



Comparative life-history traits on matrotrophy and lecithotrophy in two Poeciliidae (Cyprinodontiformes)

Comparação de atributos da história de vida na matrotrofia e lecitotrofia em dois Poeciliidae (Cyprinodontiformes)

J. W. S. Daltro; C. S. Vieira; M. F. G. Brito*

Departamento de Biologia/Laboratório de Ictiologia/Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe, 49107-230, São Cristóvão-Sergipe, Brasil

*mbrito@academico.ufs.br

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We comparatively assessed the embryonic development of *Poecilia vivipara* and *Poecilia hollandi* to define the type of energy allocation associated with viviparity. Specimens were sampled using seine nets (5 mm mesh size) deployed along macrophyte stands in the lower reaches of the São Francisco River. Following laboratory processing, morphometric data (standard length and total weight) were recorded for all females. Subsequently, ovaries were excised and assessed for weight, fecundity, and the developmental stages of oocytes and embryos. Morphometric differences were significant between species, with *P. hollandi* females displaying smaller body size and lower mass relative to *P. vivipara*. *Poecilia hollandi* exhibited lower fecundity compared to *P. vivipara*. The standard length of *P. hollandi* influenced gonad weight, while in *P. vivipara* this relationship was expressed regarding gonad weight and fecundity. The reduction in fecundity associated with organism size reflects the evolution of viviparity and environmental pressures, such as predation, as appears to occur in the study area. The late embryos of *P. hollandi* were smaller than those of *P. vivipara*. Fecundity was negatively related to offspring diameter in *P. hollandi*. The marked increase in the diameter of *P. hollandi* embryos signals the initial consumption of the yolk, followed by maternal provision, suggesting a matrotrophic pattern and predictability of resources in the environment to maintain nutrition. In contrast, a lecithotrophic pattern was observed in *P. vivipara* and appears to be conservative for this species.

Keywords: *Poecilia vivipara*, *Poecilia hollandi*, viviparity.

Avaliamos comparativamente o desenvolvimento embrionário de *Poecilia vivipara* e *Poecilia hollandi* para definir o tipo de alocação de energia associada à viviparidade. Espécimes foram coletados com redes de arrasto (malha 5 mm) em bancos de macrófitas no baixo curso do rio São Francisco. Após o processamento em laboratório, registraram-se os dados morfométricos (comprimento padrão e peso total) de todas as fêmeas. Em seguida, os ovários foram removidos e analisados quanto ao peso, à fecundidade e aos estádios de desenvolvimento de ovócitos e embriões. As diferenças morfométricas entre as espécies foram significativas, com as fêmeas de *P. hollandi* apresentando menor tamanho corporal e menor peso em relação a *P. vivipara*. *Poecilia hollandi* exibiu menor fecundidade em comparação a *P. vivipara*. O comprimento padrão de *P. hollandi* influenciou o peso das gônadas, enquanto em *P. vivipara* essa relação esteve associada ao peso das gônadas e fecundidade. A redução na fecundidade associada ao tamanho do organismo reflete a evolução da viviparidade e das pressões ambientais, como a predação, como parece ocorrer na área de estudo. Os embriões tardios de *P. hollandi* foram menores do que aqueles de *P. vivipara*. A fecundidade apresentou correlação negativa com o diâmetro da prole em *P. hollandi*. O aumento acentuado no diâmetro dos embriões de *P. hollandi* sinaliza o consumo inicial do vitelo, seguido pelo fornecimento materno, sugerindo um padrão matrotrófico e a previsibilidade dos recursos ambientais para manter a nutrição. Em contraste, um padrão lecitotrófico foi observado em *P. vivipara* e parece ser conservativo para esta espécie.

Palavras-chave: *Poecilia vivipara*, *Poecilia hollandi*, viviparidade.

