DNA metabarcoding of zooplankton communities: species diversity and seasonal variation revealed by 18S rRNA and COI

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Background. Zooplankton is an important component of aquatic organisms and has important biological and economical significance in freshwater ecosystems. However, traditional methods that rely on morphology to classify zooplankton require expert taxonomic skills. Moreover, traditional classification methods are time-consuming and labor-intensive, which is not practical for the design of conservation measures and ecological management tools based on zooplankton diversity assessment.

Methods. We used DNA metabarcoding technology with two different markers: the nuclear small subunit ribosomal RNA (18S rRNA) and mitochondrial cytochrome c oxidase (COI), to analyze 72 zooplankton samples collected in 4 seasons and 9 locations from the Sanmenxia Reservoir. We investigated seasonal changes in the zooplankton community and their relationship with water environmental factors.

Results. A total of 190 species of zooplankton were found, belonging to 12 phyla, 24 classes, 61 orders, 111 families, and 174 genera. Protozoa, especially ciliates, were the most diverse taxa. Richness and relative abundance of zooplankton showed significant seasonal changes. Both alpha and beta diversity showed seasonal trends: the diversity in summer and autumn was higher than that in winter and spring. The zooplankton diversity was most similar in winter and spring. By correlating metabarcoding data and water environmental factors, we proved that water temperature, chemical oxygen demand, total nitrogen and ammoniacal nitrogen were the main environmental factors driving the seasonal changes in zooplankton in the Sanmenxia Reservoir. Water temperature, followed by total nitrogen, were the most influential factors. This study highlights the advantages and some limitations of zooplankton molecular biodiversity assessment using two molecular markers.

Subjects: Ecology, Biodiversity, Freshwater Biology, Molecular Biology

Keywords: zooplankton diversity, metabarcoding, seasonal variation, water environmental factor, Sanmenxia Reservoir

1   **DNA metabarcoding of zooplankton communities: species diversity and seasonal**

2   **variation revealed by 18S rRNA and COI**

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14   **Abstract**

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16   biological and economical significance in freshwater ecosystems. However, traditional methods

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21   **Methods.** We used DNA metabarcoding technology with two different markers: the nuclear small

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23   72 zooplankton samples collected in 4 seasons and 9 locations from the Sanmenxia Reservoir. We

24   investigated seasonal changes in the zooplankton community and their relationship with water

25   environmental factors.

26   **Results.** A total of 190 species of zooplankton were found, belonging to 12 phyla, 24 classes, 61

27   orders, 111 families, and 174 genera. Protozoa, especially ciliates, were the most diverse taxa.

28   Richness and relative abundance of zooplankton showed significant seasonal changes. Both alpha

29   and beta diversity showed seasonal trends: the diversity in summer and autumn was higher than

30   that in winter and spring. The zooplankton diversity was most similar in winter and spring. By

31   correlating metabarcoding data and water environmental factors, we proved that water

32   temperature, chemical oxygen demand, total nitrogen and ammoniacal nitrogen were the main

33   environmental factors driving the seasonal changes in zooplankton in the Sanmenxia Reservoir.

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36   using two molecular markers.

37 **INTRODUCTION**

38 Protecting the biodiversity of freshwater ecosystems is particularly important in the 21st century.

39 Freshwater ecosystems not only contain the most concentrated biodiversity of all ecosystems on

40 the planet but are also the most endangered ecosystems worldwide (Dudgeon et al. 2006). These

41 ecosystems are susceptible to anthropogenic factors, such as climate change, eutrophication, and

42 the introduction of nonnative species, all of which will lead to a reduction in biodiversity (Dudgeon

43 et al. 2006; Revenga et al. 2005; Vörösmarty et al. 2010). Over the past few decades, the

44 biodiversity of freshwater ecosystems has decreased significantly more than that of terrestrial or

45 marine ecosystems (Sala et al. 2000). Although data on the extinction rate of freshwater

46 ecosystems are limited to North America (Ricciardi & Rasmussen 1999), these data hint at a global

47 crisis of freshwater biodiversity (Abell et al. 2008). Therefore, protecting the biodiversity of

48 freshwater ecosystems is a key priority. However, protection requires a detailed understanding of

49 this biodiversity.

50   Zooplankton, including protozoans, rotifers and microcrustaceans (cladocerans and

51 copepods) (Allan 1976; Pace & Orcutt Jr 1981), have important biological and economic value in

52 freshwater ecosystems. Not only are zooplankton the major consumers in aquatic ecosystems, they

53 are also an important food source for fish larvae and, thus, have an important role in connecting

54 primary producers with higher nutritional consumers (Allan 1976; Banse 1995; Lubzens 1987).

55 Moreover, due to the influence of abiotic factors (such as water temperature) and seasonal changes

56 in biological factors, zooplankton communities also exhibit seasonal change patterns. In addition,

57 zooplankton are very sensitive to external disturbances, and they are commonly used

58 as indicators of water quality (Branco et al. 2002; Gannon & Stemberger 1978; Oh et

59 al. 2017; Sládeček 1983). For example, *Brachionus calyciflorus* can be used as an indicator of

60 eutrophic water bodies (Gannon & Stemberger 1978), which has far-reaching importance in water

61 environment monitoring. However, the fragility of the zooplankton itself, the small size, the

62 difficulty of obtaining observations, and the species variety of zooplankton have limited the

63 understanding of zooplankton diversity (Machida et al. 2009), which is not conducive to the

64 protection of aquatic biodiversity. Among freshwater ecosystems, reservoirs have always been a

65 subject of interest. Reservoirs enable river systems to be strongly regulated while providing the

66 convenience of hydropower for human production activities (Yang & Lu 2014). However,

67 damming considerably perturbs the river's export of organic carbon to the ocean (Gao et al. 2019;

68 Maavara et al. 2017). In addition, the water residence time of the storage reservoir is

69 significantly increased and the flow velocity is obviously decreased after dam closure

70 (Friedl & Wüest 2002), which hinders the movement of aquatic organisms, and alters their

71 habitats thus has a significant effect on aquatic biodiversity (Vörösmarty et al. 2010). Direct and

72 indirect habitat changes are a major factor driving the decline or extinction of freshwater biological

73 species, especially for species with limited diffusion capacity (Pimm et al. 2014; Revenga et al.

74 2005), such as zooplankton.

75   Although zooplankton have important biological and economic value in

76 freshwater ecosystems, traditional taxonomic methods used to characterize

77 zooplankton communities are not sufficient for large-scale biodiversity research. The

78 limitations of traditional morphological identification methods cannot be completely

79 overcome in the short term (Trebitz et al. 2017). On the one hand, traditional

80 morphological classification methods are not only time consuming and labor

81 intensive but also lack resources for species identification (such as increasingly

82 limited funding to support taxonomic scientists for scientific research) (Creer et al.

83 2016; Pawlowski et al. 2016). On the other hand, traditional morphological

84 identification methods require scientific researchers to be proficient in taxonomy, but

85 academia currently lacks experienced taxonomists (Pawlowski et al. 2016). In

86 addition, a large amount of classification research work may also contain

87 classification errors (Sundermann et al. 2005). To overcome the limitations of

88 conventional morphology-based methods, improved methods are urgently needed to

89 detect zooplankton species in freshwater ecosystems more quickly, sensitively, and

90 effectively (Xiong et al. 2020). DNA metabarcoding technology can quickly alleviate

91 this situation and has proven to be a powerful tool for large-scale biodiversity

92 research (Beng et al. 2016; Ji et al. 2013; Shokralla et al. 2012). This technology

93 assigns ecological value to the taxonomic operational units (OTUs) generated by

94 high-throughput sequencing data and quickly, economically and efficiently realizes

95 the classification and identification of environmental DNA (Fernando 2002;

96 Pawlowski et al. 2016), making an important and unique contribution in the field of

97 ecology (Xie et al. 2017; Zhang et al. 2018). DNA metabarcoding technology provides

98 detailed information about zooplankton biodiversity and can support biodiversity conservation

99 (Xiong et al. 2020). In addition, it has been successfully used to track seasonal changes

100 in the zooplankton community (Berry et al. 2019; Mwagona et al. 2018; Nandini et

101 al. 2008; Tan et al. 2004; Vanderploeg et al. 2012). Metabarcoding technology sensitively

102 reflects the relationship between the abundance of the zooplankton community and environmental

103 factors and provides methodological support for exploring the water environmental factors that

104 drive seasonal changes in zooplankton (Yang et al. 2017a). Most biodiversity studies based on

105 DNA metabarcoding technology use a single molecular marker method (Amaral-Zettler et al.

106 2009; Bucklin et al. 2019; Chain et al. 2016), but the taxon scope of the study of single molecular

107 markers is limited (Drummond et al. 2015; Giebner et al. 2020). However, the multigene-based

108 metabarcoding method can increase the taxonomic breadth, which is especially important for

109 assessing the biodiversity of highly diverse groups such as zooplankton.

110   Considering the dramatic changes in ecology, water transparency, nutritional status, the

111 densities in the downstream Sanmenxia Reservoir and the strong dependence of zooplankton

112 richness on the lake conditions of the dam (creating a stable environment for the survival and

113 reproduction of different zooplankton populations), we hypothesize that the Sanmenxia Reservoir

114 has a particularly rich zooplankton community. Moreover, due to seasonal changes in the water

115 environment, the zooplankton community is likely to exhibit a seasonal change pattern in the

116 reservoir, and this seasonality is closely related to water environmental factors. However, it is not

117   clear which water environmental factors have an impact on the richness and relative abundance of

118   the zooplankton community in the Sanmenxia Reservoir or which factors have the strongest

119   influence. To verify the above hypothesis, we attempt to characterize the zooplankton diversity

120   and water environmental factors in the Sanmenxia Reservoir by obtaining seasonal (summer,

121   autumn, winter and spring in July 2017, October 2017, January 2018 and March 2018,

122   respectively) zooplankton samples and water samples. We used a multigene approach with two

123   independently evolved markers,the nuclear small subunit ribosomal RNA (18S rRNA v4 region;

124   Hadziavdic et al. 2014) and and the mitochondrial cytochrome c oxidase (COI; Leray & Knowlton

125   2015). The main purposes of our study are (1) to explore the composition of the zooplankton

126   community in the Sanmenxia Reservoir, (2) to reveal the dynamics of seasonal changes in the

127   zooplankton community, and (3) to determine the main water environmental factors that drive the

128   seasonal changes in zooplankton diversity in the reservoir area.

