Exploring ichthyofaunal richness through diving and visual census

COMPARATIVE ANALYSIS OF ICHTHYOFAUNAL RICHNESS IN A

NEOTROPICAL REGION: DIVING, VISUAL CENSUS AND

TRADITIONAL FISH COLLECTING TECHNIQUES

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Abstract: The Neotropical region harbors unique biodiversity and a vast wealth of fish species. Inventories of river basins are essential for assessing environmental impacts. In this study, we analyzed the use of diving and visual census data as complementary tools for inventorying rheophilic ichthyofauna in the Tocantins River. Compared with traditional methods, diving and visual censuses register exclusive and shared species, increasing the efficiency of detecting fish diversity in rapids. We observed that Characiformes were predominantly sampled using traditional methods whereas diving and visual censuses played important roles in sampling Siluriformes. The cluster analysis highlighted the complementarity between diving and the visual census. These findings underscore the importance of employing effective and complementary methodologies for accurate

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inventorying of rheophilic ichthyofauna in Neotropical regions, minimizing the risks of

neglect associated with conventional sampling methods.

Keywords: Conservation; Fishes; Methodologies; Tocantins; StereoDOVs.

INTRODUCTION

The Neotropical region is globally renowned for its exceptional biodiversity, especially its rich freshwater fish diversity (Lévêque et al., 2008; Antonelli, 2018; Dagosta & Pinna, 2019). Neotropical fish communities, comprising an immense variety of species (Albert et al., 2020), depend on specific microhabitats and food resources for survival (Agostinho et al., 2008; Stein et al., 2014). Among Neotropical basins, the Amazon basin is unparalleled in ichthyofaunal diversity, boasting at least 2720 validated species across 529 genera, 60 families, and 18 orders (Dagosta & Pinna, 2019). This remarkable diversity underscores the importance of cataloging hydrographic basins to provide a comprehensive strategy for evaluating the influence of anthropogenic environmental impacts on ecosystems (Silva et al., 2019).

Difficult-to-access environments, such as the rapids and waterfalls of large rivers in the Amazon region, are characterized by high velocity, highly heterogeneous water flow, oxygenrich content, and a very complex substrate matrix that harbors a diverse array of niches (Hrbek et al. 2018). These specific biotic characteristics decrease the effectiveness of traditional fish collections, highlighting a limited understanding of the habits and composition of the fish communities in these locations. This environment has especially been subjected to recurring activities such as dam construction for hydropower generation (Mol et al., 2007; Santos et al., 2017).

Unfortunately, these activities often overlook environmental concerns for freshwater biota, potentially negatively affecting river diversity and ecosystems. The Tocantins-Araguaia Waterway project is underway in Brazil, aiming to enhance navigation in a geographically significant region within the Amazon's freshwater ecoregion. This area represents Brazil's largest

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hydrographic basin, characterized by high endemism, akin to some Amazon River tributaries (Abell, 2008; Albert & Reis, 2011; Dagosta & Pinna, 2017). However, constructing this waterway could lead to habitat loss, directly impacting fish species that are closely tied to their environment. Studies have shown that environmental alterations significantly affect fish communities and their biodiversity (Casatti et al., 2012; Stein et al., 2014).

The primary drawback of bottom trawling lies in its potential adverse effects on the ecosystem, particularly on habitats and organisms associated with water bottoms (Johnson et al.,2015; Oberle et al.,2016). Similarly, hook-and-line fishing presents a significant drawback due to the considerable damage and stress inflicted upon the caught fish, often leading to their mortality (Côté Perrow, 2006; Murphy & Jenkins,2010). These concerns are juxtaposed with traditional fish sampling techniques, which have long been employed to capture, euthanize, and bring specimens to scientific collections. While these methods have played crucial roles in species description and ecological studies, their efficiency tends to diminish in regions characterized by rocky terrain and strong currents. Non-invasive methodologies such as underwater video surveys have gained prominence in fish assemblage conservation. These surveys enable ichthyofaunal inventories, diversity and abundance estimates, and assessments of environmental protection areas, providing detailed records and the potential for future analyses (Cappo et al., 2006; Langlois et al., 2006; Stobart et al., 2007; Brooks et al., 2011; Harvey et al., 2012; Coghlan et al., 2017). The progress of video equipment has allowed new methods of visual census collection, such as Diver-operated stereo-video systems (stereoDOVs), which are applied similarly to transect visual census, but the diver conducts a stereo-video system (Shedrawi et al. 2014; Watson et al. 2010; Goetze et al. 2019; Rolim et al. 2022). In environments such as the Amazon's large river rapids, which are challenging to access and characterized by dynamic and complex conditions, visual census techniques prove to be a valuable tool for sampling ichthyofauna, assisting in their management and monitoring (Russ & Alcala,1994; Rosa &Moura, 1997). Its relevance extends to fragile and hyper-diverse environments, such as coral reefs (Ferreira et al., 1995; Brotto et al., 2005; Pivetta et al., 2012; Alves Bezerra, 2022).

