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Life-history shifts in a pool-dwelling fish species across natural and artificial temporary habitats

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Dedico esse trabalho
Aos meus pais e às pessoas que amo,
Ao meu filho Pedro,
Que ainda em meu ventre, sentiu comigo cada
emoção, medo e conquista
que acompanharam a finalização desta jornada.
À Natureza, que nos sustenta,
E à ciência, farol que ilumina nosso caminho.

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``Temos pouco tempo para permanecer, como você,
Temos uma Primavera igualmente breve,
Um crescimento rápido rumo à decadência,
Como você, ou qualquer outra coisa.``

- Robert Herrick,

RESUMO

Poças temporárias estão entre os sistemas aquáticos mais sensíveis às mudanças ambientais e têm sido cada vez mais alterados ou perdidos devido às mudanças climáticas e à expansão urbana. Por outro lado, intervenções antrópicas simultaneamente têm promovido o surgimento de corpos d'água temporários artificiais, como valas à beira de estradas. Embora efêmeras, as poças naturais e artificiais diferem quanto à estabilidade hidrológica e à duração da fase aquática, podendo modular as táticas reprodutivas dos peixes. Investigamos como características da história de vida do *killifish Atlantirivulus peruibensis*, incluindo razão sexual, tamanho na primeira maturação, índice gonadossomático, fecundidade e tipo de desova, variam entre poças temporárias naturais e artificiais. As poças foram amostradas mensalmente ao longo de 2024, totalizando 260 indivíduos. A atividade reprodutiva diferiu entre os ambientes, com sazonalidade mais evidente nas poças naturais, ao passo que uma atividade reprodutiva mais intensa ao longo de vários meses foi observada nas poças artificiais. Fêmeas de poças naturais atingiram a maturidade em tamanhos maiores do que aqueles de poças artificiais. A fecundidade efetiva por lote foi maior nas poças artificiais, enquanto o diâmetro dos ovócitos vitelogênicos foi maior nas poças naturais. A distribuição de frequência dos diâmetros dos ovócitos indicou um padrão de desenvolvimento grupo-síncrono, com desova parcelada. Em conjunto, esses resultados revelam uma estratégia reprodutiva oportunista, sugerindo que *A. peruibensis* apresenta marcada plasticidade fenotípica reprodutiva, o que pode favorecer sua persistência em novos habitats temporários e cada vez mais modificados por atividades humanas.

Palavras-chave: Ecologia, Reprodução, Desova, *Atlantirivulus peruibensis*, Valas de estrada.

ABSTRACT

Temporary pools are among the systems most sensitive to environmental change and have been increasingly altered or lost due to climate change and urban expansion, which has simultaneously promoted the emergence of artificial temporary waterbodies such as roadside ditches. Although both ephemeral, natural and artificial pools differ in hydrological stability and duration of the aquatic phase, potentially modulating fish reproductive tactics. We investigated how life-history traits of the killifish *Atlantirivulus peruibensis*, including sex ratio, size at first maturity, gonadosomatic index, fecundity and spawning type, vary between natural and artificial temporary pools. Pools were sampled monthly throughout 2024, and a total of 260 individuals were collected. Reproductive activity was different between environments, with clearer seasonality in natural pools, while a more intense reproduction across multiple months was seen in artificial pools. Females from natural pools matured at larger sizes than those from artificial pools. Effective batch fecundity was higher in artificial pools, while vitellogenic oocyte diameter was larger in natural pools. The frequency distribution of oocyte diameters indicated a group-synchronous, batch-spawning pattern. Together, these results reveal an opportunistic reproductive strategy, suggesting that *A. peruibensis* exhibits marked reproductive phenotypic plasticity that may help to persistence under novel and increasingly human-modified temporary habitats.

Keywords: Ecology, Reproduction, Spawning, *Atlantirivulus peruibensis*, Roadside ditches.

LISTA DE ILUSTRAÇÕES

LISTA DE MATERIAL SUPLEMENTAR

LISTA DE ABREVIATURA E SIGLAS

AF	Tropical Without Dry Season
AIC	Akaike’s Information Criterion
CEMADEM	National Center for Monitoring and Alerts of Natural Disasters
GLMMs	Generalized linear mixed models
GSI	Gonadosomatic Index
HE	Hematoxylin and eosin
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational Analysis of Multivariate Dispersions
SD	Standard desviation
SL	Standard Length
WG	Gonad weight
WT	Total body weight

SUMÁRIO

1. INTRODUCTION

Life history refers to the set of characteristics responsible for the survival and reproduction of species, including growth patterns, age and size at first maturity, fecundity and reproductive investment (Roff, 1992; Stearns, 1992). These characteristics result from a long evolutionary trajectory frequently shaped by environmental conditions (Stearns, 1989; Winemiller, Rose, 1992). However, rapid anthropogenic and climate-driven changes in hydrology, connectivity, temperature, and water quality have increasingly altered aquatic ecosystems (Miller *et al.,* 2015; Devkota, Kathayat, 2020; Maskey *et al.,* 2022; Zolfagharpour *et al.,* 2022; Ding *et al.,* 2023; Bai *et al.,* 2025; Guedes, Araújo, 2026), leading to shifts in fish traits such as variations in size at first maturity (Pankhurst, Munday, 2011; Pilipaitytė *et al.,* 2025), reproductive period (Gao *et al.,* 2016; Hong *et al.,* 2023), fecundity (Holt, Jørgensen, 2015; Chaparro-Pedraza, de Roos, 2019) and egg size (Jia *et al.,* 2020). In this context, understanding how these strategies respond to increasingly extreme environments is central to contemporary ecology.

Among the systems most sensitive to environmental changes, especially faster drying due to high temperatures and loss of riparian vegetation, are temporary aquatic environments, such as temporary pools, seasonal ponds, and other short-lived aquatic habitats (Junk *et al.,* 2013; Polačik, Podrabsky, 2015). These habitats are characterized by intense hydrological fluctuations, strong seasonality, and an inherent risk of desiccation, imposing strong constraints on the survival and reproduction of the organisms that occupy them (Williams, 2006; Polačik, Podrabsky, 2015). Consequently, reproduction in temporary environments tends to be strongly synchronized during favorable periods (for non-annual species), such as wetter months, when the availability of aquatic habitats increases and the risk of desiccation is reduced. Thus, many fish species living in such environments display reproductive tactics that characterize an opportunistic life strategy, mostly allowing rapid colonization of the environment (Winemiller, 1989), such as accelerated growth, early maturation, and high reproductive investment. Despite their harsh conditions, temporary aquatic habitats play a fundamental role in maintaining biodiversity, supporting a wide diversity of species, often specialized and endemic (Williams, 2006; Williams *et al.,* 2010; Jeffries *et al.,* 2016; Tette-Pomárico *et al.,* 2023). However, habitats such as temporary pools have been undergoing a marked reduction in

availability, mainly resulting urban expansion and climate change (Volcan, Lanés, 2018).

Under intense anthropogenic modification, artificial temporary aquatic environments, such as roadside ditches, have become increasingly common (Clifford et al., 2025). These ditches are excavated along highway margins to prevent water accumulation on the road and may function as artificial temporary pools, exhibiting seasonal flooding and drying (Costa et al., 2025a). Although they share the ephemeral nature of natural pools, ditches are generally larger and deeper, retain water for longer periods, and often occur in areas with reduced riparian cover, facilitating contaminant input and sediment deposition. These characteristics give these environments relatively more stable hydrological regimes, but with greater thermal fluctuation and changes in water quality (Costa et al., 2025a), potentially further intensifying the nature of temporary habitats by modifying water chemistry, duration of the aquatic phase, and environmental predictability.

Such modifications may alter the life-history patterns of fish species specialized in temporary pools, which evolved under the fluctuating conditions of these natural environments. In naturally unstable systems, water availability acts as a reliable trigger for reproduction; thus, restricting the aquatic phase tends to favor concentrated and synchronized reproductive patterns during wetter periods, reducing the risk of reproductive failures associated with drought periods (Arenzon *et al.,* 1999; Cavalheiro, Fialho, 2015; Guedes *et al.,* 2023). Changes in water natural fluctuation can modulate fish reproductive tactics (García *et al.,* 2019), in artificial environments, prolonging the aquatic phase could reduce synchronization and prolong reproductive activity by offering more time for energy allocation. However, despite the increasing appearance of these structures in the landscape, little is known about the effects of these ephemeral artificial aquatic environments on fish reproduction, and how specialized taxa to natural temporary pools cope with these new challenges.

In this sense, the present study aimed to investigate how life cycle characteristics, such as sex ratio, gonadosomatic index, size at first maturity, fecundity, and spawning type, of a specie specialized in temporary environments change between natural and artificial temporary pools, evaluating this from the perspective of differences in the stability and duration of the aquatic phase between environments. We hypothesized that the reproductive traits would differ between the environments. In natural temporary pools, characterized by greater temporal restriction of the aquatic

phase, we expect to find a gonadosomatic index pattern that will be temporally marked, with peaks in rainier months, while in artificial pools, the prolongation of the aquatic phase should allow reproductive investment in a long period of the year, without evident seasonality, since they dry at a slower rate (Costa *et al.,* 2025a). Such comparisons are crucial to understand how species respond to the colonization of novel anthropogenic habitats, shedding light on the role of phenotypic plasticity in population persistence and informing conservation strategies in increasingly dynamic and extreme environments.

2. MATERIAL AND METHODS

Study area

The study was conducted in the Rio Preto sub-basin, which is part of the Itanhaém River basin, in the Coastal Atlantic Forest of the State of São Paulo, Brazil (Fig. 1). The area is characterized by a plain, low-altitude landscape with predominantly sandy soils and low clay content, poor in nutrients, with high water saturation and low moisture retention capacity. Although restinga forest predominates, small subsistence farming sites and the expansion of urban areas contribute to the reduction of natural vegetation (Camargo, Cancian, 2016). The blackwater rivers in the region have a dark color due to the high concentration of humic substances, resulting from the intense decomposition of lignin-based plant material. This results in a high concentration of dissolved organic carbon, pronounced acidity, reduced concentrations of total nitrogen and phosphorus, as well as low levels of turbidity and dissolved oxygen (Selinger *et al*. 2025).