1. INTRODUCTION

One of the most derived forms of parental care is viviparity, in which the initial stages of offspring maturation occur within the mother through trophic, osmoregulatory, excretory, respiratory, endocrinological, and immunological maternal-embryonic relations [1-3]. In bony

fishes, gestation occurs in 14 families of teleosts and differs from that in other vertebrates because it occurs inside the ovaries [2, 4, 5].

Viviparity has several advantages, including protection against predation, reduction of competition for space, mobility of offspring to suitable locations, and colonization or dispersion to new habitats [2, 6]. However, females face higher energetic costs during gestation to sustain brood metabolic rates and are more vulnerable to predation because of the increase in body volume and mass [1, 7]. Furthermore, they may be subjected to reduced fertility, depending on their body size, and the risk of total loss through the death of their offspring [2].

In lecithotrophic viviparous species, the nutrients necessary for embryonic development, such as aminoacids, proteins, and lipids, are fully provided by the yolk [3, 8]. Energy reserves are stored in advance by females before fertilization [1, 9]. This nutritional pattern allows for nutrient storage and offers a higher guarantee of reproductive success, in which the embryos continue to be nourished, even in unstable environments [10]. In contrast, in matrotrophic species, the available yolk is significantly reduced [11]. The resources demanded by embryos during pregnancy are derived from the pregnant female, which efficiently supplies specialized maternal-embryonic tissues [12, 13]. Unlike lecithotrophy, matrotrophy requires a continuous or at least predictable food supply, as an interruption in the transfer of nutrients puts the entire gestational process at risk [6].

In addition to these specialized modes of nutrient transfer, the reproductive efficiency of poeciliids is often further enhanced by the temporal overlap of successive broods, a phenomenon known as superfetation [14]. Superfetation has evolved multiple times among poeciliids [15-17] and can be described as a reproductive adaptation that allows the fertilization of new batches of oocytes shortly before the most advanced brood completes its development [18]; the new broods can be fertilized by fresh or stored sperm [19].

The Poeciliidae family comprises small neotropical fish widely distributed in fresh and brackish waters, with 275 valid species currently assigned to 27 genera [20]. They exhibit a high diversity of reproductive adaptations, such as internal fertilization, oviparity, clonality, viviparity, lecithotrophy, matrotrophy, and superfetation [6, 21]. Owing to this diversity, in addition to short generation cycles and easy management, poeciliids are widely used as models in reproductive biology [15, 22].

Among the Poeciliidae family, Poeciliinae is the most diverse subfamily, currently comprising 25 valid genera, including the genus *Poecilia* Bloch & Schneider, 1801, known for its wide distribution across the Americas, with 75 valid species [18]. *Poecilia vivipara* Bloch & Schneider, 1801 can reach a maximum length of 8 cm, and females are larger than males [23]. This species is widely distributed in rivers, lakes, and estuaries in many countries in South America [24-26]. *Poecilia hollandi* (Henn, 1916) has a maximum length of 2.5 cm in males and 4.5 cm in females [27], with recorded occurrences in the upper Paraná, Parnaíba, and São Francisco basins [28]. This study aimed to comparatively analyze the morphology and its implications for reproductive characteristics associated with matrotrophy and lecithotrophy in two closely related species in a stretch of the São Francisco River basin in northeastern Brazil. This study aimed to comparatively analyze the morphology and its implications for reproductive characteristics associated with matrotrophy and lecithotrophy in two closely related species in a stretch of the São Francisco River basin in northeastern Brazil.

2. MATERIALS AND METHODS

Fishes were sampled from the São Francisco River (10°11'06.2''S; 36°50'16.4''W) under the authorization SISBIO #42281-1, in Porto Real do Colegio, state of Alagoas. The locality is situated along a stretch bordered by dense aquatic vegetation, composed mainly of *Egeria densa* Planch. The marginal strip exhibits the characteristics of lentic environments because of the regularization of the São Francisco River flow in recent decades [29].

