DNA metabarcoding of mock communities highlights potential biases when assessing Neotropical fish diversity

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February 8, 2023

Abstract

Despite the increasing popularity of DNA metabarcoding in the assessment of aquatic ecosystems using fish eDNA or ichthyoplankton, challenges have hampered its broader application in the Neotropical freshwaters. Using five mock communities composed of fish species from two Neotropical river basins, we evaluated the influence of DNA concentration and choice of mitochondrial 12S molecular markers (MiFish, NeoFish and Teleo) on species detection and Relative Read Abundance (RRA) using DNA metabarcoding. Of the three 12S markers analysed, only MiFish detected all species from all mock communities. The performance of a taxonomy-free approach using ASV/MOTUs was not as precise as assigning DNA reads to species using a curated 12S library that includes approximately 100 fish species, since more than one ASV/MOTU was observed for the same specimen. Thus, here we showcase the importance of a custom reference database to allow precise assignment of Neotropical fish species in metabarcoding studies and that the RRA is dependent on community composition, marker and DNA concentration. We highlight the importance of controlled experiments using known species communities before large investments are made in assessing biodiversity using non-invasive methods that apply DNA metabarcoding.

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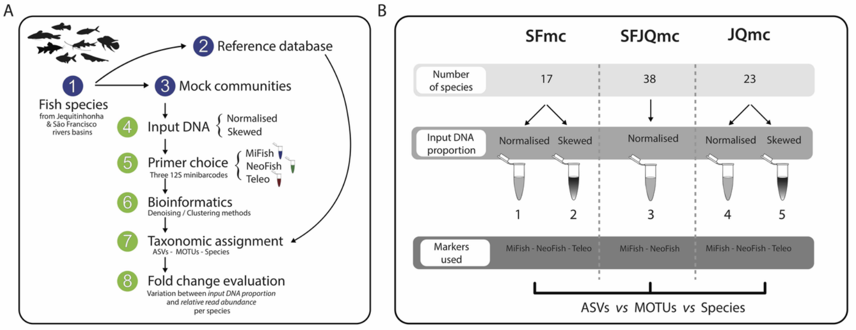
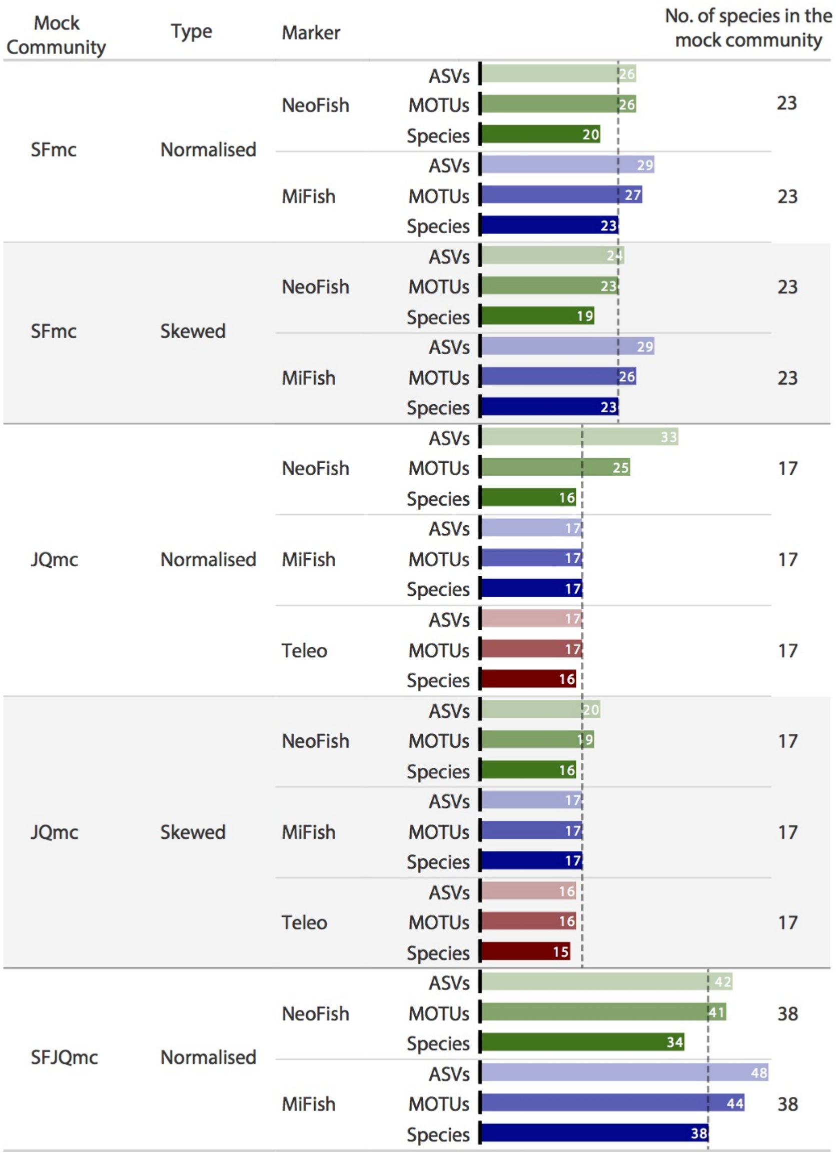
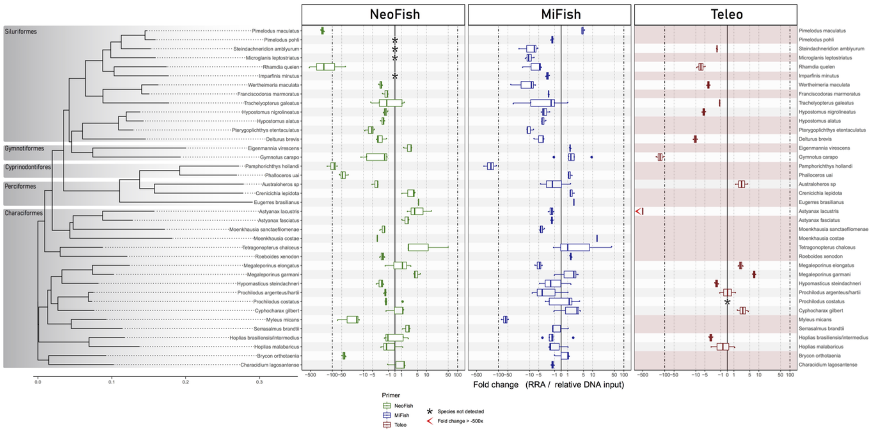
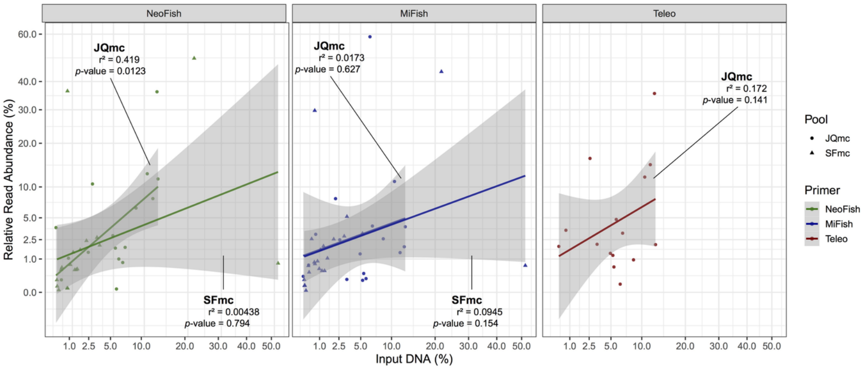



