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Reproductive biology of *Copella arnoldi* (Characiformes: Lebiasinidae), a terrestrial-spawning fish from the Amazon

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We assessed the reproductive biology of the splash tetra, *Copella arnoldi*, an ornamental species with an extraordinary reproductive strategy. We collected 171 individuals from a river in the eastern Amazon between August 2016 and June 2017. The sex ratio remained relatively stable during the study period. Gonadosomatic indices for both female and male indicated heightened reproductive activity during the rainy season. On average, each mature female produced 47 oocytes ranging from 200 to 800 µm in diameter, releasing a batch of oocytes during each reproductive period. Length-mass ratios suggested similar growth proportions for both sexes. The L_{50} was estimated at 18.09 mm for females and 18.52 mm for males. The condition factor exhibited minimal variation throughout the year. The spawning period seemed to correlate with increased precipitation rates, as evidenced by two reproductive peaks, one in December (early rainy season) and another in April (peak rainy season). Spawning in *C. arnoldi* appeared synchronized with the rainy season, likely due to the reduced risk of egg dehydration and the enhanced survival potential for juveniles.

Keywords: Amphibious fish, Breeding, Fish reproduction, Rainfall.

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Nós avaliamos a biologia reprodutiva do splash tetra, *Copella arnoldi*, uma espécie ornamental com uma tática reprodutiva extraordinária. Coletamos 171 indivíduos de um rio no leste da Amazônia entre agosto de 2016 e junho de 2017. A proporção sexual permaneceu relativamente estável ao longo do período do estudo. Os índices gonadossomáticos de fêmeas e machos indicaram maior atividade reprodutiva durante a estação chuvosa. Em média, cada fêmea madura produziu 47 oócitos, de 200 a 800 µm de diâmetro, liberando um lote de oócitos durante cada período reprodutivo. A razão massa-comprimento indica que ambos os sexos crescem em proporções iguais. Estimamos o L_{50} em 18,09 mm para as fêmeas e 18,52 mm para os machos. O fator de condição apresentou uma variação mínima ao longo do ano. O período de desova de *C. arnoldi* parece estar correlacionado a maiores taxas de precipitação, como evidenciado pelos dois picos reprodutivos, um em dezembro (início das chuvas) e outro em abril (pico das chuvas). A desova de *C. arnoldi* parece estar sincronizada com a estação das chuvas, provavelmente devido à redução do risco de desidratação dos ovos e ao aumento do potencial de sobrevivência dos juvenis.

Palavras-chave: Peixe anfíbio, Precipitação, Procriação, Reprodução de peixes.

INTRODUCTION

In fish, the timing of reproductive events is influenced by a combination of morphological traits and physiological processes, responding to favorable environmental conditions. These include variables such as egg type, resource availability, and juvenile mortality rates (Espírito-Santo *et al*., 2013). For many tropical freshwater fish, reproduction is synchronized with the hydrological cycle, especially the rainy season, allowing access flooded areas for spawning. This synchronization ensures efficient use of energy reserves, benefiting from greater availability of microhabitats and food, as well as reduced predation risk during these periods (Ballesteros *et al*., 2009; Wootton, Smith, 2014).

Terrestrial spawning, an uncommon reproductive trait among fish, involves the temporary exposure of eggs to atmospheric air. The most typical strategy of terrestrial spawning is oviposition on a substrate that is intermittently or continuously outside the water (Ishimatsu *et al*., 2018). In the Neotropical region, only three freshwater fish species, *Brycon petrosus* Meek & Hildebrand, 1913, *Kryptolebias marmoratus* (Poey, 1880), and *Copella arnoldi* (Regan, 1912), emerge from the water during the breeding process (Martin, Carter, 2013). These species have specific adaptations to prevent egg dehydration, including morphological traits and the habit of spawning in shaded or humid environments (Krekorian, 1976; Kramer, 1978; Martin, Carter, 2013).

The splash tetra, *Copella arnoldi*, a member of the family Lebiasinidae, inhabits backwaters along streams and riverbanks in the Amazonian lowlands and the Guianas (Marinho, Menezes, 2017). This species is notable for the simultaneous spawning leaps of males and females, facilitated by their broad pectoral fins. They deposit and fertilize a small batch of eggs (around 50) on foliage above the water surface (Krekorian, Dunham, 1972b). After egg deposition, males remain to splash water on the eggs at approximately one-minute intervals to prevent dehydration. Eggs hatch after three days, and the larvae are washed off the foliage and into the water by the male's splashing (Krekorian, 1976).

Although Cordeiro *et al*. (2022) recently conducted a histological assessment of the reproductive biology of *C. arnoldi*, the study did not describe key reproductive aspects, such as the length at first sexual maturity, fecundity, morphometric and frequency of the oocytes, which are essential for assessing the type of spawning (Ganias, Lowerre-Barbieri, 2018). In addition, these characteristics and ecological function can vary within the same species (Hortal *et al*., 2015), as the literature notes that the reproductive and behavioural traits of a fish species can differ significantly across river basins (Liao *et al*., 2018; Gurdak *et al*., 2019).

Moreover, *C. arnoldi* is highly sought after in the aquarium trade due to its color pattern and unique reproductive behavior in captivity (Marinho, Menezes, 2017). In the Brazilian state of Amazonas, for instance, it was estimated that approximately 504 thousand lebiasinids and 5,790 individuals of the *C. arnoldi* were exported between 2002 and 2003 (Anjos *et al*., 2009). Hence, it is crucial to gather data on the species' biology and ecology to better understand how life history processes vary across space and time. This information can inform conservation practices, such as establishing breeding season and determining minimum catch sizes.

