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Molecular Ecology
March 2022, Volume 31, Issue 6, Pages 1627-2648
https://doi.org/10.1111/mec.15944
https://archimer.ifremer.fr/doc/00693/80501/

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Abstract :

The Amazon basin holds the world's largest freshwater fish diversity. Information on the intensity and timing of reproductive ecology of Amazonian fish are scant. We use a metabarcoding method by capture using a single probe to quantify species-level ichthyoplankton dynamics. We sampled monthly for two years the Marañón and the Ucayali rivers in Peru. We identified 97 species that spawned mainly during the flood start, the flood end, or the receding periods, although some species had spawning activity in more than one period. This information was new for 40 of the species in the Amazon basin and 80 species in Peru. Most species ceased spawning for a month during a strong hydrological anomaly in January 2016, demonstrating the rapidity with which they react to environmental modifications during the breeding season. We also document another unreported event in the Amazon basin, the inverse phenology of species belonging to a same genus (Triportheus). The overall larval flow in the Marañón was more than twice that of the Ucayali, including for most commercial species (between 2 and 20 times higher), whereas the Ucayali accounts for ~ 80% of the fisheries landings in the region. Our results are discussed in the light of the main anthropogenic threats to fishes, hydropower dam construction and the Hidrovía Amazónica, and should serve as a pre-impact baseline.

Keywords : capture, fish larvae, Marañón, metabarcoding, phenology, Ucayali

INTRODUCTION

The Amazon basin is the most species-rich ecosystem on Earth. Its extensive river network holds ~16% of the world's surface, liquid fresh waters (Latrubesse et al., 2017) and provides ~ 20% of the world's freshwater discharge (Salati & Vose, 1984). It also hosts the world highest freshwater fish diversity, with 2406 described species, which represents 15 % of the world's freshwater fishes (Jézéquel et al., 2020). Once relatively pristine, the Amazon basin is now facing increasing threats from growing human activities and climate change and is considered in transition to a disturbance-dominated regime (Davidson et al., 2012). Fresh waters are the most degraded and imperilled ecosystems in the world (Carpenter, Stanley, & Vander Zanden, 2011; Dudgeon et al., 2006; Vörösmarty et al., 2010) and freshwater fishes are among the most threatened vertebrates in the world (Pimm et al., 2014). This also holds true in the Amazon basin, where the drivers of aquatic ecosystem degradation and threats to fishes are mostly the same as those menacing freshwater biodiversity worldwide: overexploitation, flow modification by dams, deforestation, destruction or degradation of habitat, water pollution, invasion by exotic species and climate change (Castello & Macedo, 2016; Castello et al., 2013).

Beside their exceptional diversity, fish represent the main source of animal protein for people of the Amazon basin, with some of the world's highest fish consumption rates per capita (up to 200 $kg.year^{-1}$ for rural populations, (Batista, Inhamuns, Freitas, & Freire-Brasil, 1998; Isaac & Almeida, 2011). The rapidly growing human Amazonian population (a 10-fold increase between 1960 and 2010 in Brazil alone, from 2.5 to 24.3 Million, (DeFries, Rudel, Uriarte, & Hansen, 2010; Tritsch & Le Tourneau, 2016) exerts increasing pressure on natural resources and on fishes in particular. Most large, highly valued species are already overexploited in the Amazon basin and have been progressively replaced by smaller, less-prized species with faster turnover rates (Castello et al., 2013). Yet, basic life history information on the reproductive periods, spawning grounds and larval recruitment of many exploited species, which are crucial determinants of fish reproductive success in an ecosystem, are often lacking for proposing sustainable fisheries management practices and conservation strategies. Traditional approaches that consist in monitoring adult populations over a complete annual cycle, at least, can be so costly and time-consuming on a lot of species or on large, high-valued and rare species, as to become unrealistic. Obtaining this information by sampling their

eggs and larvae could be an interesting alternative solution now that advances in molecular techniques, such as barcoding have solved the long-standing problems of taxonomic identification of early life stages (Becker, Sales, Santos, Santos, & Carvalho, 2015; Frantine-Silva, Sofia, Orsi, & Almeida, 2015; García Dávila et al., 2015). Obtaining individual barcodes for each egg or larvae, however, can quickly become cost-ineffective and time consuming, then unrealistic when large numbers of larvae are involved (Evans et al., 2016). Taking advantage of next generation sequencing (NGS), metabarcoding approaches allowing the massive sequencing of large numbers of larvae in bulks have recently emerged, using either PCR-based (Loh, Bond, Ashton, Roberts, & Tibbetts, 2014; Nobile et al., 2019) or capture-based methods (Gauthier et al., 2020; S. Liu et al., 2016; Maggia et al., 2017; Wilcox et al., 2018). Although these methods proved very useful and cost-effective on a qualitative basis, there were many doubts about whether they could provide reliable quantitative results, especially with PCR-based methods owing to amplification biases, barcode chimeras, and a level of bias depending on the primers used and the number of PCR cycles (Duke & Burton, 2020; Lamb et al., 2019; Piñol, Senar, & Symondson, 2019; Zinger et al., 2019). Another approach, using shotgun metagenomics successfully generated quantitative species-level estimates of larval abundance on coral reef fishes in the red see (Kimmerling et al., 2018). For Amazonian fish species, we recently proposed a new method based on hybridization capture of the COI gene using a single, almost universal probe from a species absent from the Amazon basin and approximately genetically equidistant from all Amazonian fish species (Mariac et al., 2018). This new Metabarcoding by Capture using a Single Probe (MCSP) methodology showed strong correlations with true frequencies estimated by a Sanger approach, allowing the development of a reliable quantitative approach.

Here, we use this method to quantify and compare, over two consecutive hydrological cycles, the species-level fish larval dynamics in two of the most important tributaries of the Peruvian Amazon, the Marañón and Ucayali rivers. Besides overharvesting problems common to most highly populated Amazonian regions (Castello et al., 2013), the ichtyofauna of these two rivers is also threatened by planned anthropogenic activities such as dams and waterways building (Anderson et al., 2018; Bodmer et al., 2018). We sampled larvae monthly during two consecutive annual cycles in fixed localities just upstream of the confluence of the Marañón and Ucayli rivers to answer the following research questions: What is the diversity of fish species spawning in the main channels of

the Marañón and Ucayali? What are their spawning periods and relative abundance in relation with the hydrological cycle? What is the relative contribution of these two rivers to the production of commercial fish larvae?

METHODS

Study area and sampling

Between January 2015 and December 2016, fish larvae (ichthyoplankton) were sampled monthly on the same localities in two tributaries of the Amazon River: the Ucayali and Marañón in Peru. Both the Marañón and Ucalayi rivers are white water rivers originating in the Andes. At their confluence of both rivers they form the Amazonas River. Both have beds mainly made up of sandy, silty and clayey sediments but the Ucayali is much more dynamic in its formation of meanders and channels than the Marañón. According to the SO-HYBAM dataset (www.so-hybam.org), during the two years of study (2015-2016) the average flow was 17.9 m3/s (CV=0.34) in Marañón River, 11.7 m3/s (CV=0.51) in the Ucayali River, with averaged current velocities of 1.51 m/sec (CV=0.18) and 1.16 m/s (CV=0.34), respectively. The two rivers have a marked and similar seasonality with a flooding (October to April) and a receding phase (May to September).The sampling sites were just above the city of Nauta in the Marañón (4°32’0.461” S, 73°34’30.881” W) and in the Ucayali above the confluence with the Marañón (4°29’57.249” S, 73°25’45.597” W, Figure 1). Ichthyoplankton were sampled in daylight by towing an ichthyoplankton net behind a boat with an outboard motor maintained approximately in a static position. Three nets were arranged vertically, one approximately 2 m below the surface, another ~ 3 m above the bottom and the third in between, with a distance of at least 2 m between each, along a rope weighed down by a 40 kg mass (García Dávila et al. 2015; Mariac et al., 2018). The protocol used 1.6 m long conical-cylindrical nets with an aperture diameter of 0.5 m, and a mesh size of 0.35 mm, each net containing a collector cup in its end. The nets were towed for 7 to 15 min, between 8 and 26 times a day, over a period of one to three days. It must be pointed out that our sampling by using plankton nets is limited to species with pelagic larvae, and therefore does not provide access to all the specific fish diversity present in the area.The towing duration (~fishing effort) was registered

for each individual tow (see Table S1 for details). Larvae were isolated from detritus and fixed in a 96% ethanol solution and transported to the laboratory for analysis.

