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Conference Abstract

Ichthyoplankton metabarcoding as a tool for studying fish reproductive dynamics

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Abstract

The study of ichthyoplankton composition, abundance and distribution is paramount to understand the reproductive dynamics of local fish assemblages. The analysis of these parameters allows the identification of spawning sites, nursery areas and migration routes. However, due to the lack of characters in early life stages, the morphological identification of ichthyoplankton is often impractical and many studies identify only fish larvae. Additionally, its accuracy shows great variation between taxonomists and laboratories according to their experience and specialty. DNA barcoding emerged as an alternative to provide assertive identification of fish eggs and larvae, but it becomes too expensive and laborious when the study demands the processing of huge amounts of organisms. DNA metabarcoding can overcome these limitations as a rapid, cost-effective, broad and accurate taxonomy tool, allowing the identification of multiple individuals simultaneously. Here, we present the identification of a sample containing 68 fish eggs and another containing 293 fish larvae from a single site in the São Francisco River Basin, Eastern Brazil, through DNA metabarcoding. We used a low-cost saline DNA extraction followed by PCR amplification with three primer sets targeting the 12S rRNA gene: MiFish (~170bp), Teleo_1 (~60bp), and NeoFish (~190bp). The latter was recently developed by our research group specifically for the identification of Neotropical fishes. All the amplified samples were sequenced in a single multiplexed Illumina MiniSeq run. We performed the

filtering steps and assigned Amplicon Sequence Variants (ASVs) using a DADA2/Phyloseq based pipeline and a custom 12S reference sequence database including 101 species and 70 genera from the Jequitinhonha and São Francisco basins. The species *Cyphocharax gilbert*, *Leporinus taeniatus*, *Megaleporinus elongatus*, *Prochilodus argenteus*, *P. costatus* and *Psalidodon fasciatus* were detected by all three primer sets in the larva pool, while *Pterygoplichthys etentaculatus* was detected solely by NeoFish (Fig. 1). Within the egg pool, all three markers detected the species *Characidium zebra, Curimatella lepidura, M. elongatus, Pimelodus fur* and *P. costatus*, but *Brycon orthotaenia* was detected only by NeoFish, *P. maculatus* only by Teleo, and *P. pohli* by MiFish and Teleo (Fig. 1). The consistency in species detection among all three markers underpins the credibility of this method to accurately describe the sample composition. Considering that most of species were exclusive to the larvae or egg pool, our experiment highlights the importance of including the identification of fish eggs in reproduction studies, as it can provide additional information about which species are spawning in an area. Furthermore, the application of DNA metabarcoding to the study of ichthyoplankton can help decision makers create more informed guidelines for conservation of economically and ecologically important fish species.

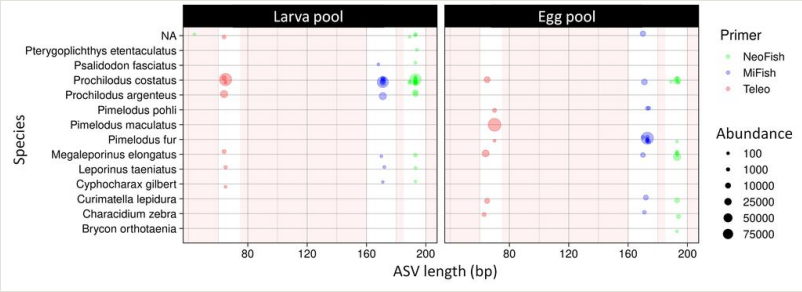

Figure 1. doi

Detected fish species and size distribution of ASVs considering 12S reads of fish larva and egg pools obtained from an Illumina Miniseq run. Each circle represents a single ASV, size is proportional to the number of sequence copies and colors correspond to each primer set: NeoFish (green), MiFish (blue) and Teleo_1 (red).

Keywords

Neotropical, 12S, fish reproduction, High throughput DNA sequencing

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