Here, we present new findings on the reproductive biology of the splash tetra, *Copella arnoldi*, using macroscopic and microscopic techniques over a full hydrological cycle in a stream in the eastern Amazon. Our study includes a histological description of gonadal development in *C. arnoldi*, determination of the mean body length at first sexual maturation, and assessments of the length-mass ratio, fecundity, and spawning type (total or partial). Monitoring the species throughout an entire hydrological cycle allowed us to determine its reproductive period. We hypothesize that optimal condition and reproductive activity in *C. arnoldi* coincide with the rainy season, when increased availability of microhabitats and higher relative humidity create more favorable conditions for egg development and offspring survival.

MATERIAL AND METHODS

Study area. The Taiassuí River (01°23'47.08"S 48°14'59.33"W) is a small tributary of the Guamá River in the eastern Amazon (MMA, 2006), located in a low latitude region in the northeastern part of the Pará State, Brazil. This area is part of the Amazon biome and is characterized by dense ombrophilous forest (MMA, 2006). The river has clear water with minimal suspended sediment and a significant amount of organic matter in its bed (Raiol *et al*., 2012). The lower Taiassuí region is influenced by the tidal cycle, causing the direction of water flow to reverse at certain times of the day.

The region has a tropical humid climate, with temperatures ranging from 26 to 32 °C throughout the year (Alvares *et al*., 2013). The seasonal movement of the Intertropical Convergence Zone (ITCZ) results in a pronounced rainy season during the first half of the year. Despite the absence of a water deficit during the dry season, the mean annual precipitation exceeds 2,500 mm (Moraes *et al*., 2005). Long-term data from the Instituto Nacional de Meteorologia (INMET) indicate that the rainy season extends from December to May, followed by a transitional period until mid-July, and a less rainy period from August to November. Flooding typically occurs between March and May, with the dry season limited to October and November.

Collection of the biological material and environmental data. Fish samples were collected every two months from August 2016 to June 2017 using a trawl and hand net at four sites along the Taiassuí River, with each site separated by 100 m. The fish were anesthetized with eugenol (Souza *et al*., 2015a) and euthanized following the guidelines of the National Council for the Control of Animal Experimentation (CONCEA), then fixed in 10% formalin. After 24 h, the specimens were transferred to 70% alcohol for storage at the Laboratório de Ecologia e Conservação (LABECO) at the Instituto de Ciências Biológicas da Universidade Federal do Pará (UFPA) in Belém, Brazil. The specimens were deposited in the ichthyological collection of the Museu de Zoologia at the UFPA in Belém (Pará, Brazil), under catalog number MZUFPA 326.

Laboratory procedures and histological description. The standard length (L_S, cm) and total mass (M_T, g) of each specimen were measured using an analog caliper with an accuracy of 0.1 cm and a precision scale with an accuracy of 0.0001 g, respectively. After fixation in 10% formalin, specimens were dissected, and the gonads were removed and weighed (M_G) on a more sensitive precision scale (0.00001 g). Subsequently, the gonads were processed using standard histological techniques for paraffin embedding. Sections of 5 µm thickness were obtained from the dissected gonads using a microtome and stained with hematoxylin-eosin (HE) (Prophet *et al*., 1995). The slides were then analyzed and photographed using a Nikon Eclipse C1 Cam DS-Ri1 optical microscope. Gonadal stages were determined based on a classification adapted from Núñez, Duponchelle (2009).

Data analysis. All data were initially checked for homogeneity, homoscedasticity and suitability for parametric tests using Levene's test. When these assumptions were not met, non-parametric analyses were employed. Firstly, we compared the sex ratio among periods using a Chi-square test (χ^2) for contingency tables with six rows (representing field expeditions) and two columns (male or female). The observed values were compared to the expected values (1:1) for each group (McHugh, 2012).

Secondly, we estimated the mean body length at first sexual maturation (L_{50}) for each sex. The L_{50} represents the length at which 50% of the individuals are capable of reproducing (Trippel, Harvey, 1991). We categorized individuals into standard length (L_S) classes of 1 mm, and determined the frequency of juveniles and adults in each class based on the logistic curve $P = \left(1 + e^{r(L_S - L50)}\right)^{-1}$, where P is the proportion of mature individuals, r is the slope of the curve, L_S is the standard length, and L_{50} is the mean length at first sexual maturation. We utilized the Microsoft Excel®365 Solver package to fit the curve.

The length-mass ratio for each sex was determined using the allometric equation $M_T = a \times L_s^{\,b}$, where M_T represents the total fish mass, a is the linear regression coefficient, L_S is the standard length, and b is the allometric coefficient of the length-mass relationship (Huxley, 1924; Froese, 1988). We then analyzed the proportional residuals of males and females (observed mass - expected mass / observed mass) using a t-test to assess potential differences in growth type (allometric or isometric) between the sexes.

The condition factor (K), which reflects the body condition of individuals over a specified period, was computed using the formula $K = (M_T/L_S^B) \times 100$ (Fulton, 1904). To assess whether the condition factor differed significantly among periods, we conducted a one-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test (Froese, 2006).

To determine the period of highest reproductive activity in males and females, we calculated the gonadosomatic index (Ig) using the equation $Ig = (M_G/M_T) \times 100$, where M_G represents the mass of the gonads and M_T is the total mass of the specimen (Nikolsky, 1963). We analyzed the variation in reproductive activity relative to the rainfall cycle using a Kruskal-Wallis analysis, followed by a multiple comparison test by ranks (Vargha, Delaney, 1998). Additionally, the reproductive period was inferred based on the frequency of different stages of gonadal development recorded during the study period. We assessed the relationship between precipitation, Ig, and K values using univariate regression analysis.

To assess fecundity, we selected eight mature ovaries and submerged them in Gilson's solution to dissociate the oocytes, which were then transferred to 70% ethanol. Using a Zeiss AxioCam ICc5 stereo microscope, we photographed the mature oocytes and counted their frequency to provide data on individual fecundity, considering only vitellogenic oocytes. Absolute fecundity (AF) was determined as the number of oocytes per ovary (Arantes *et al*., 2010).