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This study is the first to use stereoDOVs to inventory fish assemblages in an Amazon river. Our objective is to assess the utility of this technique as a complementary tool for rapid-dwelling fish inventories in the Tocantins River. This study aims to highlight the diversity of rheophilic fish using traditional techniques, diving, and stereoDOVs as complements, underscoring the importance of such methodologies in ichthyofaunistic studies.

MATERIAL AND METHODS

Study Area

Figure 1. The hydrological map shows the Tocantins-Araguaia watershed region on the border of the states of Pará and Tocantins in Brazil, and the correct map represents the diving and stereoDOV sampling points (red dots).

The Tocantins-Araguaia basin (Figure 1) includes parts of the states of Maranhão (3.8%), Tocantins (34.2%), Pará (20.8%), Goiás (26.8%), Mato Grosso (14.3%), and the Federal District (0.1%). This area accounts for approximately 10.8% of the Brazilian territory (MMA, 2006). The Tocantins River, near its confluence with the Araguaia River, flows through the Meio Norte sedimentary basin, a relatively recent formation that contributes a significant amount of sediment to the lower Tocantins River during the rainy and flood seasons (Santos et al. 1984). Along with the Araguaia River, which runs parallel and has a basin of similar magnitude, the Tocantins River

Oecologia Australis (ISSN: 2177-6199) Ahead of print (https://revistas.ufrj.br/index.php/oa/issue/view/1109/showToc) Article ID: AO#60901 Published online: 10 September 2024 covers an area of 803,250 km² (Morim et al. 2010).

Sampling Methodology

We gathered three datasets from three collection methods. The first was a fish dataset that used traditional collection methods during the Environmental Impact Report (EIA/RIMA) of dredging and rock removal works conducted in the navigable waterway of the Tocantins River (2018). The fishing gear used in this method included mesh netting, cast net, longline, net, drag, and pole. The other techniques were performed by the Laboratório de Ictiologia of Museu Paraense Emílio Goeldi team from dives during the expedition of the project funded by the Foundation for Diffuse Rights (FDD) "Reophylic Fish Fauna of the Amazon: Endangered and Unknown Natural Heritage," which took place in 2019. During diving, the divers collected fish directly from rheophilic habitats via the steroDovs technique, which consists of the diving filming process and filming specimens as many specimens as possible during the dive.

Environmental Impact Report

For this study, fish capture occurred during both flood and dry periods, and active and passive sampling techniques were employed (Figure 2). The active sampling methods included the use of trawl nets, sieves, and cast nets, whereas the passive methods involved gill nets, longlines, and fishing rods with reels and hooks and used bait such as fish pieces, fruits, and live bait (Dias, 2015).

Five trawl hauls of varying sizes were conducted on marginal beaches. The small trawl net measured 10 m x 1 m with 3 mm mesh, covering ten m² per haul. The large trawl net was 40 m x 2 m with 15 mm mesh, covering 80 m² per haul. Thus, each point had 450 m²trawled (50 m² with the small net and 400 m² with the large net) per campaign. A sieve was thrown 100 times in vegetated areas, measuring 0.60 m x 0.80 m (0.48 m² per throw), totaling 48 m² per point. Cast nets were thrown ten times for each of three sizes: small (2.4 m diameter, 28.26 m² per throw), medium (4 cm knot-to-knot, 5 m diameter, 78.5 m² per throw), and large (6 cm knot-to-knot, 6 m

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diameter, 113.04 m² per throw).

Figure 2. A - Cast nets being thrown for active capture of ichthyofauna at the Tocantins River. B - The team collects the gillnet in the early morning at the Tocantins River. Source: Environmental Impact Study: Dredging and Rock Removal Works of the Tocantins River Waterway. Volume III, DNIT, 2018.