Females of both species were collected (May/2015) using a seine net (5 mm mesh) along macrophyte banks. The specimens were anesthetized in eugenol [30], fixed in 10% formalin solution, and stored in 70% ethanol [31]. Pregnant females of each species were selected for

biometric analyses, and standard length (SL), total length (TL), body weight (BW), and dissection were performed in the laboratory. Length and weight were measured using a digital caliper (0.01 mm) and a semi-analytical precision scale (0.001 g), respectively. Dissection was performed following a left ventrolateral cut originating from the urogenital opening [32]. The ovaries were removed, weighed (OW – 0.001 g), and individually stored for oocyte and embryo characterization.

Fecundity was measured by counting the number of mature oocytes and embryos [33]. The stages of oocyte and embryonic development phases were macroscopically determined according to Haynes (1995) [34]: (1) Immature oocyte; (2) Primary growth oocyte; (3) Mature oocyte; (4) Blastodisc embryo; (5) Embryonic shield/primitive streak embryo; (6) Optic cup embryo; (7) Early-eyed/limb bud embryo; (8) Middle-eyed embryo; (9) Late-eyed embryo; (10) Very late-eyed embryo; and (11) Mature embryo. The diameters of the oocytes (mm) and embryos were measured using an ocular micrometer coupled to a stereomicroscope (Motic SMZ 140). Oocytes were classified as “mature” when they demonstrated a high concentration of yolk, a translucent golden-yellow color and oil droplets dispersed on the surface of the yolk [31].

Statistical analyses were performed using R version 4.5.1 [35] with a significance level of $p < 0.05$. The Mann-Whitney test was used to compare the distribution of values from the standard length and body weight of the specimens, weight of the ovaries, number of oocytes and embryos, and diameter of embryos at the most advanced stage of development. To assess the influence of standard length on ovarian mass and fecundity (oocytes and embryos), we employed Generalized Linear Models (GLMs) [36]. We applied a Gamma distribution for continuous variables to account for non-normal variance, while a Poisson distribution was utilized for discrete count data.

To describe the type of nutrition as lecithotrophic or matrotrophic [5], observations were based on the macroscopic characteristics of yolk presence in all recorded phases. This description was supported by a graphical representation of the increase in embryo diameter during development. The occurrence of superfetation was determined by the presence of two or more non-consecutive embryonic stages in the same female [18].

3. RESULTS

A total of 55 pregnant females were analyzed, of which 30 were identified as *P. hollandi* and 25 as *P. vivipara*. Females of *P. hollandi* (mean = 18.03 ± 1.83 mm) were significantly smaller ($W = 715$, $p < 0.05$) than those of *P. vivipara* (mean = 23.8 ± 3.16 mm), and lighter ($W = 1$, $p < 0.05$) (mean = 0.126 ± 0.040 g) than the body weight of females of *P. vivipara* (mean = 0.373 ± 0.167 g) (Table 1).

Table 1: Minimum, maximum, mean and standard deviation (SD) values of standard length (SL – mm), body weight (BW – g), ovarian weight (OW – g) and fecundity (F) of adult females of *Poecilia hollandi* and *Poecilia vivipara* collected in the lower São Francisco.

	<i>Poecilia hollandi</i> (n = 30)			<i>Poecilia vivipara</i> (n = 25)		
	Min.	Max.	Mean $\pm$ SD	Min.	Max.	Mean $\pm$ SD
SL	15	21	18.03 ± 1.83	19	30	23.8 ± 3.16
BW	0.06	0.199	0.126 ± 0.040	0.18	0.855	0.373 ± 0.167
OW	0.007	0.045	0.020 ± 0.010	0.007	0.158	0.037 ± 0.033
F	2	16	6.53 ± 3.16	2	27	9.88 ± 6.39

Ovarian weight did not differ significantly between *P. vivipara* and *P. hollandi* ($W = 489.5$, $p = 0.054$), although *P. vivipara* exhibited higher overall values. The number of oocytes and embryos was also significantly different in favor of *P. hollandi* ($W = 494.5$, $p = 0.043$). Records from females of *P. hollandi* showed an overall average of 46.97 oocytes and embryos, ranging from 25 to 153, with a total fecundity mean of 6.5. *Poecilia vivipara* exhibited a lower average

of all analyzed traits, with 31.2 embryos and oocytes in total, varying between 9 and 92, and a mean total fecundity of 9.8.