129

130   **MATERIALS AND METHODS**

131   **Study area, zooplankton sample collection and measurement of water environmental factors**

132   The Sanmenxia Reservoir, which is located in the lower reaches of the Middle Yellow River basin

133   in China, was effectively completed in 1960. The reservoir is the first large-scale water

134   conservancy project built on the Yellow River and controls 89 % of the water in the Yellow River

135   basin (Chen et al. 2017; Wang et al. 2005). The reservoir area is in a warm temperate continental

136   monsoon climate. The interannual temperature fluctuates within the range of -18.3°C~42.3°C, and

137   the average annual temperature is 14.1°C (Zhang et al. 2012). According to the geographical

138   features of the Sanmenxia Reservoir and the field investigation, nine sampling points (as 9

139   biological replicates in this study) with representative habitats (the front and rear of the dam of

140   Sanmenxia Reservoir) were set up in the reservoir area to collect samples (three replicates each

141   site) in summer, autumn, winter and spring in July 2017, October, 2017, January 2018 and April

142   2018, respectively (Fig. S1). Geographical coordinates of the sampling site were collected with a

143   global positioning system (GPS) (Garmin Legend, Garmin USA). Zooplankton samples were

144   collected by filtering 20 L of water using plankton nets (46-μm mesh). The plankton net was

145   dragged along the water surface repeatedly in a "∞" shape at a depth of 0.3 m for 5 minutes to

146   collect and concentrate samples. Then, the samples were transferred into a 50 mL centrifuge tube

147   and immediately preserved in 95% ethanol. In the laboratory, the zooplankton samples were

148   further filtered through 0.22 μm microporous filter paper (Whatman, UK) to remove excess water,

149   placed in a 5 mL centrifuge tube, and then stored at -80°C until DNA extraction could be

150   completed.

151   During the zooplankton sampling period, additional 500 mL water samples were collected at

152   each sampling site to measure chemical oxygen demand (COD), total nitrogen (TN), total

153   phosphorus (TP), ammonia nitrogen (NH_4-N) and pH. These measurements were taken in the

154   laboratory of Shaanxi Normal University. Water temperature (WT) and dissolved oxygen (DO)

155   were measured directly in the field using a BANTE820 Portable Dissolved Oxygen Meter (Bante,

156   China).

157

158   **DNA extraction, PCR amplification, library construction and high-throughput sequencing**

159   **(HTS)**

160   The total DNA of the zooplankton community was purified by mixing DNA isolated from three

161   samples from each sampling site using the DNeasy Blood and Tissue Kit (Qiagen, Germany). The

162   three biological replicates of each sampling site were pooled to yield more DNA. DNA quality

163   was monitored using a NanoDrop 2000 (Thermo Scientific, USA) and 1% agarose gel

164   electrophoresis. Mitochondrial cytochrome c oxidase I (COI) was amplified by using the universal

165   COI primers mlCOIintF and jgHCO2198 (Geller et al. 2013; Leray & Knowlton 2015), and the

166   V4 region of the nuclear small subunit ribosomal RNA (18S rRNA) was amplified by using

167   HPLC-purified PCR primers (Nolte et al. 2010) (Table S1). PCR was performed with 25   μL

168   reactions for each sample, including 1 μL of DNA template (30ng/μL), 1 μL of primers (10μM),

169   19.9 μL of ultrapure water, 2.5 μL of High-Fidelity PCR buffer (10 ×), 0.5 μL of dNTP mix

170   (10mM) and 0.1 μL of Platinum Taq DNA polymerase (Invitrogen, USA). The PCR reaction

171   procedure was as follows. 18S: 95°C for 3 min; 35 cycles at 95°C for 45 s; 50°C for 50 s; 72°C

172   for 45 s; and 72°C for 10 min; COI: 95°C for 10 min; 16 cycles at 94°C for 10 s; 62°C for 30s

173   (decreasing by 1°C per cycle); 68°C for 60 s; 25 cycles at 94°C for 10 s; 46°C for 30 s; 68°C for

174   60 s; 72°C for 10 min extension; and 4°C insulation. Then, the PCR products of the same sample

175 (three PCR replicates per sample) were mixed and subjected to 2% agarose gel electrophoresis.

176 PCR product was recycled by cutting the gel using an AxyPrepDNA gel extraction kit (Axygen

177 Biosciences, USA), and subjected to Tris_HCl elution and 2% agarose electrophoresis. After

178 purification, a standardized procedure for qualified PCR products was followed to form an

179 amplicon library, and a MiSeq Reagent Kit v2 (500 cycle) was used to perform high-throughput

180 sequencing of 2×250 bp reads on the Illumina MiSeq platform (Allwegene, China).

181 **Data processing**

182 Initial quality control was completed using Trimmomatic (Bolger et al. 2014), a 50 bp window

183 size was set, and bases with average Phred quality score less than 20 were trimmed. The primer

184 sequence was removed using Cutadapt (Martin 2011). Merging of raw paired-end reads was

185 conducted using FLASH (Magoč & Salzberg 2011) with more than 10 bp in the minimum overlap

186 sequences, and the maximum allowable mismatch ratio not exceeding 0.1. Recognition and

187 removal of chimeric sequences were conducted using UCHIME (Edgar et al. 2011). OTU

188 clustering was performed using UPARSE (namely, USEARCH was used to cluster OTUs

189 according to 97% similarity sequences, excluding single sequences, obtain representative

190 sequences and then map all their sequences according to 97% similarity to OTUs to form an OTU

191 list) pipeline (Edgar 2013). OTU annotation was conducted on the representative sequences of

192 each OTU against Silva (18S rRNA) and the BOLD reference database (COI) (Altschul et al. 1990;

193 Pruesse et al. 2007; Ratnasingham & Hebert 2007). The species names of OTUs were designated

194 as sequence identity exceeding 97%, and the genus names of OTUs were designated as sequence

195   identity between 90% and 97%. If an OTU could not be assigned to a species, it was assigned to a

196   higher taxonomic level. We also removed all bacteria, fungi, and Viridiplantae from the OTU

197   annotation tables to focus exclusively on zooplankton for the purpose of this study.

198   **Zooplankton diversity analysis**

199   Statistical analyses were performed by using the "vegan" package in R v.3.6.3 (Oksanen et al.

200   2010; Team 2013). Rarefaction curves were used to measure the saturation of zooplankton

201   samples. Venn diagrams were used to show the number of different taxa recovered by using 18S

202   (blue) or COI (yellow) at different taxonomic levels. Bar charts were used to reflect the difference

203   in OTU richness and relative abundance produced by DNA metabarcoding technology in 18S

204   (blue) or COI (yellow). Bubble plots were used to reflect the composition and seasonal variation

205   in the zooplankton community. Alpha diversity was characterized by the Shannon and ACE

206   indices, which were drawn by box plotting. In this process, since the data did not obey a normal

207   distribution, the Kruskal-Wallis nonparametric test was used to determine whether seasonal

208   changes in zooplankton diversity were significantly different. Subsequently, since the OTU

209   richness data obeyed a normal distribution and homogeneity of variance, analyses of variance

210   (ANOVA) were selected and performed using Tukey's honest significant difference (Tukey HSD)

211   tests to analyze whether the richness of each zooplankton group differed significantly among

212   seasons. Bar charts were also used to characterize the seasonal changes in the relative abundance

213   of each zooplankton group, and the Welch t test was used to determine whether their seasonal

214   changes were significant in adjacent sampling seasons. To evaluate the seasonal variation in the

215   zooplankton community between samples, a principal coordinate analysis (PCoA) was used to

216   cluster the samples by sampling season based on the Jaccard dissimilarity index and Bray-Curtis

217   dissimilarity index, and pairwise comparisons of all sampling seasons were performed using

218   PERMANOVA (Anderson 2001). An analysis of similarity (ANOSIM) was also used for further

219   evaluation. According to the characteristics of the measured water environmental factor data, the

220   relationship between the variables was analyzed using Spearman's correlation analysis with

221   Bonferroni correction to account for multiple testing. To investigate the water environmental

222   factors that drive seasonal changes in the zooplankton community, the OTU data matrix was

223   related to the water environmental factor matrix by using the partial Mantel test, and relative

224   abundance data were combined with water environmental factors for canonical correspondence

225   analysis (CCA). An Adonis analysis (permutations: 999) was used to test the contribution

226   (variation, R^2) and significance of each water environmental factor.

227

Results

229   **Results of amplicon sequencing**

230   We metabarcoded a total of 36 samples for each marker (9 locations per season, for a total of four

231   seasons). After the Illumina sequence passed all filtering procedures, 2,185,317 and 910,137 high-

232   quality reads (including non-plankton metazoans) were obtained from the 18S rRNA and COI

233   metabarcoding, respectively. In the 18S rRNA dataset, the sequencing depth per sample ranged

234   from 5510 to 116,411, with an average of 60,703. In the COI dataset, the sequencing depth per

235   sample ranged from 7057 to 68,313, with an average of 25,282 (Table S2). The OTUs of single

236   reads were excluded from further analysis to reduce the possible effects of spurious signals. This

237   exclusion could conservatively remove potential misamplified fragments, although they may

238   represent very low-abundance zooplankton groups. The rarefaction curves of 72 zooplankton

239   samples stabilized with increasing sequencing depth, which indicated that most of the sampling

240   points were properly sampled and that the data tended to be saturated (Fig. S2). DNA

241   metabarcoding of zooplankton samples resulted in the identification of 495 OTUs from the 18S

242   rRNA gene, and 1099 OTUs from the COI gene. Approximately 5.25% (26 of 495 OTUs) observed

243   in the 18S rRNA datasets could only be identified to kingdom and were labeled "Eukaryota" or

244   "Metazoa". Approximately 3.46% and 0.54% (38 and 6 of 1099 OTUs, respectively) were labeled

245   "Invertebrate environmental sample" and "Arthropoda environmental sample" in the COI datasets,

246   respectively. Approximately 64.44% (319 of 495 OTUs, 18S rRNA) and 35.94% (395 of 1099

247   OTUs, COI) of the OTUs were classified as zooplankton, and the remaining OTUs were annotated

248   as Insecta, Gastropoda, etc. (Table.S3). The number of zooplankton OTUs ranged from 26 to 148

249   (18S rRNA) and 49 to 146 (COI) in each sample (Table 1).