Choice of larval size classes for metabarcoding analysis

The plankton nets used captured larvae between 2.8 and 26 mm. Over the 24 months sampling period, a total 165,101 larvae were collected, 102,762 from the Marañón and 65,339 from Ucayali rivers. For each sampling site and month, we sub-sampled 1000 larvae, when possible. The genetic analysis was carried out on 35,842 larvae. The larvae used for the analyses had a mean length of 5.91 mm ± 0.79 (SD), a length-class that included 83% of all the larvae collected. This size range corresponds to the initial stage of ontogenic development (early flexion stage) for most species (Nakatani et al., 2001). Moreover, performing DNA extractions on bulks of larvae of homogeneous size limits the representation bias in the final DNA extract.

From the sampling of December 2015 in the Marañón River, three sub-samples of 1000 larvae each were analysed independently and compared in order to verify the representativeness (in terms of species composition and frequencies) of our sub-sampling design. There was no significant difference between the three sub-samples (Repeated measure ANOVA on ranks, $P = 0.814$).

Ethic statement

A partner of the French National Institute of Research for Sustainable Development (IRD), the *Instituto de Investigaciones de la Amazonia Peruana* (IIAP**)** is rightfully authorized by the Peruvian government to study and to sample the Peruvian Amazonian biodiversity. The DIREPRO office "*Dirección Regional de la Producción del Gobierno Regional de Loreto*" authorized collect and exportation of fish larvae DNA to IRD laboratories in France.

NGS libraries preparation and sequencing

DNA bulk extractions were performed for each larvae sample following the rapid isolation of mammalian DNA procedures (Sambrook, Fritsch, & Maniatis, 1989). The preparation of enriched libraries by capture follows Mariac et al. (2018). Briefly, the DNAs were sheared, end repaired,

ligated with adapters, nick-filled by Bst Polymerase and amplified in real time PCR. Then capture enrichment was performed using a single COI probe on two equimolar bulks of indexed libraries (see detailed protocol in Text S1). Paired-end sequencing was carried out using MiSeq v2 reagents and 2 × 250 bp at the CIRAD facilities (Montpellier, France). Four negative controls (two blank for libraries preparation and two blank process during DNA extraction), and four positive controls (mock community samples with known species composition) were included during NGS libraries preparation.

Data cleaning and taxonomic assignation

The demultiplexing of the sequencing data was performed using the Demultadapt script (https://github.com/Maillol) based on the internal 6 pair index added during the ligation of the adapters. Removal of adpter sequences was performed with Cutadapt 1.2.1 (Martin, 2011) and low quality reads (mean Phred quality score lower than 30) were discarded using a freely available PERL script (https://github.com/SouthGreenPlatform/arcad-hts/blob/master/scripts/arcad_hts_2_Filter_Fastq_On_Mean_Quality.pl). Paired-end reads were then aligned with the MALT program version 0.3.8 (Herbig et al., 2016) on our COI database (Table S2). This database contains 160,387 COI sequences of Actinopterygii extracted from GenBank and Bold (Ratnasingham & Hebert, 2007) and was also implemented with our own published sequences (271) but not yet available under GenBank (Table S2). Among the 16,270 species or sub species contained in the database, 558 species are from the Amazon basin (23.2% of the 2406 described Amazonian species, and 37.9% of described species in the Marañón and Ucayali rivers, Jezéquel et al. 2020). Given the current incompleteness of the COI database, which represents only 37.9% of the species present in the Marañón and Ucayali rivers, if some species are present in our samples but absent from the database they cannot be detected. The twenty species most landed in the Loreto area where our sampling sites are located are represented in this database. Taxonomic assignation of the alignment results was performed with MEGAN software version 6.12.3 (Huson et al., 2016) using paired reads option and weighted LCA method. The Top percent parameter was set to 7, meaning that among the several possible alignments of one read, only those within 7% of the best score are kept. Only the assignments with a score of at least 150 and 98% identity with a taxon from the database were

retained. Meeting these conditions if a sequence aligns to more than one reference it is then attributed to their lowest common ancestor (genus or family). Two additional files (Table S3) were used during the assignment with the MEGAN program in order to manage the synonymy of certain taxa and to control assignment errors linked to typographical errors in the names of some reference taxa. The assignment rate (reads assigned), the rate of reads not reaching the score and percentage identity thresholds (reads not assigned) as well as the rate of reads without hits on the COI database are reported for each samples in Table S4.

Hydrological data

Hydrological data were obtained from the SO-HYBAM dataset (www.so-hybam.org). The series of mean daily water level and discharge from 2015 to 2017 at San Regis and Requena stations were used to characterize the regime of the rivers Marañón and Ucayali, respectively. The 2 hydrometric stations are located 49 km and 132 km upstream of the larvae collection points.

The 2001 to 2017 discharge measurement series at San Regis and 2003 to 2017 discharge measurement series at Requena were used to establish the water level / wetted section relationship as a two degrees polygon. These relationships were used to provide an estimate of the daily wetted area at the two stations, which was then applied to the sampling points to compensate for the lack of this information at these points. Since the mean daily discharge is already known, this also provides an estimate of the mean daily velocity in the section.

Data analysis

For each monthly sample the total larval flow-TLF (number of larvae per second that drift through the wetted section of the river) was calculated as: TLF=(Number of collected larvae / Towing duration in seconds)*(Estimated Wetted river section in m² / Net surface). Then, the larval flow per species was obtained by multiplying the monthly TLF by the estimated frequency of each species based on the abundance of COI reads: number of COI reads per species per month/ total number of COI reads per month.

The Maximum Sensitivity plus Specificity (MaxSSS) threshold value(Liu, White, & Newell, 2013; Manel, Williams, & Ormerod, 2001), which maximizes the sum of True Positive Rate and True Negative Rate. This threshold was computed with the R package ROCR 1.0–7 (Sing, Sander, Beerenwinkel, & Lengauer, 2005) using the mock control samples by comparing their actual species composition to those obtained by NGS. Thus taxa whose frequencies were not observed at least at 0.1% in a sample were excluded.

In order to assessed if our sampling effort between river were similar, we plotted the species accumulation curves and computed the second order jackknife (Jack2) estimator (Burnham & Overton, 1979) using the packages Vegan 2.5.6 (Oksanen et al., 2016) with the functions specnumber, specpool and diversity. The Jack2 are computed in order to estimate the ratio between the observed number of species and the maximum number of identifiable species (given our database) estimate by Jack2, and the curve makes it possible to estimate at which sampling effort the plateau is reached. It must be pointed out that the accumulation curves did not represent the actual specific richness of the region since they were limited by our current COI reference database, which contains only 37.9% of the total number of species known in the Ucayali and Marañón. Moreover, many of these fish species do not have pelagic larvae and could not be collected with our sampling scheme using plankton nets. Species diversity and composition were evaluated with R packages phyloseq 1.28 (McMurdie & Holmes, 2013), heatmap3 1.1.6 (Zhao, Guo, Sheng, & Shyr, 2014), pvclust 2.2.0 (Suzuki & Shimodaira, 2006).

