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# Ichthyoplankton and plastic waste drift in a river in the Amazon Basin, Brazil

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Egg and larvae drift is a key mechanism for early fish stages to colonize nursery and growth areas and provides insights into ichthyofauna spawning times and spawning locations—crucial information for understanding fish biology. In the Tapajós River, Amazon Basin, no study has investigated the drift of ichthyoplankton along the hydrological phases nor the occurrence and dispersion of plastic waste associated with plankton. Thus, we aimed to present an overview of the spatio-temporal drift of ichthyoplankton and plastic waste in this river. Temporal sampling was carried out along a spatial gradient of approximately 300 km, covering different sectors of the river. Ichthyoplankton and plastic waste were captured through horizontal trawls on the subsurface of the water column for 10 min. Fish larvae belonging to 10 taxonomic orders and with an average size of 4.98 mm ± 3.14 mm were captured. Clupeiformes larvae occurred in all hydrological phases and reproductive peaks for other orders were recorded. Plastic waste was classified into 20 types according to color and shape and an average size of 1.55 mm ± 1.27 mm. Green fragments and blue filaments were the most abundant. We found the highest density of fish eggs and larvae drifting during the drought phase in stretches with greater environmental structure, whereas plastics were more abundant during the receding water phase in more turbulent stretches that have impacts from illegal mining. Simultaneous drift of ichthyoplankton and plastic waste was recorded in all hydrological phases and sampled sectors. This is worrying because, although we did not analyze the ingestion of plastics by fish larvae, plastic waste can enter the nursery areas of the Tapajós River and reduce the function of these areas for fish. Here we discover through the high densities of fish eggs and larvae deriving that the Tapajós River is an important reproduction site for ichthyofauna and that the presence of different landscape configurations prove to be a key factor in the dispersion, retention and development of ichthyoplankton and plastic waste.

#### KEYWORDS

aquatic pollution, dispersion, fish larvae, microplastics, Tapajós River

## 1 Introduction

Drift is a mechanism that favors the dispersion of riverine fish eggs and larvae to places that have resources for protection, feeding and development of these organisms, being considered a key and determinant process in the initial life history of fish (Pavlov, 1994; Lechner et al., 2016; Zacardi et al., 2020a). This mechanism is considered an adaptive strategy to ensure greater

survival of the offspring, thus having a direct impact on the fitness of populations, maintenance and renewal of natural stocks (Urho, 1999; Lechner et al., 2014). However, drifting ichthyoplankton in aquatic environments can vary over space and time, since it reflects favorable environmental conditions for the reproduction of adult individuals (Oliveira and Ferreira, 2008; Reynalte-Tataje et al., 2012; Cajado et al., 2022), in addition to indicating reproductive patterns of the diversity of species in each region.

In the Amazon region, which has one of the greatest diversities of freshwater fish species in the world (>2,400 validated species), ichthyoplankton drift processes have been well elucidated for nutrient-rich aquatic systems, regionally classified as whitewater rivers (Sioli, 1968; Junk et al., 2011; Jézéquel et al., 2020). However, oligotrophic blackwater (e.g., Negro and Arapiuns rivers) and clearwater rivers (e.g., Trombetas and Tapajós rivers) have been neglected in terms of studies on fish eggs and larvae. For example, the reproductive pattern of some taxonomic groups (i.e., migratory Characiformes species) in the Solimões and Amazonas rivers (whitewaters) is clarified. The species spawn during the rising water season in the main river channel and their eggs and larvae drift downstream through the flow velocity in the channel or marginal regions of the river until they reach nursery and growth sites that have environmental conditions of refuge and feeding such as lakes in floodplains (Araujo-Lima and Oliveira, 1998; Zacardi et al., 2020a).

In the Tapajós River, a clearwater tributary on the right bank of the Amazon River, processes and mechanisms that act on the reproduction of fish species and dispersion of ichthyoplankton are poorly understood. Some studies have reported that this river does not have ideal environmental conditions to support the ichthyoplanktonic community of some species, such as those that carry out reproductive migrations, but contradictorily, other studies indicate that this river system in certain locations has important reproductive sites of commercially valuable species and migratory (Lima and Araujo-Lima, 2004; Barthem et al., 2016; Nunes et al., 2019). In this case, understanding the distribution and space-time drift of fish eggs and larvae is an important and efficient step to identify periods and sites of reproduction of the ichthyofauna, in addition to allowing to precisely define the places and times of spawning, nursery and growth, and support conservation measures for fish stocks (Nakatani et al., 2001; Mounic-Silva et al., 2019; Zacardi et al., 2020a; Zacardi et al., 2020b).