To capture Abio (fish assemblage), 5-meter-high nets were initially planned, but standard height nets with 24 mesh spaces were used, varying in height on the basis of mesh size. For example, for nets with 6 cm between knots and 24 mesh spaces, the height was 1.4 m. This approach covered various depth environments, including shallower areas. Multiple nets were used at each sampling point, with mesh sizes ranging from 2.4 cm to 18 cm between knots. Each set had nine meshes, each 50 m long and up to 4.3 m high. Nets were exposed for 18 hours, from 12:00 pm to 6:00 am, covering approximately 121 m²/18 h at each point.

The study used a 200-meter rope with 50 hooks spaced at 4-meter intervals. The hooks alternated between sizes 5 and 7 and were baited with various fish pieces. The longline was deployed at 6:00 pm and retrieved at 6:00 am, capturing fish during their active night. Additional details such as the installation environment, target species, or bait types were not provided in EIA/RIMA 2018. Most fish were identified in situ and released, whereas some were euthanized with eugenol and preserved. The specimens were fixed in 10% formalin solution and housed in the following scientific collections: Emilio Goeldi Museum (MPEG) and the State University of Northern Paraná (UENP).

Diving and Underwater StereoDOVs collect methods

Diving and stereoDOVs occurred in three Tocantins-Araguaia River areas at six marked coordinates (Figure 1). The expedition ran from November 4th to November 15th, 2019, with six days of diving. Two daytime dives were typically conducted from 8:00 am to 2:00 pm, totaling 105 minutes of recorded footage. Each dive spanned 50 to 60 minutes and involved three divers, including an "acarizeiro," who specialized in capturing Loricariidae family ornamental fish (Figure 3). During diving, the fishes were collected using air compressors connected to up to 50 meter hoses, allowing collection at 40-meter depths. Head-mounted flashlights were used, and the fish collection was authorized by Authorization and Information System for Biodiversity (SISBio) under permit 70940.

Figure 3. A - Diver using a cast net for fish collection at the Tocantins River during the 2019 expedition. B - Diving tools on the boat: compressors, headlamps, and life jackets at the Tocantins River. Source: Leandro Sousa.

StereoDOVs can employ strategically placed cameras to record underwater species diversity. Cameras captured continuous images during a set time and were subsequently analyzed to identify species and assess aquatic life abundance and diversity. In this study, divers collected the fish they encountered, and once a few specimens of a species were obtained, the rest were left

Ahead of print (https://revistas.ufrj.br/index.php/oa/issue/view/1109/showToc) Article ID: AO#60901 Published online: 10 September 2024

but continued to be filmed. Tools such as gloves, a "tarrafinha" (small cast net), and PET bottles tied to the diver's body aided collection. Divers wore scuba gear, including a mask, snorkel, and head-mounted flashlights, with the professional diver equipped with oxygen cylinders, a GoPro Hero 4 camera, a flashlight, and a Suunto Zoop dive computer.

The captured fish were placed in PET bottles, identified, anesthetized, and preserved in formalin. Underwater stereoDOVs involved exploratory dives and recorded observed fish via video (Figure 4). The videos were analyzed frame-by-frame using Shotcut software to better mark and see frames. Identification guides were used for better taxonomic confirmation. Unclear cases were identified by family or genus, with the abundance estimated. The data were tabulated in spreadsheets in Microsoft Excel for further statistical analysis in R 4.0.5 (R Core Team, 2021).

Figure 4. Divers collect a specimen using flashlights with a camera for stereoDOVs and dive watches at the Tocantins River during the 2019 expedition Source: Leandro Sousa

Statistical analysis

All our analyses were performed in R software, version 4.0.5 (R Core Team, 2021). First,

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we built rarefaction individual-based curves for each sampling method, inside each fish family separately, and exclusive to the order Gymnotiformes. We extrapolated values twice the size of each curve, acknowledging that those extrapolated beyond these values can be unreliable (Colwell et al., 2012; Chao et al., 2014). To compare the species richness among the different sampling methods for each fish family and order, we calculated the confidence intervals with 999 randomizations. To assess the compositional differences among the sampling methods and their potential complementarities in collecting the fish community, we performed a cluster analysis using an abundance matrix. On the basis of this matrix, we used the Bray-Curtis dissimilarity index and UPGMA (unweighted pair-group method with arithmetic mean) linkage method to create a dendrogram. For the interpolation and extrapolation curves, we use the package iNEXT (Hsieh et al., 2016).

