# Seasonal Fish Assemblage Structure Using Environmental DNA in the Yangtze Estuary and Its Adjacent Waters

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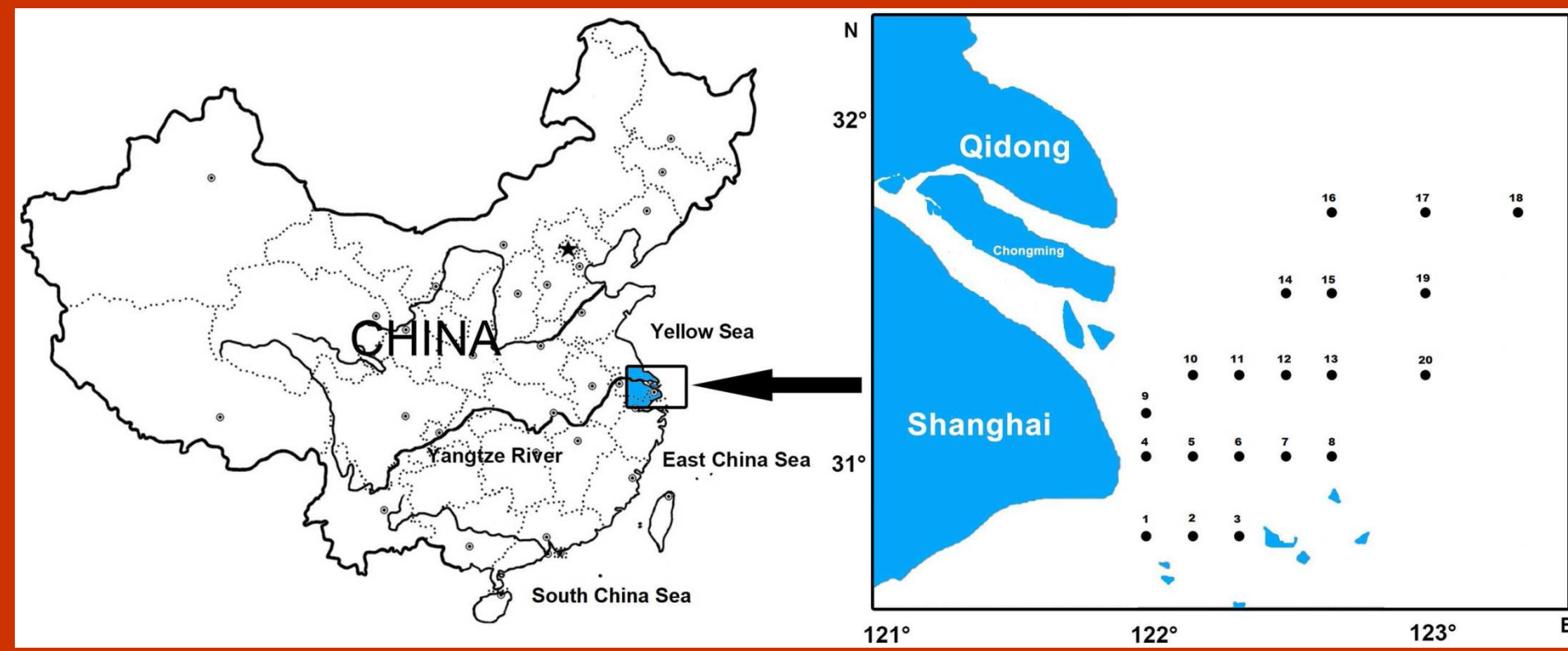
Study area and sampling stations