The frequency and diameter of oocytes were assessed using three histological slides from each maturation stage, visualized with a Nikon Eclipse C1 Cam DS-Ri1 optical microscope. These parameters were used to determine the type of spawning and the morphometric aspects of the oocytes. Morphometric variation for type I and II oocytes was analyzed using a Kruskal-Wallis test, followed by a multiple comparison test by ranks. For type III oocytes and oogonia, we conducted a t-test with separate variances. All analyses considered a significance level of 95% ($\alpha = 0.05$) (Vargha, Delaney, 1998) using the Statistica 13.3 program (license number JPZ802I302101FA-4).

RESULTS

During the study period, a total of 171 individuals of *C. arnoldi* were captured, comprising 91 females and 80 males. The standard length of the female specimens ranged from 14.4 mm to 32.0 mm (mean = 20.0 ± 3.0 mm), with body mass varying from 0.02 g to 0.45 g (mean = 0.09 ± 0.06 g). For males, the standard length ranged from 15.0 mm to 31.0 mm (mean = 21.4 ± 3.6 mm), with body mass varying from 0.03 g to 0.34 g (mean = 0.12 ± 0.06 g).

The gonads of *C. arnoldi* are paired tubular structures in both sexes, exhibiting distinct patterns of size, color, consistency, and vascularization depending on the developmental stage. Microscopic analysis of oocyte development in females revealed five stages of gonadal maturation: (i) Immature stage, characterized by oogonia and type I or perinucleolar oocytes with a central nucleus and peripheral nucleoli (Fig. 1A); (ii) Maturing stage, containing some type II oocytes with early cortical alveoli and numerous type III oocytes, distinguished by the presence of cortical alveoli filling almost the entire cytoplasm (Fig. 1B); (iii) Mature stage, predominantly featuring type

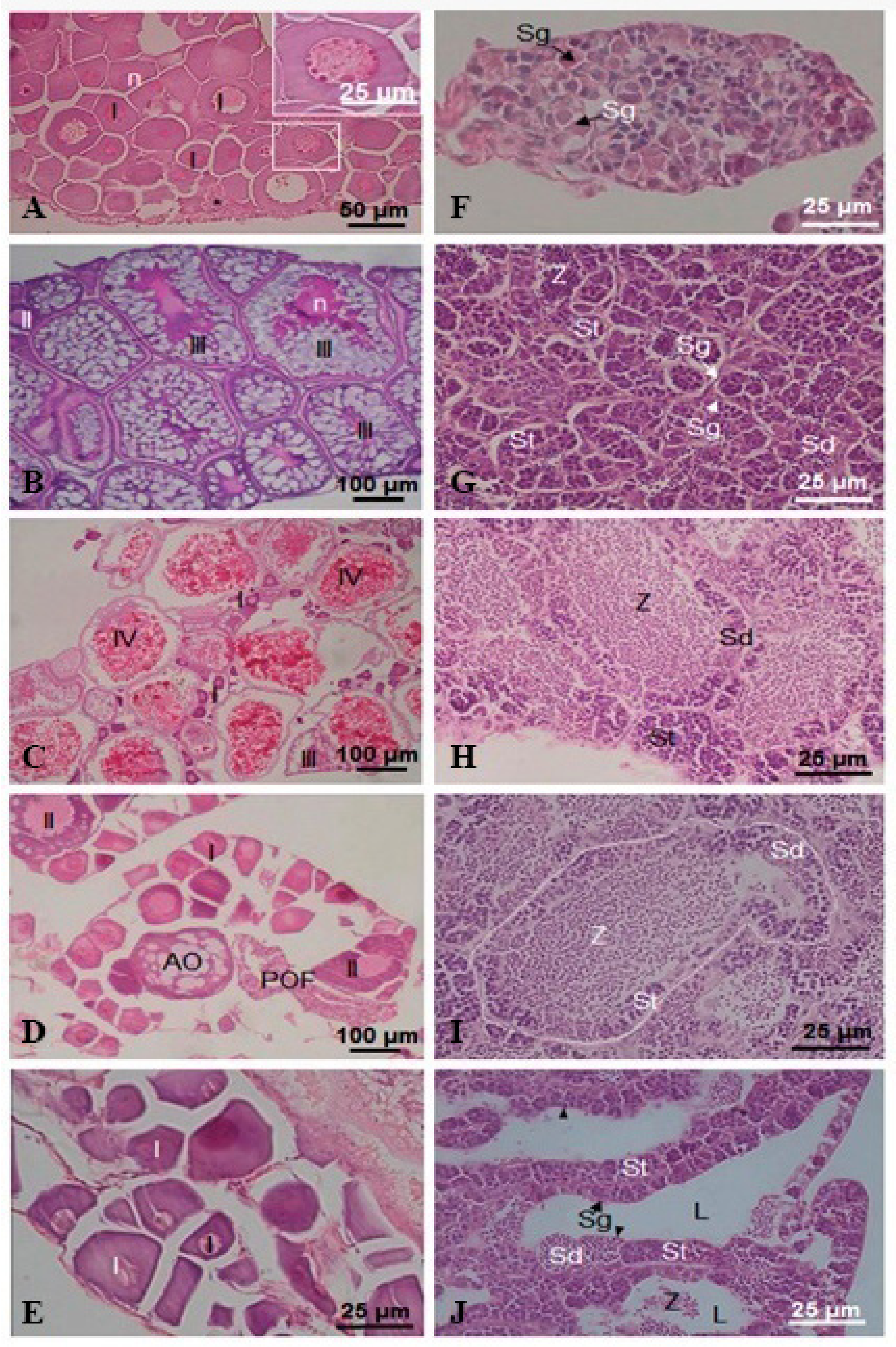

FIGURE 1 | Photomicrographs of the gonadal development of *Copella arnoldi* at different stages of maturation: **A.** Immature ovary: containing oogonia (asterisk) and type I oocytes (I) with a central nucleus (n) (insert); **B.** Maturing ovary: type II (II) and type III oocytes (III) with nucleus (n); **C.** mature ovary: predominance of type IV oocytes (IV); **D.** Spawned ovary: thin-walled ovigerous lamellae containing postovulatory follicles (POF), atretic oocytes (AO), type I and II oocytes; **E.** Resting ovary: ovigerous lamellae containing type I oocytes; **F.** immature testis: predominance of spermatogonia (Sg); **G.** Maturing testis: containing spermatogonia (Sg), spermatocytes (St), spermatids (Sd), and some spermatozoa (Z); **H**–**I.** Mature testis: anastomosis (----) of the seminiferous tubules filled with sperm; **J.** Spent testis: seminiferous tubules with continuous germinal epithelium (black triangle) and open lumen (l) containing residual sperm (Z).