Oocytes and early embryonic stages were predominant in both species. Stages 4, 5, and 6 were not found among *P. hollandi* specimens, and stage 11 was not observed in the species analyzed (Figure 1). Females of *P. vivipara* and *P. hollandi* were recorded with embryos in up to four consecutive stages of development simultaneously, with no evidence of malformation among the analyzed embryos.

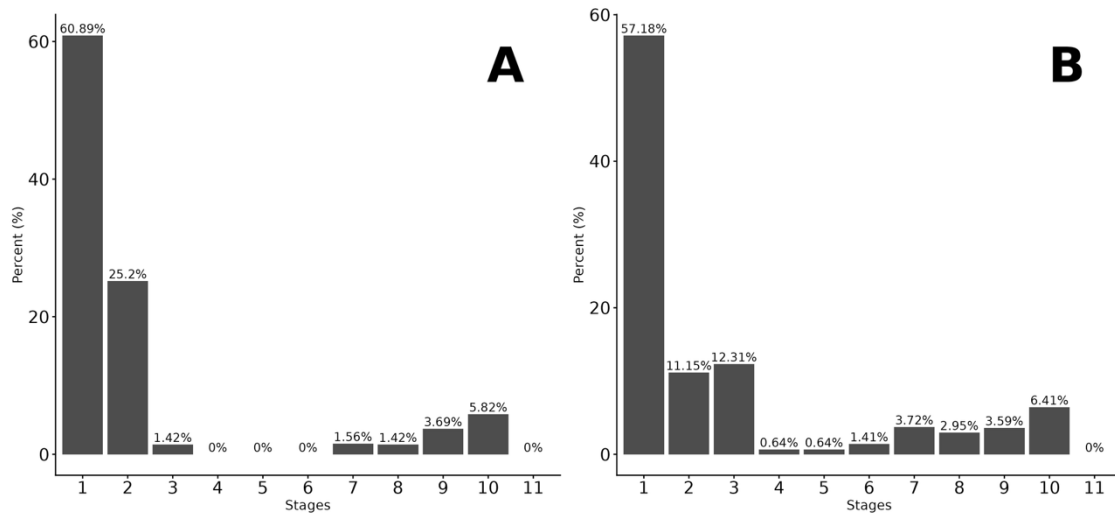


Figure 1: Frequency of oocytes and embryos per stage of development in adult females of *Poecilia hollandi* (A) and *Poecilia vivipara* (B) collected in the lower São Francisco region.

Considering the influence of the morphological development of adult females on reproductive traits, female size was significantly related to gonad weight in *P. hollandi* ($r^2 = 0.40$; $p < 0.05$) and *P. vivipara* ($r^2 = 0.49$; $p < 0.05$). Fertility was correlated with size in *P. vivipara* ($r^2 = 0.65$; $p < 0.05$); however, it was not significantly related to the examined females of *P. hollandi* ($r^2 = 0.13$; $p > 0.05$) (Figure 2).