In order to compare the spawning patterns of different fish species in the Marañón and Ucayali rivers, their respective hydrological cycles were divided into three periods of almost the same duration that best fitted the hydrological dynamics : the flood start (October-December), the flood end (January-April) and the receding (May-September) periods.

All command lines and scripts used for bioinformatic treatment, statistical analyzes and carrying out graph are available in S5 Text.

RESULTS

Sequencing and assignation results

Overall 58 libraries (48 samples, 2 repeated sub-samples in December 2015, 4 positive and 4 negative controls) were sequenced and produced 10.27 millions of raw reads. The mean number of reads per sample (excluding control and mock samples) was 199,602 (SE=120,312) with an average of 44.9 % assigned to a COI fish reference. Among the 48 samples, 38.5% of the reads were not assigned because their score or their percentage of identity were below the thresholds (minimum score of 150 and minimum percent identity of 98%) and 16.9 % of the reads found no hits in our COI database (Table S4). A blastn on the full Genebank database carried out with a subset of 10,000 "unassigned" and "no hit" reads, showed that when they could be assigned (30.2%), they are mainly of fish origin (98.5%). The 4 negative control libraries totalled only 86 reads (of which 18 were assigned to fish), representing 8.4 10^{-4}% of the sequencing run.

Species diversity patterns

The accumulation curves measures how many new taxa were added by each new monthly sample. Figure 2 shows that few additional species (considering the limitation of our database) were added after twelve months of sampling.

A hundred and twenty taxa were identified, 97 at the species level, 16 at the genus level (*Amblydoras*, *Astyanax*, *Brachyplatystoma*, *Cetopsis*, *Hoplias*, *Hypophthalmus*, *Leporinus*, *Mylossoma*, *Pimelodus*, *Plagioscion*, *Prochilodus*, *Pseudoplatystoma*, *Rhytiodus*, *Schizodon*, *Semaprochilodus* and *Sorubim*), 3 at the family level (Curimatidae, Pimelodidae, Serrasalmidae) and 3 at higher levels (Table S5). In total, the 120 taxa identified among the 48 samples analyzed cover 90.9% (Jack2=132.6) of the identifiable taxa richness (considering the limitation of our database), so that a maximum of 13 taxa might still have been detected by increasing the sampling effort. In the Marañón and Ucayali, the detected taxa (118 and 105) represented 88 and 93.8% of the identifiable taxonomic richness (134.1 and 112) using the Jack2 estimator, respectively. This difference is not

significant (p=0.19) showing that species-level ichthyoplankton dynamics could be compared between the two rivers.

The 97 species identified belong to 62 genera from 20 families and four orders. The orders Characiformes and Siluriformes accounted for most of the specific diversity: 47% and 49%, respectively. Perciformes and Clupeiformes were both represented by only 2 species (2% each). Among these 97 species, 82 were common to both the Marañón and Ucayali rivers, whereas 13 were sampled only in the Marañón (*Aguarunichthys torosus*, *Amblydoras* sp. 3 bold pattern 1312463, *Caenotropus labyrinthicus*, *Hoplias malabaricus*, *H.* aff. *intermedius*, *Leporinus friderici*, *Leptodoras cataniai*, *Nemadoras* sp. ghost 1312727, *Platynematichthys notatus*, *Rhinodoras boehlkei*, *Rhynchodoras woodsi*, *Semaprochilodus insignis*, *Sorubimichthys planiceps*) and 2 only in the Ucayali (*Ossancora punctata*, *Rhytiodus microlepis*). These 15 taxa are relatively rare, they all together represent only 1.45% of the overall larval flow (min taxa = 0.002%, max taxa = 0.93%), suggesting that their absence in one of the two rivers could be a stochastic effect of sampling.

Incidence of hydrology on larval distribution, abundance and diversity

During the two-year sampling period and in both rivers, the larval flow was higher during the flooding periods (both flood start and flood end; Figure 3), as was the number of taxa identified (Figure 4).

In both the Marañón and Ucayali, the number of taxa sampled during the flooding periods (flood start and flood end) was about twice as much as that sampled during the receding period (Figure 4). Within each of these hydrological periods, however, taxa diversity did not significantly differ between the Marañón and Ucayali rivers (p=0.52, p=0.98 and p=0.99 between flood end, flood start and receding periods, respectively). The lowest larval flow and number of taxa were observed during the receding period. The larvae collected during the flooding periods represented 77.7% (Ucayali) and 87.5% (Marañón) of the total flow.

Interestingly, the sharp discharge decline in January 2016 in the Marañón River (Figure 3) resulted in a strong decline in larval flow at the same month. The similar, but much less intense discharge decline in the Ucayali River at the same month resulted in a smaller, if any, decline in larval flow.

During the hydrological cycles, the larval flow varied between 153 and 14.982 larvae.sec^{-1} (mean 2918 ± 3684 SD) in the Marañón and between 51 and 6.664 larvae.sec^{-1} (mean 1312 ± 1786) in the Ucayali (Figure 3). Over the study period, the Marañón River produced 2.2 times higher larval flow than the Ucayali River (70.027 vs 31.491 larvae.sec^{-1}, respectively). Among the taxa identified, 64 have a significantly different larval flow between the two rivers ($p<0.01$, see Table S5 for details). The larval flow was higher in the Marañón River (between 2.3 and 951 times) for 39 taxa and only higher in the Ucayali River (between 0.5 and 9.9 times) for 25 taxa ($p=0.02$, Table S5).

Out of the 97 species identified, 20 accounted for nearly 90% of total larval flows and 5 of them only (*Triportheus angulatus*, *Potamorhina altamazonica*, *Pimelodus blochii*, *Brachyplatystoma vaillantii*, *Schizodon fasciatus*) together accounted for over 50% of total larval flow (54% in the Marañón, 67.4% in the Ucayali and 58.4% as a whole, Table 1). Interestingly, two of the 20 species with the highest larval flow correspond to undescribed species: *Prochilodus* sp aff. *costatus* and *Pimelodus* sp. C CGD-2016. Absent from this list are the 3 largest and highly commercial Characiformes species, *Colossoma macropomum*, *Piaractus brachypomus* and *Brycon melanopterus*. Overall, Characiformes accounted for 63% of the total larval flow (59.7% and 69.6% in the Marañón and Ucayali, respectively), Siluriformes 36.6% (40% and 29.5%), Perciformes 0.3% (0.2% and 0.6%) and Clupeiformes only 0.2% (0.04% and 0.39%). Between rivers differences in larval flows observed for these 4 Orders were significant (p value <0.001). Only 3 families (Pimelodidae, Triportheidae and Curimatidae, in order of decreasing importance) accounted for 79.2% of the total larval flow (78.2% and 81.4% in the Marañón and Ucayali, respectively), and 6 families (+ Anastomidae, Doradidae and Prochilodontidae) accounted for 92.1% of the total larval flow (91.3% and 93.9% in the Marañón and Ucayali, respectively).

Most of the 97 species detected hold commercial interest for human consumption or for the ornamental trade, or for both (Table 2). The overall trend of higher larval flow in the Marañón than in the Ucayali was also observed for most commercial species, whose larval flows were between 1.7 (*P. fasciatum*) and 19.6 times (*A. elongatus*) higher in the Marañón than in the Ucayali (Table 1).

Only three of the most abundant commercial species (*Potamorhina altamazonica*, *Pterodoras granulosus* and *Psectrogaster rutiloides*) had slightly higher larval flow in the Ucayali than in the Marañón.

The larval flow of the emblematic goliath catfishes were also higher in the Marañón than in the Ucayali: 3.8 times for *Brachyplatystoma capapretum* (3.05 vs 0.80), 4.6 times for *B. vaillantii* (4465 vs 974), 8.2 times for *B. filamentosum* (2368 vs 287), 18.7 times for *B. platynemum* (23.22 vs 1.24) and 270.6 times for *B. rousseauxii* (10.03 vs 0.04).