The method uses the UPGMA algorithm with the Bray-Curtis coefficient to analyze fish abundance data from three sampling methods: diving, stereoDOVs, and traditional collection with fishing gear. UPGMA is a hierarchical clustering method that groups similar samples, and the Bray-Curtis coefficient measures dissimilarity between samples on the basis of species composition. This approach allows for a comparative analysis of fish diversity and distribution across different sampling methods.

RESULTS

In 2017-2018, 5583 individuals were collected using the traditional methods for the EIA/RIMA assessment (Table 1). In 2019, the expedition collected 636 individuals via diving and 143 via stereoDOVs. The most abundant groups observed through different collection methods were as follows: in traditional collections, the Characiformes group comprised 58.51% of the total individuals, followed by Siluriformes at 20.51%; during diving activities, Characiformes accounted for 64.89% of the total individuals, with Siluriformes at 14.77%; and via StereoDOVs, Siluriformes represented 60.22% of the total individuals, followed by Gymnotiformes at 9.9% (Figure 8). We found 115 species belonging to the Characiformes order: 105 in Siluriformes, 36

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in Cichliformes,22 in Gymnotiformes, and 11 in Clupeiformes (Table 1).

Table 1. Sampling results by fish order and family from the Tocantins River. Data include the number of species and individuals collected using traditional methods, diving, and stereoDOVs, based on information gathered during the 2019 expedition.

Among the traditional methods, four species were most represented, comprising 25.79% of the total individuals analyzed: *Cyphocharax leucostictus* (Eigenmann & Eigenmann, 1889) (13.32%), *Geophagus proximus* (Castelnau, 1855) (5.10%), *Acestrorhynchus microlepis* (Jardine, 1841*)* (3.77%), and *Lycengraulis batesii* (Günther, 1868) (3.58%), which belong to the orders Characiformes, Cichliformes, and Clupeiformes. Regarding the diving method, four species were most represented, accounting for 31.76% of the individuals analyzed, including rheophilic species sampled via traditional methods: *Peckoltia* sp. (16.03%), *Ancistrus* sp. (6.28%), *Parancistrus aurantiacus* (Castelnau, 1855) (5.34%), and *Baryancistrus longipinnis* (Kindle 1895) (4.08%) (Figure 5).

With respect to the stereoDOVs method, four species were most represented (31.4%), with Loricariidae present in three of the four most representative species: *Parancistrus* sp. (9.79%), *Acanthicus hystrix* Spix & Agassiz, 1829 (8.39%), *Baryancistrus* sp. (6.99%), and *Leporinus* sp. (6.29%) (the latter belonging to the family Anostomidae) (Figure 6).

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Figure 5. Images of the most represented species during the dive in the Tocantins River: A - *Ancistrus* sp. (85.1 mm); B - *Baryancistrus longipinnis* (93.4 mm); C - *Parancistrus aurantiacus* (87.2 mm); D - *Peckoltia* sp. (63.5 mm), based on data collected during the 2019 expedition.

Oecologia Australis (ISSN: 2177-6199) Ahead of print (https://revistas.ufrj.br/index.php/oa/issue/view/1109/showToc) Article ID: AO#60901 Published online: 10 September 2024

Figure 6. Images of the most represented species in the stereoDOVs from the Tocantins River: A - *Parancistrus* sp.; B - *Acanthicus hystrix*; C - *Baryancistrus* sp.; D - *Leporinus*sp., based on data collected during the 2019 expedition. Source: Leandro Sousa

Loricariidae was more abundant under both traditional and diving methods, while StereoDOVs recorded a greater number of species. Characidae exhibited more extraordinary richness and abundance in the Traditional approach when comparing the three methods. Cichlidae also used three methods, which were more effective than traditional ones Anostomidae had a higher abundance using the Traditional method and a greater species richness when compared with the others. Gymnotiformes showed higher abundance during diving, with species richness similar to that observed using traditional methods, comparable to stereoDOVs (Figure 7) The similarity analysis, using the UPGMA linkage method, revealed that the diving and stereoDOV methods had more remarkable similarities than did the traditional methods (Figure 8).