250   **Zooplankton community composition**

251   There were differences in the number of zooplankton taxa identified by the two markers at different

252   taxonomic levels (Table 2, Fig. 1). A total of 12 phyla were identified in this study, including 24

253   classes, 61 orders, 111 families, 174 genera and 190 species. Of the 319 nonsingleton zooplankton

254 OTUs in the 18S rRNA gene, 12 phyla were represented across 23 classes, 54 orders, 83 families

255 and 110 genera (Table 2). Of the 395 nonsingleton zooplankton OTUs in the COI gene, 4 phyla

256 were represented across 7 classes, 19 orders, 42 families and 80 genera (Table 2). At the phylum

257 level, the four phyla detected by the COI were also be recovered with 18S. The corresponding

258 percentages were 85.71% for class, 63.16% for order, 33.33% for family, 20.00% for genus, and

259 9.4% for species-level taxa (Fig. 1).

260   The richness (Fig. 2A) and species diversity (Fig. 1GHI) of the same zooplankton groups

261 showed differences in the results identified by the two markers, with Copepoda richness the only

262 exception (Fig. 2A). Protozoa was the most abundant group in the 18S rRNA, accounting for

263 68.03% (217 OTUs) and 76.19% (64 species) of zooplankton richness and species diversity,

264 respectively, and only 14.18% (56 OTUs) and 18.80% (22 species), respectively,in COI. Rotifera

265 was the most abundant group (57.47%, 227 OTUs; 51 species, 43.59％) in COI but only accounted

266 for 8.46% (27 OTUs) and 11.90% (10 species) of richness and diversity, respectively, in 18S

267 rRNA. Copepoda was the second most abundant taxon between the two markers; its OTUs richness

268 was similar (73, 71OTUs),and 11 and 30 species were identified in the COI and 18S rRNA

269 samples, respectively. The least diverse taxonomic groups was Cladocera (2 OTUs, 18S rRNA;

270 41 OTUs, COI) using both markers, and 0 and 14 species were identified respectively. In addition,

271 11 species were identified by both markers (Fig. 1GHI): 2 species of Protozoa, namely,

272 *Vermamoeba vermiformis* and *Reclinomonas americana*, 4 species of rotifers, namely, *Adineta*

273 *vaga, Asplanchna sieboldi, Brachionus calyciflorus* and *Testudinella clypeata*, and 5 species of

274   copepods, namely, *Megacyclops viridis*, *Mesocyclops dissimilis*, *Mesocyclops pehpeiensis*,

275   *Microcyclops varicans* and *Pseudodiaptomus inopinus*.   *Brachionus calyciflorus* was detected in

276   all (72) zooplankton samples (Fig. S3). *Eucyclops macruroides*, *Sinocalanus sinensis* and

277   Brachionidae sp. SHDT150710, and *Keratella cochlearis* were also present in each zooplankton

278   sample of 18S rRNA or COI genes (Fig. S3).

279   The relative abundance of the same zooplankton group differed between the two

280   markers.(Fig.2B). 18S Copepoda had significantly higher relative abundance than other

281   zooplankton taxa, but 18S Cladocera had the lowest relative abundance. In COI, Rotifera had the

282   highest relative abundance, which was similar to that of Copepoda. However, the relative

283   abundance of Protozoa was low according to both markers.

284   **Seasonal dynamics of zooplankton diversity**

285   The Shannon diversity and ACE index observed in the metabarcoding datasets of each molecular

286   marker were similar, and all showed a maximum in summer or autumn and a minimum in spring

287   or winter (Table 1). Moreover, the entire zooplankton community assemblages showed significant

288   seasonal changes, including the richness estimators ACE, and the diversity estimator Shannon

289   (Fig. 3). The overall trend showed high diversity in summer and autumn and low diversity in winter

290   and spring.

291   To explore seasonal changes in each zooplankton group, we performed ANOVA on the

292   richness datasets of zooplankton at OTU levels (Fig. 4). The results showed that the richness of

293   zooplankton groups, except for the 18S Cladocera, had significant seasonal changes (Tukey HSD,

294   *p* < 0.01, Fig. 4), and the species diversity of most zooplankton groups was significantly higher in

295   summer and autumn than in winter and spring. In addition, the trend in seasonal changes in the

296   relative abundance of Rotifera was consistent in the two molecular markers, and Copepoda was

297   no exception. From summer to autumn in 2017, the relative abundance of Rotifera decreased from

298   21.57% (18S) and 32.78% (COI) to 3.36% and 11.49% , respectively (Fig. 5AD), and then

299   increased to 42.22% and 67.20% in winter 2018 (Fig. 5BE) and finally decreased to 35.20% and

300   59.92% in spring 2018 (Fig. 5CF). The change tendency of the relative abundance of 18S Protozoa

301   was consistent with that of Rotifera, and the trend was only consistent with that of the COI

302   Protozoa from winter to spring (Fig. 5F). The relative abundance of Cladocera was consistent with

303   the change trend in 18S Copepoda. However, for COI, the relative abundance increased from

304   summer (8.37%) to winter (15.41%) and then decreased in spring, when it reached a minimum

305   (6.31%) (Fig. 5DEF). In fact, the relative abundance of most zooplankton groups, except for the

306   richness, also showed significant seasonal variation between adjacent sampling seasons (Welch *t*

307   test, p < 0.05). However, the relative abundance of Cladocera showed no significant seasonal

308   changes between adjacent sampling seasons (Welch *t* test, p > 0.05), although its relative

309   abundance based on COI was obviously higher than that of the 18S rRNA gene.

310   To assess the seasonal variations in the entire zooplankton community assemblages between

311   samples, we performed a PCoA at the OTU level (Fig.6). The results showed that within the ellipse

312   with a 75% confidence interval, the samples for most sampling seasons were divided into three

313   distinct clusters. Two distinct clusters were formed separately in summer and autumn, and another

314   large cluster was formed in winter and spring, and there was no overlap between seasons other

315   than between winter and spring (Fig. 6). Thus, the zooplankton communities in winter and spring

316   were more similar to each than were those in the summer and autumn.

317   In terms of relative abundance, the zooplankton communities during winter and spring were

318   dominated by Rotifera and Copepoda in the 18S metabarcoding datasets, and their relative

319   abundances were 42.22% and 35.20% (Rotifera) and 54.15% and 63.39% (Copepoda) (Fig. 5C),

320   respectively, within which *Eucyclops* and *Brachionus* were predominant (Fig. 7A), contributing

321   36.57% and 33.59% to the similarity of the zooplankton community in winter and spring,

322   respectively. Conversely, the autumn zooplankton samples were nearly all annotated as Copepoda

323   (95.81%) (Fig. 5A), including *Eucyclops* and *Mesocyclops* (Fig. 7A). The summer zooplankton

324   samples also had a small number of Rotifera (21.57%), but the abundance was far lower than the

325   relative abundance of Rotifera in winter and spring, except for a large number of Copepoda

326   (*Mesocyclops* and *Undinula*) (77.16%). Although the dominant group changed slightly with

327   different markers, it did not affect the trend. In the COI metabarcoding datasets, the zooplankton

328   communities in winter and spring were dominated by Rotifera (67.20%, 59.92%) (Fig. 5F), mainly

329   *Brachionus*, which contributed 27.92% to the community similarity between the two seasons. In

330   autumn, 78.59% of the sequences were annotated as Copepoda (Fig. 5D), mainly including

331   *Eucyclops* (contribution rate of 20.44%) (Fig. 7B), but the relative abundances of Rotifera and

332   Cladocera were similar, accounting for 11.49% and 8.95%, respectively. The relative abundance

333 of dominant groups in summer COI showed similar trends to those of 18S rRNA.

334   In general, the entire zooplankton community assemblages showed significant seasonal

335 changes (Fig. 6, PERMANOVA, $p < 0.001$). In addition, the ANOSIM results further indicated

336 that the zooplankton community had significant seasonal variations (Table S4).

337 **Seasonal changes in zooplankton diversity driven by water environmental factors**

338 The measured values (mean values) of water environmental factors in the Sanmenxia Reservoir

339 during all four seasons (time) in the study period are shown in Table 3. Each water environmental

340 factor showed statistically significant differences between seasons (Kruskal-Wallis, $p < 0.01$).

341 With the change in sampling season, the WT fluctuated, ranging from 2.0 (winter) to 33.2°C

342 (summer); the recorded TN concentration showed an increase and reached the highest

343 concentration in spring (5.3 ± 0.16 mg/L); the DO increased from summer and reached the highest

344 concentration in winter (8.31 ± 0.49 mg/L) and then decreased to the lowest concentration in spring

345 (6.16 ± 0.16 mg/L). The pH, COD, TP, and NH_4-N showed synchronous changes that fell from

346 summer to autumn, rose again from autumn to winter, and eventually fell from winter to spring.

347   To understand the environmental factors that drive seasonal changes in zooplankton diversity,

348 we performed partial (geographic distance-corrected) Mantel tests to correlate the DNA

349 metabarcoding datasets and the distance matrix of water environmental factors based on Euclidian

350 distances. The results showed that WT, COD, TN and NH_4-N were the main water environmental

351 factors that drive seasonal changes in zooplankton richness (Fig. 8). WT had a significant positive

352 correlation (Spearman's, $p < 0.05$) with the richness of most zooplankton groups. Moreover, the

353 water environmental factors that drive seasonal changes in zooplankton groups changed slightly

354 with different groups in the same molecular marker, such as Rotifera and Copepoda. Due to the

355 different taxa and richness identified by different molecular markers, the environmental factors

356 that drive the seasonal changes in these four zooplankton groups were not completely consistent

357 between the two molecular markers.

358   The CCA results showed that six water environmental factors explained 59.67% and 50.29%

359 of the zooplankton community variance based on the relative abundance in CCA axis 1 and 32.22%

360 and 29.39% in axis 2, respectively (Fig. 9). Among all the factors, WT was the most powerful

361 driving factor, followed by TN (Table S5). However, DO was not found to directly affect the

362 seasonal variation in zooplankton diversity in the current analysis (partial Mantel test, $p > 0.05$;

363 Adonis, $p > 0.05$).

364

365 **Discussion**

366 **Zooplankton diversity**

367 DNA metabarcoding technology is a powerful tool for large-scale biodiversity research. Multi-

368 gene metabarcoding is a particularly promising approach. A total of 111 families, 174 genera and

369 190 species were identified in the four sampling seasons by using 18S and COI as markers.