A total of 50 eDNA samples were collected from the Yangtze Estuary and its adjacent watersWe collected 15 eDNA samples in spring and summer,and 20 in autumn.
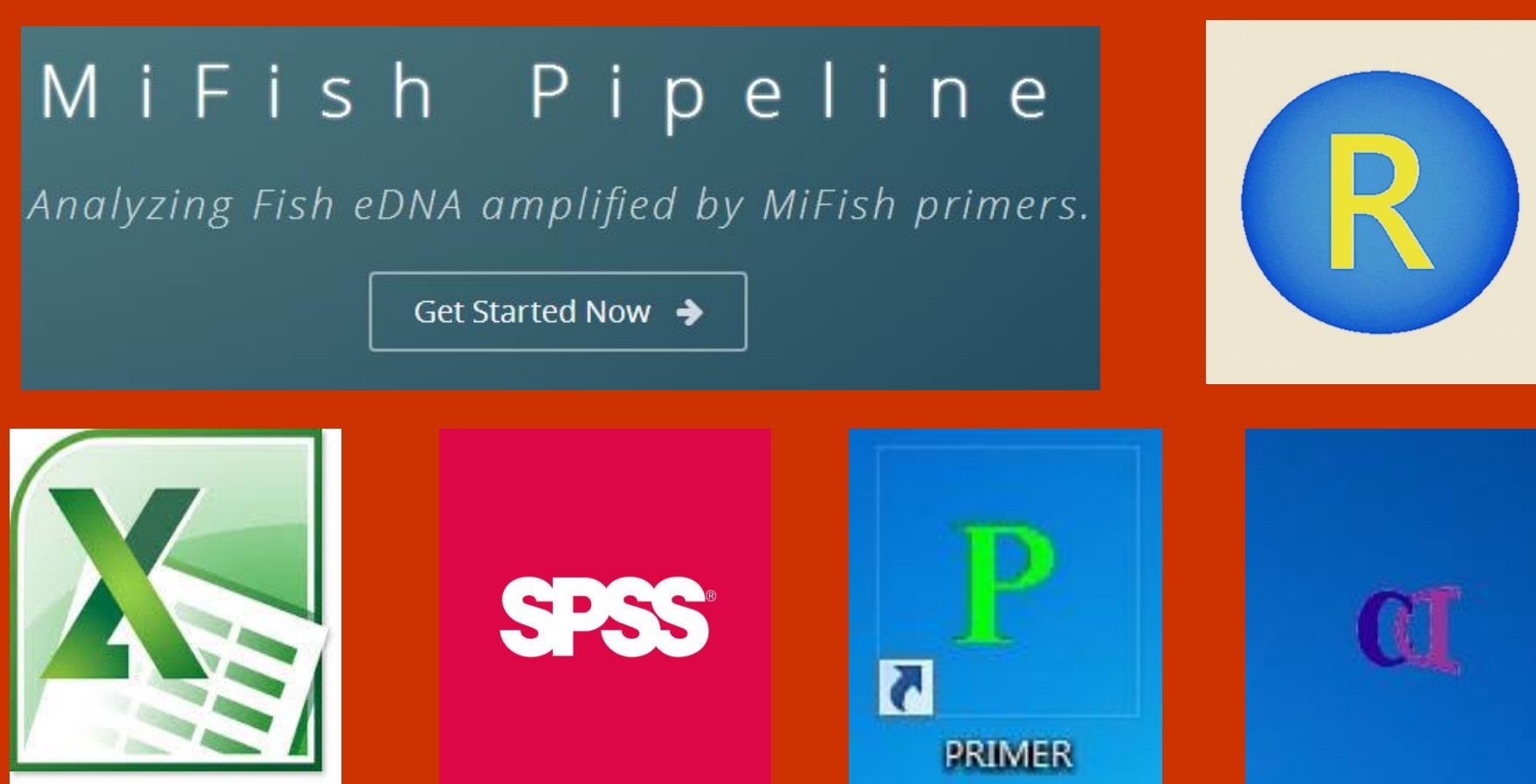
Data analysis
Biological and Environmental data

Introduction

The fisheries resources of the Yangtze Estuary and its adjacent waters have undergone dramatic declines as a consequence of environmental changes and human activities, with traditional ecological investigations demonstrating progressive decreases in species diversity and annual numbers in the fisheries resource. Environmental DNA technology has been demonstrated as an effective tool by many studies for detecting fish species, monitoring fish biodiversity, and indicating the abundance of fish. In the present study, we chose the Yangtze Estuary and its adjacent waters as a primary research area to investigate fish assemblage structure using eDNA technology.

Materials and Methods

Sample Collection

A total of 50 eDNA samples were collected from the Yangtze Estuary and its adjacent waters.

Paired-End Library Preparation on the MiSeq Platform

Amplicon libraries of partial 12S rRNA genes were obtained by PCR amplification using the universal primer pairs for fish eDNA, MiFish-U/E (Miya et al., 2015).

Data Quality Control and Reads Assembly

After quality check, the data processing was implemented by the MitoFish database (Iwasaki et al., 2013) and MiFish pipeline (Sato et al., 2018; available at http://mitofish.aori.u-tokyo.ac.jp/).