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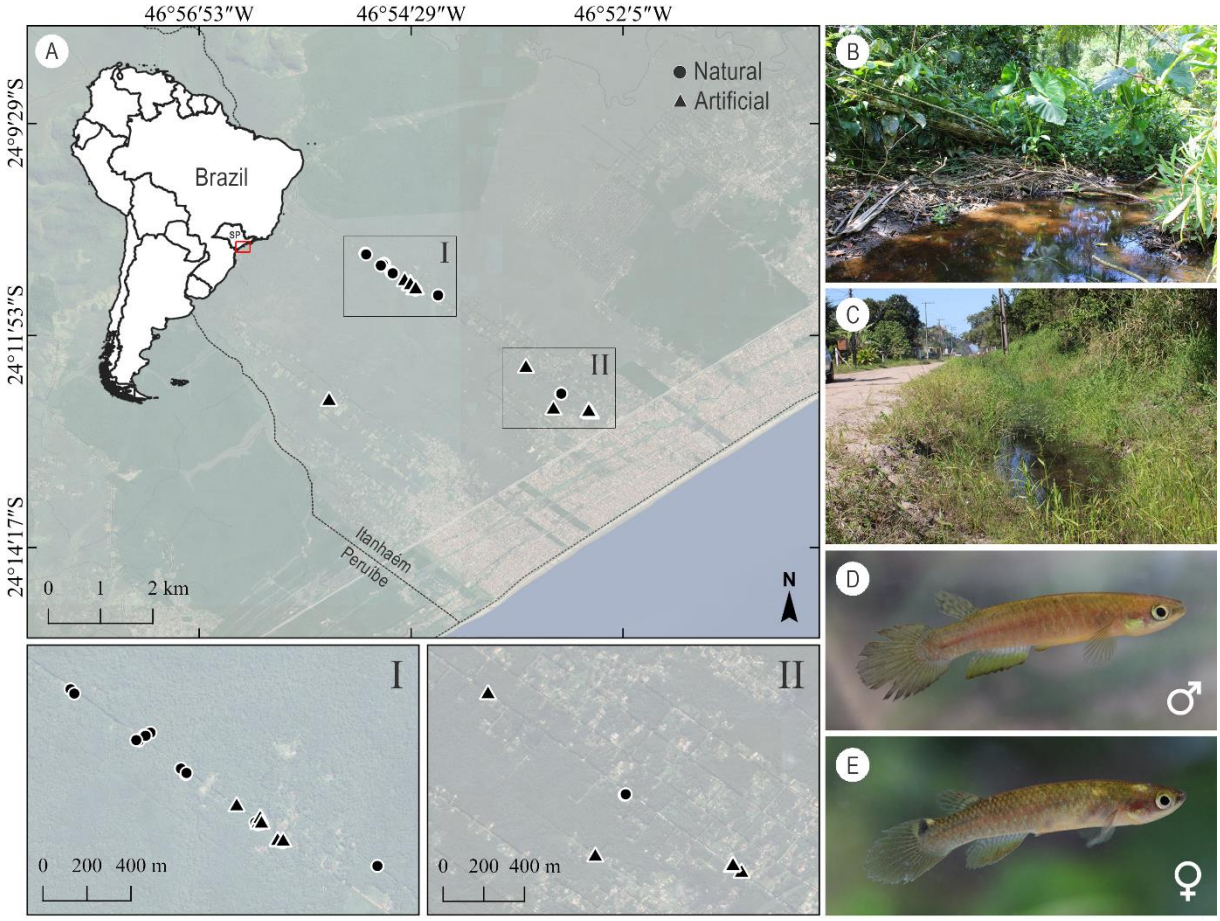

Figure 1. (A) Sampling points of natural pools (circles) and artificial pools (triangles) in the Rio Preto sub-basin, Itanhaém-SP, Brazil. Examples of a natural (B) and an artificial (C) temporary pool sampled. Male (D) and female (E) specimens of *Atlantirivulus peruibensis* (Photo: Amanda Selinger).

The study region lies within the humid tropical zone and is classified as Af according to the Köppen climate classification refined for Brazil by Alvares *et al*. (2013). The rainfall regime is marked by periods of high and low rainfall (Fig. 2), as can be seen by the data obtained from the National Center for Monitoring and Alerts of Natural Disasters (CEMADEN), specifically from the Balneário Gaivota station (24°14’27.6” S, 46°53’34.8” W), located in the municipality of Itanhaém-SP, near the sampled areas. For details on the filtering of rainfall data (2016 to 2024), see Costa *et al.* (2025).

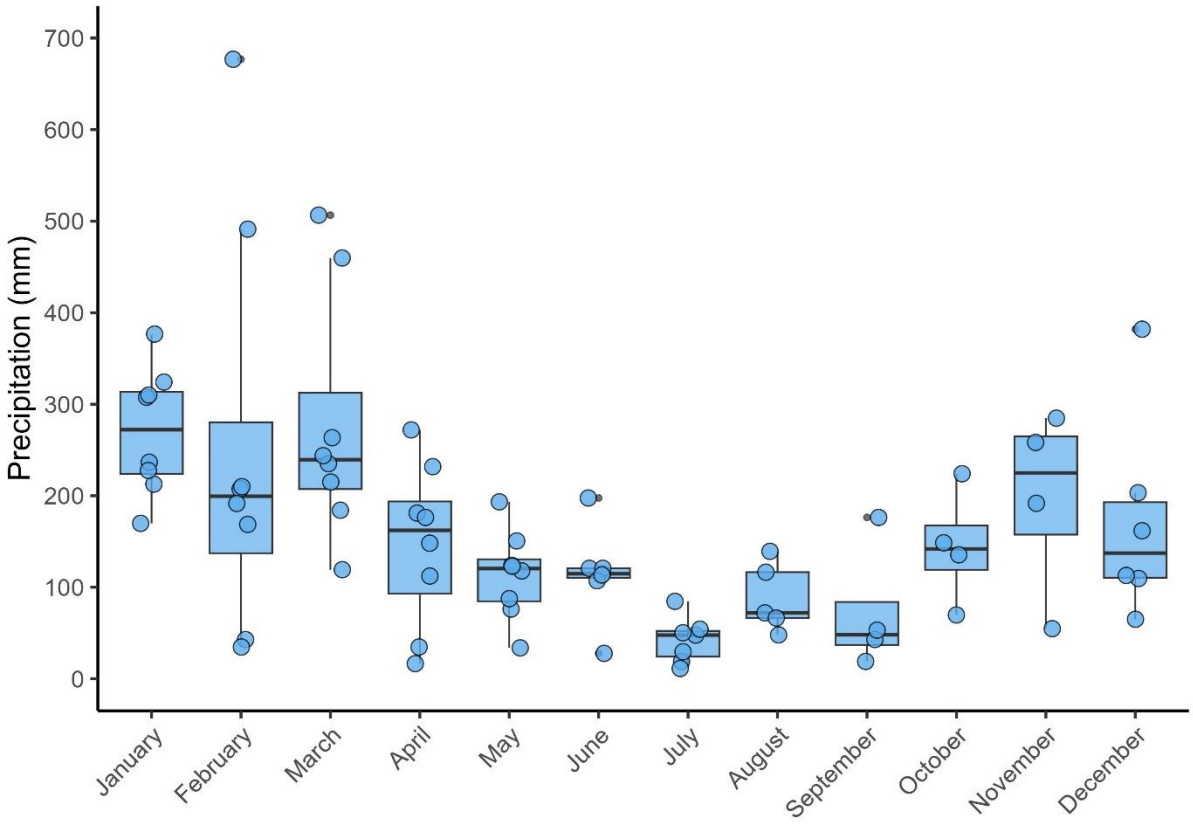

Figure 2. Average monthly precipitation (mm) in the Rio Preto sub-basin region, Itanhaém-SP, Brazil, based on monthly precipitation data from 2016 to 2024. Boxplots represent the minimum, maximum, quartiles, and median values, while points correspond to the observations. Data were obtained from the National Center for Monitoring and Alerts of Natural Disasters (CEMADEN), using records from the Balneário Gaivota station (24°14’27.6” S, 46°53’34.8” W).

Study species

To test the hypothesis, *Atlantirivulus peruibensis* Ywamoto, Nielsen & Oliveira, 2025 was used, a non-annual rivulid that inhabits temporary pools distributed along the southern coast of the state of São Paulo, Brazil. In standard length and exhibit sexual dimorphism related to body size and coloration, more evident in live specimens, with males showing more contrasting coloration, with greenish, bluish and brownish tones, while females have a more cryptic coloration, varying between yellowish and translucent tones (Ywamoto, Nielsen ,Oliveira, 2025) .

Similar to other aplocheiloids associated with temporary environments, the species presents reproductive characteristics related to hydrological dynamics and has been observed occupying shallow pools with a substrate composed of leaf litter

(Espírito-Santo *et al*., 2017; Espírito-Santo, Zuanon, 2017; Costa et *al*., 2025a, 2025b). In the Itanhaém River basin, the species appears to be strongly associated with temporary habitats, with only occasional records in streams (Ferreira, Petrere, 2009; Ferreira et al., 2014; Costa et al., 2025a, 2025b). Therefore, this study focused exclusively on individuals inhabiting natural and artificial temporary pools.

Habitat characterization and sampling

Two types of temporary aquatic environments were sampled in 2024: natural pools and artificial pools (= road ditches) (Fig. 1). Both environments exhibit hydrological dynamics marked by flooding and drying events. Natural pools form in depressions in the terrain after intense rainfall events and are frequently associated with floodplains. These habitats occur predominantly in forested areas, where fine sediment deposits result in substrates with low water permeability, favoring temporary water retention. On the other hand, road ditches are environments excavated along the edges of dirt roads, intended for rainwater drainage and to prevent water accumulation on the roadbed. Like natural pools, these environments are temporary and most of them were also observed to dry seasonally, although they take longer to dry (Costa et *al*., 2025a). Also, although non-annual rivulids are often associated with perennial environments such as small streams, this association does not appear to be strong for *A. peruibensis* in the Itanhaém River basin. In this study, we therefore focused exclusively on individuals inhabiting pools formed within the dynamic floodplain landscape, rather than assuming recruitment from nearby streams. This decision is supported by the fact that only a few individuals of this species have been historically recorded in streams of the basin (Ferreira, Petrere, 2009; Ferreira *et al*., 2014), a pattern that contrasts sharply with its frequent occurrence in ephemeral aquatic habitats in the region (Costa *et al*., 2025a, 2025b). Moreover, during parallel sampling of nearby streams conducted in the same year (2024) as part of another project, only four individuals were recorded (JHAC & AS, personal observation). Finally, despite the heterogeneous terminology applied to these types of wetlands in the literature (Richardson *et al*., 2022), we opted to use the term “pools” to maintain consistency with studies describing fish communities in shallow, half-ellipsoid-shaped depressions filled with water, typically with leaf-litter substrates (Espírito-Santo *et al*.,

2017; Espírito-Santo, Zuanon, 2017), as well as with recent studies conducted in similar environments in the same region (Costa *et al*., 2025b).