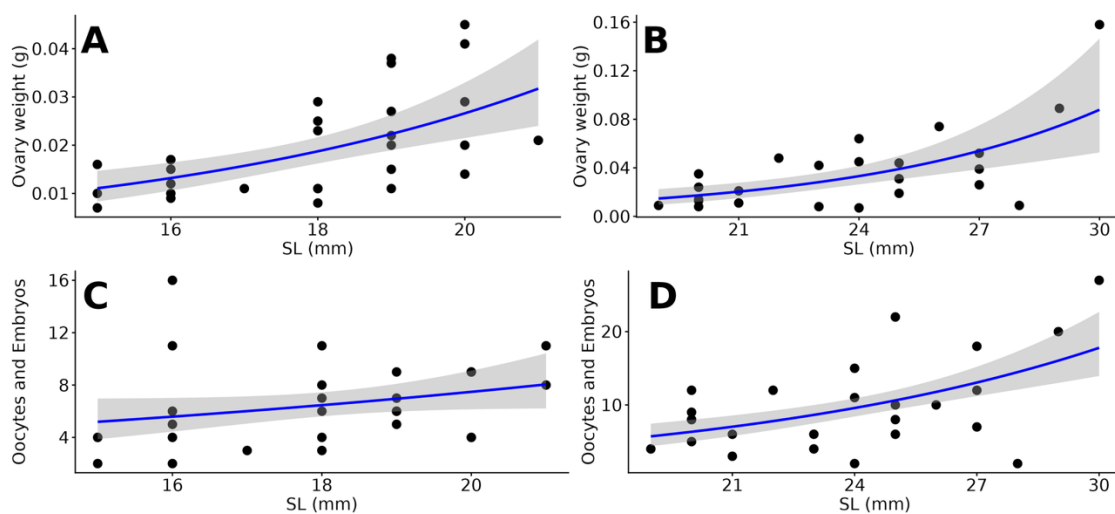


Figure 2: Ovarian weight (OW, g) and fecundity (F) related to standard length (SL, mm) of adult females of *Poecilia hollandi* (A, C) and *Poecilia vivipara* (B, D) collected in the lower São Francisco River.

The average diameter of the embryos recorded in the very late-eyed embryo phase (stage 10) was considered smaller ($W = 3348$; $p < 0.05$) for *P. hollandi* (mean = 2.61 mm) than for *P. vivipara* (mean = 3.04 mm). Total fecundity had a negative effect on the diameter of the offspring in *P. hollandi* ($r^2 = 0.32$; $p < 0.05$), whereas in *P. vivipara*, offspring size was not significantly affected ($r^2 = 0.067$; $p > 0.05$) (Figure 3).

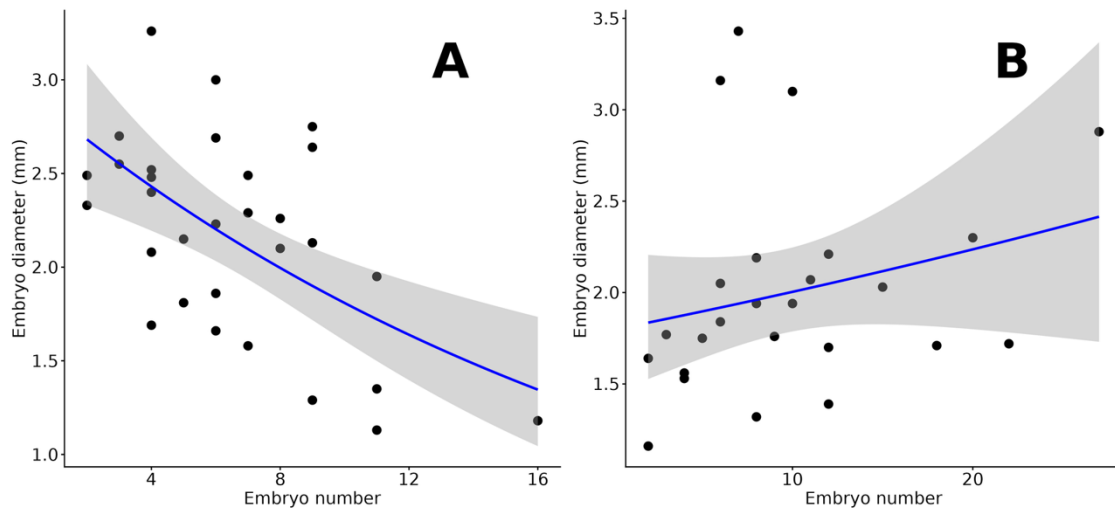


Figure 3: Relationship between the number and average diameter of embryos from pregnant females of *Poecilia hollandi* (A) and *Poecilia vivipara* (B) collected in May/2015 in the lower São Francisco River.