Moreover, ichthyoplanktonic studies can help detect the influence of climatic events and environmental impacts at local and regional levels on the structure and early life history of species (Bonecker et al., 2019; Cajado et al., 2022; Oliveira et al., 2022). Because many Amazonian rivers have become vulnerable to anthropic pressures, the impacts of these actions have been directly observed on fish communities, which show a decrease in their species richness, functional diversity and increased ingestion of plastic waste (Arantes et al., 2018; Andrade et al., 2019; Ribeiro-Brasil et al., 2020; Keppeler et al., 2022). In the latter case, many studies have been dedicated to investigating the consequences of the interaction of plastic waste on fish communities at different stages of the life cycle (Lima et al., 2015; Lima et al., 2016; Steer et al., 2017; Pegado et al., 2018; Andrade et al., 2019; Ribeiro-Brasil et al., 2018; Andrade et al., 2019; Ribeiro-Brasil et al., 2018; Andrade et al., 2019; Ribeiro-Brasil et al., 2020).

Plastic waste is retained in aquatic vegetation in riverside regions or even adrift for long kilometers undergoing a degradation process. During the drift, they become smaller particles that are classified according to their size range into macroplastics (which include megaplastics, macroplastics themselves and mesoplastics) and microplastics (microplastics and nanoplastics themselves) and interact in different ways with the community of fish at different stages of the life cycle (Blettler et al., 2017; Andrade et al., 2019; Bancin et al., 2019; Blettler et al., 2019; Heinlaan et al., 2020).

Although it is necessary to investigate the direct interactions of plastic waste on ichthyoplankton (e.g., ingestion of plastics by larvae), information on the drift of both can elucidate many aspects that help conservation measures, such as identifying areas and critical periods for the development of the early life stages of fish. Thus, this study presents an overview of the occurrence and drift of ichthyoplankton and plastic waste along the Tapajós river basin, Brazilian Amazon, with the goals of i) evaluating the spatial-seasonal distribution of ichthyoplankton and plastic waste, ii) visually classify these inorganic materials according to their color, type and size. We carried out samplings on a spatial scale of approximately 300 km in length with different landscape configurations and during four phases of the Amazonian hydrological cycle to answer the following prediction: The highest densities of fish eggs and larvae drifting occur during rising water phase of the river Tapajós as observed in other Amazonian rivers (Zacardi et al., 2020b), while the highest densities of plastic waste occur during receding water phase due to greater water runoff.

# 2 Materials and methods

## 2.1 Study area

The study area is located in the middle and lower reaches of the Tapajós River, state of Pará, Brazil (Figure 1). Tapajós River is in central part of the Amazon Basin and is the fifth-largest tributary of this basin. It has low concentrations of nutrients and sediments and is considered a clearwater river. The highest rainfall occurs from December to June (>100 mm/month), while the lowest is observed from July to November (<100 mm/month). Along its length, Tapajós River presents a geomorphological variation that configures an environment of numerous waterfalls and rapids in its upper portion, while its lower stretch has a wider alluvial plain, characterizing it as a semi-lentic system (Scoles, 2016; Fróis et al., 2021).