370 Compared with the 48 species (29 species of Protozoa, 16 species of Rotifera, 2 species of

371   Copepoda, 1 species of Cladocera) surveyed in the reservoir area in 1985 (Qiaoyu & Zhifeng

372   2005), the number of zooplankton species identified by the metabarcoding method was

373   significantly higher. Based on this significant increase in zooplankton species, we believe that a

374   significant change has occurred in the species composition of zooplankton in the Sanmenxia

375   Reservoir. However, we realize that the previous research used a classification method based on

376   traditional morphology, which was different from the metabarcoding method we used, and the data

377   analysis methods and sampling locations were also inconsistent. Therefore, studies on zooplankton

378   diversity   should be conducted by using the same method and sampling location to obtain more

379   comprehensive and reliable results (Li et al. 2019b)

380   We found that the number of zooplankton species identified by the two molecular markers is

381   larger than that of any single molecular marker in this study. The simultaneous use of two

382   molecular markers can improve the detection of species (Zhang et al. 2018), and make the results

383   of zooplankton diversity research more comprehensive. Different markers not only have different

384   taxonomic resolution but also complement one another (Bucklin et al. 2016; Giebner et al. 2020).

385   The taxa identified by the two markers were different, and we suspect two principal reasons for

386   this difference. Firstly, it could be caused by the different amplification ability of PCR primers for

387   different organisms. Each molecular marker gene has different amplification efficiency for each

388   taxonomic group under the amplification of different universal primers, resulting in different

389   amplified taxa. In this process, PCR amplification bias may be introduced due to universality of

390   the primers, annealing temperature, and the number of replication cycles, etc. (Engelbrektson et

391   al. 2010). Secondly, the use of different reference databases can also lead to differences

392   in taxonomic identification. To more comprehensively identify zooplankton taxa, we need to

393   use both a reference database with taxonomic integrity and that is geographically comprehensive

394   (Bucklin et al. 2016), but we did not choose the NCBI nt database. Instead, we selected the

395   SILVA, and BOLD databases as references for 18S rRNA and COI sequences,

396   respectively. The SILVA database is suitable for annotating 18S rRNA sequences to

397   a more refined taxonomic level compared to the NCBI nt database(Lindeque et al.

398   2013); the BOLD database is mainly based on COI barcodes and currently contains

399   over 4 million sequences of more than 5 million different species (Wangensteen et

400   al. 2018) Moreover, the purpose of our research was to more comprehensively identify and

401   classify zooplankton diversity rather than compare the diversity and taxonomy of different

402   molecular markers, although the NCBI nt database can facilitate the comparison of multiple

403   molecular markers in the same database (Djurhuus et al. 2018). Therefore, we chose two different

404   reference databases. Interestingly, all 18 classes found only in the 18S rRNA identification results

405   were protozoans, such as Dinophyceae, Phyllopharyngea, and Litostomatea so and on. We think

406   that this sensitivity to protozoans may arise from their closely connection with the PCR primers

407   and reference database we selected. 18S rRNA datasets were mainly used to identify Protozoa in

408   this study, and the PCR primers and reference database of the 18S rRNA datasets we selected were

409   also biased to detect Protozoa, which further indicates that the choice of reference database and

410   primer has a great impact on the detection results. Moreover, it should be pointed out that no matter

411   which database is used, the annotation of the OTU may be biased or blocked due to the absence of

412   the reference sequence(Djurhuus et al. 2018). The 18S marker has a low taxonomic resolution

413   (Gibson et al. 2014), and previous studies have shown that it is not suitable for determining the

414   species-level richness of environmental samples, which was also found in our study (Tang et al.

415   2012). However, COI is more suitable for taxonomic identification at the species level because it

416   has a higher mutation rate, and its high resolution is usually not achieved by the highly

417   conservative 18S (Dupuis et al. 2012; Tang et al. 2012). Although the resolution of 18S rRNA

418   genes is lower than that of COI at the species level, its resolution is higher than that of COI for

419   taxonomic levels other than the species level. Therefore, when the research is not aimed at the

420   species level, 18S is sufficient. Otherwise, COI is more suitable. We also found that the lower the

421   taxonomic level, the less overlap between the zooplankton taxa identified by the two markers

422   (Wangensteen et al. 2018). In addition, 18S is superior to COI in identifying Protozoa, but the

423   situation is the opposite when identifying rotifers, copepods and cladocerans. It should be noted

424   that the 18S rRNA datasets only identified 2 OTUs of Cladocera, and neither of them were

425   identified at the species level, which is in sharp contrast with the results of COI identification.

426   Amplicon length polymorphisms have been shown to cause differential bias (amplification and

427   taxonomic) in metabarcoding studies of bacteria and fungi (Ihrmark et al. 2012; Ziesemer et al.

428   2015), which may explain why only 2 cladoceran OTUs were detected in 18S. If we used only 18S

429   to analyze our samples instead of two markers (nuclear and mitochondrial gene), we would have

430   failed to detect the important influence of cladocerans on the observed beta-diversity (Clarke et al.

431   2017). This finding emphasized the benefits of using multiple markers metabarcoding methods for

432   biodiversity assessment. It also illustrates that the use of a single molecular marker for the

433   taxonomic identification of environmental DNA is limited. Although inconsistent results may

434   occur by using multiple markers in metabarcoding technology (Dupuis et al. 2012), it can

435   maximize the information needed for restoring the biodiversity of the ecosystem.

436   Protozoa, especially ciliates, were the most diverse zooplankton community group, which

437   was consistent with the survey of zooplankton diversity in the Xiangxi River of the Three Gorges

438   Reservoir (Li et al. 2019b). Compared with other zooplankton groups, the protozoan

439   species were dominant, but their relative abundance was low throughout the year,

440   which may be caused by amplification and primer bias. The usefulness of DNA

441   metabarcoding technology for quantitative analysis of species abundance is limited

442   by biological and technical biases, which can affect sequence reads counts (Thomas

443   et al. 2016). The PCR step may introduce chimeric sequences, biasing in taxonomic

444   representation (primer bias) (Bista et al. 2018). Primer-template mismatches can

445   reduce the amplification efficiency, and the resulting bias is further enhanced by the

446   number of PCR cycles (amplification deviation) (Giebner et al. 2020; Piñol et al.

447   2019). Moreover, sequencing bias may also affect the abundance estimation in metabarcoding

448   research (Esling et al. 2015; Lee et al. 2012). Although the read counts of metabarcoding

449   technology are extremely dependent on the amount of DNA as well as the number of gene copies

450   and primer bias (Elbrecht & Leese 2015), it can provide a preliminary or semi-quantitative

451   estimation of relative abundance (Yang et al. 2017b). While the relative abundance of reads is not

452   an accurate measure of abundance at the community level, we assumed that these biases would

453   not have a significant impact on intra-taxon comparisons between samples, so these data can be

454   used to examine the comparative differences in the identified taxa between different sampling

455   dates (Banerji et al. 2018).

456   Rotifers have high values of both richness and relative abundance because they are r-

457   strategists, or opportunists with a small size and short life cycle, and they are tolerant to various

458   environmental factors; thus, rotifers were dominant in the reservoir environment (Neves et al.

459   2003; Nogueira 2001). Moreover, the phenotypic plasticity and strong applicability masticatory

460   apparatus of Rotifera also created favorable conditions for its success (Segers 2007). A large

461   number of studies showed that Rotifera can be used as indicators of water nutrition status, where

462   the presence of *Brachionus* indicates that water bodies are at moderate to high levels of organic

463   pollution (Sládeček 1983; Whitman et al. 2004). In our study, *Brachionus* was found in all

464   zooplankton samples with high diversity, which shows that the water in the reservoir was in a

465   medium or high trophic state. Of course, other genera (*Keratella* and *Rotaria*) used to indicate

466   eutrophication status were also found in this study (Mageed 2007; Tackx et al. 2004). Therefore,

467   it is necessary to strengthen the monitoring of pollution sources in the reservoir area, strictly

468   control the increase of new pollution sources, improve the water quality of the reservoir area, and

469   provide strong support for the sustainable development of water ecology in the reservoir area

470   (Liang et al. 2015)

471 **Seasonal changes in the zooplankton community**

472 Metabarcoding technology can also detect distinct seasonal patterns of taxa that are generally not

473 detectable using traditional methods (Banerji et al. 2018).The analysis shows that the zooplankton

474 community has obvious seasonal changes in Sanmenxia Reservoir. This finding is consistent with

475 previous studies of significant seasonal changes in the zooplankton community based on

476 traditional morphological monitoring and metabarcoding techniques in other freshwater reservoirs

477 (Mwagona et al. 2018; Nandini et al. 2008; Tan et al. 2004; Vanderploeg et al. 2012) However,

478 the relative abundance identified for Cladocera did not differ significantly between seasons despite

479 its relative abundance in COI being significantly higher than that in 18S. According to reports, the

480 seasonal changes in Cladocera were controlled by the seasonal changes in nutrients (Xiao-Jun et

481 al. 2014). We found that the seasonal variation in TP was not large in our study, which explains

482 the nonsignificant seasonal variation in Cladocera. Biodiversity patterns were strongly structured

483 temporally, which was reflected in alpha- and beta-diversity measures (Banerji et al. 2018).Their

484 change patterns throughout seasons indicated that zooplankton responded to changes in the water

485 environment of the reservoir. Moreover, when we explored the seasonal variation pattern, we

486 found similar beta-diversity in the two markers, which was consistent with the findings of Clarke

487 et al.(Clarke et al. 2017).

488 **Effects of water environmental factors**

489 Water environmental factors can directly or indirectly affect the zooplankton community. In this

490   study, we found that WT, COD, TN and ammonia nitrogen were the main water environmental

491   factors driving seasonal changes in the richness of the zooplankton community, and six water

492   environmental factors other than dissolved oxygen had important effects on the relative abundance

493   of zooplankton. We also found that WT was the most powerful environmental factor driving the

494   seasonal changes in zooplankton (partial Mantel test, Adonis, $p < 0.01$) (Sellami et al. 2016;

495   Sunagawa et al. 2015), followed by TN. This result confirmed that temperature and nutrient

496   accumulation were the most important water environmental factors that affected the seasonal

497   changes in the zooplankton community (Li et al. 2019a). Different zooplankton species have

498   specific optimal temperatures (Li et al. 2019a). Appropriate temperature conditions promote the

499   growth and predation of some zooplankton groups, resulting in seasonal changes in zooplankton

500   groups.

501   Some important variables (chlorophyll a, phytoplankton biomass, particulate organic carbon,

502   precipitation) were not monitored, although we tested multiple water environmental factors. These

503   factors may also have significant effects on the zooplankton community structure, but they

504   exceeded the scope of this study. In addition, it has been observed that climate change and fishery

505   pressure can also cause changes in the populations and composition of zooplankton in aquatic

506   ecosystems around the world (Richardson 2008; Sarma et al. 2005). Unfortunately, understanding

507   these factors is beyond the scope of this study because of the lack of relevant data on climate

508   change, fish farming, and predation in the Sanmenxia Reservoir.