Fish Species Identification

MitoFish, NCBI Organelle Genome Resources were used for the BLAST search. After blast, we confirm the species name according to supplementary of Miya et al. (2015).
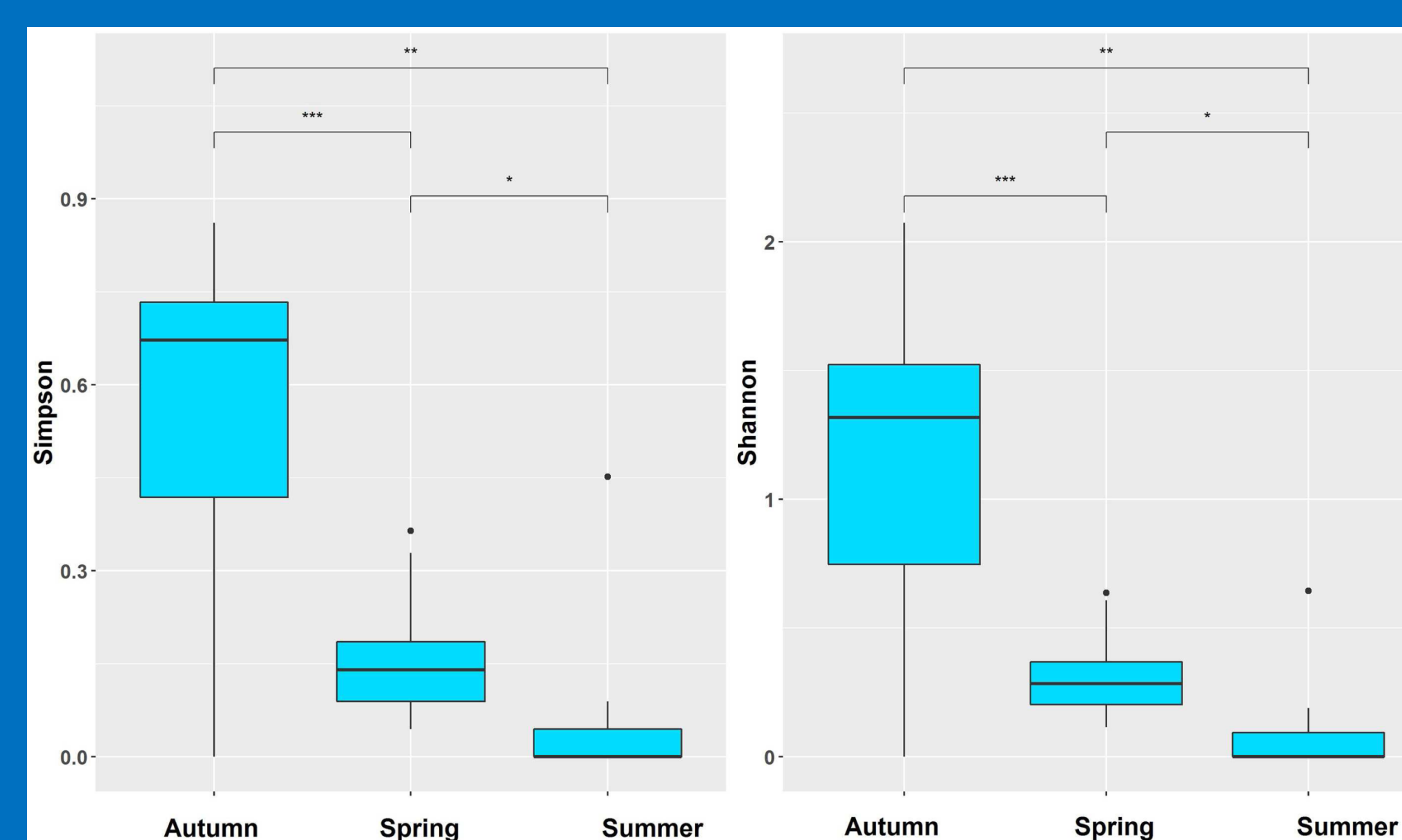
Results