# 2.2 Data sampling

Ichthyoplankton and plastic waste were collected during the four phases of the regional hydrological cycle: Flooding (June/2021), receding water (September/2021), drought (November/2021) and rising water (February/2022) along a spatial gradient of approximately 300 km across 12 sampling sites. The stations were categorized into three sectors according to their landscape settings. Sector 1 (S1, S2, S3, and S4) has an average distance between its banks of approximately 2.4 km, has numerous rapids making the water turbulent, and tributaries that drain mining regions causing high sediment discharge into the main river. In this sector is located the rapids of São Luiz do Tapajós (S4)-a region that has been threatened in relation to the implementation of hydroelectric plants for energy production. Sector 2 has an average distance of 3.4 km between the banks of the river (S5, S6, S7, and S8), has greater landscape structure due to alluvial islands and sandbanks along the main river channel and a large center urban (Itaituba city-S5). Sector 3 (S9, S10, S11, and S12) is the widest section along the entire length of the Tapajós river



Location of the study area with an emphasis on the sampled sectors and collection stations along the middle and lower reaches of the Tapajós River, Amazon Basin, Brazil. (A): characteristic environment of sector 3. (B): characteristic environment of sector 2. (C): characteristic environment of sector 1.

basin, with an average distance of 14 km between the banks, without the presence of alluvial islands, rapids or sandbanks. This section of the river has a lower flow, having a semi-lentic system as a characteristic (Irion et al., 2006; Lobo et al., 2017); it has environmental protection areas on both banks, numerous marginal lagoons connected to the main river and influenced by the flood pulse. Samplings were carried out during the daytime (3–6 p.m.) and nighttime (8–11 p.m.; UTC -3), on the left and right banks of the river, totaling 192 final samples.

The captures of ichthyoplankton and plastic waste were carried out horizontal trawls on the subsurface of the water column for 10 min using a conical-cylindrical plankton net (300  $\mu$ m mesh) with a coupled flowmeter to measure the volume of filtered water. To ensure efficiency in the captures the trawls were carried out using a motorized vessel at low speed. After the trawling, the samples were euthanized according to the guidelines of the Conselho Nacional de Controle e Experimentação Animal do Brasil (Conselho Nacional de Controle e Experimentação Animal, 2015) and fixed in a 4% formalin solution. The license for the collection of biological material was granted by the Sistema de Autorização e Informação em Biodiversidade (SISBIO) of the Instituto Chico Mendes de Conservação da Biodiversidade and Ministério do Meio Ambiente of Brazil, authorization number 75271-1/2020, issued based on the Normative Instruction n<u>o</u>. 154/2007.

## 2.3 Sample processing

In the laboratory, ichthyoplankton and plastic waste were sorted, separating them from the rest of the material present in the samples and stored in glass vials. The inclusion of inorganic material as plastic waste was performed according to Ribeiro-Brasil et al., 2020. Subsequently, the material was quantified, and the larvae were taxonomically identified at the order level. To avoid contamination of microplastics in the laboratory samples and ensure quality assurance and quality control, air contamination was checked. For this, Petri dishes were distributed on the screening bench for 24 h to collect particles from the air. Only cotton fibers were captured, thus they were disregarded during the sorting process. During laboratory procedures, 100% cotton clothing and metal/aluminum equipment were used for sorting, classifying and identifying samples. Plastic waste was classified according to its shape: filaments-when they presented elongated and filamentous shape; spherical-classified as having a rounded shape; fragments-those with irregular, square, or rectangular shapes; foams-they have the appearance of almost spherical or granular particles that can deform under pressure and can be partially elastic, depending on the weathering state (Ribeiro-Brasil et al., 2020; Queiroz, 2022) and also about its color. The total length of the larvae and plastic waste was measured in millimeters (accuracy of 0.01 mm) using a binocular stereoscopic microscope (Leica S9i) coupled with an integrated digital color camera.

## 2.4 Data analysis

Ichthyoplankton and plastic waste abundances were standardized for the density per 10 m<sup>-3</sup> of filtered water according to Tanaka (1973), modified by Nakatani et al. (2001):  $Y = (X/V) \times 10$ , where Y represents the density in 10 m<sup>-3</sup>, X represents the number of individuals captured, and V represents the volume of filtered water. Ichthyoplankton and plastic waste density data were Box-Cox transformed (Box and Cox, 1964) to achieve normality (Shapiro-Wilk test) and homoscedasticity assumptions (Cochran's test). To detect differences in the density of ichthyoplankton and plastic waste

Density (10 m <sup>-3</sup> )	ing		Receding water			Drought			Rising water			Total density	
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	
Fish eggs	0.28	0.35	0.00	0.25	0.00	27.65	12.33	77.90	0.64	2.34	6.26	1.18	129.18
Fish larvae	16.21	341.13	351.66	13.45	133.17	63.72	143.08	1671.86	280.15	11.45	121.37	216.10	3363.36
Plastic waste	3.08	5.61	2.42	200.44	50.02	80.58	2.24	6.72	26.22	2.12	2.55	2.85	384.86