Reproductive ecology

Given the very similar hydrological cycles of the Marañón and Ucayali rivers, most species had a similar spawning periodicity in both rivers. Most Characiformes had their main spawning activity during the flooding periods (flood start, flood end or both), whereas Siluriforms were observed spawning during all periods (Figure 5). This pattern was consistent in both the Marañón and Ucayali rivers.

In both the Marañón and Ucayali, the clustering analyses identified three main groups supported by steep inertia drops (see Figure S1): species with a peak spawning activity during the flood start (C1), during the flood end (C2) and during the receding period (C3). Although species were clustered into these three groups according to their main spawning peak, many species also presented spawning activity in another or in all hydrological periods (Table 2, Figure S2). The flood end was the hydrological period when most species had their main spawning activity (over 50%), followed by the flood start. A relatively large number of species also had their main spawning activity during the receding period (19 in the Marañón and 12 in the Ucayali). Figure S2 also illustrates that many species had their reproductive activity over two hydrological periods (most commonly during the flood start and the flood end) and that seventeen species had reproductive activity during the three hydrological periods in at least one of the two river basins: *Brachyplatystoma filamentosum*, *B. vaillantii*, *Cetopsis coecutiens*, *Curimata cyprinoides, Hypophthalmus edentatus*, *H. marginatus*, *Leporinus affinis, L. lacustris*, *Pellona flavipinnis*, *Plagioscion squamosissimus*, *Pimelodus sp. B CGD-2016*, *P. sp. C CGD*-2016, *Pinirampus pirinampu*, *Psectrogaster rhomboides*, *P. rutiloides*, *Pseudostegophilus nemurus*, *Tetragonopterus argenteus*. Ten were Siluriformes of the family Pimelodidae, the others were Characiformes (5), Clupeiformes (1) and Perciformes (1).

During the 2015-2016 flooding period, the discharge of the Marañón River suddenly dropped from 20.680 to 9.300 $m^3.sec^{-1}$ during January 2016, before rising abruptly again to 22.480 $m^3.sec^{-1}$ in

February 2016 (Figure 3). This spectacular hydrological anomaly had a negative effect on the larval flow. As evidenced in Figure 5, most species of Characiformes spawn during the flooding periods. For most of these species, the strong drop in discharge resulted in a strong reduction or in the complete cessation of larval production at the exact same month (Figure 6). Larval production then returned to normal values when river discharge returned to normal in February, illustrating how sensitive species are to environmental cues and the rapidity with which they can react to environmental modifications during the breeding season. At the exact same period, a hydrological event of a much lesser amplitude (16,100 m^3 to 12,500 m^3) was observed in the Ucayali River, but did not result in similar decreased larval flow (Figure 3).

One of the many advantages of being able to analyse the spawning patterns of so many species at the same time is that it allows the identification of phenological differences even between species of the same genus. In both the Marañón and the Ucayali rivers, *Triportheus albus* spawned mainly during the receding period, whereas *T. angulatus* and *T. auritus* reproduced during the flooding periods (Figure 7). The latter two species also strongly reacted negatively to the hydrological anomaly of January 2016 in the Marañón.

DISCUSSION

Diversity

We identified 120 taxas, among which 97 to the species level. These 97 species belong to 62 genera from 20 families, accounting for over one third of all known families in the Amazon basin (N=56, Jézéquel et al., 2020) and ~ 43 % of all known families in the Peruvian Amazon (N=42, Ortega, Hidalgo, Trevejo, Correa, & Cortijo, 2012). Our sampling retrieved 44.3% of the 221 species (from 146 genera and 37 families) reported from the lower reaches of the Marañón and Ucayali (Guerra, Alcantara, & Sanchez, 1990). However, the larvae of many species are not expected to be present in the river main stem because they provide parental care or because they spawn in lakes. In the 90's, Pavlov, Nezdoliy, Urteaga, and Sanches, (1995) performed extensive sampling of ichthyoplankton in approximately the same locations as ours, plus other tributaries of the Amazon. They reported, based on morphological identification, representatives of 6 orders and 20 families. The

2 orders not identified in our study, the Beloniformes and Pleuronectiformes, were only represented by 1 and 3 specimens, respectively, out of a total of 20,657 specimens sampled.

Some of the observed patterns, such as the higher larval flow during the flooding periods or the dominance of Characiformes and Siluriformes in both diversity and larval flow, are consistent with the findings of previous studies in the upper Amazon River in Peru (Pavlov et al., 1995) and in the lower (R. Barthem, da Costa, Cassemiro, Leite, & da Silva, 2014) or upper (Cañas & Pine, 2011) Madeira River. They differed, however, from other studies in Central Amazon near Manaus, where Characiformes, Clupeiformes and Perciformes dominated the ichthyoplankton (Araujo-Lima & Oliveira, 1998; Lima & Araujo-Lima, 2004), or in the lower Amazon near Santarem, where Characiformes, Clupeiformes and Siluriformes dominated the ichthyoplankton (Diego Maia Zacardi et al., 2017).

One of the most interesting outcomes of our study is the precise identification and quantification of close to a hundred species, allowing the comparison of the species' phenology, within and between hydrological basins. Studies based on the morphological identification of larvae that have succeeded in reaching a specific resolution in the Amazon basin usually concerned only a few species (e.g. Araujo-Lima, 1994; Chaves, Carvalho, Ferreira, & Zacardi, 2017; Lima & Araujo-Lima, 2004; Ponte, Silva, & Zacardi, 2017; Zacardi, Ponte, Chaves, Oliviera, & Cajado, 2018). One notable exception is the study of Zacardy et al. (2017), who identified 45 species and 63 taxa from the Lower Amazon near Santarem. There have been a few attempts at specific identification using individual barcoding (Sanger) of each fish larvae, but these were limited to a few hundred larvae (García Dávila et al., 2015, 2014). Here our metabarcoding approach by capture using a single COI probe (MCSP, Mariac et al., 2018) allowed the analysis of over 35,000 larvae. We identified 97 species and compared their relative production (larval flows) and dynamics in two of the main river basins of the Peruvian Amazon concentrating most of the commercial fishery activities (García Vásquez et al., 2009; Tello-Martín, 1995; Tello-Martín & Bayley, 2001). These 97 species, however, represent only 13% of the 882 species currently described in the Marañón and Ucayali rivers. Of course, many of the 882 species do not have pelagic larvae and were not expected to be present in our sampling. But considering that only 334 of the 882 species recorded in the Marañón and Ucayali rivers basins (Jézéquel et al., 2020) are present in our current COI database, it is likely that with a more complete

reference database we might have identified some additional species among the 38.5% of sequences that remained not assigned. This emphasizes the need for incrementing the number of barcode reference sequences in public databases (BOLD, GenBank) of properly identified nominal species, in order to make the most of rapidly developing metabarcoding methods.

Larval flow and spawning periods

In the large rivers of the Amazon basin, reproductive activity is usually associated with the flooding periods, which provide increased availability of shelters and food for most fish species and their progeny (Goulding, 1980; Lowe-McConnell, 1987; Vazzoler & Menezes, 1992). Our results are consistent with this pattern, but nevertheless show that an important proportion of the species spawn mainly during the receding period in both the Marañón (19.8%) and Ucayali (14.1%) rivers, or during the receding and one of the flooding periods. They also show that 17 out of the 97 species (17.5%) have reproductive activity during the three hydrological periods. This indicates that a significant proportion of the species do not directly synchronize its breeding activity with the flooding periods, suggesting that patterns of fish phenology are more complex and distributed across the hydrological periods than originally described. This may partly result from the fact that our knowledge of fish phenology in the Amazon basin is essentially based on results obtained for the most commercial Characiformes and Siluriformes species, which happen to reproduce mainly during the floods. For 40 out of these 97 species (41%), no prior information about their spawning activity or breeding patterns had been published in the Amazon basin. Moreover, it must be pointed out that in many instances, the information available for the 57 other species often refers to spawning seasons without demonstrating data. Taking into account only the Peruvian Amazon, our results provide new information for 80 species (82.5%).