Between January and December 2024, *A. peruibensis* was recorded at 50 distinct sampling sites, of which 25 were located in natural temporary pools and 25 in artificial temporary pools (Fig. 1A). Because these habitats are highly dynamic, the number of sites sampled per month within each habitat type varied between one and four and depended on the occurrence of the species rather than on a fixed monthly design. On average, we sampled 2.3 ± 1.1 natural pools and 2.1 ± 0.9 artificial pools per month (see Table S1 for the number of sites sampled per month in each habitat type). No natural pools yielded records of *A. peruibensis* in December. Sampling effort was standardized at 15 minutes per site, with two researchers using hand nets (50 × 40 cm, 1-mm mesh), following protocols previously applied in aquatic temporary habitats of the region (Costa *et al.,* 2024, 2025a). A given pool (natural or artificial), or its flooded area, was only re-sampled in a subsequent month if it had completely dried and/or been newly re-flooded, reconnecting the flooded plain. Under these conditions, the area was considered to have undergone full recolonization, and was therefore treated as a new sampling site within our dataset, preventing carry-over effects of previous sampling on site-abundance patterns.

To characterize environmental differences between natural and artificial pools, physical and chemical variables (pH, water temperature (°C), dissolved oxygen (mg/L), conductivity (µS/cm) and total dissolved solids (mg/L) were measured during sampling using a multiparameter probe (Hanna HI98194). Pool volume (m^3) was estimated assuming a half-ellipsoid shape using the formula: $V = 2/3 \times \pi \times a \times b \times c$, where a is maximum length, b is maximum width, and c is maximum depth.

The sampled specimens were anesthetized and euthanized with eugenol, fixed in 10% formalin for 48 hours, and preserved in 70% ethanol. Selected specimens were deposited in the São José do Rio Preto Fish Collection of UNESP – DZSJRP (DZSJRP 25056). All procedures were approved by the Ethics Committee on the Use of Animals of the São Paulo State University "Júlio de Mesquita Filho" - Litoral Campus (CEUA - IB/CLP nº 15/2023), and sampling was carried out under SISBIO licenses 90241-1 and 90241-2.

Laboratory procedures

In the laboratory each individual had its standard length (SL, in mm) measured using an ichthyometer, and its total weight (WT, in g) recorded on an analytical balance. After the biometric measurements, the specimens were eviscerated to confirm sex. The gonads were removed, weighed (in g, precision: 0.0001 g) and macroscopically evaluated for the stage of gonadal maturation, following criteria proposed by Vazzoler (1996).

To complement the macroscopic analysis, microscopic analysis of the gonads was performed. A total of 40 gonads (19 females and 21 males), previously classified macroscopically into different stages of development, were submitted to routine histological analysis. The material was dehydrated in an increasing sequence of ethanol and infiltrated with historesin (Leica), sectioned to 5 µm thickness slices and stained with Harris hematoxylin and eosin (HE) (Junqueira Junqueira, 1983). The microscopic definition of the maturation phases was based on the presence and frequency of the different cell types of the oogenic and spermatogenic lineages, following the proposal of Brown-Peterson *et al.* (2011).

Reproductive activity was analyzed using gonads from mature females, which were stored in Gilson's solution to promote the detachment of epithelial and ovarian follicles from the oocytes. Subsequently, the oocytes were subjected to three washes in 70% alcohol to eliminate tissue residues and were kept in the same solution. The oocyte mass from each female was then transferred to a grid plate, applying mechanical dissociation in cases where the ovaries were not fully dissociated. The diameters of all oocytes were measured, including oocytes reserve and vitellogenic oocytes, the latter characterized by larger size and yellowish coloration. All steps were performed under a stereomicroscope with a micrometric eyepiece, using lower magnifications for counting, and a 10× eyepiece with 4× magnification for measurements.

The microanatomy of the chorion was analyzed with the aim of inferring possible structural adaptations related to adhesion, protection and development of eggs, which could be crucial for survival in both natural and artificial temporary environments. For this purpose, mature oocytes were subjected to scanning electron microscopy (Phenom ProX, Thermo Scientific) at the Multiuser Ultrastructure Laboratory of the Federal Rural University of Rio de Janeiro. The comparison of the observed structures for *A. peruibensis* followed the approach of Thompson *et al.* (2017).

Data analysis

Environmental (physicochemical and structural) variables were summarized using a Principal Component Analysis (PCA) in order to characterize and visualize patterns of environmental variation between the two habitat types, with the aid of the *FactoMineR* R package (Lê et *al*., 2008). The analysis also incorporated the comparison between dry and wet periods within each habitat type, following the rainfall-based seasonal classification proposed by Costa *et al*. (2025a). Differences in multivariate environmental conditions between habitat types and hydrological periods were formally tested using a Permutational Multivariate Analysis of Variance (PERMANOVA), while differences in within-group environmental heterogeneity were evaluated using a Permutational Analysis of Multivariate Dispersions (PERMDISP), both implemented with the *vegan* R package (Oksanen et *al*., 2025).

Sex ratio was evaluated separately for natural and artificial pools, considering all individuals sampled in each habitat type. Observed sex ratios were compared with the expected 1:1 proportion using a Chi-square test (χ^2). Reproductive activity of *A. peruibensis* was assessed using the Gonadosomatic Index (GSI), calculated as GSI = (*Wg* / *Wt*) × 100, where *Wg* is gonad weight (g) and *Wt* is total body weight (g). Analyses were conducted separately for males and females. For each sex, we evaluated whether the GSI varied between habitat types and among months using generalized linear mixed models (GLMMs) with a Gamma error distribution and a log link function. Habitat type (natural vs. artificial pools) and month of sampling (treated as a categorical factor) were included as fixed effects. Sampling point was included as a random intercept to account for repeated temporal observations at some of the sites. We initially tested models including an interaction between habitat and month, however, interaction terms did not significantly improve model fit and resulted in rank-deficient parameters due to unbalanced sampling across habitat-month combinations. Model comparisons based on likelihood ratio tests and Akaike's Information Criterion (AIC) indicated that additive models were equally or more parsimonious than interaction models ($\Delta AIC < 2$ for both sexes). Therefore, we retained additive models to test whether GSI values differed overall between habitat types and whether temporal variation followed general seasonal patterns within habitats, rather than focusing on month-specific differences between habitats. Estimated marginal means and pairwise comparisons among habitat types and months were obtained from the selected models

using the *emmeans* package in R (Lenth, 2025), with Tukey-adjusted p-values for multiple comparisons.

Model assumptions were evaluated using simulation-based residual diagnostics implemented in the *DHARMa* package (Hartig, 2024). For both sexes, scaled residuals were generated from 1,000 simulations and inspected using quantile-quantile plots and residuals plotted against fitted values and predictors. Formal tests indicated no significant deviations from uniformity (Kolmogorov-Smirnov test; females: $p = 0.14$; males: $p = 0.95$), no evidence of over- or underdispersion (females: $p = 0.76$; males: $p = 0.50$), and no excess of outliers (females: $p = 0.18$; males: $p = 1.00$). Residuals were homogeneous across habitat types and months, and no systematic patterns were detected across fitted values. Minor deviations detected within some random-effect levels were attributed to low sample sizes within certain sampling points and are expected in mixed models with unbalanced repeated measures. Overall, residual diagnostics supported the adequacy of the selected GLMMs for both females and males. Generalized linear models were implemented with the *glmmTMB* R packages (Brooks et *al*., 2017).

The standard length at which 50% of individuals were sexually mature (L_{50}) and the length at which 100% of individuals were mature (L_{100}) were estimated using logistic regression, by fitting a binomial model relating the probability of sexual maturity to standard length (mm). Estimates were calculated separately for males and females and for each habitat type, using 2-mm length classes. Uncertainty around L_{50} and L_{100} estimates was quantified using nonparametric bootstrap resampling (5,000 iterations), from which 95% confidence intervals were derived using the percentile method.

Fecundity of *A. peruibensis* was assessed separately for each habitat type, based on the analysis of 37 mature gonads from artificial pools and 16 from natural pools. Total fecundity was calculated as the total number of oocytes per female, regardless of oocyte developmental stage. The fecundity of the most advanced modal group, corresponding to the batch of oocytes available for imminent spawning, was estimated from the group of largest oocytes identified using a Gaussian mixture model fitted to the distribution of oocyte diameters, with the aid of the *mclust* R package (Scrucca *et al*., 2023). The separation point between oocyte groups was defined by the intersection of the fitted component distributions for each habitat type, allowing an objective delimitation of the reproductive batch.

Differences in batch fecundity between habitat types were tested using a generalized linear model with a negative binomial error distribution and a log link function, including habitat type as a fixed effect and the logarithm of standard length (mm) as an offset. This formulation allowed fecundity to be interpreted on a per-unit-length basis, effectively controlling for differences in female size while modeling count data with overdispersion. To investigate whether habitat type also influenced the size of mature oocytes independently of female body size, we analyzed the diameter of individual oocytes retained after the cutoff procedure using a Gaussian generalized linear mixed model. Habitat type was included as a fixed effect, the logarithm of standard length (mm) was incorporated as an offset, and female identity was included as a random intercept to account for the non-independence of multiple oocytes measured from the same individual. This approach allowed us to test whether mean oocyte diameter differed between environments after accounting for body size and repeated measurements within females. Model assumptions for both analyses were evaluated using simulation-based residual diagnostics implemented in the *DHARMa* package. For the batch fecundity model, dispersion and zero-inflation tests indicated no significant deviations from model assumptions. For the oocyte diameter model, residual diagnostics showed no evidence of over or underdispersion and no excess of outliers. Although uniformity tests detected deviations from the expected residual distribution, these were interpreted cautiously given the large number of observations and the absence of other diagnostic issues. Overall, diagnostic results supported the adequacy of the selected models for inference. Spawning type was determined from the full set of 53 mature gonads, based on the frequency distribution of oocyte diameters, allowing the identification of one, two, or more modal groups (Heins, Brown-Peterson, 2022). All analyses were performed in R version 4.5.1.

3. RESULTS

Environmental variables varied between hydrological periods and habitats (Tab. S2). In general, artificial pools exhibited higher values of conductivity, total dissolved solids, temperature, volume, and pH compared to natural pools in both dry and wet seasons. Dissolved oxygen was lower during the dry season in both environments and increased during the wet season.

The PCA revealed a clear separation between the sampled periods, but with habitats overlapping. However, natural pools were characterized by lower values of

volume and pH compared to artificial pools, especially during the dry season (Fig. S3). This visual pattern supports the PERMANOVA, which indicated significant differences in environmental conditions between habitat types ($F = 7.84$, $R^2 = 0.12$, $p = 0.001$) and between hydrological periods ($F = 12.70$, $R^2 = 0.19$, $p = 0.001$), while the interaction between habitat and period was not significant ($F = 0.48$, $p = 0.731$). Tests of multivariate dispersion revealed differences in within-habitat environmental heterogeneity (PERMDISP: $F = 5.75$, $p = 0.02$), indicating that PERMANOVA results reflect both shifts in environmental centroids and differences in variability among habitats.