Using a Venn diagram (Figure 9), we grouped the methods and the number of species per method, revealing that 181 (59.3%) species were exclusive to traditional methods, 60 (19.7%)

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species were exclusive to diving, and 21 (6.89%) were exclusive to stereoDOVs. Additionally, 4

(1.31%) species were detected via all three methods, 14 (4.59%) were detected via both diving

and stereoDOV, and 22 (7.21%) species were detected via both diving and traditional techniques.

Finally, 3 (0.98%) species were detected via both the conventional and stereoDOV methods

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Figure 7. Accumulation curves for different tropical fish families from the Tocantins River, depicting abundance and species richness across the three sampling methods. (A) Loricariidae, (B) Characidae, (C) Cichlidae, (D) Anostomidae, and (E) Gymnotiformes. The curves highlight differences in abundance and species richness distribution among traditional sampling, diving, and underwater stereo camera (stereoDOVs) methods, based on data collected during the 2019 expedition.

15

Distance Bray-Curtis

Figure 8. UPGMA method (Unweighted Pair Group Method with Arithmetic Mean), using the Bray-Curtis coefficient with fish abundance data from three sampling methods (diving, stereoDOVs, and traditional collection with fishing gear), based on data collected during the 2019 expedition in the Tocantins River.

Figure 9. Venn diagram illustrating the quantity and proportion of fish species sampled exclusively and shared among various methods (diving, stereoDOVs, and traditional fishing gear collection), based on data collected during the 2019 expedition in the Tocantins River.

16

DISCUSSION

Collection Methods

The selection of proper methods for ichthyofaunal inventories should consider the environment and species' habits. This study emphasized the complementarity of stereoDOVs in river and rheophilic habitats and compared it with traditional methods such as gill nets. Traditional methods captured more species due to an increased sampling effort, spanning a greater diversity of habitats. StereoDOVs were limited due to their maximum operating depth and water visibility, but they explored a range of aquatic depths, increasing precision. By their design, video methods are non-invasive and non-extractive and, therefore, have minimal impact on the marine environment. They can be applied in almost every habitat (Hammerl *et al.* 2024). To improve stereoDOVs, a comprehensive, layered approach, and targeted dives are crucial for precise fish diversity and ecological assessment in rapids.

The dives were directed, allowing for a more comprehensive exploration of different levels of the aquatic environment. The divers could observe species inhabiting intermediate layers and the bottom, contributing to a more accurate understanding of the diversity of fish in the rapids. Stereo-DOVs were not conducted perfectly, as they were considered a 'pilot' in the expedition and did not record videos on every dive. This resulted in less diversity of fish in the rapids from the filming than normally happens when the technique is used.

The stereoDOV limitations and dive-focused approach underscore the need for a more comprehensive and integrated method. Improving stereoDOVs involves considering diverse habitat layers and targeted dives for precise fish ecology assessment in rapids. Data interpretation suggests that traditional methods such as gill nets capture more taxa due to their non-selective nature. Colton & Swearer (2010) favored stereoDOVs because of their relatively high species richness despite differences in effort, suggesting the need for multiple methods for diversity cataloging. Combining techniques, such as those in Murphy & Jenkins (2010), proves more effective for monitoring, supporting the idea that active and passive methods complement each

other and prevent the underestimation of species richness.

Our results, similar to those of Esteves (2006), were due to drier conditions, affecting visibility due to turbid water. The Venn diagram highlights how the combined methods minimize selection differences, achieving a more accurate representation of the local fish assemblage (Greene & Losos 1988). Diverse life habits should be considered via distinct selection methods for assemblage inventories (Backiel et al. 1980, Sale & Sharp 1983). Traditional methods are comprehensive, capturing 59.3% of species (Elliott& Bagenal 1972, Degerman et al. 1988, Jensen 1990, Hubert et al. 2012). Generalist sampling depends on individual behavior, as observed in Anostomidae (Hayes 1989, E. G. Reis & Pawson 1999, Olin et al. 2009).

Comparatively, stereoDOVs excel in reaching specific species and habitats, providing greater diversity, as supported by Colton & Swearer (2010), Murphy & Jenkins (2010), Zeller & Russ (1998), Willis & Babcock (2000), and Stobart et al. (2007). Divers capture nuances in complex habitats missed by stationary cameras, which is vital for rheophilic environments (Watson et al. 2005, Stobart et al. 2007).