IV or vitellogenic oocytes (with some type I and III oocytes), with cytoplasm filled with yolk granules (Fig. 1C); (iv) Spawned stage, containing few oocytes (types I and II), with the presence of post-ovulatory follicles (POFs) and atretic oocytes (AOs) (Fig. 1D); and (v) Resting stage, characterized by ovigerous lamellae containing type I and II oocytes and a well-defined fibrillar network (Fig. 1E).

For males, we identified four stages in the gonadal development: (i) Immature stage, characterized by seminiferous tubules with a sealed lumen and the presence of only spermatogonia (Fig. 1F); (ii) Maturing stage, where the tubule size increases and cysts representing various spermatogenic lineages, including spermatogonia, spermatocytes, spermatids, and a few spermatozoa in the tubule lumen (Fig. 1G); (iii) Mature stage, exhibiting seminiferous tubules filled entirely with spermatozoa along with spermatocytes and spermatid cysts, and evident tubular anastomosis (Figs. 1H, I); (iv) Spent stage, characterized by seminiferous tubules with an open lumen and a continuous germinative epithelium, containing a few spermatogonia, spermatocytes, spermatid cysts, and residual spermatozoa (Fig. 1J). The development of germ cells in *C. arnoldi* was asynchronous in both males and females.

The sex ratio remained relatively stable throughout the study period (χ^2 = 6,428, d.f = 5, p = 0.266), with no significant variation observed. The relationship between gonadal maturation stages and body length indicated that the mean length at first sexual maturation (L_{50}) for females was 18.09 mm (Fig. 2A), while for males, it was 18.52 mm (Fig. 2B).

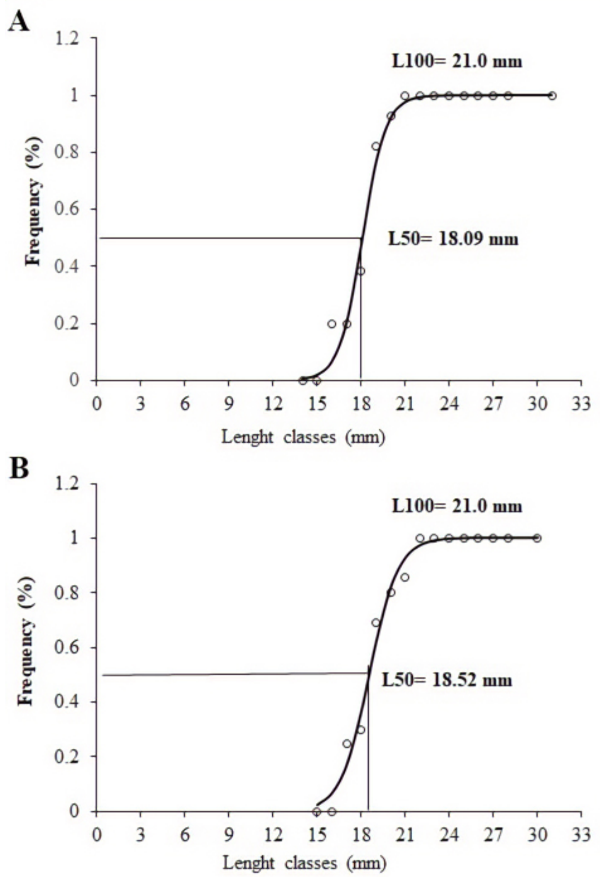

FIGURE 2 | Mean length (mm) at first sexual maturation (L_{50}) and at which 100% are sexually mature (L_{100}) in **A.** Female and **B.** Male *Copella arnoldi* collected from a stream in eastern Brazilian Amazon, between August 2016 and July 2017.

The analysis of proportional residuals revealed no differences in growth patterns between males and females (t = 0.020; d.f. = 168; p = 0.682). Consequently, the length-mass ratio of the population was described by the equation $M_T = 0.0103\ L_S^{3.10}$, indicating a tendency toward positive allometric growth pattern, where mass and length increase at different rates. The species' condition factor (K) did not vary throughout the sampling period ($F_{(2.167)} = 2.30$, p = 0.103), and precipitation had no significant influence on K values ($R^2 = 0.001$; p = 0.675).

Female and male of *C. arnoldi* exhibited greater reproductive activity during the rainy season, specifically in December and April (Female: $H_{(2.63)} = 23.05$, p < 0.001; Male: $H_{(2.59)} = 20.05$, p < 0.001 (Fig. 3). Differences were observed when comparing the *Ig* values of August with those of December and April for females, and June with those of December, February, and April for males (see pairwise comparison values in Tab. **S1**).