| OTU ID	Species	Spring	Summer	Autumn
AB969962	*Pennahia argentata*			†
AB970004	*Harpadon nehereus*	†	†	†
AB972202	*Upeneus japonicus*			†
AB972205	*Larimichthys crocea*			†
AB972229	*Trichiurus japonicus*	†		†
AB972233	*Nibea mitsukurii*			†
AB974486	*Benthosema pterotum*	†		†
AB974524	*Acropoma japonicum*			†
AB974683	*Liparis tanakae*	†		†
NC002333	*Danio rerio*			†
NC003196	*Pagrus major*			†
NC006131	*Acanthogobius hasta*	†	†	†
NC006291	*Carassius carassius*			†
NC009579	*Coilia nasus*	†	†	†
NC010194	*Hypophthalmichthys nobilis*			†
NC011707	*Pampus sp.*	†	†	†
NC011710	*Larimichthys polyactis*	†	†	†
NC014263	*Collichthys niveatus*		†	†
NC014350	*Collichthys lucidus*			†
NC014351	*Miichthys miiuy*	†		†
NC015205	*Nibea albiflora*	†		
NC016693	*Trypauchen vagina*			†
NC018347	*Bahaba taipingensis*			†
NC020466	*Cyprinidae sp.*			†
NC020468	*Setipinna taty*	†	†	†
NC021130	*Johnius grypotus*			†
NC021460	*Psenopsis anomala*			†
NC022464	*Johnius belangerii*	†		†

△ OTUs / Species information for three seasons
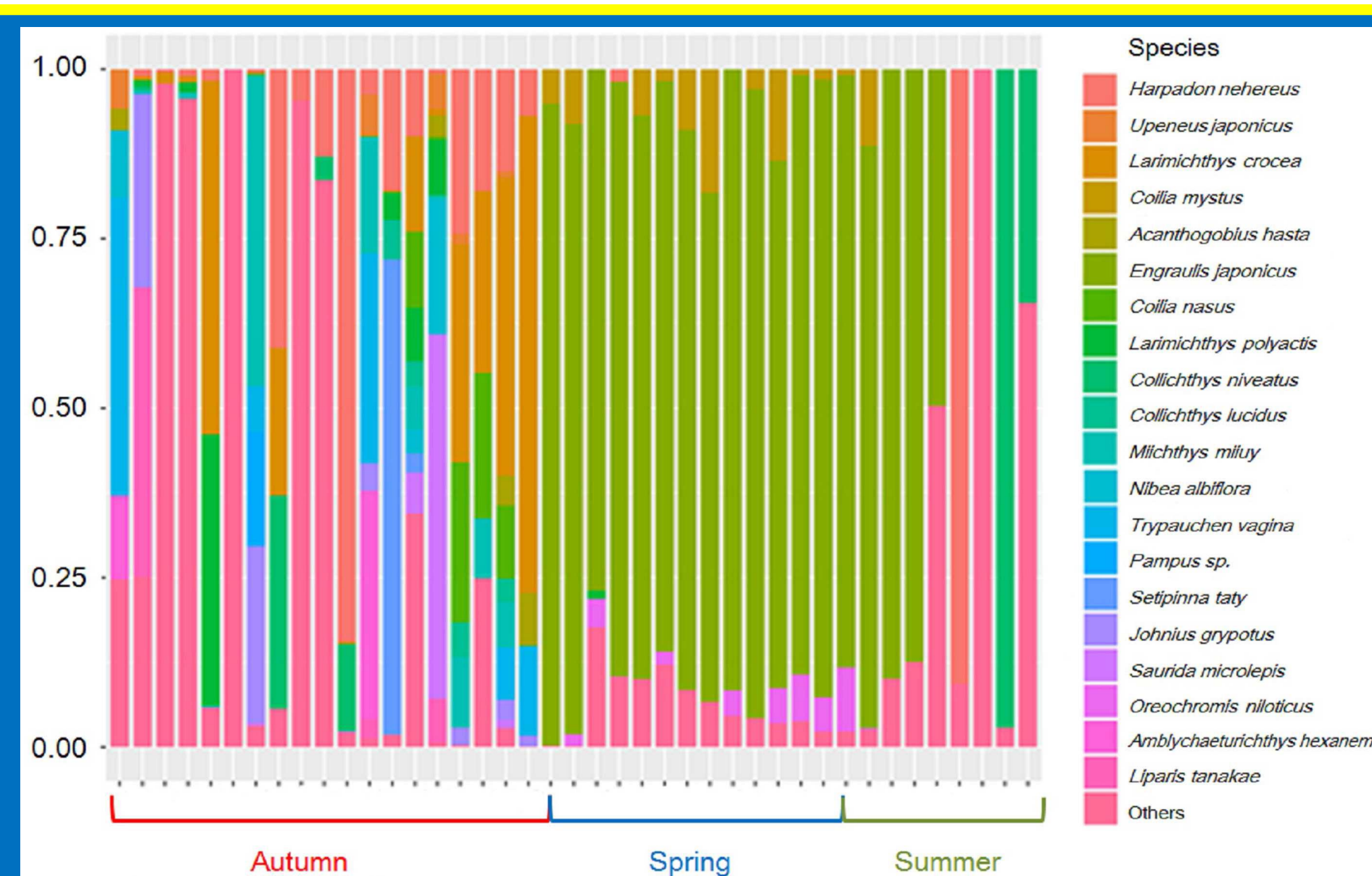
△ Species distribution in three seasons.