Diving and remote underwater video methods,each offer advantages (Longo & Floeter 2012). Videos provide permanent records and behavioral insights but face visibility and identification challenges (Colton & Swearer 2010, Leonard 2020). Diving allows live specimen collection, aiding in behavioral and physiological studies (Mourão et al. 2019). Observations are vital for understanding the habits of species such as the psamophilic *Pygidianops amphioxus* (Pinna & Kirovsky 2011), for example (Carvalho et al. 2014). However, divers can impact territorial fish, altering feeding and refuge-seeking behaviors (Benevides et al. 2019). Studying such behavioral changes due to human influence is crucial (Hodgson 2000, Lang et al. 2010, Wong & Candolin 2015) as they are related to adaptation and survival strategies. Long-term sensitization and varying tolerance levels require comprehensive assessments, not just species abundance and length measurements (Geffroyet al. 2015, Hodgson 2000, Lang et al. 2010). Monitoring fish behavior in rapid environments akin to coral reefs aids in understanding

anthropogenic impacts for better conservation and management (Gregor et al. 2015).

Fish Assemblage

Among the endemic species, according to the technical report 02001.000809/2013-80 from the Museu Paraense Emílio Goeldi (MPEG), issued after the 2019 expedition, several species were considered threatened with extinction in the Tocantins River, especially in the Pedral do Lourenço region. This classification follows the List of Threatened Fauna (D.O.U. 445 from 2014). The species at risk include: *Baryancistrus niveatus* (Castelnau, 1855), *Baryancistrus longipinnis*, *Crenicichla cyclostoma* Ploeg, 1986, *Crenicichla jegui* Ploeg, 1986, *Lamontichthys parakana* Paixão & Toledo-Piza, 2009, *Potamobatrachus trispinosus* Collete, 1995, *Scobinancistrus pariolispos* Isbrucker & Nijssen, 1989, *Teleocichla cinderella* Kullander, 1988, *Mylesinus paucisquamatus* Jégu & Santos, 1988, and *Paratrygon aiereba* (Walbaum, 1792). It should be noted that *Baryancistrus longipinnis* was found only in the Pedral do Lourenço region and was absent in other areas of the Tocantins River. In conjunction, the endemism of 24 species in the TO-AR Basin over the last 14 years has been reported (Akama 2017).

The traditional method revealed Loricariidae diversity, but complementary methods improved species coverage. The fish captured depend on habitat characteristics, as shown by Uieda and Barreto (1999). These results can be attributed to the use of selective gear and inadequate sampling periods (Castro 1997). In the Xingu River rapids, Loricariidae, Cichlidae, and Anostomidae are strongly rheophilic (Zuanon 1999). Anostomidae and Loricariidae predominated in the diving samples, such as *Baryancistrus* Rapp Py-Daniel, 1989 and *Leporinus* Agassiz, 1829. Gymnotiformes thrive in diverse habitats accessible through diving but can escape detection by stereoDOVs because of their sensory perception abilities, which could influence observer bias. Gymnotiformes possess the unique ability to perceive their surroundings through electrical fields. This sensitivity allows them to identify prey, avoid predators, and navigate aquatic environments efficiently. Complementary inventories are vital for understanding ichthyofaunal diversity, especially in the face of human activities such as dam construction (Lima 2020). Conservation efforts are crucial in light of the threats faced by freshwater biodiversity (Dudgeon 2006).

Considering the current situation of the Tocantins-Araguaia River, as described by Akama (2017), which anticipates significant harm to the ichthyofauna of this basin, supporting Dudgeon's (2006) findings, freshwater biodiversity faces global threats, with habitat destruction and degradation being among the leading causes. The authors point to the need to carry out impact assessments in these environments for continuous efforts to conserve them, including future ventures in the basin.

The conservation of rapid-dwelling fishes is vital for Tocantins River biodiversity. Complementary studies are needed to assess ichthyofaunal diversity and mitigate human-induced impacts. Despite our study's sampling limitations, methodological complementarity highlights the importance of combined approaches for accurate biodiversity inventories and freshwater fish impact analyses. Diving should complement traditional methods such as gillnets, traps, and trawls for comprehensive habitat and behavior coverage. StereoDOVs aid in counting fish, offering valuable data for monitoring, performing conservation programs, and understanding distribution patterns. Integrating diving, traditional methods, and stereoDOVs efficiently collects diverse data, aiding aquatic ecosystem understanding and conservation.

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19

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