P. reticulata* population from Maringá Stream corroborate with the results aforementioned.

Males from Maringá Stream presented wider mouths, and males from Jaboti Lake presented more protractible mouths (greater values of protrusion index). Both characteristics are related to feeding behavior and foraging performance (Watson & Balon, 1984; Wikramanayake, 1990). In addition, females of Jaboti Lake were characterized by having wider heads, which are directly related to the size of the food consumed (Winemiller, 1991; Oliveira *et al*., 2010). These morphological characteristics suggest that other factors in addition to water current can influence the ecomorphological structure of the populations, such as the food resource availability.

Studies concerning the trophic ecology of *P. reticulata* observed a wide range in food resources consumed by this species, occurring in some cases considerable consumption of detritus (Rocha *et al.*, 2009). Unpublished data performing stomach analysis content of *P. reticulata* from Maringá Stream verified great consumption of detritus and Diptera larvae (mainly from Chironomidae family). The presence of detritus in the diet of *P. reticulata* from streams may be related to anthropic interferences, as observed by Oliveira & Bennemann (2005). The habitat homogenization caused by the removal of the riparian vegetation and other human activities can cause negative influences on the contribution of allochthonous resources, such as terrestrial insects. In homogeneous habitats, the food resources available for fish are mostly autochthonous, mainly algae, detritus and aquatic insects (Zeni & Casatti, 2014).

The presence of predators is other factor that may cause variation in body shape in *P. reticulata* (Langerhans & DeWitt, 2004; Torres-Dowdall *et al.*, 2012). Morphological characteristics in *P. reticulata* populations under predation pressure, according to Langerhans & DeWitt (2004), are more elongated bodies, larger caudal peduncle and lower position of the eye. However, predation pressure was low in Maringá Stream, with only one predator species [*Rhamdia quelen* (Quoy & Gaimard, 1824)]. In Jaboti Lake, *P. reticulata* seems to suffer predation from two species, *Hoplias malabaricus* (Bloch, 1794) and *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (Souza *et al.*, 2015). However, the morphological characteristics of the studied populations do not match the ones mentioned in the literature.

The differences on ecomorphological characteristics between the populations suggest a set of environmental variables influencing the fish in each studied habitat, such as water current, availability of food resources and predation. Then the initial hypothesis could be corroborated. It is important to emphasize that the variables mentioned above can act simultaneously. In this way the correlation between the morphology and ecological aspects can only be verified through experimental studies focused only in one variable. Even though, an integrative analysis including data of trophic ecology, swimming performance and observations concerning habitat occupation are important to comprehend the responses of the populations in natural conditions where the environmental variables interact.

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