A total of 260 individuals were examined, comprising 157 females (52 from natural pools and 105 from artificial pools) and 103 males (50 from natural pools and 53 from artificial pools). Body size varied between habitats and sexes. In natural pools, females ranged from 18 to 39.5 mm standard length (SL), with a mean of 29.2 mm (SD = 4.7 mm), whereas males ranged from 19 to 44 mm SL, with a mean of 29.8 mm (SD = 5.9 mm). In artificial pools, females showed a wider size range, from 10 to 44 mm SL, with a mean of 24.2 mm (SD = 7.8 mm), while males ranged from 18 to 40.0 mm SL, with a mean of 26.9 mm (SD = 5.8 mm). Sex ratio differed between habitats: it did not deviate from 1:1 in natural pools ($\chi^2 = 0.039$, $p = 0.843$), whereas artificial pools showed a predominance of females 1:1.98 ($\chi^2 = 17.114$, $p < 0.001$).

Abundance patterns differed between habitat types. In natural pools, abundance fluctuated a little across months, subtly following rainfall patterns. Females were present in most months, showing a slight peak in January and September, but were absent in May, November and December. Males, in turn, reached their highest abundance in February and were also absent in December (Fig. 3). In contrast, abundance varied more markedly and without following rainfall patterns in artificial pools. Female abundance fluctuated over the year, with peaks in January, July and October. Males were less abundant than females and showed a more even distribution across months, with a pronounced peak in January, in February and May only males were sampled (Fig. 3). The macroscopic gonadal maturation class-abundance by sex, month and habitat type is shown in Tab. S3. The frequency of mature individuals was high between January and March in natural pools, whereas in artificial pools mature individuals were recorded throughout most of the year (Tab. S4).

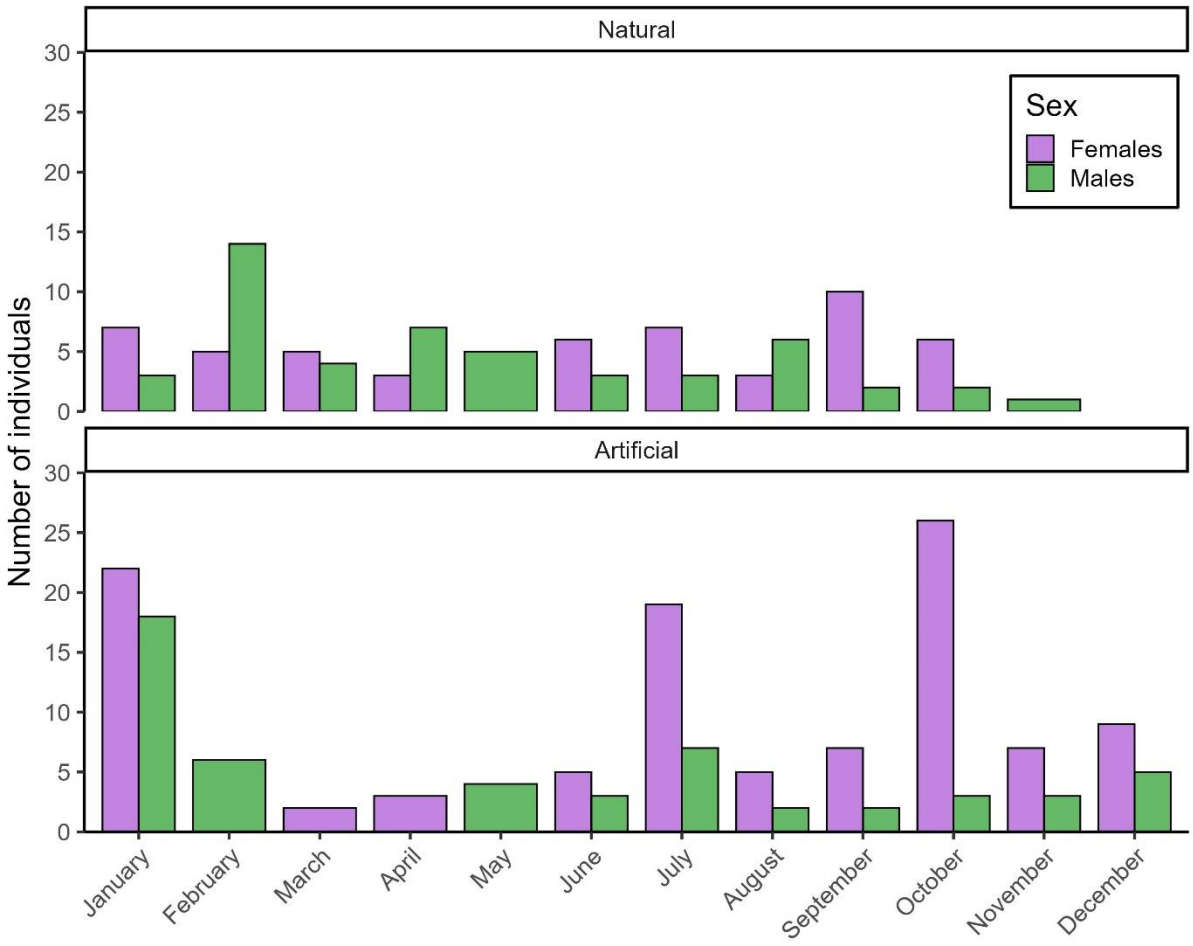

Figure 3. Monthly abundance of females and males of *Atlantirivulus peruibensis* sampled from January to December 2024 in natural and artificial temporary pools in the Rio Preto sub-basin, Itanhaém, São Paulo, Brazil.

Regarding the gonadosomatic index (GSI), generalized linear mixed models revealed clear differences between sexes. For females, GSI varied significantly between habitat types and across months. Females inhabiting artificial pools exhibited higher GSI values than those from natural pools (GLMM, estimate = 0.57 ± 0.29 SE, z = 2.01, p = 0.044), a pattern corroborated by estimated marginal means averaged across months (emmeans on the log scale: natural = 0.77 [95% CI: 0.36–1.18], artificial = 1.34 [0.95–1.74]). Temporal variation was also evident, with significantly lower GSI values observed during mid-year months, particularly April, June, July, and August, relative to January (all $p < 0.05$), indicating a seasonal decline in female reproductive investment. The random intercept associated with sampling point explained a moderate proportion of variance ($\sigma^2 = 0.27$; SD = 0.52), with consistent site-level differences in female GSI across repeated temporal observations.

For males, GSI showed a weaker response to habitat type and limited temporal structuring. Males from artificial pools tended to exhibit higher GSI values than those from natural pools, however this effect was marginally significant (estimate = 0.45 ± 0.23 SE, z = 1.94, p = 0.052; emmeans contrast: natural – artificial = −0.45). Monthly variation was generally low, with only November differing significantly from the reference month (January), showing higher GSI values (p = 0.047). In contrast to females, the random effect of sampling point accounted for negligible variance in male GSI ($\sigma^2 \approx 0$), suggesting limited site-level heterogeneity in male reproductive investment. Model comparisons supported additive models without interaction between habitat and month for both sexes (ΔAIC < 2). These results indicate that female GSI is structured by both habitat type and seasonal dynamics, whereas male GSI exhibits weaker habitat and temporal variation (Fig. 4).

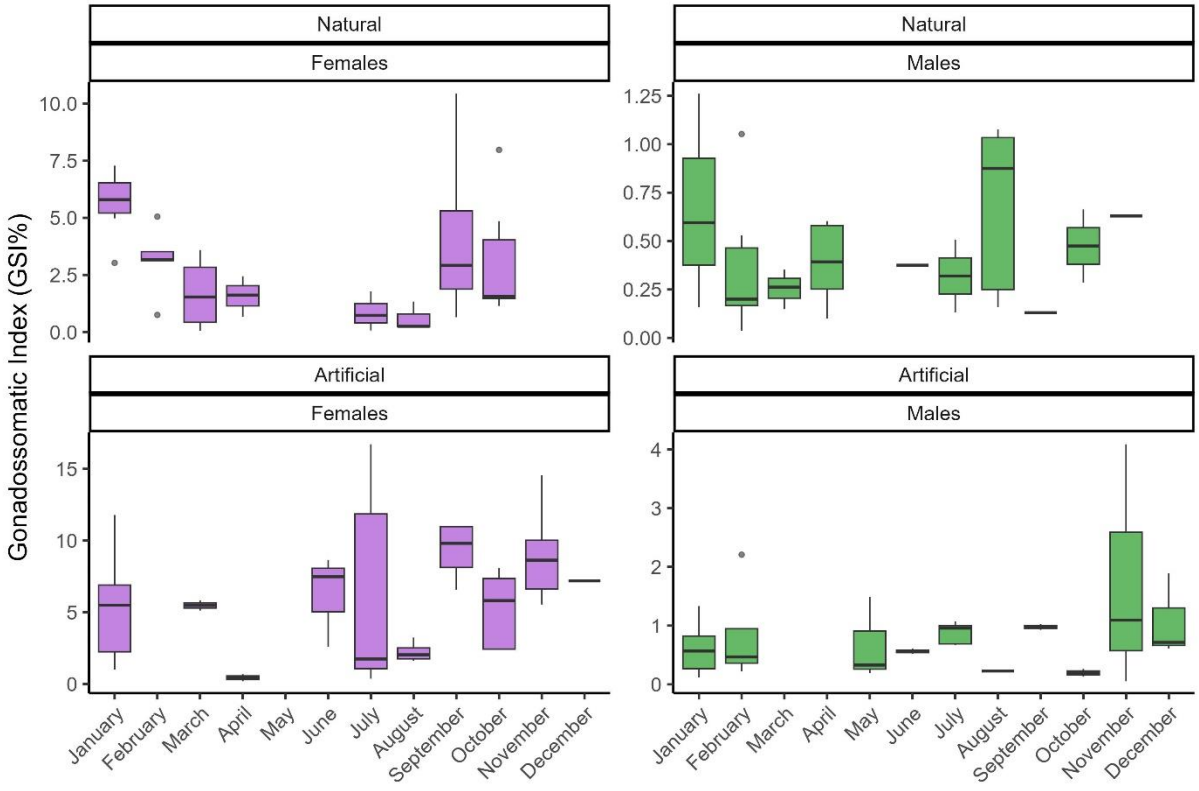

Figure 4. Monthly variation in Gonadosomatic Index (GSI%), for females and males of *Atlantirivulus peruibensis* sampled in natural and artificial temporary pools throughout 2024.

Size at sexual maturity could only be estimated for females, since male data did not adequately fit the logistic model due to the wide overlap in body size between immature and mature individuals (Fig. S5). In natural pools, females reached 50% maturity (L_{50}) at 31.74 mm SL (95% CI: 29.52–37.73 mm) and near-complete maturity (L_{100}) at 52.14 mm SL (95% CI: 43.07–88.29 mm). In contrast, females from artificial pools matured at smaller sizes (Fig. 5), reaching L_{50} at 27.79 mm SL (95% CI: 26.34–30.43 mm) and L_{100} at 44.41 mm SL (95% CI: 38.39–52.01 mm).