TABLE 1 Total density (10 m<sup>-3</sup>) of ichthyoplankton and plastic waste along the hydrological phases and sectors of the Tapajós River, Amazon Basin, Brazil. S1: Sector 1; S2: Sector 2 and S3: Sector 3.



between the phases of the hydrological cycle, as well as between the sectors studied, a One-Way ANOVA was applied. Tukey's *a posteriori* test was performed whenever significant differences were detected. All analyzes were performed in the statistical environment R Studio (R Development Core Team, 2022) with significance of p < 0.05.

# **3** Results

A total density of 129.18 eggs 10 m<sup>-3</sup>, 3363.36 larvae 10 m<sup>-3</sup>, and 384.86 plastic waste 10 m<sup>-3</sup> were obtained. In all hydrological phases and sectors of the Tapajós River were recorded fish eggs and larvae and plastic waste (Table 1). Larvae had an average standard length of 4.98 mm  $\pm$  3.14 mm and were identified in 10 taxonomic orders, being Characiformes (53.27%), Clupeiformes (20.58%), and Siluriformes (11.43%) most abundant (accumulated percentage) (Figure 2). About 7.39% of the captured larvae could not be taxonomically identified because they were damaged or at a very early stage of development and without formed morphological structures that would allow identification. Based on the proportion of larval density, different reproductive patterns of taxonomic orders were observed in relation to hydrological phases. In the flooding phase, Clupeiformes (37,78%), Siluriformes (21.76%), Gobiiformes (17.30%), and Perciformes (12.88%) were the most representative orders. During the receding water phase, Clupeiformes remained abundant (67.51%), as well as Perciformes (16.56%), but followed by Carangiformes (5.45%), and only in this phase were Beloniformes larvae captured. Characiformes (73.23%) and Clupeiformes larvae (11.25%) predominated during the drought phase. In the rising water phase, Siluriformes (43.88%) were the most abundant, as well as Characiformes and Clupeiformes (33.09% and 20.67%, respectively) with the exclusive occurrence of Gymnotiformes larvae (Table 2).

Egg distribution was significantly different between the four hydrological phases (ANOVA; F = 4.018; p = 0.023), with contrasting values between drought and flooding (Tukey test; p = 0.013). The highest total egg densities were recorded during drought phase with 90.87 eggs 10 m<sup>-3</sup>, followed by the receding and rising water with 27.90 eggs 10 m<sup>-3</sup> and 9.78 eggs 10 m<sup>-3</sup>, respectively (Table 1). The larvae also presented a heterogeneous distribution between hydrological phases (ANOVA; F = 5.113; p = 0.004) with the highest total densities during the drought and the flooding (2095.10 and 709.00 larvae 10 m<sup>-3</sup>). The drought season differed significantly from the rising water and receding water seasons (Tukey test; p = 0.012 and 0.005, respectively).

The spatial distribution of fish eggs and larvae showed a pattern in which the highest total densities were recorded in sector 2 (88.04 eggs 10 m<sup>-3</sup> and 2,496.96 larvae 10 m<sup>-3</sup>) and 3 (54.38 eggs 10 m<sup>-3</sup> and 992.63 larvae 10 m<sup>-3</sup>), respectively (Table 1). However, egg density did not vary significantly along this scale (ANOVA; F = 1.221; p = 0.340). On the other hand, larval density showed a heterogeneous distribution (ANOVA; F = 20.20; p = 0.001). This difference was the result of variations between sectors 3 and 1 (Tukey test; p = 0.017) and between sectors 2 and 1 (Tukey test; p = 0.0003).