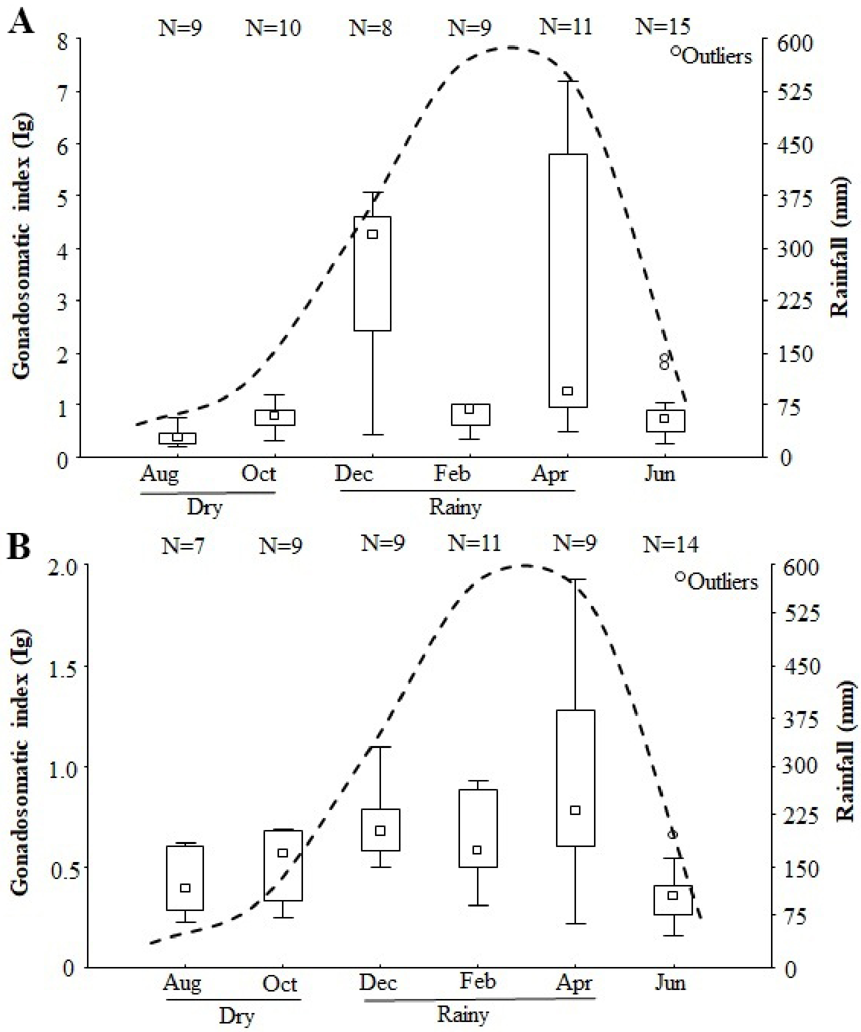

FIGURE 3 | Bimonthly variation in the gonadosomatic index of **A.** Female and **B.** Male *Copella arnoldi* (open box = median; bar = non-outlier range) and the accumulated monthly precipitation recorded at a stream in eastern Brazilian Amazon, between August 2016 and July 2017.

The stages of gonadal maturation corroborated the gonadosomatic index values, with the frequency of mature females (Fig. 4A) and males (Fig. 4B) being higher during the rainy season (December and April). Regression analyses revealed a significant (but weak) relationship between precipitation and the gonadosomatic index of both females ($R^2 = 0.202$; $p < 0.001$; $y = 0.1342 + 0.0041x$) and males ($R^2 = 0.231$; $p < 0.001$; $y = 0.309 + 0.0008x$) of *C. arnoldi*.