Although close to a hundred species were identified, only about a fifth of them accounted for ~ 90% of the total larval flows of the two rivers and 5 species alone accounted for more than 50%. Most of these 20 species are among the most landed species by commercial fisheries in the Loreto region (main city port of Iquitos). Although the number of larvae produced by a species is difficult to link directly to the adult's abundance because of inter-specific variations in relative fecundity and mortality patterns, a high correlation between the landed production of adults and the abundance of

larvae in the river was observed for *Mylossoma* spp. in the lower Amazon (D.M. Zacardi et al., 2018). This correlation was not evident in our sampling as the most landed species in the Loreto at the same period, *Prochilodus nigricans* (with over 30% of total landings, see Table S6), only accounted for 1.4% (1.9 and 0.4% in the Marañón and Ucayali, respectively, Table 1) of the larval flow over two hydrological cycles. Similarly, *Triportheus angulatus*, which represented 24.4% of the total larval flows (Table 1), only accounts for 3.6% of total landings, together with *T. elongatus* (from which it is not differentiated in the landings, see Table S6).

Interestingly, two overexploited goliath catfishes, *Brachyplatystoma vaillantii* and *B. filamentosum* (Alonso & Pirker, 2005; R. Barthem & Petrere, 1995; Petrere, Barthem, Córdoba, & Gómez, 2004), were among the species producing the highest larval flows. If *B. vaillantii* still represents about 1% of total catches in the Loreto (Table S6), *B. filamentosum*, the largest catfish of the Amazon basin (~ 3.8 m), has long disappeared from the most landed species' list of the Loreto (García Vásquez et al., 2009) and is becoming increasingly rare (García Vasquez et al., unpublished data). Their presence, and in particular that of *B. filamentosum*, is surprising. Although these species are highly fecund owing to their large sizes (García Vásquez et al., 2009), other species, particularly among Characiformes, have much higher relative fecundities (per unit body mass; e.g. García Vásquez, Vargas, Sánchez, Tello, & Duponchelle, 2015). Hence, their size-related fecundity cannot account for the observed larval flows. The fact that they reproduce almost throughout the year might be a better explanation.

Some other goliath catfish species, such as *B. rousseauxii* and *B. platynemum*, which are more abundant than *B. filamentosum*, were relatively rare in our samples. This might be related to the fact that these species are expected to spawn much higher in the river networks (Barthem et al., 2017). Hence, by the time they reach our sampling locations, their larvae might already be above the size limit we fixed in our analyses. Complementarily, *Brachyplatystoma* larvae in advanced development stages are most frequently caught in bottom trawl samples than in plankton net samples (Barthem et al., 2014; Cella-Ribeiro et al., 2015; Leite, Canas, Forsberg, Barthem, & Goulding, 2007), suggesting a specific behaviour resulting in lower susceptibility to plankton nets.

One worrisome finding was the very low larval flow of the three largest Characiformes species, *Colossoma macropomum*, *Piaractus brachypomus* and *Brycon amazonicus*. These species are

among the most commercial and appreciated species in the Loreto, as anywhere else in the Amazon basin. Although *B. amazonicus* and *P. brachypomus* are still among the most landed species in the Loreto, they only account for 1.3 and 1.2 % of the landings, respectively (Table S6). With only 0.4% of the total landings, *C. macropomum* is no longer among the most landed species, whereas it accounted for up to 6% in the past decades (García Vasquez, Tello, Vargas, & Duponchelle, 2009). *Brycon amazonicus* and *P. brachypomus* have accounted for up to 4.9 and 2.5%, respectively. Although no specific investigation about their population dynamics was carried out in the Peruvian Amazon, their decreasing catches and low larval flow might suggest overexploitation in the Peruvian Amazon, as observed in central Amazonia (Campos, Costa Sousa, Catarino, de Albuquerque Costa, & Freitas, 2015; Isaac & Ruffino, 1996). Low larval densities of *C. macropomum* were also observed and similar conclusions were drawn in the lower Amazon (Zacardi et al., 2017).

Influence of hydrology on spawning

As mentioned earlier, the close relationship between the reproduction of Amazonian fishes and the hydrological cycle, in particular with the flooding period, is well documented and our results provide further information on an unprecedented number of species. Less studied is the speed with which species can react to unexpected hydrological variations. During our two years sampling, we witnessed an important hydrological anomaly in January 2016 in the Marañón River, during which the discharge suddenly dropped before rising abruptly again in February 2016. The larval flow of most species spawning during this period (flood end) abruptly decreased or completely stopped during January 2016. Spawning activity and larval production were back to normal in February, illustrating an acute perception and capacity of most species to adapt to unfavourable environmental conditions by adjusting their reproductive effort. This adjustment may be mediated via different potential mechanisms. Depending on reproductive strategies (single, multiple or continuous spawning) fish species may be able to hold the gonadal maturation process (recrudescence and vitelogenesis) for a few weeks and resume it when suitable environmental conditions are back. Or, more likely, in this special situation individuals ready to spawn when the abrupt hydrological anomaly occurred resorbed (atresia) their maturing or mature oocytes, which is a common response in fishes (Brown-Peterson, Wyanski, Saborido-Rey, Macewicz, & Lowerre-Barbieri, 2011; Lowerre-Barbieri,

Ganias, Saborido-Rey, Murua, & Hunter, 2011; Serrat et al., 2019). After that, the same individuals might have developed a new batch of oocytes to spawn in February when the water level was back to normal, or the spawning events of February resulted from other individuals that were not yet ready to spawn at the moment of the anomaly. Another explanation could be that spawning did occur but eggs were either unfertilized or unviable owing to unfavourable conditions and quickly degraded, explaining the absence of larvae, as described in other tropical teleosts (e.g. Legendre, Slembrouck, Subagja, & Kristanto, 2000). Either ways, such a generalized and fast reaction of spawning individuals to temporally unfavourable environmental conditions had never been reported so far in the Amazon basin or anywhere else, as far as we know.

Interspecific difference of phenology within congeneric species

Species within a same genus usually share similar life history traits, including spawning seasons, which often justify using the information of a congeneric species when biological information lacks for a species (e.g. Espírito-Santo, Rodríguez, & Zuanon, 2013). Intra-genus differences of spawning season have been reported in species of *Alestes* spp., although most other species within a same genus (*Barbus* spp. *Labeo* spp., *Hemichromis* spp.) had similar breeding season in the studied rivers of Côte d'Ivoire, Africa (Albaret, 1982). Variations in the reproductive periods among species belonging to the same genus have also been reported in the Neotropics, in the Sinamari River (Ponton & Mérona, 1998). In both studies, these differences were more or less extended periods around a common breeding season rather than really distinct breeding seasons.

Here we provide evidence, in two different rivers and over two consecutive annual cycles, of complete inverse phenology between species of the genus *Triportheus* (Figure 7). This is particularly interesting as the breeding seasons of three species of *Triportheus* in the lower Tocantins was previously reported to widely overlap: November to March for *T. angulatus* and *T. albus* and September to January for *T. elongatus* (Santos, Jegu, & de Merona, 1984). A study based on the morphological identification of *Triportheus* larvae in the middle Amazon River, near Mamirahua reserve, only succeeded in identifying *T. auritus* at the species level (Ponte, Ferreira, Bittencourt, Queiroz, & Zacardi, 2016). Nevertheless, they reported that all *Triportheus* species, which are supposed to be the same as in the upper Amazon in our sampling area, spawned at the same period of

the year, mainly during the flooding season. A fourth species, *T. trifurcatus*, studied in the Araguaia River, Tocantins basin in Brazil also indicated a reproductive season between November and January during the flooding period (Martins-Queiroz, Mateus, Garutti, & Venere, 2008), similar to the other species in the Tocantins and to *T. angulatus* and *T. auritus* in our study. The reason why *T. albus* would have an inverse phenology in the Peruvian Amazon is beyond the scope of the present study, but emphasize the potential of our barcoding approach for investigating the spawning patterns of Amazonian fishes.