Visually, plastic waste was grouped into 20 classifications based on color (white, blue, transparent, black, pink, green, red, yellow, gray, and orange) and shape (fragments, filaments, film, styrofoam, and foam) and showed length average of  $1.55 \pm 1.27$  mm (Figure 3). During the flooding period, 11.11 plastic 10 m<sup>-3</sup> was recorded drifting in the Tapajós River, with greater occurrence in sector 2 (5.61 plastic 10 m<sup>-3</sup>), while in receding water there was 331.04 plastic 10 m<sup>-3</sup> mainly in sector 1 (200.44 plastic  $10\,m^{-3}).$  In the drought, the density was 35.19 plastic 10 m<sup>-3</sup> with greater drift in sector 3. On the other hand, during rising water, the total density did not exceed 7.52 plastic 10 m<sup>-3</sup>, being more abundant in sector 3 (2.85 plastic 10 m<sup>-3</sup>) (Table 3). As for the classifications, the highest densities were observed for green fragments, which predominated in the receding water phase (207.27 plastic 10 m<sup>-3</sup>) in sector 1 (153.38 plastic  $10 \text{ m}^{-3}$ ) and for blue filaments that were abundant in receding water (68.74 plastic 10 m<sup>-3</sup>) in sector 1 (32.94 plastic 10 m<sup>-3</sup>) (Table 3). The total density of plastic waste varied significantly along the spatial scale (ANOVA; F = 6.699; p = 0.017) due to the differences between sectors 2 and 1 (Tukey test; p = 0.016). Likewise, significant differences were also registered between hydrological phases (ANOVA; F = 8.828; p = 0.00012), with a clear difference between the receding water phase and the others (Tukey test; p < 0.005).

Receding Rising Orders Flooding Drought Water Water Beloniformes Percentage % Characiformes 0 0.01 to 1.00 Clupeiformes Gobiiformes 1.01 to 10.00 Gymnotiformes 10.01 to 20.00 Unidentified 20.01 to 30.00 Perciformes 30.01 to 40.00 Carangiformes 40.01 to 50.00 Siluriformes 50.01 to 60.00 Synbranchiformes 60.01 to 70.00 Tetraodontiformes >70 Total Density  $(10 \text{ m}^{-3})$ 709.00 210.34 2095.10 348.93

TABLE 2 Cumulative percentage of larval density (10 m<sup>-3</sup>) of taxonomic orders captured along hydrological phases of the Tapajós River, Amazon Basin, Brazil.

along hydrological phases of the Tapajós River, Amazon Basin, Brazil.



# 4 Discussion

The ichthyoplanktonic assemblage in the Tapajós River along its studied sectors was represented mainly by specimens of Characiformes, Clupeiformes, and Siluriformes, taxonomic orders that harbor commercially valuable species and importance in aquatic trophic structuring. Variations in ichthyoplankton capture spatial and temporal scales indicate that the Tapajós River is an important spawning and nursery site for regional ichthyofauna and that different taxonomic orders have their reproduction peaks at different hydrological phases. Our prediction was supported in part since the densities of fish eggs and larvae, contrary to what we expected, were higher during the drought phase. However, as we had predicted, plastic waste