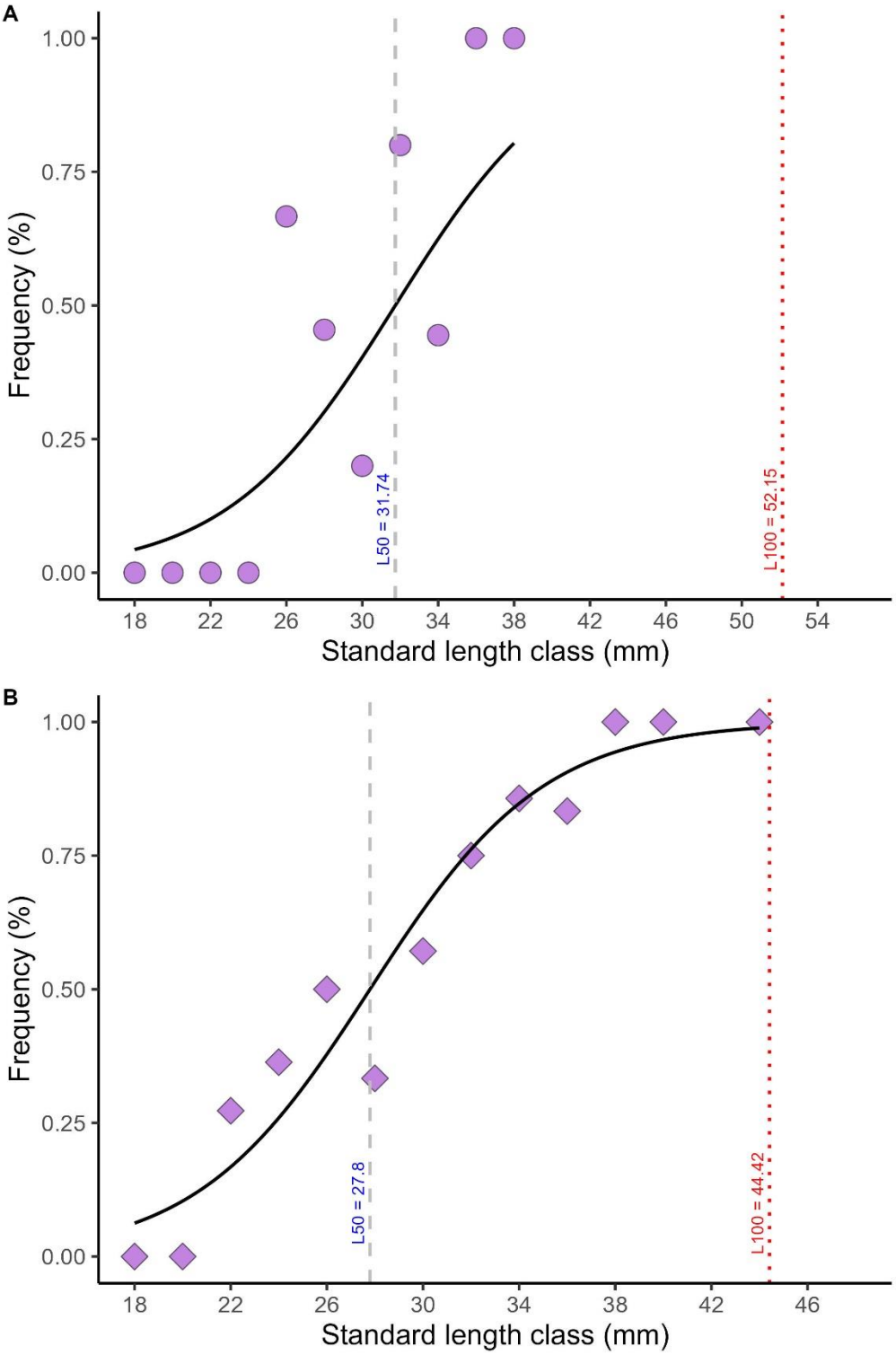

Figure 5. Logistic regression curves estimating the standard length at which 50% (L_{50}) and 100% (L_{100}) of females of *Atlantirivulus peruibensis* reach sexual maturity in (A) natural pools and (B) artificial pools. Points represent maturity proportions by 2 mm length classes, and solid lines indicate the fitted logistic models. Estimates are based on individuals sampled in temporary aquatic habitats of the Rio Preto sub-basin, Itanhaém, São Paulo, Brazil.

The total number of oocytes in mature females ranged from 5 to 112 in natural pools, with a mean of 42.8 ± 26.8 oocytes per female, whereas in artificial pools it ranged from 22 to 167, with a mean of 76.2 ± 42.6 oocytes per female. Oocyte diameter ranged from 0.125 to 1.88 mm in natural pools (mean = 0.56 ± 0.38 mm) and from 0.125 to 1.80 mm in artificial pools (mean = 0.67 ± 0.40 mm). The frequency distribution of oocyte diameters was bimodal in both environments, with one group of smaller, less-developed oocytes and another of larger, more-developed oocytes, indicating a batch-spawning pattern. The separation points between these groups, occurred at approximately 0.863 mm for females from natural pools and 0.780 mm for females from artificial pools (Fig. 6).

The fecundity of the vitellogenic batch ranged from 1 to 22 oocytes in natural pools (mean = 6.22 ± 5.26) and from 4 to 72 oocytes in artificial pools (mean = 20.2 ± 15.5). When accounting for female body size using a length-based offset, batch fecundity differed strongly between habitat types. Females from natural pools exhibited substantially lower batch fecundity per unit of body length than females from artificial pools (negative binomial GLM with offset, estimate = −1.11 ± 0.19 SE, $z = -5.73$, $p < 0.001$). The diameter of oocytes within the reproductive batch ranged from 0.87 to 1.87 mm in natural pools (mean = 1.42 ± 0.26 mm) and from 0.80 to 1.80 mm in artificial pools (mean = 1.25 ± 0.25 mm; Fig. 6). After accounting for female body size using a length-based offset, mean diameter of mature oocytes differed significantly between habitat types. Females from artificial pools produced smaller mature oocytes than females from natural pools (Gaussian GLMM with offset, estimate = −0.141 ± 0.059 SE, $z = -2.37$, $p = 0.018$), while the random intercept associated with individual identity accounted for a moderate proportion of variance ($\sigma^2 = 0.030$; SD = 0.17). Oocytes at advanced developmental stages exhibited a chorion composed of a network of thin, long filaments with rounded, perforated protrusions (Fig. 7).

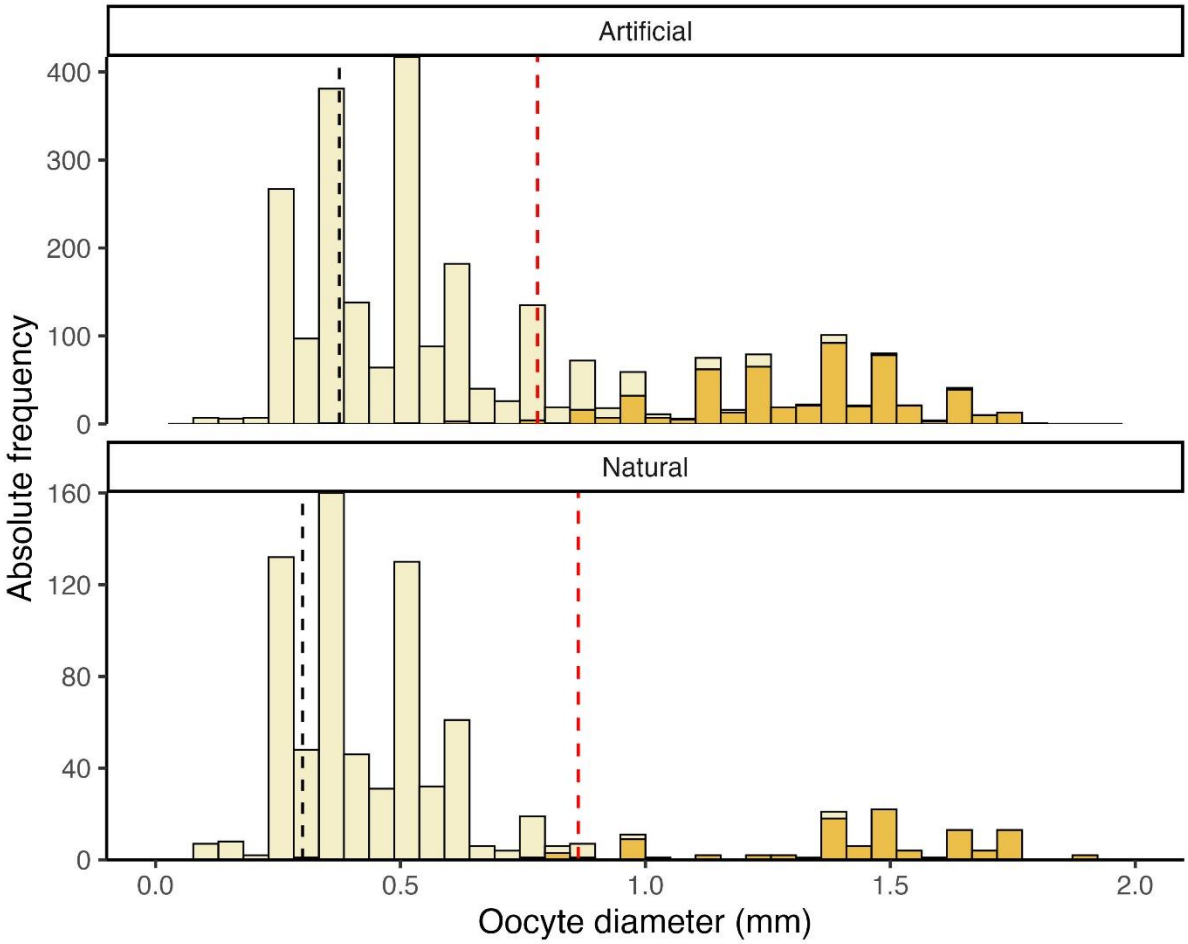

Figure 6. Frequency distribution of oocyte diameters of *Atlantirivulus peruibensis* females from natural and artificial temporary pools in the Rio Preto sub-basin, Itanhaém, São Paulo, Brazil. Light yellow bars represent previtellogenic oocytes, whereas yellow bars correspond to vitellogenic oocytes. The black dashed line indicates the minimum diameter of vitellogenic oocytes, and the red dashed line represents the lower size limit of the reproductive batch.