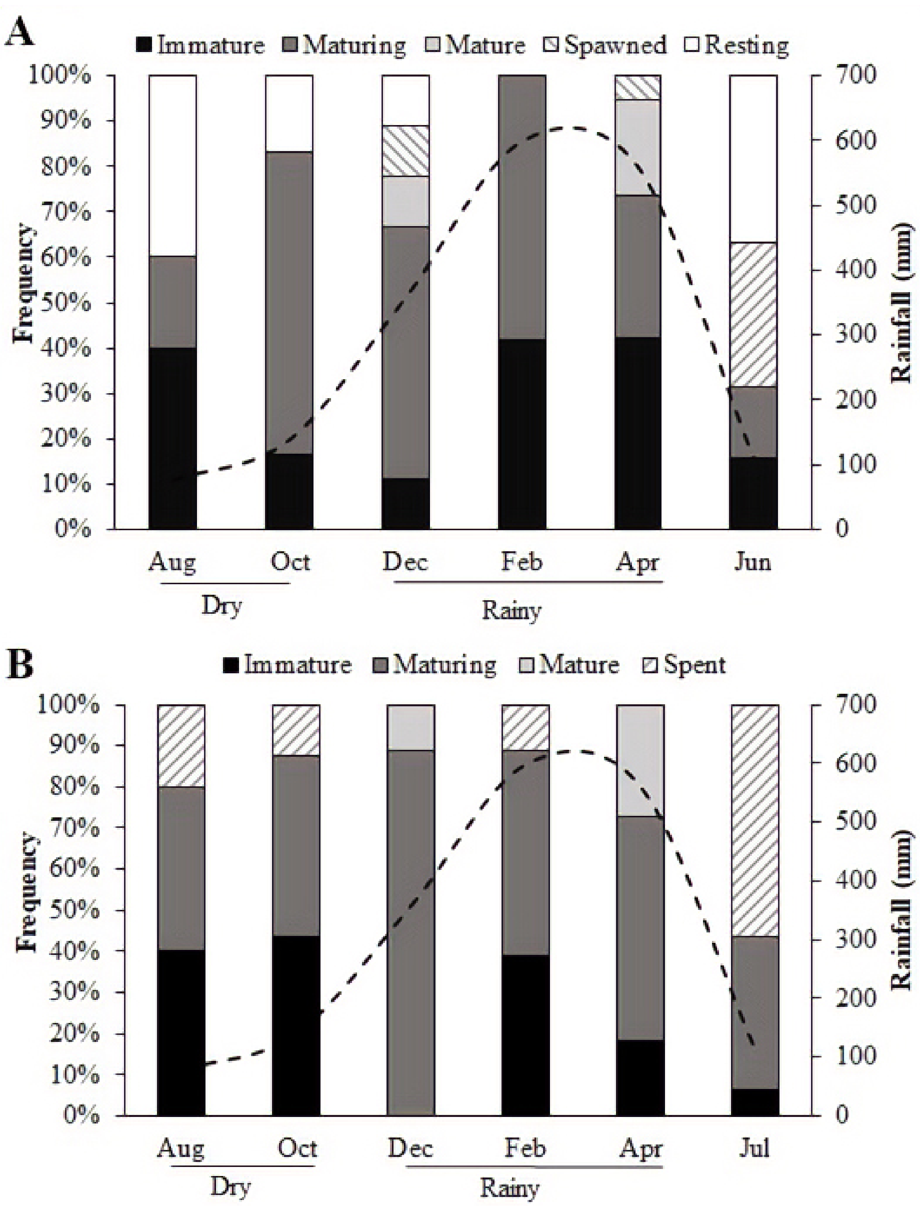

FIGURE 4 | Bimonthly variation in the frequency of the gonadal maturation stages of **A.** Female and **B.** Male *Copella arnoldi* and the accumulated monthly precipitation recorded at a stream in eastern Brazilian Amazon, between August 2016 and July 2017.

The mature ovaries of *C. arnoldi* contained a mean of 148 ± 45 total oocytes, with 47 ± 14 of these being mature oocytes. The diameters of type I oocytes were largest in the mature and resting stages ($H_{(4, 254)} = 91.78$; $p < 0.001$). While type II oocytes had the largest diameters in the mature and maturation stages ($H_{(4, 251)} = 142.05$; $p < 0.001$). Type III oocytes also showed a larger diameter at the mature stage ($t = -2.946$; d.f = 99; $p = 0.004$). No significant differences were observed in the diameter of oogonia ($t = -5.483$; d.f. = 98; $p = 0.651$) between the different stages of ovarian maturation (Tab. 1). The frequency of oocyte types in each maturation stage displayed a pattern similar to that of total spawners (single batch) (Fig. 5). An infographic summarizing the results obtained in this study is shown in Fig. 6, along with schematic illustrations of the species reproductive behavior.

TABLE 1 | Diameter of the types of oocytes (mean ± standard deviation) in all stages of ovarian development in *Copella arnoldi*. N = 50. In a column, the same superscript letter over the result indicates that there was no significant difference, while different letters indicate a statistical difference. – Means no data. *Insufficient data for performing statistical test.

	Oogonia	Type I	Type II	Type III	Type IV
Immature	15.18 ± 2.25[a]	41.21 ± 8.35[a]	80.65 ± 12.38[a]	–	–
Maturing	–	41.79 ± 10.11[a]	109.12 ± 16.34[b]	313.12 ± 94.27[a]	398.12 ± 65.11*
Mature	–	61.03 ± 11.85[b]	137.11 ± 24.48[b]	359.77 ± 64.69[b]	433.05 ± 92.21
Spawned	–	44.35 ± 10.89[a]	94.81 ± 21.50[a]	–	–
Resting	12.66 ± 2.37[a]	53.77 ± 10.75[b]	77.66 ± 18.28[a]	–	–

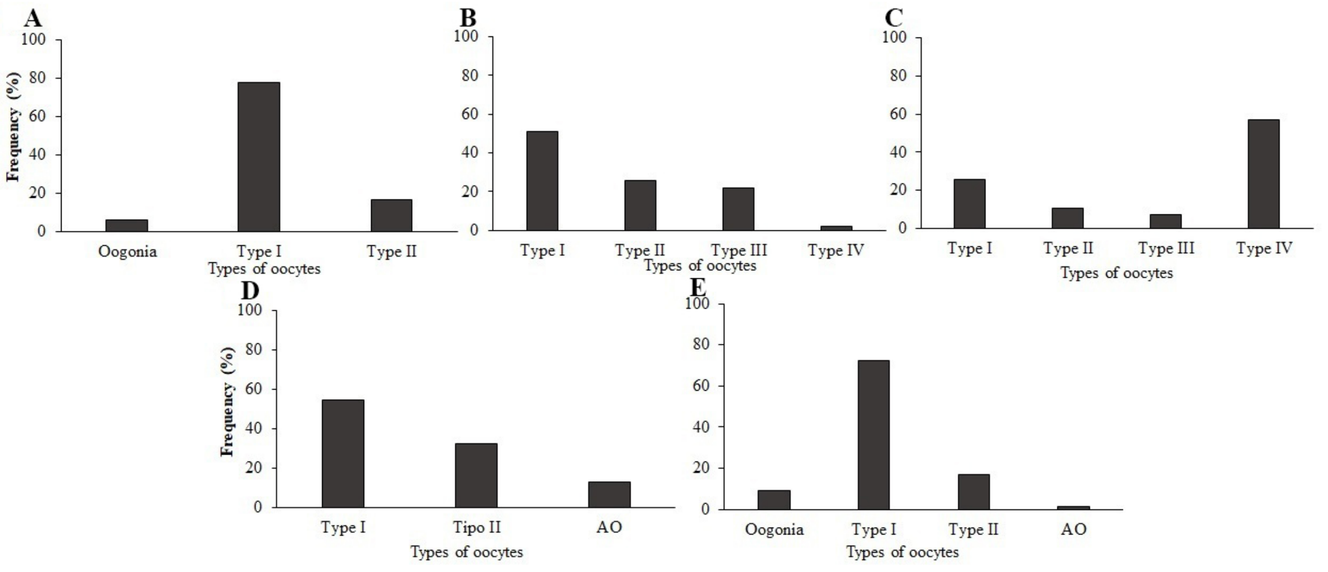

FIGURE 5 | Frequency of types of oocytes in all stages of ovarian development (**A.** Immature; **B.** Maturation; **C.** Mature; **D.** Spawned; **E.** Resting) of specimens of *Copella arnoldi* collected in a stream in the eastern Brazilian Amazon.