Plastic waste (10 m <sup>-3</sup> )	Flooding			Receding water			Drought			Rising water		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
White foam	0.07	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00
Blue filament	0.00	1.88	0.06	32.94	11.87	23.93	0.00	0.14	7.88	0.13	0.00	1.21
White filament	0.00	0.54	0.00	0.38	0.77	0.26	0.00	0.24	0.00	0.10	0.00	0.00
Transparent film	0.00	0.00	0.00	0.49	0.32	1.25	0.00	0.00	0.00	0.00	0.16	0.00
Black filament	0.00	0.00	0.00	1.80	0.25	3.39	0.00	0.00	0.00	0.00	0.00	0.00
Pink filament	0.16	0.00	0.00	1.99	5.16	1.99	0.00	0.00	0.00	0.00	0.00	0.00
Transparent filament	0.00	0.00	0.00	0.91	1.74	0.26	0.28	0.00	0.25	0.00	0.00	0.00
Green filament	0.16	0.26	0.06	0.45	0.16	0.11	0.12	0.11	0.00	0.00	0.00	0.00
Red filament	0.00	0.55	0.00	0.74	0.53	0.00	0.00	0.29	1.97	0.04	1.79	0.00
Yellow fragment	0.21	0.00	0.00	0.83	0.08	3.84	0.00	0.09	9.85	0.00	0.00	0.00
Blue fragment	0.28	0.24	0.58	2.33	5.02	2.93	0.97	2.82	0.13	1.46	0.48	0.00
White fragment	0.50	0.76	0.29	2.30	2.45	1.03	0.62	0.43	0.22	0.00	0.00	0.00
Grey fragment	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.09	0.00	0.00	0.00	0.00
Orange fragment	0.00	1.19	0.00	0.17	2.59	1.50	0.00	1.94	0.00	0.00	0.12	0.00
Black fragment	0.00	0.19	0.00	0.32	0.07	1.21	0.00	0.00	0.00	0.00	0.00	0.00
Pink fragment	0.00	0.00	0.00	0.08	0.00	0.39	0.00	0.00	0.00	0.00	0.00	0.00
Transparent fragment	0.00	0.00	0.58	1.02	0.09	0.72	0.00	0.00	0.00	0.13	0.00	0.00
Green fragment	0.66	0.00	0.65	153.38	16.45	37.44	0.00	0.45	5.91	0.12	0.00	1.64
Red fragment	1.03	0.00	0.00	0.15	2.21	0.27	0.13	0.13	0.00	0.00	0.00	0.00
White styrofoam	0.00	0.00	0.09	0.15	0.10	0.07	0.12	0.00	0.00	0.00	0.00	0.00
Total density	3.08	5.61	2.42	200.44	50.02	80.58	2.24	6.72	26.22	2.12	2.55	2.85

TABLE 3 Total density per 10 m<sup>-3</sup> and classification of plastic waste captured in different hydrological phases and sectors of the Tapajós River, Amazon Basin, Brazil. S1: Sector 1; S2: Sector 2 and S3: Sector 3.

was abundant during the receding water phase, surpassing ichthyoplankton densities.

Although some taxonomic orders occurred in all hydrological phases, others were more prevalent in specific phases indicating a reproductive association with environmental conditions. In the case of Clupeiformes, which occurred and were abundant in all hydrological phases, they have adaptations that allow them to reproduce throughout the year and under different hydrological conditions (Bloom and Egan, 2018; Reis et al., 2020; Oliveira et al., 2021). Variations in the captures of larvae of different taxonomic orders throughout distinct hydrological phases may reflect an adaptive life history strategy relevant to species perpetuation. For example, some species of Characiformes reproduce before the reproductive peak of other species so that their larvae can develop and capture their prey (i.e., larvae of other fish) (Leite et al., 2006; Cajado et al., 2018). On the other hand, some species start their reproductive activities when the river level rises so that their larvae reach the recently flooded plains and find in these environments' favorable places for growth (Zacardi et al., 2020a; Oliveira et al., 2020). Therefore, variations observed in larval densities throughout hydrological phases are directly related to the natural environmental changes and the landscape mosaics that occur associated with the modulation of local hydrological cycle.

Clupeiformes and Perciformes larvae (family Sciaenidae) contributed to the highest larval densities during receding water. Larvae of these two orders are abundant and found during all months of the year drifting in the Amazonian aquatic systems (Araujo-Lima and Oliveira, 1998; Oliveira and Ferreira, 2008; Zacardi et al., 2017). In the case of Perciformes larvae, their highest densities when the water level is receding are related to the productivity that occurs in the main river channel at this stage, such as a greater abundance of zooplankton and organic material (Schöll et al., 2012; Chaves et al., 2019). In addition to Clupeiformes, larval contribution of Siluriformes and Gobiiformes was important for the highest density observed in the flooding phase. For Siluriformes, highest records of larval drift occur mainly during January, February, March, and December in the Amazon River, when water levels are rising and coincide with the reproductive aspects of migratory species (Chaves et al., 2017; Zacardi et al., 2017). This abundance of Siluriformes during the flooding phase, contrasting with the pattern observed in some Amazonian rivers, may be due to the distinct functional composition of species of this order, which varies according to the hydrological cycle (Silva et al., 2021).