Production difference between the Marañón and Ucayali & conservation implications

The presence over two annual cycles of larvae of a large number of the region's commercially valuable fishes emphasizes the importance of the study area for their reproduction, development, and dispersal. One interesting outcomes of our study is the fact that, at our sampling locations, we observed more than twice as much larvae in the Marañón as in the Ucayali River, including of the most commercial species. This is intriguing as the Ucayali basin, with the Puinahua channel (Figure 1), is supposed to be the most productive fishing area of the Peruvian Amazon, accounting for 60-80% of fisheries landings, whereas the Marañón accounts for less than 10% (Tello-Martín & Bayley, 2001; Tello-Martín, 1995). Several hypotheses may explain why larval flow in the Marañón was more than twice that of the Ucayali.

Fishing pressure is considered higher in the Ucayali (Tello-Martín, 1995; Tello-Martín & Bayley, 2001), which might result in a depleted number of large adult fishes in the Ucayali and as larger fish are more fecund in most species, to a decreased larval production. Another explanation is that our sampling area is located at the collector of an extensive flooded area (Figure 1), which is likely to act as a nursery area for the larvae produced upstream in the Marañón and Ucayali. Floodplain habitats are known to play key nursery and feeding roles (Bayley, 1995; Castello, Bayley, Fabré, & Batista, 2019; Castello, Isaac, & Thapa, 2015). The larvae collected at our sampling sites might be just a fraction of what is produced upstream, most of it being retained in the floodplains. The higher number of larvae in our Marañón samples might reflect the higher discharge of the Marañón that would flush out a higher proportion of larvae, or a higher retention rate in the area of the Puinahua channel in the

Ucayali. The situation might also be explained by a combination of these hypotheses. These alternative explanations are amenable to testing combining plankton sampling upstream and downstream of specific areas, such as the Puinahua channel for instance, with our metabarcoding approach.

But whatever the explanation, the much higher larval flows observed in the Marañón River at our sampling locations over two hydrological cycles indicates that the Marañón basin contributes at least twice as much as the Ucayali to fish recruitment downstream in the floodplains of the Amazon River, which has important implications for fisheries management and conservation.

Peru is the Andean country with the major number of proposed dams for construction in the coming years. Unlike the already existing ones, which are mostly small (<50 MW), planned dams are between 100 and 1000 MW and some could even exceed this capacity (Anderson et al., 2018). More than twice as much new dams are planned for the Marañón (82) when compared to the Ucayali (37), several of which in the lowlands (<500 m altitude), including some large (>1000 MW) dams such as that of the Pongo de Manseriche (Anderson et al., 2018), a few hundreds of kms upstream of our sampling area. However, even dams built on tributaries located in the uplands (>500m) are likely to significantly impact lowland fishes by strongly modifying discharge, water level variations and sediment transport, hence the geomorphology (which might alter spawning habitats) and productivity of downstream portions of the tributaries (Forsberg et al., 2017). For instance, alteration of granulometry and geomorphology resulted in strongly decreased abundance of iliofagous species, such as the migratory Curimatidae (one of the most abundant Families in our sampling) below Samuel and Tucuruí dams in the Amazon basin (Agostinhho, Julio, & Petrere, 1994; Merona, 1987; Santos, 1995). Upstream dams can also alter the hydrological and physico-chemical cues used by the fish to initiate spawning (Agostinho, Gomes, Verissimo, & Okada, 2004; Agostinho, Pelicice, & Gomes, 2008; Bailly, Agostinho, & Suzuki, 2008; Lytle & Poff, 2004) and our results have illustrated the importance of discharge variations on larval production.

The existing dams have already reduced the connectivity networks (among tributaries of a river basin) of the Marañón and Ucayali by approximately 20%, but connectivity losses could increase by >50% if planned dams were to be completed (Anderson et al., 2018). The Marañón and Ucayali are considered the most vulnerable Amazonian rivers to dam construction (Latrubesse et al., 2017, 2020). Increased

river fragmentation has been shown to decrease fish diversity (alpha and beta) and abundance downstream from dams in other regions (Freeman, Pringle, & Jackson, 2007; Pringle, Freeman, & Freeman, 2000). These connectivity losses could also have dramatic consequences for the migrations (reproductive or trophic-driven) of fishes and ultimately for the fisheries of the region (review in Duponchelle et al., in press), which provide the main animal protein source of Peruvian Amazon people (Tello-Martín & Bayley, 2001), particularly in the more vulnerable riverine communities (Coomes, Takasaki, Abizaid, & Barham, 2010).

Additionally, our sampling area is in the heart an on-going mega-infrastructure project with huge potential for biodiversity (particularly aquatic biodiversity), habitat and productivity degradation in the region: the Hydrovía Amazónica, which involves dredging and channelization in several portions of a ~2700 km stretch along the Amazon, Marañón, Huallaga and Ucayali rivers (Figure 1) to improve navigation of goods between the Atlantic ocean and the western Amazon (Anderson et al., 2018; Bodmer et al., 2018). Our monthly sampling over two consecutive hydrological cycles has demonstrated the importance of the area for the production of commercial fish larvae during the three hydrological periods. It can be anticipated that the repeated dredging and channelization activities will seriously disrupt the reproduction of many species by destructing spawning and nursery sites associated with instream woody habitats (Gurnell, Tockner, Edwards, & Petts, 2005; Zalewski, Lapinska, & Bayley, 2003) and affect the survival of their eggs and larvae, which are the most likely to suffer lethal damages (Wenger et al., 2017). If the Hydrovía Amazónica were to be implemented despite the numerous oppositions (e.g. https://www.dw.com/es/hidrov%C3%ADa-amaz%C3%B3nica-una-amenaza-para-per%C3%BA-y-el-planeta/a-51679653), our results would contribute to a baseline for evaluating post-dredging and channelization impacts.

We recommend that further studies with sampling designs adapted to test specific questions be carried out with our metabarcoding approach. For instance, besides sampling upstream and downstream of the Puinahua channel to test its potential retention effect on larvae (see above), it would be particularly interesting to test the relative contribution of the main tributaries of the Marañón (Tigre, Pastaza, Huallaga, upper Marañón) to the larval production of commercial species to inform decision-making about the potential consequences of dams' construction in these tributaries. Similarly, systematic monitoring of fish eggs and larvae in the specific river portions where dredging

and channelization are planned in the Hydrovía Amazónica project would provide precise information on the fish species reproducing upstream of or within these so-called “mal pasos” (sand banks and associated instream woody habitats) to be dredged, but also in which proportion. Although delimiting spawning areas was outside the scope of this study and its original design, we could have attempted to do it using larvae. However, incertitude regarding the development times between species of different families and orders (information is available for a limited number of Amazonian species only: e.g. Andrade et al., 2016; Nakatani et al., 2001), the changes in water velocity and temperature (development time is directly linked to water temperature) during the hydrological cycle would have likely resulted in large confidence intervals and ultimately in hundreds of kms of potential spawning areas upstream of our sampling locations (see Miranda-Chumacero et al., 2020). We instead recommend using our metabarcoding approach on eggs, whose developmental stages are more constant between species (Andrade et al., 2016; Nakatani et al., 2001) to which should also be added a special effort to increase the number of reference barcodes available. Collecting further upstream in the river basins would also likely results in larger proportion of eggs in the samples, which would allow the precise determination of important spawning areas for conservation. Carrying out further studies on the diversity, reproductive ecology and spawning areas of fishes using highly effective new molecular tools such as MSCP (Mariac et al., 2018) is particularly important in the Marañón River, whose upper portion remains understudied (Anderson et al., 2018; Jézéquel et al., 2020). Combined with specifically designed sampling schemes to address particular research and conservation issues, metabarcoding approaches like the one implemented here have the potential to greatly improve the knowledge of the reproductive dynamics and recruitment of fishes in the Amazon basin. It can also prove an invaluable tool for fisheries management and conservation but also to help decision-making by providing crucial information about the relative contributions of tributaries to commercial fish recruitment.