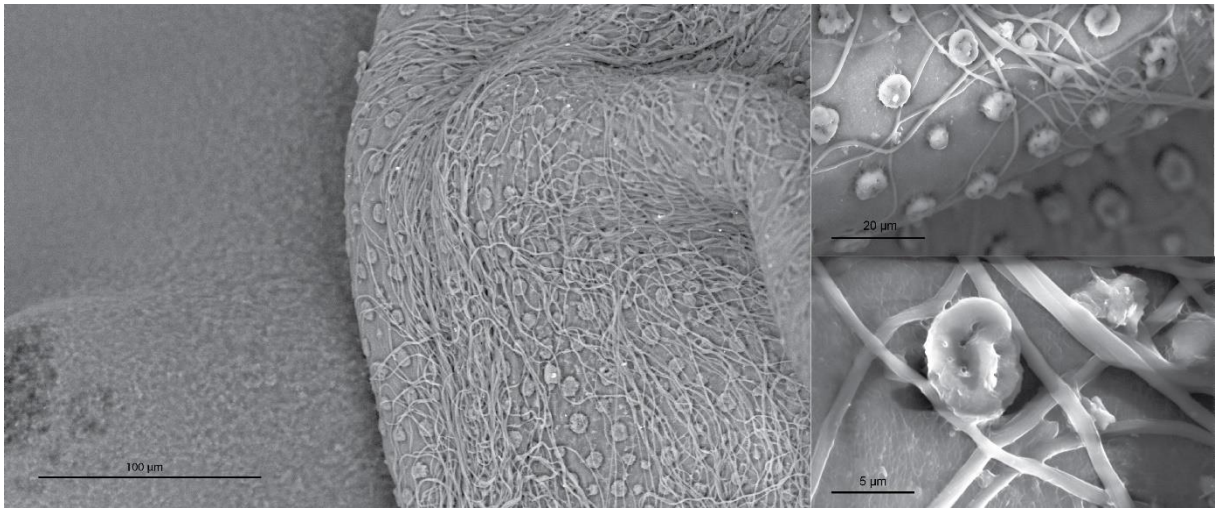

Figure 7. Micrograph of the chorion of mature and unfertilized eggs of *Atlantirivulus peruibensis*, showing numerous thin and long filaments, as well as rounded projections with small perforations.

Histological analyses confirmed the patterns previously identified through macroscopic classification of female gonads. Ovaries classified as immature were characterized exclusively by the presence of oogonia and primary-growth oocytes, with reduced intercellular space and absence of associated muscle tissue (Fig. 8A–B). Developing ovaries were identified by the presence of primary and secondary vitellogenic oocytes, without tertiary vitellogenic oocytes, mature oocytes, or post-ovulatory follicles (Fig. 8C–D). Females classified as mature exhibited enlarged ovaries containing tertiary vitellogenic oocytes, mature oocytes, and post-ovulatory follicles (Fig. 8E–F). In contrast, histological examination of males revealed evidence of spermatogenic activity even in individuals macroscopically classified as immature or developing. Early maturation stages were characterized by a continuous germinal epithelium with spermatogonia located at the periphery and the presence of primary spermatocytes, spermatids, and spermatozoa within the lumen (Fig. 9A). Intermediate stages exhibited a discontinuous epithelium near the sperm ducts and the presence of secondary spermatocytes (Fig. 9B–C). In more advanced stages, testes displayed a discontinuous germinal epithelium throughout their length, anastomosed lobules, and reduced abundance of spermatogonia (Fig. 9D). Regressing and regenerating testicles showed patterns consistent with post-spawning depletion followed by the resumption of spermatogenesis, as indicated by the reappearance of spermatogonia, spermatocytes and spermatids (Fig. 9D–H).

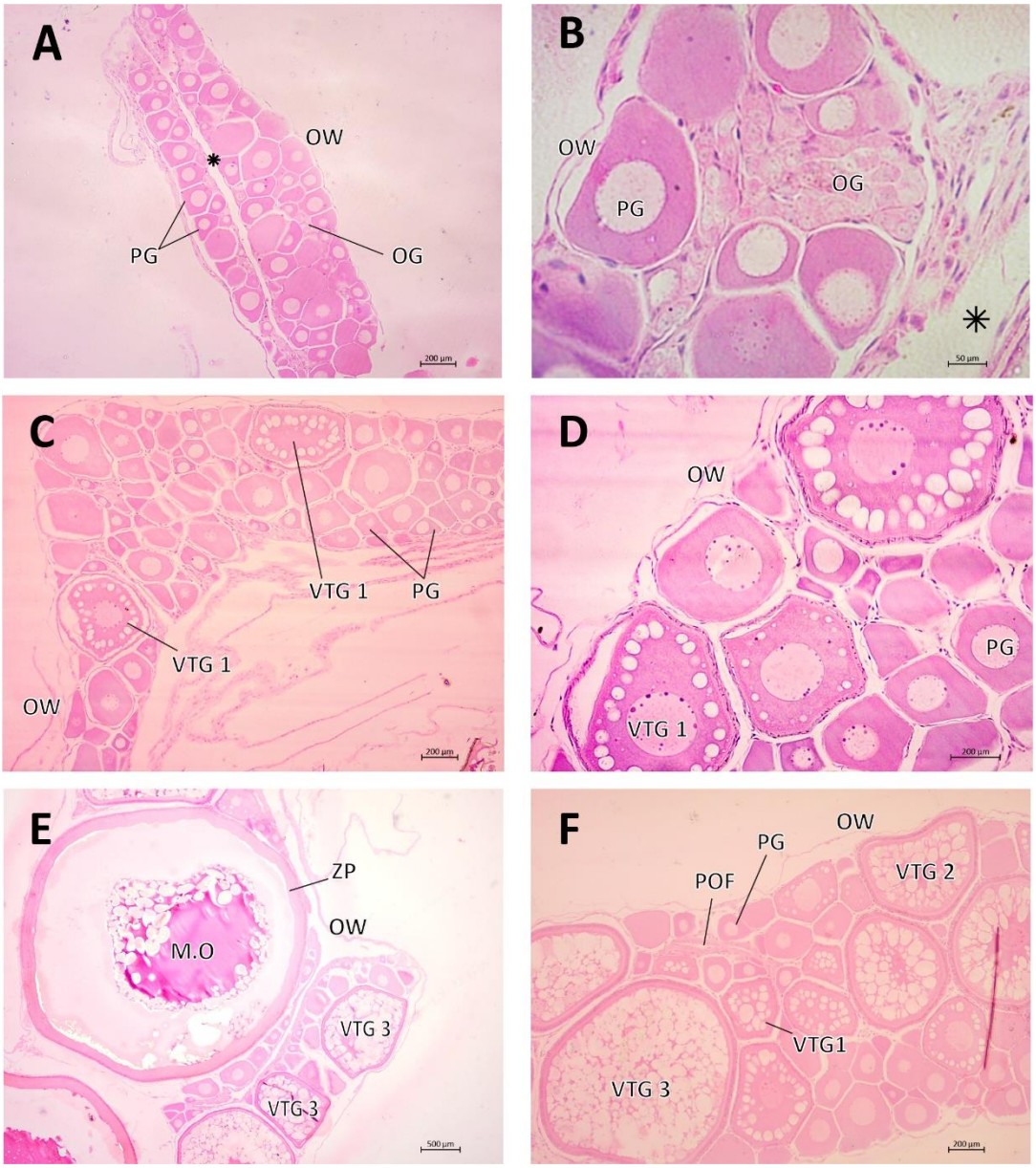

Figure 8. Histological sections of the ovaries of *Atlantirivulus peruibensis* stained with hematoxylin and eosin (HE), illustrating the different stages of oogenesis. Immature ovaries (A–B): thin ovarian wall (OW), reduced intercellular space, and the exclusive presence of oogonia (OG) and primary-growth oocytes (PG). Developing ovaries (C–D): onset of primary vitellogenic oocytes (VTG1). Spawning-capable ovaries (E–F): predominance of secondary (VTG2) and tertiary vitellogenic oocytes (VTG3), together with post-ovulatory follicles (POF). Mature oocytes (M.O) exhibit a well-developed zona pellucida (ZP). Asterisk = lumen.

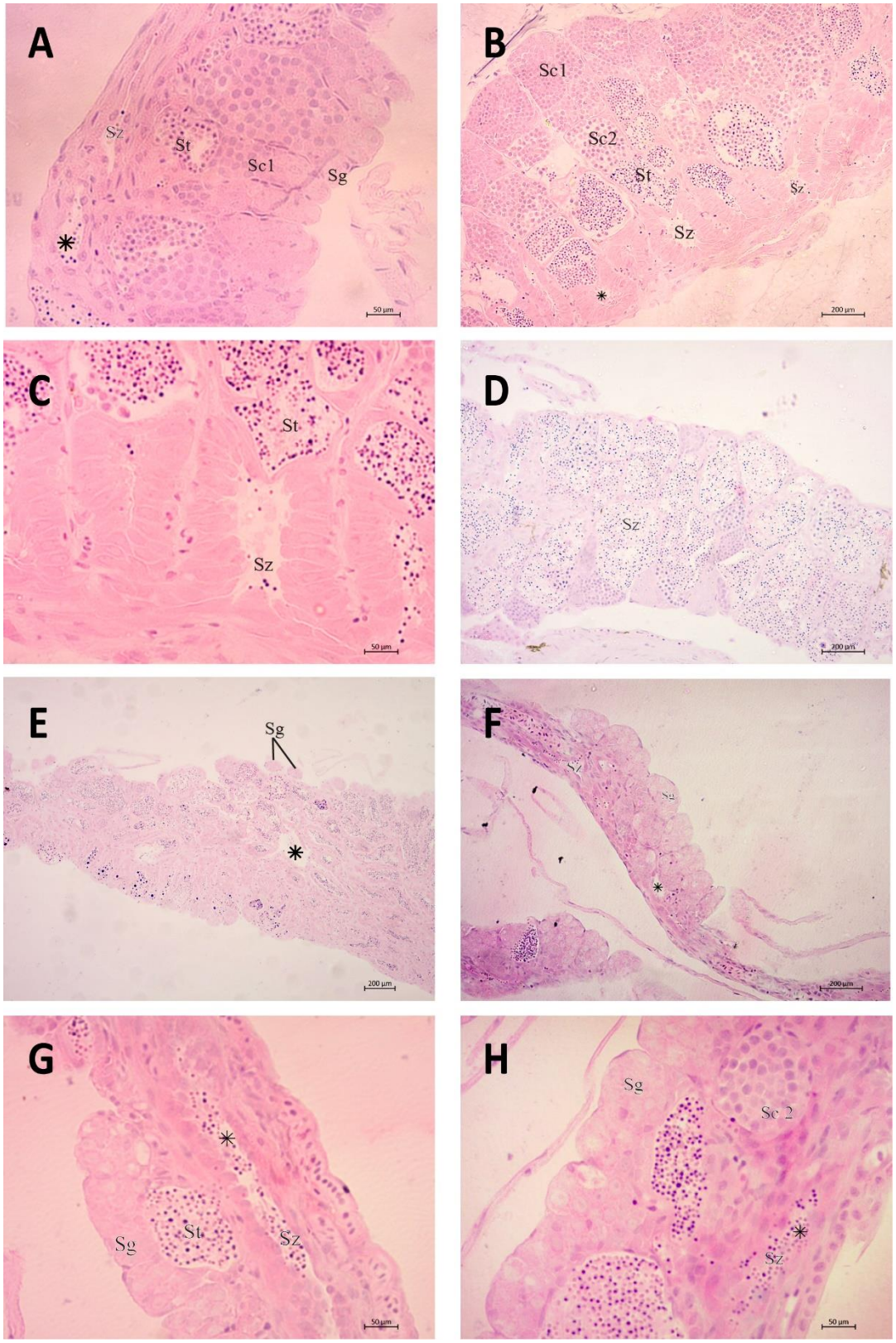

Figure 9. Histological sections of testes of *Atlantirivulus peruibensis* stained with hematoxylin and eosin (HE), illustrating the stages of the reproductive cycle: spermiating (A–D), regression (E), and regeneration (F–H). In early maturation stages

(A), the germinal epithelium is continuous, with spermatogonia (Sg) arranged in cysts, primary spermatocytes (Sc1), and spermatids (St); the lumen contains some spermatozoa (Sz). In intermediate stages (B–C), spermatogenesis is partially interrupted in lobules adjacent to the sperm ducts, and the germinal epithelium becomes discontinuous, the formation of secondary spermatocytes (Sc2) occurs. In advanced spermiating stages (D), the epithelium is widely discontinuous, with anastomosed lobules and a reduced abundance of spermatogonia. During regression (E), spermatozoa are depleted, spermatocytes and spermatids decrease, and spermatogonia reappear along the periphery. In the regeneration phase (F–H), spermatogonia proliferate throughout the testis; residual spermatozoa may persist in the lumen, and, in more advanced phases, primary spermatocytes (Sc1) and spermatids (St reappear. Asterisk = lumen of the sperm ducts.