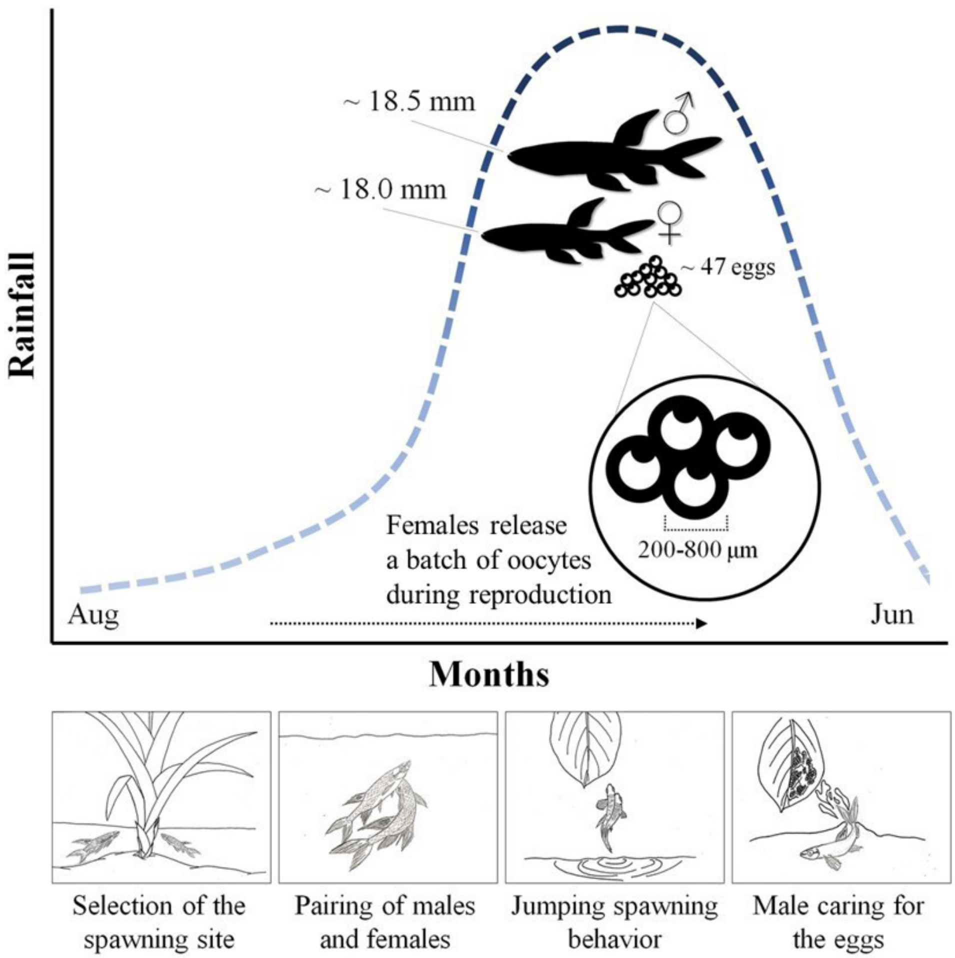

FIGURE 6 | Summary illustrating reproductive aspects (L_{50}, spawning period, fecundity, oocyte diameter and spawning type) and terrestrial spawning of *Copella arnoldi* in relation to rainfall.

DISCUSSION

The histological analysis of *C. arnoldi* specimens revealed five gonadal stages in females (immature, maturing, mature, spawned and resting) and four stages in males (immature, maturing, mature and spent). The species exhibited a positive allometric growth pattern, with both males and females reaching sexual maturity at similar body length. Variations in the gonadosomatic index and the relative frequency of mature specimens indicated a peak of reproductive activity during the rainy season. Females of *C. arnoldi* had ovaries containing a few mature oocytes, which seemed to be released over two reproductive events.

The ovarian histology of *C. arnoldi* is consistent with that described by Cordeiro *et al*. (2022), except for the resting phase, which was not observed by those authors. This suggests that sexually mature specimens remain inactive until the subsequent reproductive period (Brown-Peterson *et al*., 2011; Prudente *et al*., 2015). Immature and resting ovaries exhibit similarities but can be distinguished by features such as a thicker ovarian wall, increased spacing between gametes, and the presence of atretic oocytes (Brown-Peterson *et al*., 2011).

The testicular analysis of *C. arnoldi* revealed a continuous proliferation of germ cells, with seminiferous tubules displaying a persistent germinal epithelium. This process involves the release of spermatogonia or germinal cysts into the seminiferous tubule, where they differentiate into spermatozoa (Grier, 2002; Cordeiro *et al*., 2022). Combined with the species' sexual behavior, this suggests that males undergo rapid reorganization of germ cells to facilitate mating with multiple females during the reproductive season (Marinho, Menezes, 2017; Cordeiro *et al*., 2022).

Cordeiro *et al*. (2022) noted a predominance of females in a population of *C. arnoldi*, particularly during the rainy season, which corresponds to the reproductive period. This may be attributed to the territorial behavior of males. Marinho, Menezes (2017) observed that males of *C. arnoldi* can exhibit their fins as a sign of competition, with dominant individuals forming harems and excluding competing males. Additionally, males engage in parental care, making them more vulnerable to predation, potentially leading to areas with higher concentrations of female (Cordeiro *et al*., 2022).

Females and males of *C. arnoldi* reached L_{50} at similar sizes (18.09 and 18.52 mm, respectively). This is the first estimation of this parameter for the species and is applicable to both sexes, as they did not exhibit morphometric differences. The L_{50} is a crucial ecological trait for determining a minimum catch size in the wild, helping to prevent the capture of immature individuals and optimize reproductive management in aquariums and conservation centers (Godinho, 2007).