509   It should be noted that although DNA metabarcoding technology has been widely used in

510   biodiversity research (Deiner et al. 2017; Lim et al. 2016), many technical issues have not been

511   resolved or properly resolved (Xiong et al. 2016a; Xiong et al. 2016b). A large number of studies

512   have extensively discussed the ways in which some technical biases related to DNA extraction,

513   PCR conditions, primer specificity, library preparation and sequence analysis could affect the

514   analysis results (Esling et al. 2015; Kermarrec et al. 2013; Lee et al. 2012). Therefore, there is still

515   a great need for a complete and standardized protocol (Majaneva et al. 2018). In addition, the gaps

516   and misidentifications in the reference database can cause obstacles to the assignment of taxonomy

517   to environmental sequences (Visco et al. 2015). Considering the advantages and limitations

518   of DNA metabarcoding technology, we propose using both 18S and COI as markers to reveal

519   the breadth of zooplankton diversity in the Sanmenxia Reservoir to overcome the inherent

520   limitations of using a single marker.

521

522   **CONCLUSION**

523   Zooplankton communities show obvious seasonal variations in the Sanmenxia Reservoir.

524   Temperature and nutrients are the most important water environmental factors affecting the

525   seasonal changes in zooplankton communities. The results of this study provide data support for

526   aquatic biodiversity protection in the Sanmenxia Reservoir and for sustainable ecological

527   management.

528

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533

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Alpha diversity of the entire zooplankton assemblages in each sample.

Samples	18S				COI			
	OTUs	reads	Shannon	ACE	OTUs	reads	Shannon	ACE
SUM_1	101	31787	1.73	110.83	130	22340	2.6	155.41
SUM_2	142	58326	2.1	160.11	139	22131	2.76	156.38
SUM_3	144	56263	1.95	170.04	135	21489	3.08	141.1
SUM_4	131	42619	2.23	143.83	145	20755	2.85	169.79
SUM_5	145	87908	2.16	174.61	119	21384	2.58	137.52
SUM_6	148	84124	2.27	179.04	122	21456	2.62	147.47
SUM_7	81	48914	1.45	92.75	136	19656	2.58	164.43
SUM_8	99	34553	1.59	118.58	98	18675	2.34	115.29
SUM_9	104	51766	1.57	123.03	96	18814	2.3	110.99
AUT_1	123	30108	1.67	168.92	112	5882	2.81	140.85
AUT_2	106	18216	1.54	136.19	116	3695	2.87	138.27
AUT_3	104	27958	1.19	129.64	112	5316	2.83	131.47
AUT_4	103	34195	1.81	140.86	120	9537	2.85	164.59
AUT_5	117	44036	1.48	162.35	101	4024	2.72	133.37
AUT_6	80	26275	2.08	98.48	146	8723	3.09	181.54
AUT_7	71	52425	1.64	85.9	118	14766	2.85	153.54
AUT_8	63	69162	1.58	86.26	102	19037	2.57	139.55
AUT_9	68	66918	1.57	91.2	103	16720	2.55	128.24
WIN_1	77	31180	1.66	103.39	76	17500	2.03	80.94
WIN_2	66	16455	1.63	76.45	49	2307	2.27	63.35
WIN_3	56	11396	2.05	98.19	56	4512	1.72	74.09
WIN_4	53	71055	1.14	108.61	71	21905	2.31	84.43
WIN_5	42	55232	1.13	60.79	77	21181	2.06	93.86

WIN_6	45	28844	1.11	52.05	74	21706	2.02	92.39
WIN_7	47	8251	1.8	65.28	57	2694	2.64	68.53
WIN_8	57	8569	1.69	82.21	65	6018	2.52	81.96
WIN_9	43	12647	1.34	65.47	72	5332	2.7	91
SPR_1	40	29215	0.74	60.15	66	7367	1.72	78.51
SPR_2	32	36306	0.64	34.9	59	19461	1.02	67.62
SPR_3	43	36212	0.87	85.35	79	11795	2.15	97.03
SPR_4	31	15650	1.36	50.68	87	14348	2.09	114.91
SPR_5	41	9195	1.6	69.85	78	12331	1.75	86.24
SPR_6	26	3916	1.84	35.99	79	8985	2.35	82.05
SPR_7	39	35357	0.92	46.03	68	21177	1.5	99.43
SPR_8	87	105749	0.92	113.01	85	22711	1.44	114.4
SPR_9	83	104381	1.12	111.27	93	20955	1.62	131.48

Results of taxonomic assignment of different metabarcoding datasets.

	OTUs	Phylum	Class	Order	Family	Genus	Species
18S	319	12	23	54	83	110	84
COI	395	4	7	19	42	80	117
18S+COI	/	12	24	61	111	174	190

Water environmental conditions (mean value ± SE) of Sanmenxia Reservoir. WT, water temperature; pH, pH; COD, chemical oxygen demand; TP, total phosphorus; NH4-N, ammonium; DO, dissolved oxygen; TN, total nitrogen.

Sample	WT(°C)	pH	COD (mg/L)	TP (mg/L)	NH_4-N (mg/L)	DO (mg/L)	TN (mg/L)
SUM	30.41 ± 0.38	8.11 ± 0.00	48.73 ± 3.71	0.04 ± 0.00	0.45 ± 0.04	6.97 ± 0.67	3.90 ± 0.48
AUT	15.97 ± 0.42	7.68 ± 0.04	22.98 ± 1.17	0.02 ± 0.00	0.20 ± 0.02	7.77 ± 0.42	4.79 ± 0.03
WIN	2.67 ± 0.13	8.10 ± 0.05	35.50 ± 3.71	0.07 ± 0.01	0.79 ± 0.05	8.31 ± 0.49	5.05 ± 0.08
SPR	16.66 ± 1.55	7.81 ± 0.04	30.56 ± 1.69	0.05 ± 0.00	0.23 ± 0.05	6.16 ± 0.16	5.30 ± 0.16

Figure 1

The number of different taxa recovered by using 18S (blue) or COI (yellow) under different taxonomic levels. (A) Phylum, (B) Class, (C) Order, (D) Family, (E) Genus, (F) species, (G) Protozoa, (H) Rotifera, (I) Copepoda.
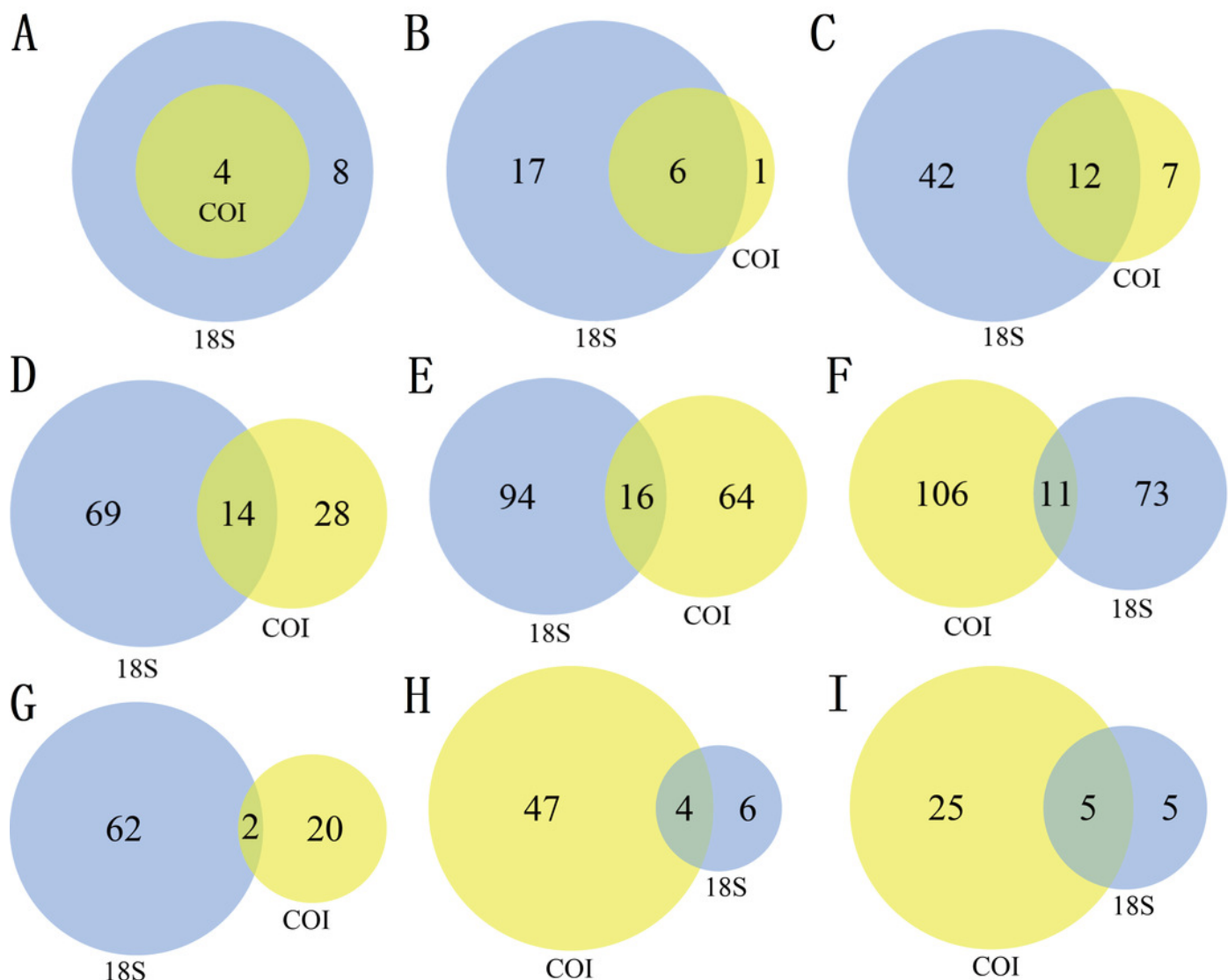
Figure 2