4. DISCUSSION

In this study, we investigated how the reproduction of *A. peruibensis*, a non-annual rivulid associated with temporary pools, responds to the occupation of artificial temporary environments with hydrological and physicochemical characteristics distinct from its natural habitat. The results showed that the species modulates its reproductive activity in the new environmental conditions, adjusting key parameters of its life history. These adjustments included maturation at smaller sizes, prolongation of reproductive activity throughout the year and greater investment in fecundity at the expense of the average size of oocytes, evidencing a certain degree of plasticity in the species.

The abundance of *A. peruibensis* fluctuated in the artificial pools, with peaks in January, July and October, while it remained relatively close to rainfall patterns in the natural pools. This variation may be related to increased connectivity between pools, as a consequence of the high volume of rainfall in January and October (Ruiz *et al.,* 2014; Abrial *et al.,* 2019), and to the role of artificial pools as refuges during drier periods, due to their greater depth and capacity to store larger volumes of water (DeAngelis *et al.,* 2010; Gatto, Trexler, 2019), resulting in delayed dying. However, the abundance results presented here serve more to clearly show the number of individuals sampled per month for reproductive analyses, and do not serve as a concrete parameter for the variation in abundance of the species, since in this study we only considered sites with the presence of the species (i.e., its absence is poorly

represented), and also regionally clustered sites, as the aim of the work is to answer life history shifts and not abundance changes or their drivers.

The sex ratio also differed between environments. In natural pools, a balanced ratio (1:1) was observed, similar to that described for other Rivulidae (Laufer *et al.,* 2009; Lanés *et al.,* 2014; Cavalheiro, Fialho, 2015). On the other hand, artificial pools showed a predominance of females. Deviations in the sex ratio may result from environmental differences (Lanés *et al.,* 2012; Gonçalves *et al.,* 2011), variation in birth and mortality rates (Nomi *et al.,* 2018), selective predation (Reichard *et al.,* 2014; López-Rodríguez *et al.,* 2021), and spatial segregation between the sexes (Pettersson *et al.,* 2004; McKellar *et al.,* 2009; Trouchet *et al.,* 2016). In the present study, the predominance of females in artificial pools may reflect the greater tolerance or competitiveness of females in the face of new and probably more adverse conditions in these environments, including the presence of other species and potential predators due to larger water volume in ditches (Costa *et al.,* 2025a), while males possibly avoid competitive situations, as observed for another Amazonian rivulid (Espírito-Santo *et al.,* 2019).

The reproductive activity of *A. peruibensis* was more intense and continuous in individuals from artificial pools, reflected by the higher values observed for the gonadosomatic index compared to natural pools. This pattern suggests that artificial environments can provide favorable conditions for reproductive investment throughout the year. Factors such as variations in temperature and water availability constitute important environmental signals capable of triggering endocrine mechanisms and stimulating reproductive investment in fish, especially in environments subject to seasonal droughts (Lanés *et al.,* 2016; Podrabsky *et al.,* 2016; Grégoir *et al.,* 2017; Oliveira *et al.,* 2023). Thus, the greater hydrological stability and the prolongation of the aquatic phase in artificial pools seem to favor the extension of reproductive activity throughout the year. This pattern was similar to that observed in *Melanorivulus rossoi* (Costa, 2005), a species associated with locations subject to drying and which exhibits constant gametogenesis in both sexes (Cassel *et al.,* 2023), but contrasts with *Melanorivulus* aff. *punctatus* (Boulenger, 1895) (Cassel *et al.,* 2013), a species from a perennial environment, in which females exhibit reproductive activity restricted to shorter periods of the year, despite the continuous availability of water.

The reproductive seasonality of females was more pronounced in natural pools, as expected by our hypothesis, where water fluctuations are more intense and restrict

reproduction to periods of higher rainfall, a pattern consistent with that found for *Atlantirivulus riograndensis* (Cavalheiro, Fialho, 2015). Reproductive activity is more intense during periods of higher rainfall, when resources are more abundant and may ensure greater fish survival (Williams, 1987; Whittier, Crews, 1987; Gatto, Texler, 2019). In contrast, no evident reproductive seasonality was observed in artificial pools, possibly due to greater water stability throughout the year, which directly reduces the dependence on rainfall for reproduction. For males, the temporal effect was not significant, which is consistent with the fact that in fish, male gametogenesis is generally less sensitive to environmental variables, since reproduction is less costly compared to females (Souza *et al.,* 2015; López-Rodríguez *et al.,* 2021; Oliveira *et al.,* 2023), a result corroborated in this study by the observation of continuous gametogenesis in histological analyses.

The size at first maturity also differed between females from natural and artificial pools. Females from artificial pools were observed to mature at smaller sizes compared to females from natural pools, indicating that distinct environmental conditions and cues regulate this trait. Changes in this trait in response to environmental alterations have been observed in Rivulidae (García *et al*., 2019) and in other groups (Thorpe, 2007; Watson *et al*., 2013; Nunes *et al.,* 2024), and constitute a fundamental part of maintaining fitness (Stearns, 1986). In species from temporary environments, reproductive activity is strongly regulated by water availability (Arenzon *et al.,* 1999; Cassel *et al.,* 2013; Cavalheiro, Fialho, 2015), and the risk of desiccation is the dominant predictor of phenotypic responses (Grégoir *et al.,* 2017). Thus, the observed pattern also reflects differences in the duration and stability of the aquatic phase between environments, where, females in artificial pools appear to be able to detect more stable water availability and invest rapidly in reproduction, a typical tactic of opportunistic species that depend on the rapid recolonization of favorable environments to maximize fitness (Winemiller, 1989).

For males, the L_{50} and L_{100} estimates were unreliable, likely due to the wide size variation among mature individuals and the absence of a clear boundary between immature and mature stages, in addition to the rapid advancement of gametogenesis observed in histological analyses. This pattern is consistent with the phenotypic plasticity reported for species in temporary environments (Blažek *et al.,* 2013; Vrtílek *et al.,* 2018). Besides plasticity, growth in fish is also a labile trait influenced by local conditions, such as resource availability and population density (Vrtílek *et al.,* 2019);

in this sense, environmental heterogeneity among the pools may have accentuated the size variation among mature males. In addition, the fact that reproduction is less costly for males (Cassel *et al.,* 2013) means that, in this scenario, maturation even at reduced sizes can be viable in temporary environments, allowing for the rapid exploitation of favorable reproductive windows. However, it cannot be ruled out that the small sample size of males within each habitat type contributed to the imprecision of the estimates, and future sampling with a larger sample size would be necessary to confirm this pattern.

In addition to the timing of maturation, the spawning pattern also highlights the opportunistic nature of the reproductive strategy of *A. peruibensis*. The bimodal distribution of oocyte diameter indicates the presence of two cohorts of developing oocytes, consistent with a batch spawing in both habitat types. This batch spawning pattern has also been observed in other rivulids, such as *Leptopanchax opalescens* (Myers, 1942) (Guedes *et al.,* 2023), *A. riograndensis* (Cavalheiro, Fialho, 2015), and *M.* aff. *punctatus* (Cassel *et al.,* 2013). Batch spawning is frequently observed in fish with opportunistic reproductive strategies (Winemiller, 1989; Winemiller, Rose, 1992), as seems to be the case for *A. peruibensis* in both natural and new artificial environments. This reproductive tactic may confer adaptive advantages by reducing intraspecific competition among juveniles; minimizing the risk of brood loss in stochastic events such as sudden droughts; increasing offspring's genetic variability; and decreasing adult mortality, since the effort is distributed over time, avoiding metabolic depletion that could lead to post-reproductive death, as occurs in annual rivulids (Godinho *et al.,* 2010; Hočevar *et al.,* 2021).

Fecundity and egg size are strongly influenced by environmental conditions, such as resource availability and environmental predictability, which modulate energy allocation for reproduction (Hutchings, 1991; Jobling, 1993; Winemiller, 2005). In natural pools, which present greater temporal constraints, but are relatively predictable throughout the evolutionary history of the species, females showed lower fecundity associated with the production of larger eggs, a strategy that may increase offspring survival under conditions of water stress and resource limitation, since larger yolk reserves provide greater energy under adverse conditions (Winemiller, Rose, 1992; Duarte, Alcaraz, 1989). A similar pattern, in which restrictive environments favor lower fecundity and larger eggs, has been reported, for example, in *Salmo salar* Linnaeus, 1758, where egg size was larger in poorer quality environments (Rollinson, Hutchings,

2013). On the other hand, in artificial pools, where the aquatic phase is more prolonged and hydrologically more stable, females showed higher fecundity associated with smaller eggs, reflecting an opportunistic strategy based on the rapid colonization of favorable environments (Winemiller, 1989). Thus, although both environments are temporary, differences in hydrological predictability seem to act as a selective filter that modulates the reaction norms (Stearns, Koella, 1986) of the species, alternating between maximizing colonization in artificial pools and ensuring the individual survival of juveniles in natural pools.