Mock Community
Type
Marker
No. of species in the mock community
SFmc
Normalised
NeoFish
ASVs 26
MOTUs 26
Species 20
23
MiFish
ASVs 29
MOTUs 27
Species 23
23
SFmc
Skewed
NeoFish
ASVs 24
MOTUs 23
Species 19
23
MiFish
ASVs 29
MOTUs 26
Species 23
23
JQmc
Normalised
NeoFish
ASVs 33
MOTUs 25
Species 16
17
MiFish
ASVs 17
MOTUs 17
Species 17
17
Teleo
ASVs 17
MOTUs 17
Species 16
17
JQmc
Skewed
NeoFish
ASVs 20
MOTUs 19
Species 16
17
MiFish
ASVs 17
MOTUs 17
Species 17
17
Teleo
ASVs 16
MOTUs 16
Species 15
17
SFJQmc
Normalised
NeoFish
ASVs 42
MOTUs 41
Species 34
38
MiFish
ASVs 48
MOTUs 44
Species 38
38


NeoFish
MiFish
Teleo
Siluriformes
Gymnotiformes
Cyprinodontiformes
Perciformes
Characiformes
Pimelodus maculatus
Pimelodus pohli
Steindachneridion amblyurum
Microglanis leptostriatus
Rhamdia quelen
Imparfinis minutus
Wertheimeria maculata
Franciscodoras marmoratus
Trachelyopterus galeatus
Hypostomus nigrolineatus
Hypostomus alatus
Pterygoplichthys etentaculatus
Delturus brevis
Eigenmannia virescens
Gymnotus carapo
Pamphorichthys hollandi
Phalloceros uai
Australoheros sp
Crenicichla lepidota
Eugerres brasilianus
Astyanax lacustris
Astyanax fasciatus
Moenkhausia sanctaefilomenae
Moenkhausia costae
Tetragonopterus chalceus
Roeboides xenodon
Megaleporinus elongatus
Megaleporinus garmani
Hypomasticus steindachneri
Prochilodus argenteus/hartii
Prochilodus costatus
Cyphocharax gilbert
Myleus micans
Serrasalmus brandtii
Hoplias brasiliensis/intermedius
Hoplias malabaricus
Brycon orthotaenia
Characidium lagosantense
0.0
0.1
0.2
0.3
−500 −100−50 −10 −5 −1 0 1 5 10 50 100
Fold change (RRA / relative DNA input)
Primer
NeoFish
MiFish
Teleo
* Species not detected
< Fold change > -500x


NeoFish
MiFish
Teleo
Relative Read Abundance (%)
60.0
40.0
30.0
20.0
10.0
5.0
2.5
1.0
0.0
JQmc
r² = 0.419
p-value = 0.0123
SFmc
r² = 0.00438
p-value = 0.794
JQmc
r² = 0.0173
p-value = 0.627
SFmc
r² = 0.0945
p-value = 0.154
JQmc
r² = 0.172
p-value = 0.141
1.0 2.5 5.0 10.0 20.0 30.0 40.0 50.0
Input DNA (%)
Pool
JQmc
SFmc
Primer
NeoFish
MiFish
Teleo