The length-mass ratio is a useful tool for estimating the weight corresponding to a given length and the growth pattern, which is influenced by environmental conditions, the sex of the individual, and the developmental state of the species (Le Cren, 1951; Froese, 2006). The positive allometric growth pattern observed in both sexes of *C. arnoldi* aligns with findings reported by Freitas *et al*. (2019) but contrasts with the negative allometric growth observed in other studies of the same species (Machado *et al*., 2020; Cordeiro *et al*., 2022). Discrepancies in these recorded values could be attributed to variations in habitat conservation levels, as investments in weight or length may represent a species' strategy to optimize physiological activities in response to changes in habitat structure and functioning (Froese, 2006; Cordeiro *et al*., 2022).

The condition factor (K), also derived from the length-mass ratio, provides valuable insights into the nutritional status and reproductive condition of fish populations. It is as a useful descriptor in fish biology and facilitates comparisons among aquatic systems with varying levels of human impact (Cifuentes *et al*., 2012). In the study, the K values of *C. arnoldi* exhibited minimal variation between the rainy and dry seasons, which may be attributed to the local environmental conditions. The study area experiences more intense rainfall compared to other Amazonian regions where the species is found, with sporadic rainfall even during the dry season. Such rainfall patterns may provide access to food resources even during drought periods (Souza *et al*., 2015b). Thus, features related to trophic dynamics of *C. arnoldi* may remain balanced throughout the hydrological seasons, preventing a more prominent variation in the condition factor.

Despite the consistent condition factor, the variation in gonadosomatic index indicates that both females and males of *C. arnoldi* exhibit two reproductive peaks: one in December (at the onset of the rainy season) and another in April (towards the end of the rainy season). In small forested streams, fish spawning typically occurs during the rainy season when larger rivers flood (Carvalho *et al*., 2007). This flooding leads to a brief period of inundation in smaller rivers and streams, resulting in a predictable increase in the availability of microhabitats and food supply for rapidly growing fish larvae (Carvalho *et al*., 2009). For terrestrial spawners like *C. arnoldi*, the influence of rainfall may be even more pronounced due to the trade-off between the need for atmospheric oxygen and the risk of egg dehydration during embryonic development (Martin, 2014).

Our findings also align with those of Krekorian (1976), who observed that *C. arnoldi* spawns exclusively during the rainy season. Rainfall periods enable males to reduce the frequency of tail-spraying behaviour required to hydrate the eggs (Krekorian, 1976). This reduction in energy expenditure on parental care may also allow males to temporarily leave the nest to avoid potential predators (Wootton, Smith, 2014; Ishimatsu *et al*., 2018).

However, terrestrial spawning and parental care behaviour may have negative implications for the fertility of *C. arnoldi* due to the increased investment in egg quality and offspring care (Wootton, Smith, 2014). The presumed low fecundity observed in this study (47 ± 14 oocytes per female) is consistent with findings from Krekorian, Dunham (1972a) on *C. arnoldi* in captivity, where 69% of females produced between one and 100 eggs. The diameter of oocytes in *C. arnoldi* follows a similar pattern to that observed in *Pyrrhulina semifasciata* Steindachner, 1876, where oocytes typically range between 300 and 700 µm in mature ovaries. Although *P. semifasciata* reaches larger sizes and has higher fecundity compared to *C. arnoldi* (Favero *et al*., 2010), both species have mature oocytes of similar diameter. Literature also indicates that larger females produce more offspring than smaller ones (Domínguez-Petit *et al*., 2022) and that relative fecundity decreases as more energy is invested in each offspring by having larger egg sizes (Smalås *et al*., 2017). Thus, the low fecundity and larger egg diameter are attributed to the unique reproductive behavior of *C. arnoldi* among lebiasinids.

The morphometric analysis of oocyte distribution in *C. arnoldi* revealed a pattern of synchronous development, characterized by the presence of dominant oocyte classes, which is typical of species exhibiting total spawning. Our results contrast with the histological assessment by Cordeiro *et al*. (2022), who suggested that *C. arnoldi* displays asynchronous development and partial spawning. However, this difference may be a matter of interpreting oocyte distributions. Fish species with long spawning intervals may exhibit oocyte distributions similar to those of total spawners because it would not be possible to visualize the oocyte batches typical of partial spawners, but in reality they have synchronous grouping or asynchronous development with partial spawning (Ganias, Lowerre-Barbieri, 2018), which may be the case for *C. arnoldi*.

In contrast to findings of Cordeiro *et al*. (2022), we did not observe mature individuals throughout the entire study period. Therefore, we cannot conclude that *C. arnoldi* is capable of spawning throughout the year, independent of the hydrological cycle. Even partial spawners may exhibit more favourable times for spawning (Ganias *et al*., 2014). Moreover, reproductive behaviours such as terrestrial spawning, site selection, territorialism, mate choice, and parental care are energetically costly (Wootton, Smith, 2014). Thus, it is plausible to posit that a fish with these traits may not exhibit multiple consecutive reproductive events during periods of adverse environmental conditions.

Overall, *C. arnoldi*'s reproductive period appears to be synchronized with the rainy season, as indicated by the greater number of individuals with mature ovaries during this period. This synchronization likely represents a strategic adaptation to reduce the risk of egg dehydration and to minimize the effort required for parental care and offspring survival. These factors are crucial determinants of reproductive success, as observed in other fish species that reproduce terrestrially (Sayer, 2005; Martin, Carter, 2013).

Studying fundamental aspects of population biology, such as fecundity, size at maturity, and spawning patterns, provides valuable insights for developing effective conservation and management strategies for fish populations. Based on our findings, we recommend that *C. arnoldi* be preferably caught during the ebb and dry hydrological periods, while avoiding fishing during the flood and inundation periods, which coincide with the species' reproductive period. Furthermore, we propose that the minimum capture size be set no less than 2.1 cm, in which 100% of the individuals are already sexually mature, to ensure adequate and continuous recruitment of juveniles into the population. These measures can help sustain the population and support the long-term conservation of *C. arnoldi*.

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The authors declare no competing interests.

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