Poecilia hollandi embryos exhibited a more pronounced increase in diameter than *P. vivipara* embryos (Figure 4). Considering the developmental phases and energetic reserves, most yolk consumption in *P. hollandi* embryos was observed during the early developmental stages. In contrast, *P. vivipara* embryos continued to use their energy reserves until the final stages (Figure 5).

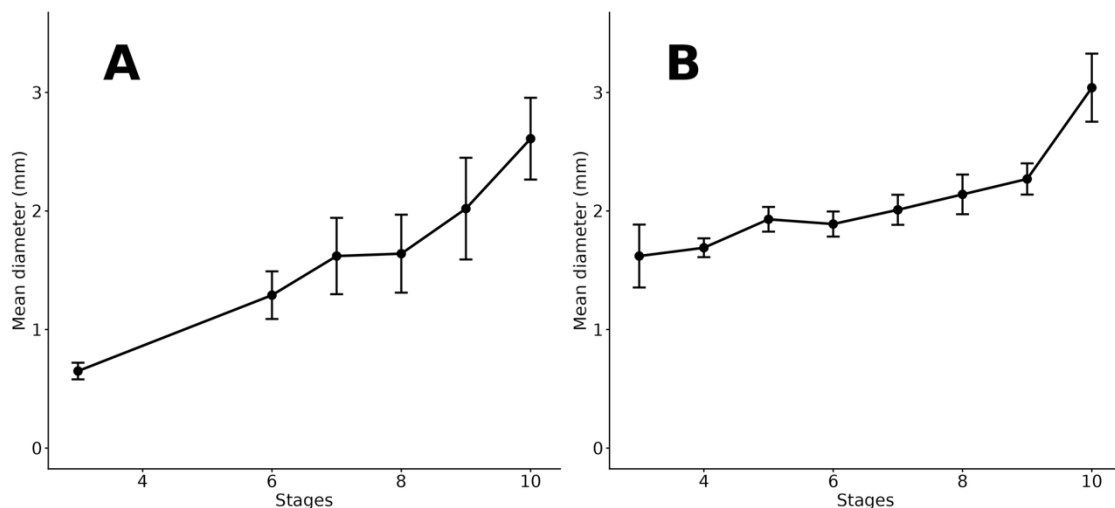


Figure 4: Average diameter (mm) of mature oocytes and embryos during embryonic development from adult females of *Poecilia hollandi* (A) and *Poecilia vivipara* (B) collected in May/2015 in the lower São Francisco River.