Environmental changes occasioned over time, such as the increase in river level, pH, temperature, and dissolved oxygen in water, are

crucial for the reproduction processes and their predictors to be guaranteed (Baumgartner et al., 2008; Rosa et al., 2018; Humphries et al., 2020). The peak of ichthyoplankton capture in the Tapajós River basin was during drought phase. This differs from the pattern of greater reproductive activity observed for the ichthyofauna in Amazon region, in which the highest densities of ichthyoplankton are captured during rising waters phase (Ponte et al., 2016; Zacardi and Ponte, 2016; Zacardi et al., 2017; Mariac et al., 2022). The highest ichthyoplankton densities during the drought phase may have been strongly influenced by the occurrence of rainfall since, during November 2021 (drought phase), there was precipitation before the rainy season. As rainfall is considered a key environmental factor for the gene flow of species, it directly influences the abundance of fish eggs and larvae (Ferreira et al., 2016; Teixeira et al., 2019; Sanches et al., 2020; Badú et al., 2022). Furthermore, temporal variability of ichthyoplankton drift is related to functional characteristics and life history of each species (Nagel et al., 2021; Gogola et al., 2022).

The occurrence of drifting eggs in all sampled sectors indicates that the Tapajós River has multiple spawning sites. Some studies investigating the traditional knowledge of fishers observed that the area known as the São Luiz do Tapajós rapids (sector 1 in our study) would be one of the main sites of breeding for migratory fish species (Barthem et al., 2016; Nunes et al., 2019; Runde et al., 2020). Cajado et al. (2020), when studying the ichthyoplankton community in the confluence zone between the Tapajós and Amazon rivers, indicated that a probable spawning region would be near the São Luiz do Tapajós rapids. Due to the capture of eggs in development having been recorded close and even in these rapids, we confirm that this region should be considered an essential area of gene flow for the ichthyofauna of the Tapajós River. This result was expected since stretches with high water flow are conducive to intense reproductive activity, especially of migratory species (Silva et al., 2012; Ávila-Simas et al., 2014; Makrakis et al., 2022). However, more in-depth studies investigating the embryonic stages are needed to define spawning areas with greater precision.

The existence of islands and sandbanks may have been one of the main factors that favored the highest densities of ichthyoplankton in sector 2. For example, islands can act as biological filters, retaining fish larvae during floods and favoring their survival and growth, as they can act as important sources of nutrients and sustain the high abundance of fish larvae (Chaves et al., 2019; Oliveira et al., 2020; 2021). The highest larval density in sector 3 is due to reproductive activity in stretches upriver. This sector has numerous marginal lagoons connected with the main river and influenced by the flood pulse. Moreover, protected areas on both riverbanks maximize productivity and fish conservation (Keppeler et al., 2017; Silvano, 2020; Rocha et al., 2022). These conditions can increase the survival of larvae since it facilitates their access to the river's marginal lagoons, which are considered nursery and fish recruitment sites in the Tapajós River (Oliveira et al., 2023).

Although mosaics of environments may favor the survival of the larvae, the plastic waste that drifts concomitantly with these organisms can cause deleterious effects on this biological community, mainly through the ingestion of inorganic particles (Steer et al., 2017; Gove et al., 2019). However, the impacts of plastic waste are not restricted to the trophic ecology of the species but also its fitness. For example, Pannetier et al. (2020), when investigating the possible effects of microplastics on fish larvae, found that environmental exposure to plastic waste induces sublethal effects on larval behavior and growth. In addition, these authors also observed DNA damage, which indicates the absorption of pollutants sorbed from microplastics by the larvae, as well as physical damage, revealing some of the impacts of inorganic materials on the early life stages of fish. Here we identified the importance of the Tapajós River as a spawning, drifting, and nursery area for the early life stages of fish and detected a major ichthyoplankton density. Although we did not assess the ingestion of plastic waste by fish larvae the simultaneous occurrence of ichthyoplankton and plastic waste in this river is a problem. According to Lima et al., 2016 the function of nursery areas may be reduced if larvae are ingesting plastics. During the receding water phase, the density of plastic waste exceeded about 1.5 times the larval density. In this phase, individuals born in drought and rising water and managed to survive already have well-developed locomotion structures and, consequently, the ability to swim against the current and capture prey (Oliveira et al., 2020; Silva et al., 2022), which can facilitate the ingestion of plastic waste.