The difference in richness and relative abundance recovered in 18S or COI .
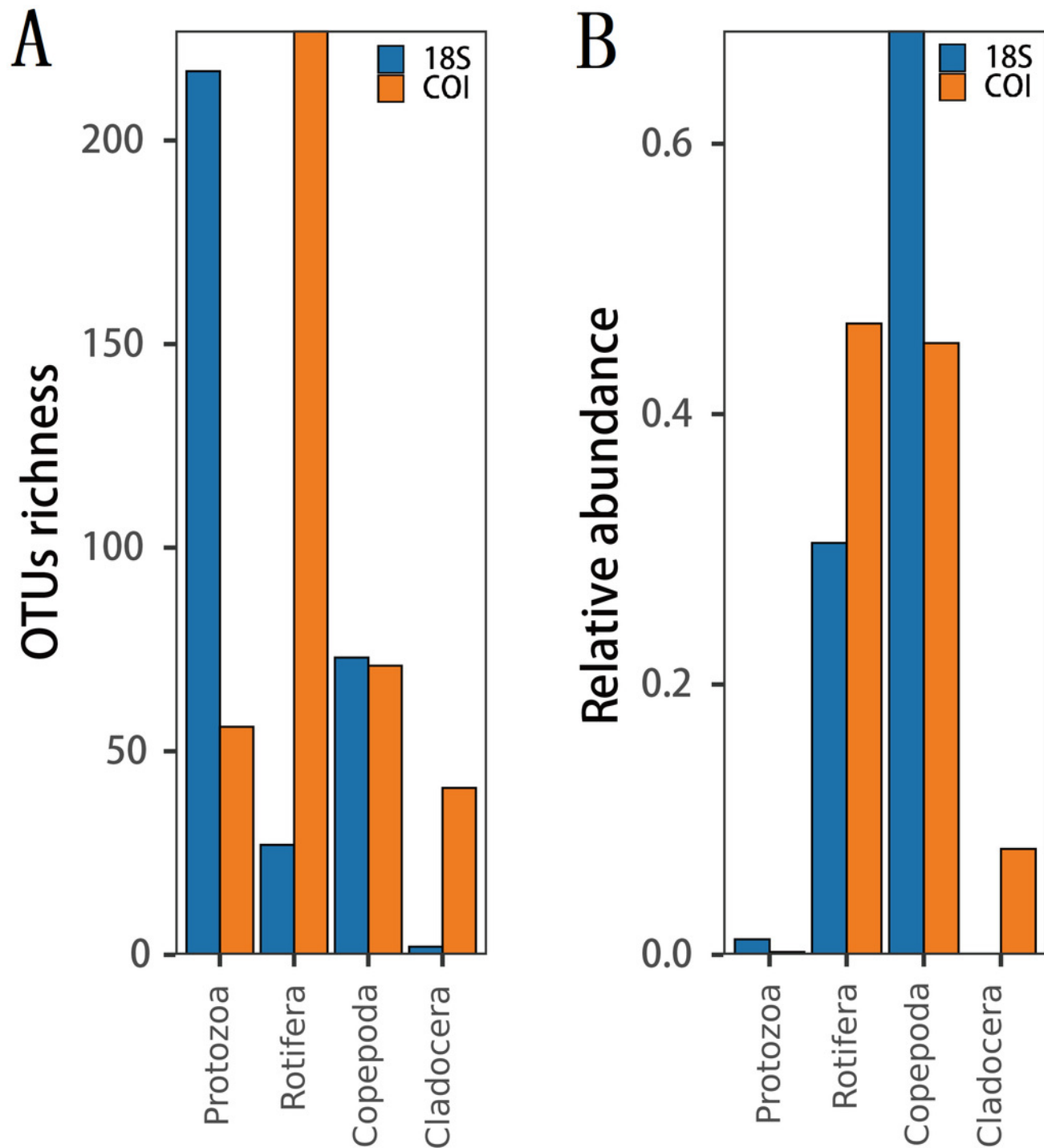
Figure 3

Different diversity estimators show significant differences in seasonal changes in zooplankton community based on different metabarcoding datasets.

(A) 18S Shannon diversity index (B) COI Shannon diversity index, (C) 18S ACE index, (D) COI ACE index.
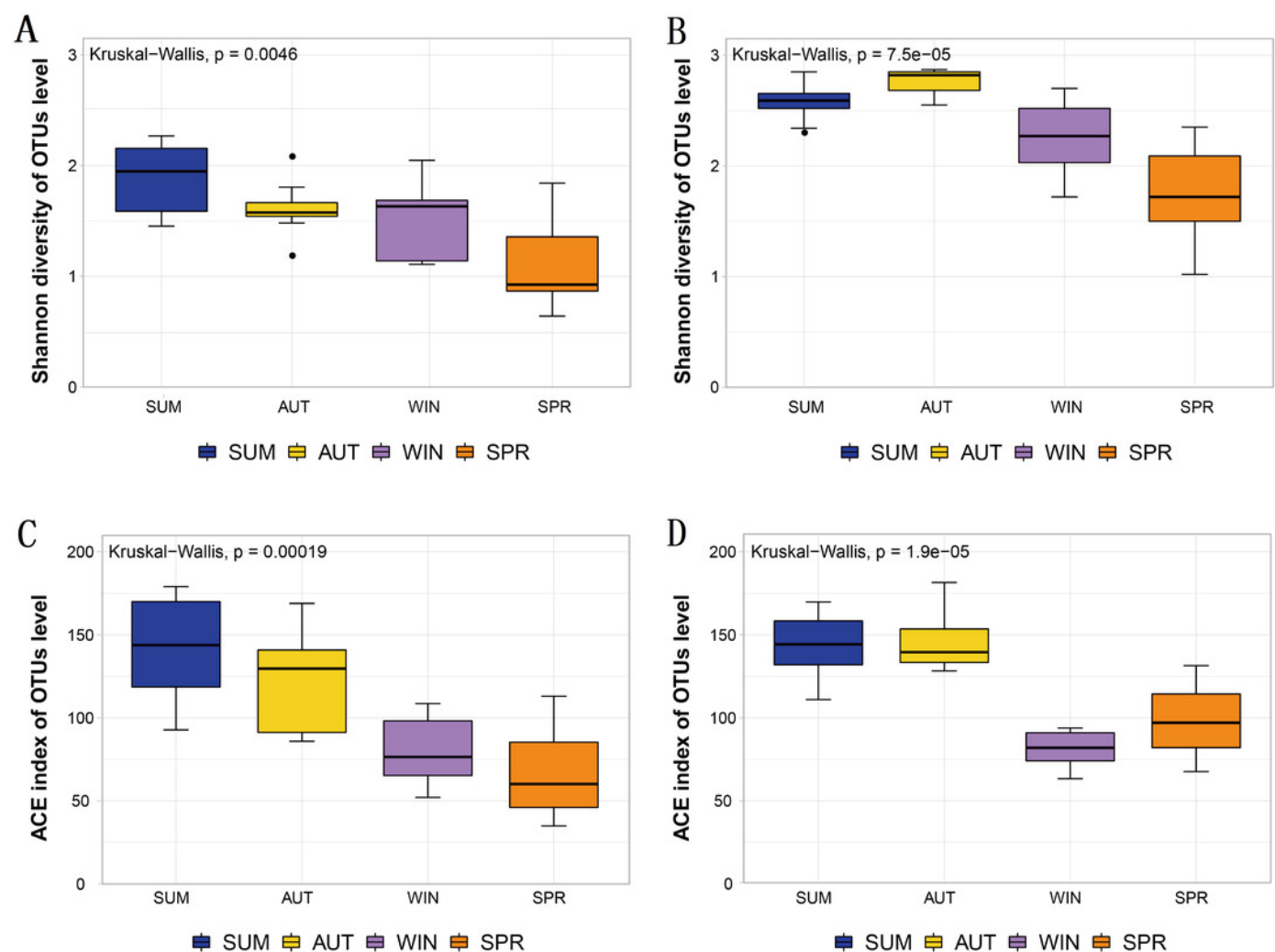
Figure 4

The richness of zooplankton groups in different seasons shows significant differences based on different metabarcoding datasets.

(A) 18S Protozoa, (B) 18S Rotifera, (C) 18S Copepoda, (D) 18S Cladocera, (E) COI Protozoa, (F) COI Rotifera, (G) COI Copepoda, (H) COI Cladocera. Seasonal changes in zooplankton groups were significant according to ANOVA and multiple comparisons between sampling seasons by using the Tukey HSD test. *, p≤0.05; **, p≤0.01; ***, p≤0.001.
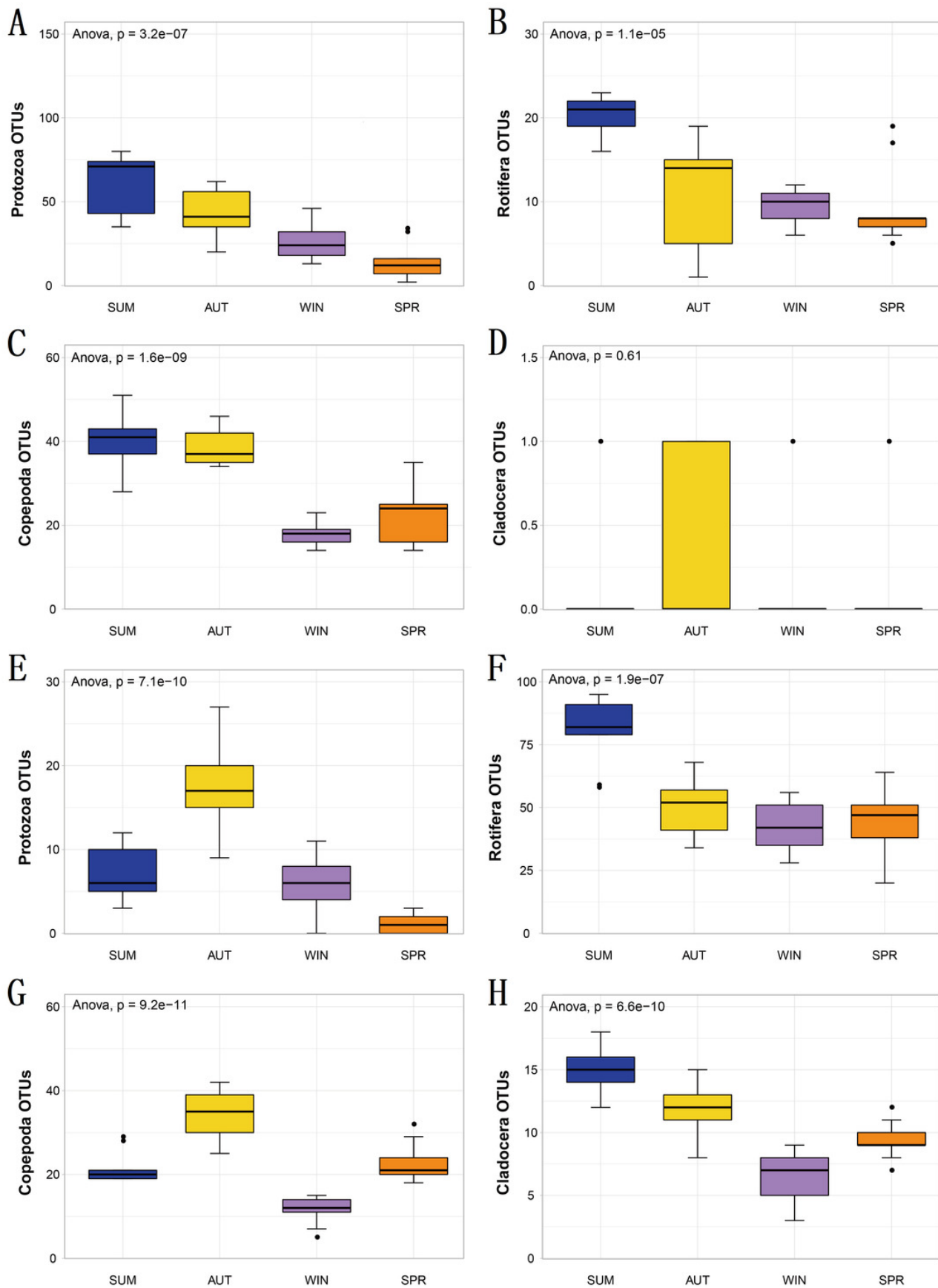
Figure 5

The seasonal variation in relative abundance of different zooplankton groups in different metabarcoding datasets.