△ The seasonal difference based on Simpson and Shannon-Wiener index; *p < 0.05, **p < 0.01, ***p < 0.001.
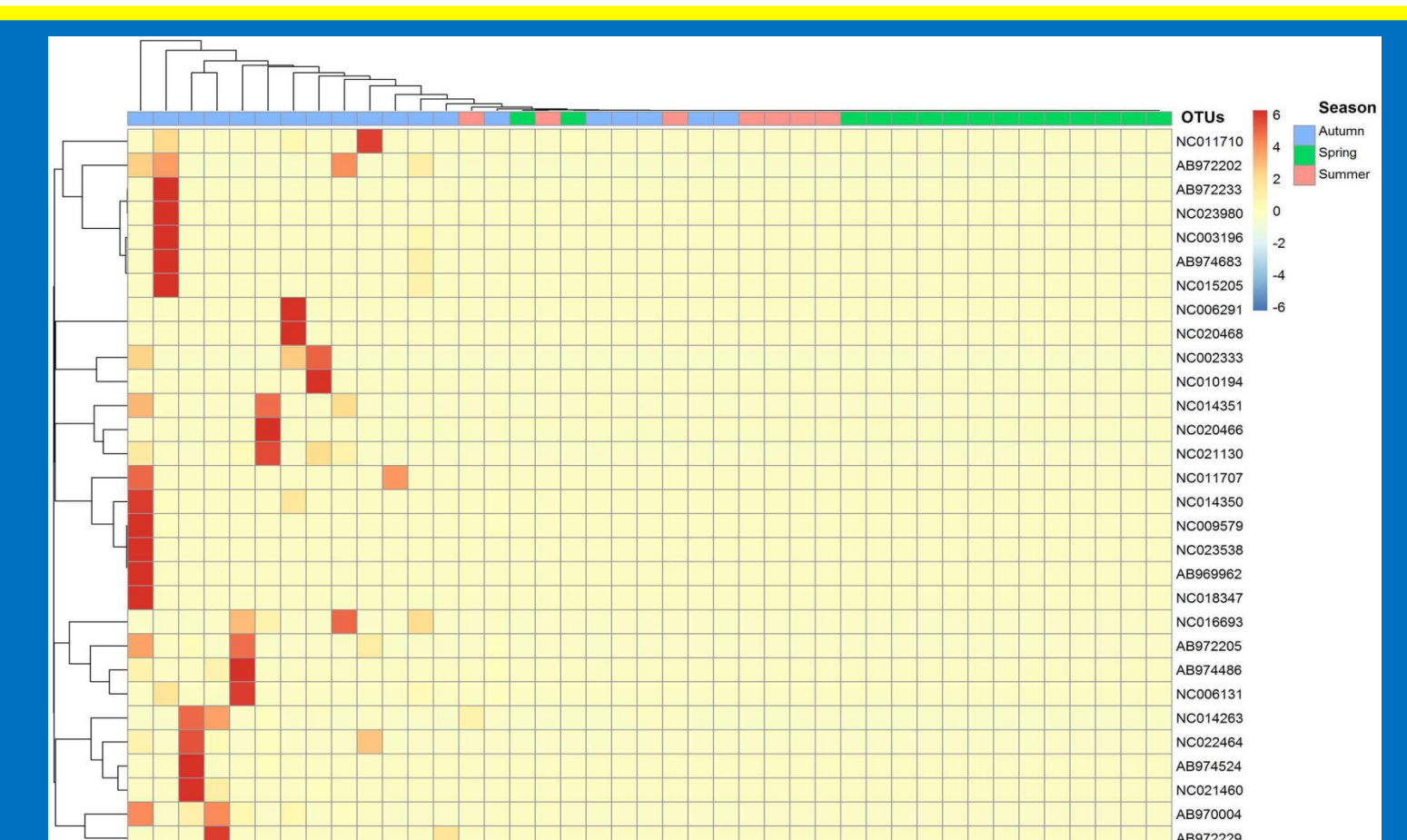
△ Thermal image shows the difference of assemblage composition
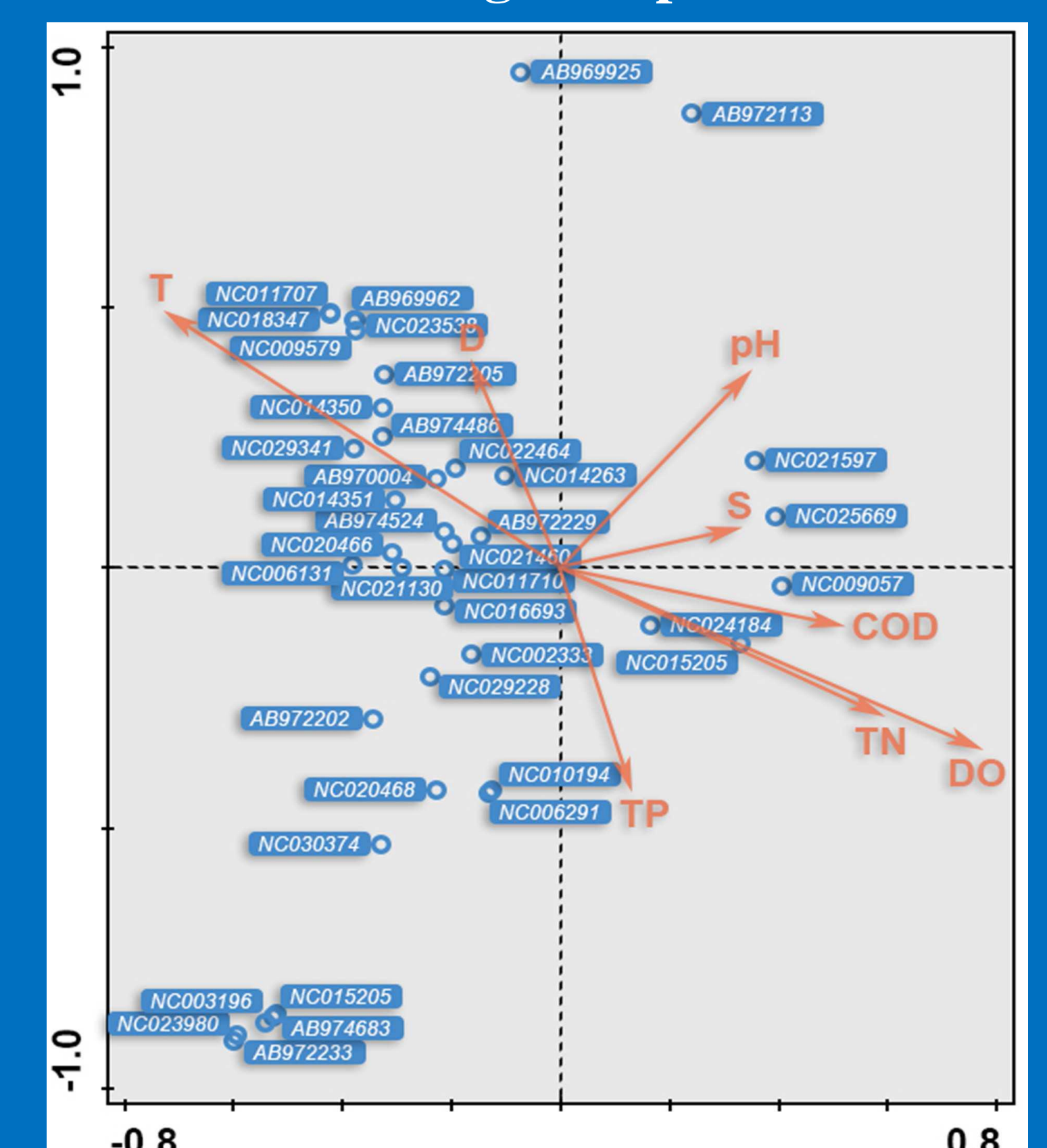
△ The CCA biplot of fish species

Discussion

➢The results of the present study demonstrate that eDNA can likewise be an effective tool to estimate fish species diversity, abundances, biomass, and spatial distributions in the Yangtze Estuary and its adjacent waters.

➢A total of 41 fish species identified in the 2018 samples across three seasons. This number is a reasonable estimate when compared with traditional surveys. Besides the detection of dominant species, our eDNA analysis also distinguished several rarer fishes,

➢Significant seasonal differences occur in the Yangtze Estuary and its adjacent waters. CCA indicated that T, S, and DO were the main environmental factors affecting the seasonal fish assemblages.