Acknowledgments

The two years of larvae collection in the Marañón and Ucayali rivers were financed by the Concejo Nacional de Ciencia y Tecnología (CONCYTEC) of Peru, who through the Fondo Nacional de

Desarrollo Científico, Tecnológico y de Innovación Tecnológica (FONDECYT), project 088-2014-FONDECYT-DE. The LMI EDIA and UMR DIADE financed the metabarcoding analyses. The authors acknowledge the IRD itrop HPC (South Green Platform) at IRD Montpellier for providing HPC resources that contributed to the research results reported within this paper (URL: https://bioinfo.ird.fr/- http://www.southgreen.fr). We are also grateful to Domingo García for his assistance during field work. We are also grateful to Juan José Palacios Vega (IIAP) for the elaboration of Figure 1.

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Data Accessibility

Sequencing reads were deposited in the National Centre for Biotechnology Information Sequence Read Archive (BioProject ID: PRJNA660964, BioSample accessions: SAMN15965655-SAMN15965706).

Author Contributions

CGD, CM, FD and JFR conceived this study. CA, EM, GE, JA, JV, WC, CGD, FD and JFR collected and processed the samples. CM, CDG, DCR and MF carried out laboratory analyses. CM, FD and JFR analysed the data. FD, CM and JFR wrote the manuscript with revision by all authors. All authors worked on the interpretation of the data.

Titles for Tables

TABLE 1: Total larval flow of the most abundant species. We reported the larval flow of species representing more than 1% total larval flow and their respective proportions in the Marañón, Ucayali, and as a whole. Mar and Uca stand for Marañón and Ucayali, respectively. Species underlined are among the most landed species (>1% of total landings) in Iquitos (See S6 Table), whereas † refers to an undescribed species likely sold together with its commercial relative (Pimelodus blochii).

TABLE 2: Frequency of larval flows in the three hydrological periods for the 97 fish species caught in the Marañón and Ucayali rivers during the hydrological cycles of 2015 and 2016. Columns C

indicates the corresponding cluster in the heatmaps (Figure 5). The intensity of the color is proportional to the larval flow in each hydrological period. Species underlined are important for human consumption, whereas † indicate species exported for the ornamental trade.

Figure captions

FIGURE 1: Map of the Amazon basin with a close-up on the study area in the Peruvian Amazon, illustrating the main cities (black squares) and the monthly sampling locations (red dots) in the Marañón and Ucayali rivers. The map is a courtesy of the SO HYBAM (https://hybam.obs-mip.fr/fr/website-under-development-2/).

FIGURE 2: Accumulation curves for the Marañón and Ucayali rivers (legends for curves and). The blue lines represent the species accumulation when samples are added in the order of collecting date. The red area is the confidence interval obtained with 1000 permutations (samples in random order).

FIGURE 3: Evolution of the larval flow (orange lines) and of the number of taxa sampled (grey bars) over two hydrological cycles in the Marañón and Ucayali rivers. The flood start, flood end and receding periods are represented by green, blue and red lines, respectively. Black dots on the hydrological cycles correspond to the larval sampling dates. The shaded vertical rectangles highlight the hydrological event that occurred in January 2016.

FIGURE 4: Boxplots of the taxa diversity per hydrological periods in the Marañón and Ucayali rivers.

FIGURE 5: Heatmap of the 97 species clustered into the three hydrological periods according to the intensity of monthly larval flow during 2015 and 2016 in the Marañón (A) and Ucayali (B) rivers. Cluster C1 corresponds to species with a peak spawning activity in during the flood start, C2 during the flood end, and C3 during the receding period.

FIGURE 6: Effect of a sudden hydrological anomaly on the larval flow of some species of Characiformes belonging to the families Hemiodontidae. Serrasalminae. Characidae and Anostomidae (from top to bottom) in the Marañón River.

FIGURE 7: Inverse phenology (reproduction in receding vs flooding period) in closely related species of the genus Triportheus (Characidae) in the Marañón and Ucayali rivers.

Family	Species	Marañón Flow	Ucayali Flow	Total Flow	% Mar	% Uca	% TOT
Triportheidae	*Triportheus angulatus*	15923	6814	22737	25.5	22.2	24.4
Curimatidae	*Potamorhina altamazonica*	6177	**9216**	15394	9.9	30.0	16.5
Pimelodidae	*Pimelodus blochii*	4303	2234	6537	6.9	7.3	7.0
Pimelodidae	*Brachyplatystoma vaillantii*	4465	974	5439	7.2	3.2	5.8
Anostomidae	*Schizodon fasciatus*	2793	1439	4232	4.5	4.7	4.5
Doradidae	*Pterodoras granulosus*	1142	**1780**	2922	1.8	5.8	3.1
Pimelodidae	*Sorubim elongatus*	2511	342	2852	4.0	1.1	3.1
Hemiodontidae	*Anodus elongatus*	2542	130	2672	4.1	0.4	2.9
Pimelodidae	*Brachyplatystoma filamentosum*	2368	287	2655	3.8	0.9	2.9
Pimelodidae	*Pimelodina flavipinnis*	1789	330	2120	2.9	1.1	2.3
Pimelodidae	*Sorubim lima*	1591	247	1838	2.6	0.8	2.0
Pimelodidae	*Pseudoplatystoma fasciatum*	1048	607	1655	1.7	2.0	1.8
Pimelodidae	*Hypophthalmus edentatus*	1357	239	1596	2.2	0.8	1.7
Curimatidae	*Psectrogaster amazonica*	1317	204	1521	2.1	0.7	1.6
Curimatidae	*Psectrogaster rutiloides*	490	**928**	1417	0.8	3.0	1.5
Prochilodontidae	*Prochilodus nigricans*	1213	46	1259	1.9	0.2	1.4
Triportheidae	*Triportheus albus*	674	578	1252	1.1	1.9	1.3
Prochilodontidae	*Prochilodus* sp aff. *costatus*	853	326	1179	1.4	1.1	1.3
Pimelodidae	*Pimelodus* sp. C CGD-2016 †	713	450	1163	1.1	1.5	1.2
Serrasalmidae	*Mylossoma albiscorpum*	877	135	1012	1.4	0.4	1.1