(A) 18S summer & autumn, (B) 18S autumn & winter, (C) 18S winter & spring; (D) COI summer & autumn, (E) COI autumn & winter, (F) COI winter & spring. Variations from summer to autumn, autumn to winter and winter to spring are shown. Welch's t-test was used to compare the variation between two adjacent sampling seasons. *, P≤0.05; **, P≤0.01; ***, P≤0.001.
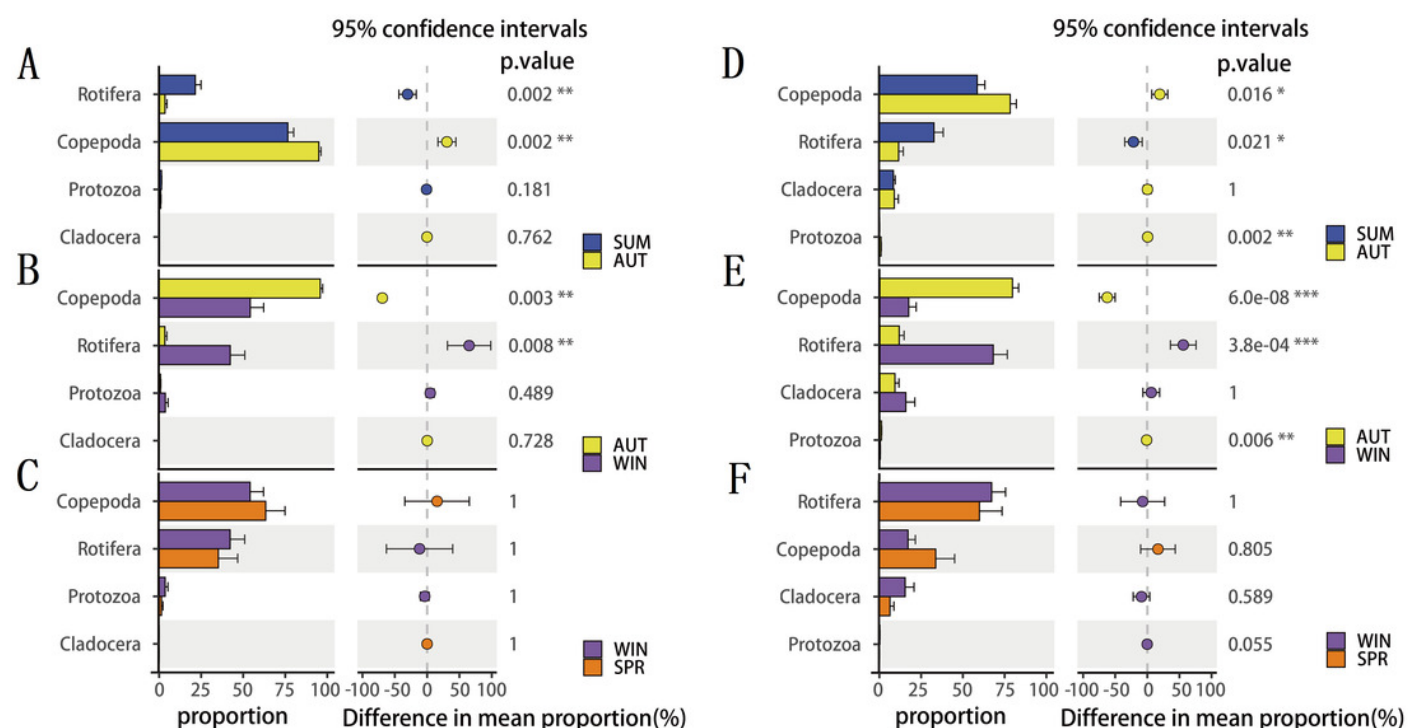
Figure 6

Clustering of the entire zooplankton community assemblages by sampling season using principal coordinate analysis (PCoA) based on the Jaccard dissimilarity index and Bray-Curtis dissimilarity index.

(A) 18S Jaccard dissimilarity index; (B) 18S Bray-Curtis dissimilarity index; (C) COI Jaccard dissimilarity index; (D) COI Bray-Curtis dissimilarity index.
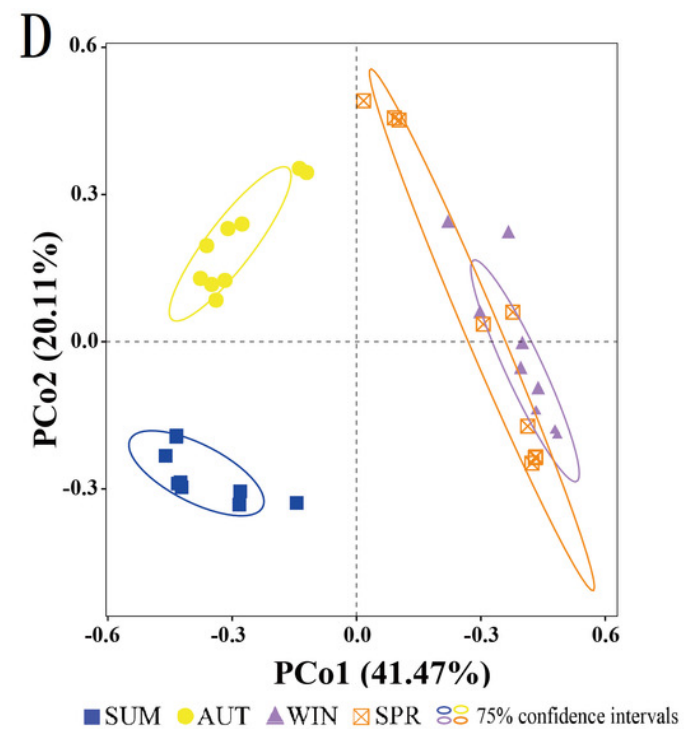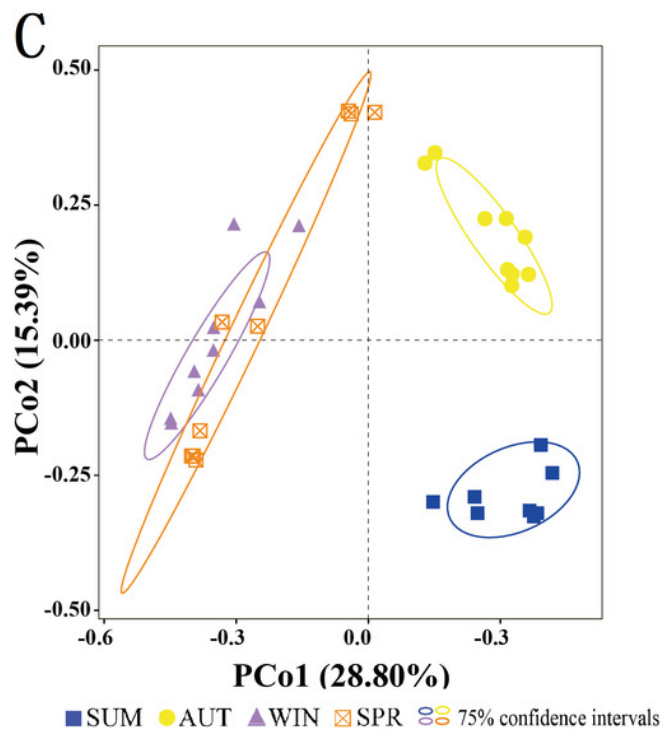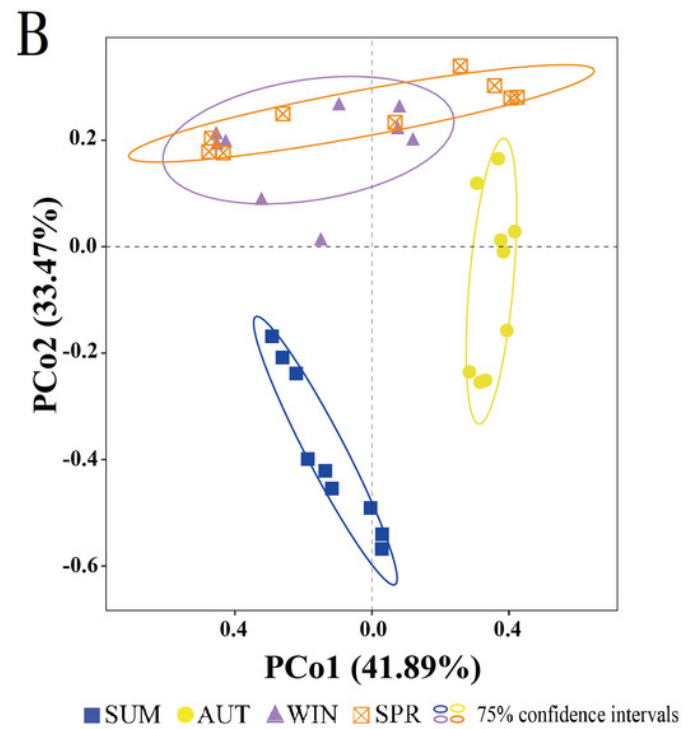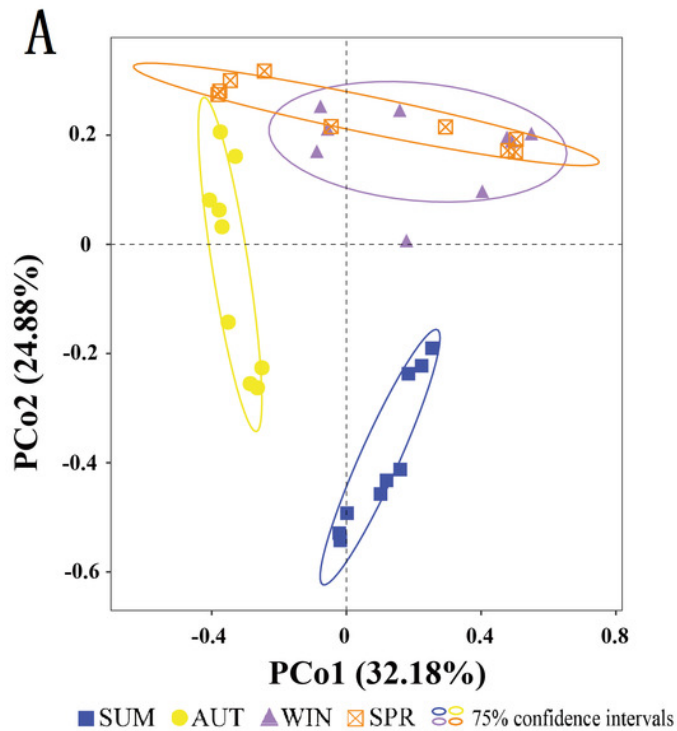
Figure 7

Relative abundance of all samples in different sampling seasons at the genus level.