In the receding water phase there is a change in the direction of water flow (from floodplain to river), carrying organic and inorganic materials to the river channel (Sousa and Freitas, 2008). Plastic waste densities exceeding that of eggs and fish larvae during this phase can be attributed to the unfavorable time of reproduction of the local ichthyofauna and the movement of flow and concentration of water in the main channel of the river, contributing to input, suspension and dispersion of plastic waste along with plankton in the lotic system. This is similar to what was hypothesized for the Goiana estuary, in which the entry of microplastics into the estuary is associated with river flow, which, together with rainfall, induces the runoff of terrestrial plastic waste into the aquatic system (Lima et al., 2014). Although the density of plastic waste was higher in the receding water phase, their drift in the other phases is also worrying.

Although we did not identify the polymers in plastic waste, most of the filaments captured during sampling seemed to come from nylon cables and lines—a material widely used in fisheries and to moor medium and large vessels in regional ports (Dantas et al., 2012; Ramos et al., 2012). These findings suggest that small-scale fisheries can contribute a portion of the entry of plastic waste into this aquatic system, although there are other sources such as waterway ports, domestic and industrial effluents. This shape of plastic waste was observed to contaminate the gills and the gastrointestinal tract of fish species from Amazonian streams, indicating the vulnerability of this community to plastic pollution arising mainly from urbanization (Garcia et al., 2020; Ribeiro-Brasil et al., 2020).

The diversity of plastic waste classes drifting can be the result of physical, chemical and biological fragmentation of plastic bags, paint chips, snack packaging, bottles, jars, cosmetics and other inorganic sources (Lima et al., 2014; Giarrizzo et al., 2019; Azevedo-Santos et al., 2021; Parker et al., 2021). This variety of plastic waste sources can be considered a predictive factor of the diversity of colors captured in the samples along the Tapajós River. However, this result is an alert, as the small size (mean =  $1.55 \pm 1.27$  mm) and the different colors of the plastic waste can be easily confused as prey and ingested by fish larvae. This can cause deleterious consequences in the larvae, such as the sensation of false satiation, reduction in feeding rates, blockage of the digestive system and, consequently, negative effects on growth, in addition to chemical stress reflecting in altered feeding behavior (Wright et al., 2013; Sá et al., 2015; Welden and Cowie, 2016).

# 5 Conclusion

This study is the first to present an overview of the distribution and drift processes of ichthyoplankton and plastic waste along the Tapajós

River, Amazon Basin. Our findings indicate that this river system is of great importance for the reproduction of fish species and its different sectors have specific relevance such as breeding sites, areas of dispersion, nursery and growth. The largest captures of ichthyoplankton during the drought phase is an indication that at this phase there is a great interaction and gene flow of species in the Tapajós River, and the capture of eggs in all sectors allows us to infer that there is reproduction of fish throughout in all sampled area. The first information on plastic waste in the Tapajós River provided here can serve as a baseline for future studies. In addition, the different shapes, and colors of microplastics found are worrisome, since there may be a negative interaction of these residues with fish larvae communities and negatively influence their physiological and ecological processes. Because plastic waste shares the same environment with the ichthyoplankton community in the Tapajós River and presents different sizes, studies that investigate the trophic ecology of larvae are necessary to verify the direct impacts of the presence of these wastes on larval assemblages in this Amazonian river system. Finally, management actions aimed at minimizing and reducing the entry of plastic waste into the Tapajós River are necessary and must be developed in agreement with environmentalists, public agents and residents.

# Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

# **Ethics statement**

Ethical review and approval was not required for the animal study because all specimens used in this study were captured under the authorization issued by the Sistema de Autorização e Informação em Biodiversidade (numbers 775271-1/2020) and followed the euthanasia protocols in accordance with the norms of the Conselho Nacional de Controle e Experimentação Animal (Conselho Nacional de Controle e Experimentação Animal, 2015).

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# Author contributions

Conceptualization: LO, DZ, and JO-J. Development methods: LO, DZ, FS, and RC. Conducting the research: LO, FS, and RC. Data analysis: LO. Preparation of figures and tables: LO. Conducting the research, data interpretation, writing: LO, DZ, JO-J, RC, and FS. Writing-review and editing: DZ and JO-J.

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# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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