	Marañón				Ucayali			
	C	Flood start	Flood end	Receding	C	Flood start	Flood end	Receding
Characiformes								
Anodus elongatus	1	0.9	0.1	0.0	1	0.9	0.1	0.0
Astyanax bimaculatus †	1	0.9	0.1	0.0	1	0.7	0.3	0.0
Brycon melanopterus	1	1.0	0.0	0.0	1	1.0	0.0	0.0
Caenotropus labyrinthicus	1	1.0	0.0	0.0				
Hemiodus microlepis †	1	0.8	0.0	0.2	1	0.9	0.0	0.1
Leporinus affinis	1	0.7	0.3	0.0	1	0.8	0.1	0.1
Leporinus friderici	1	0.7	0.3	0.0				
Leporinus octofasciatus	1	1.0	0.0	0.0	1	0.7	0.0	0.3
Leporinus piau	1	0.9	0.1	0.0	1	1.0	0.0	0.0
Megaleporinus macrocephalus	1	0.9	0.1	0.0	1	0.9	0.0	0.0
Megaleporinus trifasciatus	1	0.8	0.2	0.0	1	0.8	0.1	0.0
Mylossoma albiscorpum	1	0.5	0.5	0.0	2	0.1	0.9	0.0
Mylossoma aureum	1	0.8	0.2	0.0	2	0.3	0.7	0.0
Piaractus brachypomus	1	0.9	0.1	0.0	1	0.7	0.3	0.0
Potamorhina latior	1	0.7	0.3	0.0	2	0.2	0.8	0.0
Psectrogaster rhomboides	1	1.0	0.0	0.0	1	0.7	0.1	0.2
Psectrogaster rutiloides	1	0.9	0.1	0.0	1	0.7	0.1	0.1
Pygocentrus nattereri †	1	1.0	0.0	0.0	1	0.7	0.3	0.0
Roeboides margareteae †	1	0.8	0.2	0.0	1	0.5	0.5	0.0
Roeboides myersii †	1	0.8	0.2	0.0	1	0.6	0.4	0.0
Schizodon fasciatus	1	0.6	0.4	0.0	2	0.3	0.6	0.0
Semaprochilodus kneri	1	0.9	0.0	0.1	1	1.0	0.0	0.0
Triportheus angulatus	1	0.5	0.5	0.0	1	0.8	0.2	0.0
Triportheus auritus	1	0.9	0.1	0.0	1	1.0	0.0	0.0
Curimata macrops	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Cynodon gibbus †	2	0.0	1.0	0.0	1	1.0	0.0	0.0
Cyphocharax vanderi	2	0.2	0.8	0.0	2	0.1	0.9	0.0
Hoplias cf *intermedius*	2	0.0	1.0	0.0				
Hoplias malabaricus	2	0.0	1.0	0.0				
Leporinus trimaculatus	2	0.2	0.7	0.0	1	0.9	0.1	0.0
Prochilodus nigricans	2	0.3	0.7	0.0	1	0.4	0.6	0.0
Prochilodus sp. aff. *costatus*	2	0.2	0.8	0.0	2	0.2	0.8	0.0

Psectrogaster amazonica	2	0.4	0.6	0.0	2	0.0	1.0	0.0
Rhaphiodon vulpinus	2	0.3	0.7	0.0	2	0.1	0.9	0.0
Thoracocharax stellatus †	2	0.2	0.8	0.0	2	0.0	1.0	0.0
Curimata cyprinoides	2	0.4	0.5	0.1	2	0.0	1.0	0.0
Curimatella meyeri	2	0.4	0.6	0.0	2	0.0	1.0	0.0
Hydrolycus scomberoides	2	0.4	0.6	0.0	2	0.3	0.7	0.0
Leporinus lacustris	2	0.4	0.5	0.1	1	0.8	0.1	0.1
Potamorhina altamazonica	2	0.4	0.6	0.0	2	0.0	1.0	0.0
Tetragonopterus argenteus †	2	0.4	0.6	0.1	1	0.9	0.1	0.0
Colossoma macropomum	3	0.3	0.0	0.7	1	0.9	0.0	0.1
Leporinus fasciatus	3	0.2	0.0	0.8	1	0.6	0.4	0.0
Semaprochilodus insignis	3	0.1	0.0	0.9				
Triportheus albus	3	0.0	0.0	0.9	3	0.1	0.0	0.9
Rhytiodus microlepis					1	0.0	1.0	0.0
Clupeiformes								
Pellona castelnaeana	3	0.4	0.0	0.6	1	0.9	0.1	0.0
Pellona flavipinnis	3	0.3	0.2	0.5	3	0.1	0.2	0.7
Perciformes								
Plagioscion auratus	3	0.3	0.0	0.7	3	0.0	0.0	1.0
Plagioscion squamosissimus	3	0.1	0.4	0.5	3	0.2	0.2	0.6
Siluriformes								
Calophysus macropterus	1	0.8	0.2	0.0	1	1.0	0.0	0.0
Hypophthalmus edentatus	1	0.7	0.1	0.2	1	0.9	0.1	0.0
Pimelodus sp. B CGD-2016 †	3	0.4	0.1	0.4	3	0.2	0.1	0.7
Pimelodus sp. C CGD-2016 †	1	0.7	0.1	0.3	3	0.4	0.0	0.6
Pseudoplatystoma tigrinum †	1	0.8	0.2	0.0	1	0.4	0.6	0.0
Amblydoras gonzalezi †	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Amblydoras sp. 3 bold pattern †	2	0.0	1.0	0.0				
Hemidoras stenopeltis †	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Hemisorubim platyrhynchos	2	0.0	1.0	0.0	2	0.1	0.9	0.0
Leiarius marmoratus	2	0.3	0.7	0.0	1	1.0	0.0	0.0
Leptodoras cataniai	2	0.0	0.8	0.2				
Lithodoras dorsalis †	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Nemadoras elongatus †	2	0.1	0.9	0.0	2	0.0	1.0	0.0
Nemadoras humeralis †	2	0.0	1.0	0.0	2	0.0	1.0	0.0

Oxydoras niger †	2	0.3	0.7	0.0	2	0.0	1.0	0.0
Phractocephalus hemioliopterus †	2	0.1	0.8	0.0	2	0.1	0.9	0.0
Pimelodus blochii †	2	0.1	0.9	0.0	2	0.1	0.9	0.0
Pimelodus maculatus †	2	0.1	0.9	0.0	2	0.0	1.0	0.0
Platysilurus mucosus †	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Pseudoplatystoma fasciatum †	2	0.1	0.9	0.0	2	0.1	0.9	0.0
Pseudorinelepis genibarbis †	2	0.1	0.9	0.0	2	0.0	1.0	0.0
Pterodoras granulosus †	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Rhinodoras boehlkei	2	0.0	0.7	0.3				
Rhynchodoras woodsi †	2	0.0	0.8	0.2				
Sorubim cuspicaudus	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Sorubim elongatus	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Sorubim lima	2	0.0	0.9	0.0	2	0.0	1.0	0.0
Sorubimichthys planiceps †	2	0.0	1.0	0.0				
Trachelyopterus galeatus †	2	0.0	1.0	0.0	2	0.0	1.0	0.0
Trachydoras steindachneri †	2	0.0	0.9	0.1	2	0.0	1.0	0.0
Zungaro zungaro	2	0.2	0.8	0.0	2	0.0	1.0	0.0
Hypophthalmus sp. aff. *oremaculatus*	2	0.4	0.6	0.0	1	0.5	0.5	0.0
Pimelodina flavipinnis	2	0.4	0.6	0.0	2	0.2	0.8	0.0
Pinirampus pirinampu	2	0.1	0.5	0.4	3	0.4	0.1	0.5
Platystomatichthys sturio †	2	0.0	0.5	0.5	3	0.0	0.5	0.5
Aguarunichthys torosus †	3	0.0	0.3	0.7				
Brachyplatystoma capapretum †	3	0.0	0.1	0.9	1	0.6	0.0	0.4
Brachyplatystoma filamentosum †	3	0.1	0.2	0.7	3	0.2	0.3	0.6
Brachyplatystoma platynemum †	3	0.4	0.0	0.5	1	0.8	0.0	0.2
Brachyplatystoma rousseauxii †	3	0.0	0.1	0.9	3	0.0	0.0	1.0
Cetopsis coecutiens †	3	0.2	0.2	0.6	3	0.1	0.4	0.5
Hypophthalmus marginatus	3	0.3	0.1	0.6	3	0.2	0.1	0.8
Nemadoras sp. ghost †	3	0.0	0.1	0.9				
Platynematichthys notatus †	3	0.0	0.2	0.8				
Pseudostegophilus nemurus †	3	0.1	0.0	0.9	1	0.6	0.1	0.3
Brachyplatystoma vaillantii †	3	0.3	0.3	0.4	3	0.1	0.5	0.4
Ossancora punctata †					2	0.0	1.0	0.0