(A) 18S rRNA; (B) COI.

A

Reads relative abundance (18S)

SUM   AUT   WIN   SPR

Brachionus   Cyclops   Eucyclops   Megacyclops   Mesocyclops   Microcyclops   Pseudodiaptomus   Sinocalanus
Others(<0.5%)   unidentified   Undinula   Daphnia   Keratella   Moina   Synchaeta   Cyrtonia
Macrothrix   Trichocerca   Rotaria   Chydorus   Epiphanes   Tortanus   Polyarthra   Asellopsis
Simocephalus   Scapholeberis   Diaphanosoma   Vermamoeba   Diacyclops   Platyias   Plationus   Lecane
Leptodiaptomus   Drepanopus   Paracalanus   Acanthocyclops   Sinodiaptomus   Kellicottia

B

Reads relative abundance (COI)

SUM   AUT   WIN   SPR

Brachionus   Cyclops   Eucyclops   Megacyclops   Mesocyclops   Microcyclops   Pseudodiaptomus   Sinocalanus   Others(<0.5%)
unclassified   Eudiaptomus   Vorticella   Testudinella   Peridiniopsis   Notholca   Ectocyclops   Asplanchna   Hexarthra
Gregarina   Collotheca   Rhogostoma   Carchesium   Acineta   Heteromita   Macrocyclops

Figure 8

Water environmental factors driving seasonal changes in the zooplankton community.

Pairwise comparison of water environmental factors was achieved by using Spearman tests, and the color gradient represents Spearman's correlation coefficient. OTUs from different metabarcoding datasets (18S rRNA, COI) were related to water environmental factors by using partial (geographic distance-corrected) Mantel tests. Edge width: Mantel's R statistic; edge color: statistical significance (based on 9,999 permutations). (A) 18S rRNA; (B) COI.
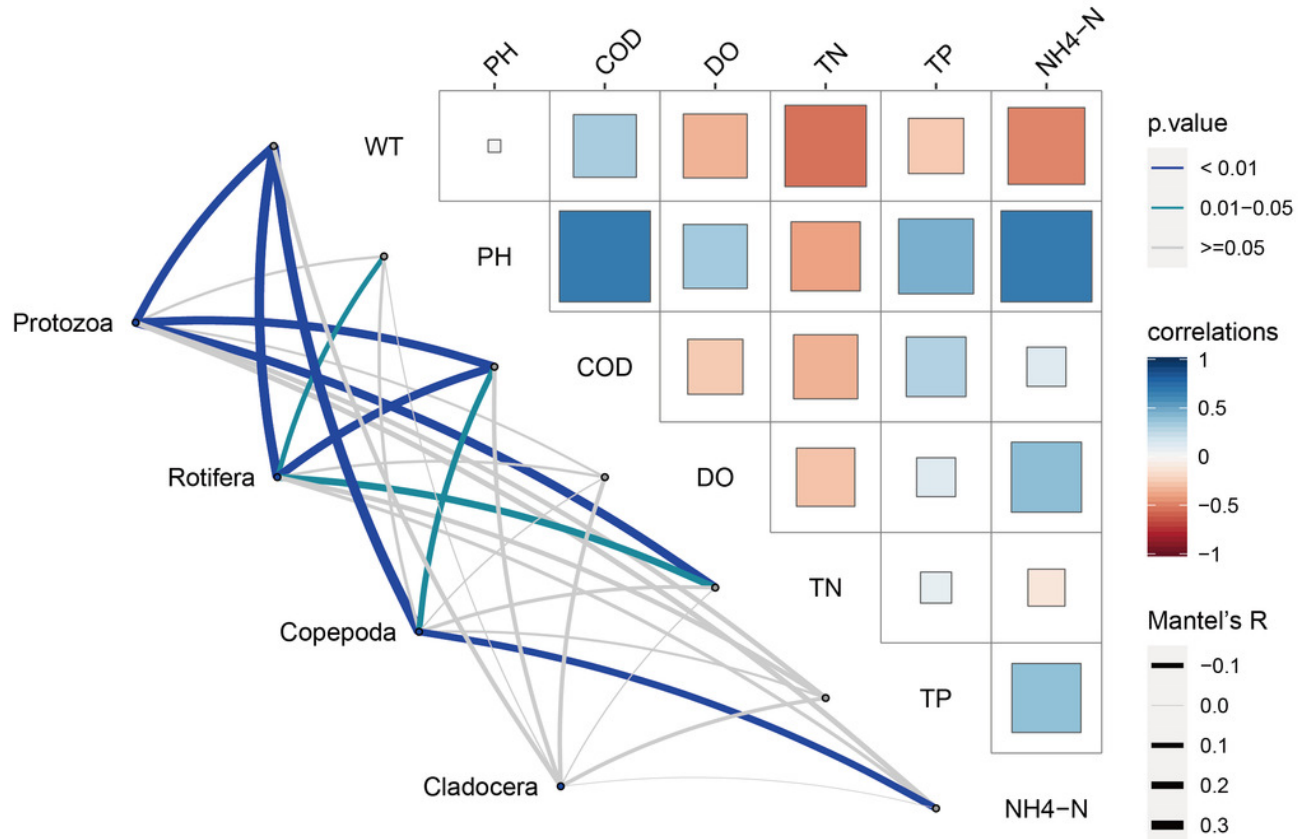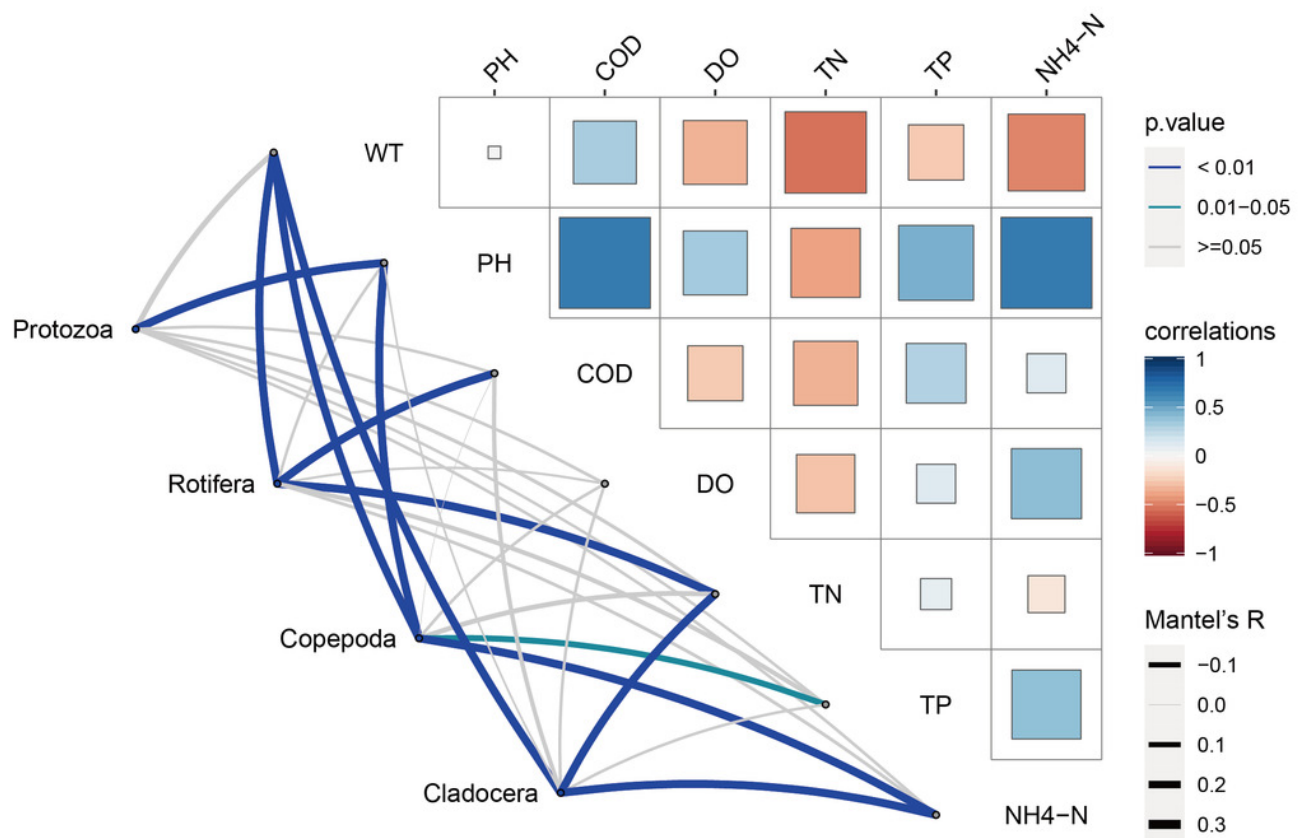
Figure 9

Canonical correspondence analysis (CCA) of the relative abundance of the zooplankton community in different metabarcoding datasets.

WT, water temperature; pH, pH; COD, chemical oxygen demand; TP, total phosphorus; NH4-N, ammonium; TN, total nitrogen. (A) 18S rRNA; (B) COI.