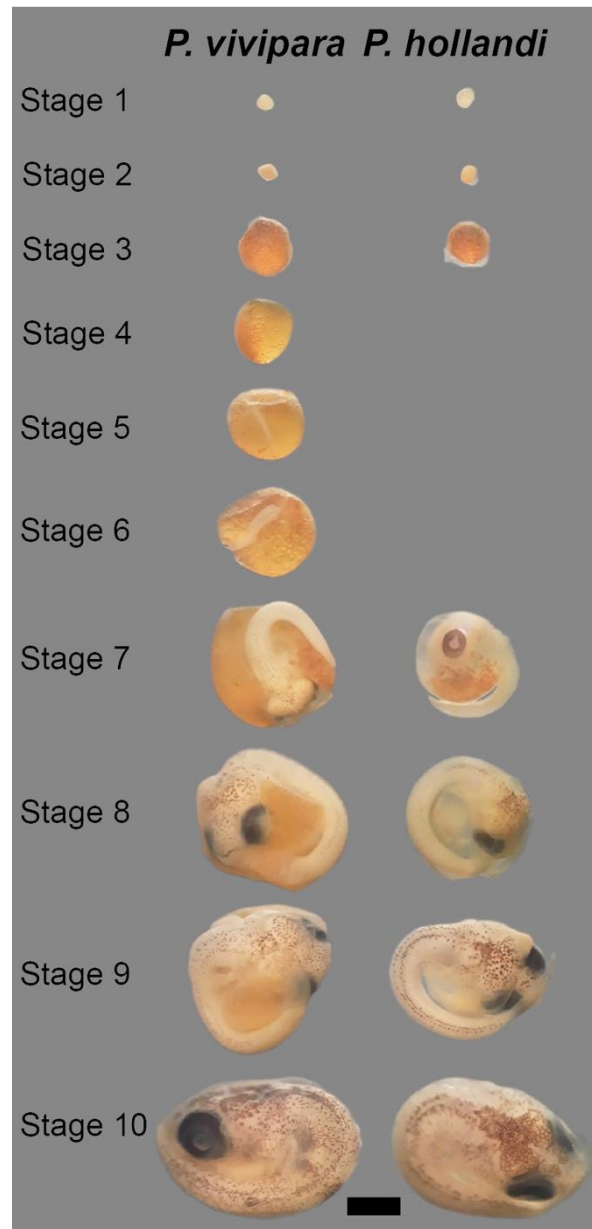


Figure 5: Embryonic development in two *Poecilia* species according to HAYNES (1995): (Stage 1) Immature oocyte; (Stage 2) Primary growth oocyte; (Stage 3) Mature oocyte; (Stage 4) Blastodisc embryo; (Stage 5) Embryonic shield/primitive streak embryo; (Stage 6) Optic cup embryo; (Stage 7) Early-eyed/limb bud embryo; (Stage 8) Middle-eyed embryo; (Stage 9) Late-eyed embryo; (Stage 10) Very late-eyed embryo. Scale bar = 1 mm.

4. DISCUSSION

The size at the first maturation stage is a life history trait that can be shaped by the biotic conditions of the environment [37]. Early maturing individuals gain a reproductive advantage in environments with a high rate of size-selective predation [38, 39]. Although a larger size provides higher fecundity and the largest diameter of embryos during the final developmental stages, it can also increase the risk of predation related to the female's difficulty in swimming [7, 40]. This vulnerability is offset by the choice of habitats with a high density of macrophytes, as the composition of aquatic vegetation guarantees greater spatial heterogeneity and higher chances of offspring escaping and hiding [41]. The size of adult females of *P. hollandi* and *P. vivipara* observed in this study was smaller than that reported in the literature [24, 42]. Early maturation associated with the occurrence among macrophytes could be linked to a life cycle strategy in

response to the higher local predation rate [43], considering the presence of piscivore native [e.g., *Acestrorhynchus lacustris* (Lütken, 1875), *Acestrorhynchus britskii* Menezes, 1969, *Hoplias malabaricus* (Bloch, 1794)] and non-native species [e.g., *Astronotus ocellatus* (Agassiz, 1831), *Cichla kelberi* Kullander & Ferreira 2006].

As noted in the literature, one of the outcomes of viviparity evolution in teleosts was a decrease in fecundity associated with the size of the organism, where we could assume that smaller females have a lower fecundity than larger ones [2]. Our findings suggest that the smaller size recorded for adult females of *P. hollandi* is related to lower fecundity values. Previous analyses indicated an average fecundity of 4.7–18 embryos for *P. hollandi* [42], while for *P. vivipara* an average of 22 was determined [24]. The results showed a correlation between standard length, gonad weight, and fecundity in adult females of *P. vivipara*. Furthermore, evidence from adult females of *P. hollandi* established significant relationships between standard length and gonad weight, where the influence of length over fecundity was not observed but previously recorded in an earlier study [44]. Based on these findings, it is possible to determine that a larger body size allowed *P. vivipara* females to bear heavier ovaries, higher fecundity, and larger embryos than *P. hollandi*.