Mature eggs of *A. peruibensis* exhibited a large number of long, thin filaments, as well as rounded and perforated structures distributed over the surface of the chorion. Similar structures have been described in other non-annual rivulids, such as *Anablepsoides hartii* (Boulenger, 1890), *A. urophthalmus* (Günther, 1866), and *A. rubrolineatus* (Fels & de Rham, 1981) (Thompson *et al.,* 2017). These characteristics are frequently interpreted as functional adaptations associated with survival in temporary environments and the amphibious habit of the species (Podrabsky *et al.,* 2010; Messaddeq *et al.,* 2018). The long, thin filaments act as anchors, promoting greater adhesion of the eggs, mainly to submerged vegetation, which prevents their dispersal during floods or burial in the sediment as the water volume decreases. This attachment may also provide additional protection for the offspring against predators, since the plants act as shelters for the eggs (Thompson *et al.,* 2017). As the volume of the pools decreases and environmental conditions become more severe, the likelihood of egg exposure to air increases. Thus, the perforated protuberances observed in the chorion appear to be related to facilitating gas exchange (Thompson *et al.,* 2017), suggesting that, although it is a non-annual rivulid, *A. peruibensis* has mechanisms that allow the eggs to tolerate brief periods of air exposure, probably in still humid substrates. This set of characteristics possibly helps the species' eggs to develop in both environments, one with a supposed higher risk of exposure to air (natural pools), and the other with a hypothetical higher risk of being carried away or found by predators (artificial pools).

This study provides pioneering evidence on how the occupation of artificial temporary environments influences the reproduction of non-annual fishes inhabiting ephemeral aquatic systems. Our results show that *A. peruibensis* displays pronounced reproductive phenotypic plasticity, adjusting key life-history traits in response to differences in both overall conditions of the aquatic environment (e.g. water quality,

predation pressure, food availability, competition) and the duration of the aquatic phase between natural and artificial pools. The species sustained reproductive activity through shifts in size at first maturity, reproductive timing, fecundity and per-offspring investment. These findings indicate that artificial environments can reshape reproductive tactics and that the plasticity observed in *A. peruibensis* likely represents an important mechanism for maintaining fitness under novel or altered hydrological conditions, an ability that may become increasingly relevant in scenarios of climate change, loss of natural habitats and the expansion of human-made waterbodies.

Despite these advances, important limitations remain. Because *A. peruibensis* is an amphibious killifish able of short-distance terrestrial movements, it remains unclear whether individuals actively select natural or artificial pools, or whether their occupancy is constrained by hydrological or landscape barriers. Accordingly, future studies incorporating mark-recapture or individual-tracking approaches will be essential to disentangle habitat selection from habitat constraint, and to evaluate how movement behavior, site fidelity and dispersal interact with reproductive plasticity in shaping long-term population dynamics. Furthermore, as our study is based on a single year of sampling, long-term investigations will be necessary to determine whether prolonged exposure to artificial pools, where seasonal regimes may be altered and additional stressors such as invasive species, roadside contaminants and chronic pollution are present, leads to cumulative, delayed, or irreversible changes in reproductive traits.

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6. SUPPLEMENTARY MATERIAL

Table S1. Number of sampling sites where *Atlantirivulus peruibensis* was sampled per month in natural and artificial temporary pools throughout 2024.

Month	Natural	Artificial
January	1	4
February	4	2
March	3	1
April	2	2
May	1	1
June	3	2
July	3	3
August	3	2
September	3	1
October	1	2
November	1	2
December	0	3
Total	25	25

Tabela S2. Mean (± standard deviation) of environmental variables measured in artificial and natural pools during the dry and wet periods. Variables include conductivity (µS/cm), dissolved oxygen (mg/L), total dissolved solids (mg/L), temperature (°C), volume (m^3), and pH.

	Dry Period		Wet Period	
Variable	**Artificial Pool**	**Natural Pool**	**Artificial Pool**	**Natural Pool**
Conductivity (µS/cm)	92.77 ± 38.96	65.88 ± 29.19	95.25 ± 39.92	61.22 ± 24.60
Dissolved oxygen (mg/L	0.35 ± 0.86	0.58 ± 1.09	2.23 ± 1.73	1.71 ± 1.28
Total dissolved solids (mg/L)	48.77 ± 20.42	34.44 ± 14.31	61 ± 25.28	45.33 ± 12.75
Temperature (ºC)	20.16 ± 2.92	19.39 ± 2.54	26.82 ± 2.84	25.92 ± 1.68
Volume (m^3)	17.18 ± 10.36	2.57 ± 4.01	56.77 ± 70.93	19.33 ± 26.63
pH	4.98 ± 1.16	3.86 ± 0.99	5.44 ± 0.80	4.65 ± 1.04

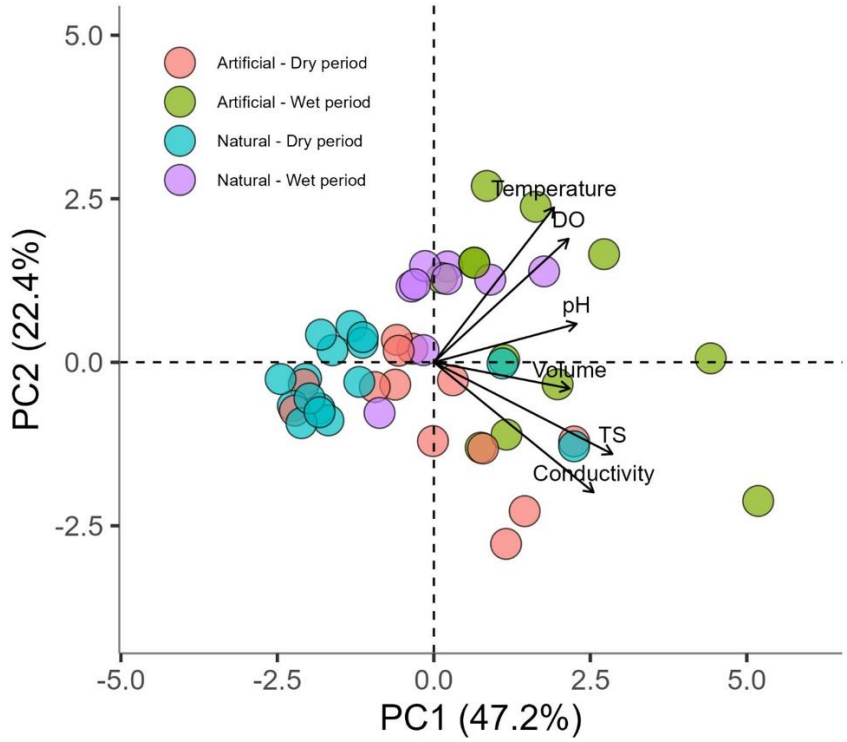

Figure S3. Principal Component Analysis (PCA) summarizing the environmental variation of natural and artificial temporary pools across wet and dry periods. Symbols represent individual sampling sites colored according to habitat type and hydrological period. Environmental vectors indicate the strength and direction of the variables contributing to the ordination.

Table S4. Absolute abundance of gonadal maturation stages of *Atlantirivulus peruibensis* by month sex and habitat (natural and artificial temporary pools). Maturation stages are classified as A = immature B = developing and C = mature F = females M = males.

Sex	Month	NATURAL POOLS			ARTIFICIAL POOLS		
		Maturation phase					
		A	**B**	**C**	**A**	**B**	**C**
F	January	0	1	6	6	5	11
M		0	1	2	7	5	6
F	February	0	3	2	0	0	0
M		0	1	13	1	2	3
F	March	0	2	3	0	0	2
M		1	1	2	0	0	0
F	April	0	2	1	1	2	0
M		0	7	0	0	0	0
F	May	0	0	0	0	0	0
M		0	4	1	0	2	2
F	June	6	0	0	1	2	2
M		2	1	0	0	2	1
F	July	0	6	1	6	7	6
M		0	2	1	1	4	2
F	August	0	3	0	1	4	0
M		0	5	1	0	0	2
F	September	3	3	4	3	0	4
M		1	0	1	0	0	2
F	October	0	4	2	20	3	3
M		0	1	1	0	1	2
F	November	0	0	0	1	0	6
M		0	0	1	0	2	1
F	December	0	0	0	6	1	2
M		0	0	0	0	5	0

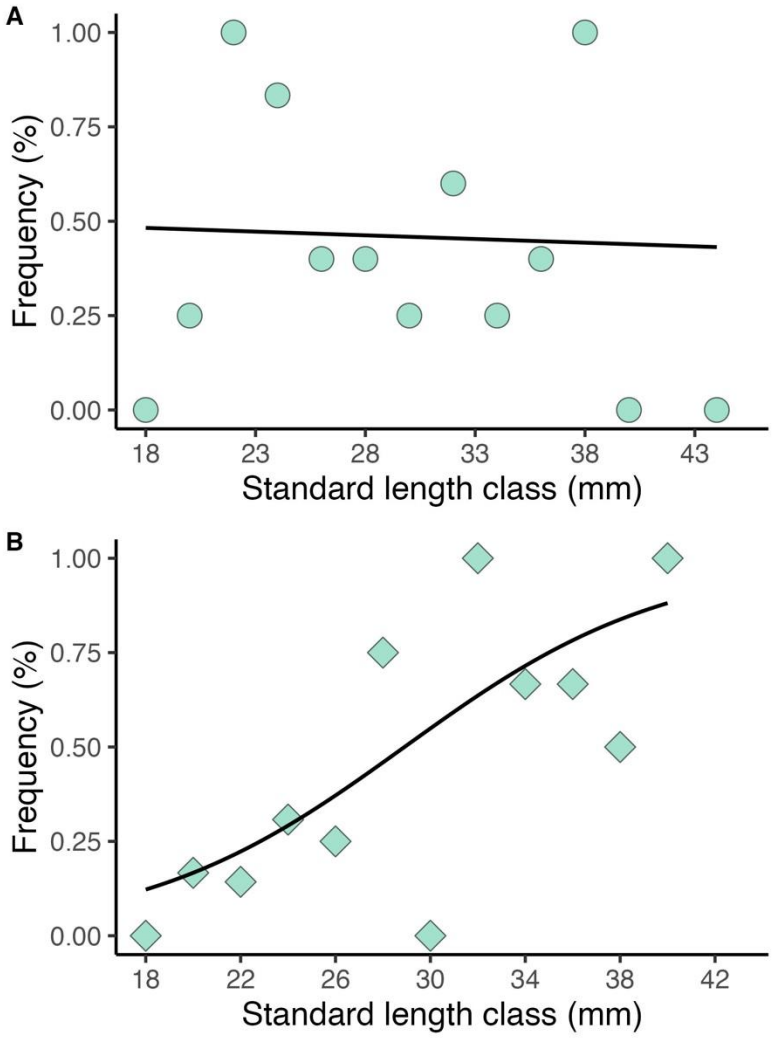

Figure S5. Logistic regression models describing the relationship between body length and sexual maturity for males of *Atlantirivulus peruibensis* in (A) natural pools and (B) artificial pools. Points represent maturity proportions by 2 mm length classes and solid lines indicate the fitted logistic curves. In both cases the models showed weak or inconsistent relationships between size and maturity with high overlap in body size between immature and mature individuals; therefore reliable estimates of L_{50} and L_{100} were not derived.