The increase in fecundity implies greater volume and body mass, which can reduce the hydrodynamics and chances of survival of mature females [7]. Nevertheless, the size of the oocytes and embryos of viviparous species may respond to fecundity to ensure reproductive success [2, 45]. This influence was observed in *P. hollandi*, where pregnant females with more fertilized eggs also carried the smallest embryos. This result suggests a reproductive response to predation [43], generating more broods from a decrease in offspring size.

The data strongly suggest that the mode of embryonic nutrition demonstrated by *P. hollandi* was matrotrophic, as it was possible to observe that most of the yolk initially available to the embryos undergoes a rapid decrease in the early stages of development. During the developmental stages of “Blastodisc embryo” (stage 4) and “Embryonic shield/primitive streak embryo” (stage 5), the biomass was possibly converted into metabolic energy, and nutrients were provided by the mother [6, 46]. The entire previously genus *Pamphorichthys* Regan, 1913, which has been reassigned to *Poecilia* [20], is known to have developed matrotrophy [42].

However, it is worth mentioning that this strategy is highly plastic and can be abandoned if the food supply is not continuous or at least predictable, as an interruption in nutrient transfer can interrupt embryo development [6]. The occurrence of matrotrophy in this region of the São Francisco River suggests that food resource availability is stable or predictable for these populations. Moreover, the high density of macrophytes in the region serves as an important resource for providing food and shelter for fish [26]. In contrast, *P. vivipara* displays a lecithotrophic mode of nutrition, with the maternal-embryonic relationship limited to the incubation of the offspring. The embryos presented sufficient yolk to be individually nourished throughout their development, which was visible by the permanence of the yolk sac until the most advanced stage was observed. This lecithotrophic mode was previously described in *P. vivipara*, which seems to be a conservative trait in this species [5].

In the present study, there was no evidence of superfetation for *P. hollandi* and *P. vivipara*. While a few pregnant females carried four different developmental stages simultaneously, oocytes/embryos were observed in consecutive phases, making it difficult to ensure the occurrence of superfetation in these species. Such cases can occur due to the gradual fertilization of oocytes and asynchronous development, phenomena made possible by the storage of sperm by females [6, 34]. The literature recorded superfetation in *P. hollandi* [44] corroborating the premise that the occurrence of this mode of reproduction is usually associated to matrotrophy [47]. Superfetation can change depending on ecological conditions, such as unstable environments related to food availability [6]. The absence of superfetation in *P. hollandi* in the present study showed that the predictability of resources in the sampled space and time was sufficient to sustain the matrotrophic mode without superfetation in this species.

5. CONCLUSION

Our data revealed that closely related viviparous species show morphological differences in early development and reproductive tactics, where early maturation in both species seems to indicate greater exposure to predation risk. The larger body size of *P. vivipara* allowed females to bear heavier ovaries, higher fecundity, and larger embryos than pregnant females of *P. hollandi*. Corroborating the literature, the matrotrophic mode has been suggested in pregnant females of *P. hollandi*, indicating that important resources, such as food availability, were stable or predictable in the stretch and period sampled in the lower São Francisco River. Our findings also support the notion that *P. vivipara* is exclusively lecithotrophic, with the presence of long-lasting energetic resources provided by the yolk through the final developmental stages of the embryos.

Studies considering different environmental conditions and larger sample sizes can help identify the different pressures to which a population may be exposed. Likewise, addressing the nutritional patterns of these animals at a macroscale level may elucidate how they respond and adapt to different environments, allowing comparisons between conserved sites and those subject to anthropogenic actions, or even between different biomes. These life-history attributes make these species good bioindicators of environmental conditions.

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